UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

BIOLOGY

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DEVELOPMENTAL AND EVOLUTIONARY IMPLICATIONS OF COLD SHOCK EFFECTS IN THE SPECKLED WOOD BUTTERFLY

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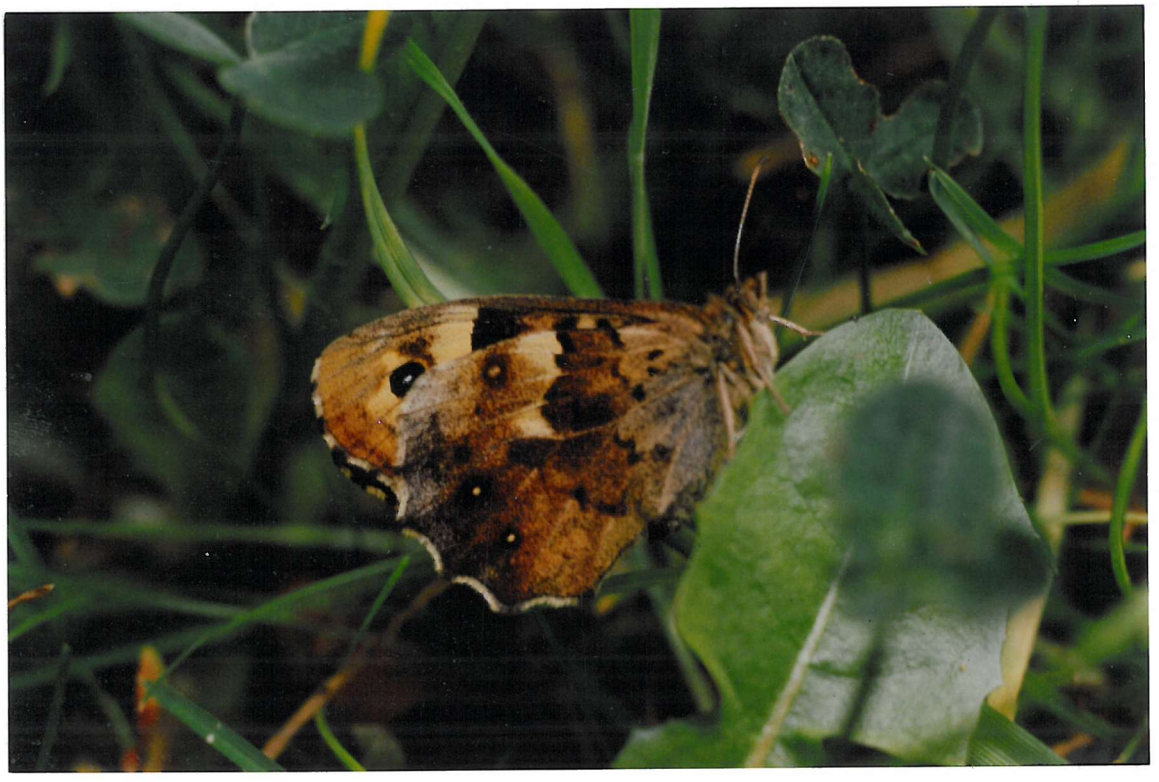

PLATE 1 (Frontispiece, preceeding page, above and below). Male and female Speckled Wood butterflies, basking.

PLATE 2 (Frontispiece, opposite, above and below). Male and female Speckled Wood butterflies, at rest.

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

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Doctor of Philosophy

DEVELOPMENTAL AND EVOLUTIONARY IMPLICATIONS OF COLD SHOCK EFFECTS IN THE SPECKLED WOOD BUTTERFLY

by Leonard Winokur

The effects of pupal cold shock on the life cycle and wing morphology of the Speckled Wood butterfly are examined and their genetic assimilation is investigated. Metamorphosis is modelled in terms of changes in stability, and the mediation of cold shock effects by hormones is considered.

Current theories of pattern formation are evaluated for the species, and pattern is analysed using manual, photographic and digital methods. The development of wing morphology is modelled, and cold shock effects understood by comparison with normal development.

Developmental canalisation is estimated as variability and fluctuating asymmetry. An index is developed that predicts the extent of assimilation. Likely modes of inheritance are suggested, and the possibility of natural cold shock and assimilation in the species is considered.

Recent trends in biology indicate that neo-Darwinian concepts cannot adequately account for certain developmental and hereditary phenomena and that a new paradigm is emerging. The two schools are compared with particular reference to Weismann and Waddington, and the phenomenology is re-examined in the light of the new findings.

The central theme of this thesis - the inheritance of acquired characteristics - is one that most biologists and non-biologists regard as long since dead. The reasons for this have a somewhat long and intricate history, but require examination if the real issues involved are to be correctly understood. This is particularly pertinent today, because the theory of evolution itself is now under threat - from the very phenomena that present to it and which call for a fundamental revision of both its philosophical foundations and investigative aims. Hence the thesis will attack the theme from *both* directions.

The experimental basis of the project concerns the inheritance of acquired characters, or in technical parlance, genetic assimilation. This is examined at progressive levels of organisation, starting with the relevant thermodynamic principles, and concluding with its place in understanding adaptation. Prior to these are the epistemological considerations which form an underlying thread that helps interpret the phenomenology; these considerations are largely centred on the introduction to avoid cluttering the empirical sections.

The thesis consists of two parts and opens with an introduction to the main aims and methods that outlines only the core essentials of the background theory. Since the aims and background are elaborated in the following chapters, literature citations are omitted here for brevity and clarity.

The first part overviews the main tenets of neo-Darwinism and the emerging school of thought, and compares both their similarities and differences. Concepts are illustrated with biological examples wherever possible and particular emphasis is given to this. It continues with a chapter on seasonal polyphenism with special reference to Lepidoptera, which is followed by a chapter which reconsiders the work and ideas of C.H. Waddington in the light of subsequent developments. The fourth chapter examines a number of epistemological problems facing evolutionary theory and their implications.

The biology of the Speckled Wood butterfly is reviewed in the fifth chapter which leads on to the empirical text. Each chapter concludes with suggestions as to how the findings might apply more

generally.

Certain terminologies have in the past led to much confusion and misunderstanding. Thus particular care is taken to define them adequately here, and these definitions are adhered to throughout. Examples include the precise meanings of 'form' and 'prepattern', and the distinctions between 'mechanism' and 'reductionism' and between 'genetic' and 'hereditary'.

Two additional frameworks - physics and theology - have had tremendous bearing on modern biology, the former because it has long been assumed the lower level 'cause' of biological phenomena, the latter because it provides higher level metaphysical bases into which paradigms may fit. Modern physics and chemistry have made a substantial contribution towards understanding biological complexity, and their concepts are taken up again in a move towards resolving some of the conceptual difficulties that confront the study of morphogenetic fields and their regulation.

Religion has stood largely in opposition to biological thought. Recent trends in theology itself, however, call for a reconsideration of its ontological foundations that brings it into accord with Natural process. These trends have been acknowledged by physics, but biology still tends to see religion as one of a hotchpotch of ideas that do not really require connecting up. Since this lack of connection also ties in with other reasons for the resistance to change, it is felt fitting to outline them after introducing the new paradigm. However for those interested in pursuing particular areas in more depth, a list of selected readings is given following the literature citations.

The traditional stance in relation to naïve Lamarckism - the inheritance of acquired characteristics - is that it cannot happen. However, in the early twentieth century, C.H. Waddington carried out experiments showing that it could. He demonstrated that environmental stimuli could alter various traits, and, by selectively breeding from those individuals that reacted most strongly and then repeating the treatment at each generation, was able to produce the characters in the absence of the original stimuli.

His explanation was that the characters in question were under the control of several genes; their number was such that under standard conditions they did not exceed a critical threshold for expression. The eliciting stimulus lowered this threshold so that the underlying genetic variation was revealed, enabling selective breeding to build up a gene complex in which their number did exceed the threshold for expression under standard conditions. He called this process genetic assimilation, and it represented the framing of Lamarckian phenomena in neo-Darwinian terms.

He drew the analogy of possible courses of development with a landscape of peaks and troughs - the epigenetic landscape - whose topology was set up by the genome and therefore modifiable by selection. Development was conceptualised as the path of a ball rolling over the landscape, whose particular course or 'pathway' depended along which trough or 'canal' it progressed. Perturbation (genic or environmental) could dislodge the ball, which then would either return to its original pathway or enter another, depending on the severity of the stimulus and how deeply into the landscape the pathway was set or 'canalised'. Selection could modify the landscape topology so that a pathway became more or less canalised, *i.e.* stable to perturbation.

Genetic assimilation was now understood as follows:- Given pathways are canalised to varying extents in different individuals. Those individuals that are least canalised, *i.e.* most evidently express some *alternative* pathway in the presence of a particular perturbation or 'stimulus', are selectively bred, which increasingly deepens canalisation of the alternative so that *it* is eventually

expressed in the absence of the original stimulus. The epigenetic landscape thus provided a scheme whereby organisms could register environmental stimuli and take them on board as hereditary phenomena.

The empirical component examines the possibility of genetic assimilation in a common British butterfly - the Speckled Wood, Pararge aegeria L. Most Lepidoptera produce regular seasonal forms, and their genetic assimilation has been suggested in the evolution of certain geographical races. These forms and races are adaptive, this concerning mainly thermoregulation and predation.

The environmental stimulus used in the present study is a freezing temperature during the early pupal stage, which it is known can alter wing pattern in several other species and has also been invoked as a cause of some natural aberrations. Yet the possible influence of photoperiod, and the effects of temperature shock on the pupa itself and subsequent life cycle parameters, have been generally omitted from such studies. Thus the present study also controls for the dark period that the treated pupae undergo.

The study commences with an investigation into the relationships between the size and duration parameters of the main life cycle stages. A scheme is developed that models metamorphosis in terms of stability and complexity changes. It is generally understood that metamorphosis is guided by the relative concentrations of juvenile hormone and ecdysone, and although there are some anomalies, the above model can account for these and also explain why young pupae are particularly sensitive to perturbation.

The actual effects of cold shock on pupal and subsequent development are examined, and discussed in relation to ecdysone turnover. The possibility of natural exposure to cold shock by the species is also considered.

The assimilation of cold shock effects is then explored. Experimental studies of assimilation in Lepidoptera have not previously been done owing to the poor fecundity of laboratory phenocopies, but this may have been due to the rearing conditions, since the insects are extensively farmed and breeding programmes have proved successful with genetic aberrants. Thus a number of rearing methods are used and evaluated. A coefficient is developed that measures the extent to which lineages have previously experienced assumed perturbatory influences and can assess the extent to which

responses have become assimilated. The assimilation of characters not directly affected by cold is also examined.

Possible modes of inheritance are evaluated. The extent to which the protocol reflects the phenology and breeding structure of wild P. aegeria, and the possibility of natural assimilation in the species is considered.

Wing development is investigated. Size parameters are measured by microscopy and a chart is constructed for scoring colour. Venation is examined and its current nomenclature adjusted so as to bring forewing and hindwing into homology and unify various descriptive schemes.

Interactions between wing features are explored in relation to venation and groundplan elements. Sex and family differences are then examined. These are discussed with reference to current theories of development, which include progressive division of the wing into distinct regions and pattern organisation around the wing veins and eyespots, and the P. aegeria wing morphology is modelled.

The extent of canalisation as evidenced by variability and asymmetry is evaluated. The latter assumes that both sides are of identitical (genetic) constitution so that any difference between them must be due to perturbation.

Further examination involves the use of (monochrome) photography to avoid excessive handling of the specimens. Deformity and total wing development failure are examined at this stage. The photographs are perused and the total set of cold shock effects is collated. These are understood by comparison with normal development and the means by which they are mediated is considered. The extent of assimilation as evidenced by changes in their frequency and expression is evaluated.

The number of unaffected features, however, precludes their individual analysis. A digital technique is therefore developed to analyse overall pattern that yields statistical descriptions of the wing surface; the method can also be used to examine shape. These descriptions are amenable to quantitative comparison and have the advantage that they automatically capture contingent features without prior reference to their individual description.

The effects of treatment and their assimilation are again examined. The likely modes of mediation and inheritance are evaluated in connection with the behaviour of life cycle aspects. A general model of evolution in the species is presented and the role of genetic

assimilation here is considered.

In contrast to Popper who viewed science as being composed of contingent tenets and progressing through the piecemeal process of conjecture and refutation, Kuhn viewed science as comprising conceptual structures - paradigms - that superceded their predecessors when the latter proved refractory to the problems confronting them, that is, reached crisis state. Paradigms are the conceptual and methodological frameworks that comprise the respective disciplines; indeed it is the implementation of science within their guidelines that distinguishes it from non-science.

Evolutionary theory is currently in the throes of Kuhnian crisis, and a new paradigm is emerging. Recent considerations have shown that the neo-Darwinian concept of inheritance, which arose out of the Weismann doctrine, provides but a partial picture of hereditary phenomena. In addition, it views the genes as the a priori cause of phenotypic traits, whose hereditary variations are thus to be ultimately traced back to mutation. Together with the notion that recombination can bring together any assortment of genes - and hence traits - it has led to the view that the range of possible phenotypes is unlimited, the only costraint on the actual range being that of subsequent adaptive efficacy, through selecting which will enter for heredity.

Darwin was not an oracle, although he is often set up as such within neo-Darwinism. This is unfortunate, for it has resulted in a biology that does not define itself as 'that which treats of living things' - the dictionary definition, but in a manner that implies that concepts and even phenomena presented by organisms themselves which might falsify Charles Darwin, be framed within his terms, or better still, be avoided; in other words, in a biology that emphasises the political rather than the biological.

Crisis states are typically characterised by frequent appeals to philosophy, but biology over the past century has shunned virtually all such appeals, pretending that no such crisis state exists. As Waddington poignantly remarked, 'sociobiologists are just running scared of ferocious philosophers', yet nothing at all ferocious is involved. All observation is *necessarily* made in the light of some theory (for how else could one make sense of it?); and as experimentation is simply a means of extending its unaided limits, it

too must be presupposed by theory (for how else would one decide which parameters to control?) - theory whose basis for inception represents an epistemology.

Waddington was one of the first to put his epistemology to use in actual experimental situations, and reopened the door to a conceptual framework that was buried alive about the time of Weismann. This framework is one which acknowledges the role of development in evolution, and which draws upon the ideas of several workers including Piaget, Lamarck and Weismann.

Rather than pathways exploring a landscape set up a priori by the genome, the topology is a thermodynamic one which itself arises epigenetically in an attempt to minimise the reactivity (total potential energy of) the system (which may be increased by perturbation), pathways representing the means through which this minimisation, *i.e.* canalisation, is achieved.

The set of available pathways depends on both initial conditions and certain formal constraints on differentiation and organisation, the latter mainly presenting themselves as morphogenetic fields. The immediate source of these conditions and constraints is inherited factors, which can involve modes of inheritance other than just DNA and with environmental influences allow for the transmission of epigenetic potential.

Self-organisation is a central tenet of biological structuralism, born of Piaget, in which traits are understood to develop in relation to one another. Each component is a 'form' whose identity is derived from its position within a set of relations or 'type', the evolution of which involves changes in the presence or quality of selective components as mediated via *ontogeny*. An example of such a type is the Nymphalid wing pattern groundplan.

Such relatedness is relevant to canalisation, both in normal development because it constrains the pathways concerned by limiting their individual potential; and under perturbation conditions because it serves to organise the pathways, so providing them some power of self-correction; in other words, because it determines the kinds of transformations that are possible.

The new paradigm however, still lacks a formal scheme of adaptation, and this is a crucial issue because natural selection - the only one biology has yet come up with - remains its major bone of

contention. The new school argues, justifiably, against its a priori creative role - a cornerstone of neo-Darwinism - on the grounds that it can act only on forms already extant. But it currently appears to be relegating *any* role that it may play, so running the serious risk of forcing the two paradigms into diametric opposition. Yet rather than standing in opposition, the new paradigm should be more encompassing.

The problem of adaptation is met by extending structural principles to external relations. Pointers in this direction have already been indicated by several workers from both paradigms.

Adaptiveness refers to the stability of forms as *replicative* entities whose adaptive function derives from their role within an organism-environment interaction; ontogeny is a necessary constraint as it delimits the set of functional and structural possibilities available for entry into such relationships *i.e.* adaptive solutions.

Since organisms can choose their niche, organism-environment interactions too are self-constructing. Again the component members tend towards stability, as replicative entities within the interaction, whose evolution involves changes in the quality or number of selective components as mediated via *heredity*. It is this process that is encapsulated by the concept of natural selection, which, since the reproduction of forms necessarily involves reproduction of the entire organism, concerns evolution at the species level; and whose 'types' include Darwin's 'common ancestors'. The combination of structural and replicative possibility also resolves certain paradoxes concerning the definition of 'species'.

AIMS OF THE THESIS

The essential aim is to examine the effects of early pupal cold shock on the life cycle and wing morphology in the butterfly Pararge aegeria, and their possible genetic assimilation. This insect was chosen for study rather than, say, Drosophila, as many Lepidoptera, including aegeria, show seasonal differences in the life cycle and wing pattern - a phenomenon known as seasonal polyphenism, the genetic assimilation of which has been implicated in the origin of certain geographical forms yet never tested in the laboratory. Cold shock was chosen as the experimental stimulus as it has already proven able to modify wing phenotype in other species: such phenotypes often resemble naturally occuring aberrants, and in species whose pupae likely experience frost in nature. Here too genetic assimilation has been implicated in the origin of certain wild, genetically determined, forms but again never tested. Moreover, the effects of cold shock on the life cycles have yet to be examined, although at least one species is known to have undergone a change in voltinism.

The first chapter explains how the study of development can further our understanding of evolution: since evolution involves the evolution of phenotypes, and since phenotype arises through embryonic development, evolution must consider development. Weismann's hereditary scheme of germ plasm and soma is elaborated to allow for levels above the gene and environmental influences; and models of pattern formation are described. Chapter Two reviews Lepidopteran seasonal polyphenism.

The third chapter explains Waddington's concept of developmental canalisation, namely that the ability of environmental and genetic factors to influence development depends on development being more or less labile to potential disturbances. Genetic assimilation - an increase towards heredity in the relative importance of heredity and environment in determining phenotype, is discussed in depth; and Waddington's evolutionary scheme is put into current context.

Chapter Four focuses on the philosophical problems facing modern evolutionary theory. A new paradigm is emerging, but one usually seen as antithetical to traditional Neo-Darwinism. This chapter examines how traditional evolutionary thought arose, aims to meet criticisms

levelled against the emerging school, and attempts to construct a more unified understanding of evolution. This chapter necessarily goes into considerable depth and readers already convinced by the empirical arguments may wish to skim or skip this section.

Chapter Five reviews the natural history of P. aegeria and opens by describing its particular suitability for experiment: namely that it exhibits seasonal polyphenism; occurs as several geographical races differing in phenotype and voltinism; has a variable life cycle strategy dependent on both environmental and genetic factors; and is also easy to rear.

Chapters Six to Ten present the experimental findings; and Chapters Eight and Nine, especially, are very long since the complex and voluminous data required lengthy dissection in order that they be sufficiently critical. This has resulted in the conclusions becoming seemingly intertwined with their respective analyses. Thus each chapter opens with a short summary of the overall aims, methods and findings before going on to justify these in depth.

Chapter Six examines the relationships between linear dimension at each moult and the time between moults. A model of metamophosis is presented in which linear growth describes a sigmoid form with a pre-pupal deviation (towards maximum length). This deviation is understood in terms of the properties of larval integument; and is conceptualised as a disturbance which triggers adult development and which might render early pupal development susceptible to environmental perturbation. The evolution of pupal polyphenism, and of holo- versus hemi-metabolous metamorphosis, are discussed.

Chapter Seven examines the effects of early pupal cold shock, and of the concomitant darkness, on pupal and post-pupal life cycle parameters; which had hitherto not been examined in Lepidoptera. Sex and family differences are examined under both control and experimental conditions to ascertain the role of genetic factors in development. A number of timings of treatment application are tested. The effects of cold and dark are understood in terms of their influence on ecdysone and juvenile hormone turnover; the possibility that aegeria experiences natural frosts is assessed.

Chapter Eight explores the potential for genetic assimilation of cold shock effects. An index is developed to quantify the extent to which any lineage has previously experienced (in this case) cold shock; and life cycle parameters are compared with this index to evaluate the progress of any genetic assimilation. A number of stocks of the butterfly are used and these are examined both overall and individually. Survival is assessed, as are the possibilities that selection and the various culture regimes might bias the findings. Inbreeding and assimilative effects are compared and distinguished. The merits of each culture regime are examined; and the extent to which the protocol reflects the species' natural ecology and breeding structure is assessed.

Chapter Nine examines the effects of cold shock on wing morphology. The wing pattern is described in terms of Schwanwitsch's Nymphalid Groundplan; and each element is examined. Venation is also considered. Differences and correlations among the surfaces are examined with respect to wing compartmentalisation. Developmental stability as evidenced by random asymmetry and variability is also assessed. Sex and Family differences are examined to ascertain the role of genetic factors in patterning. Wing development under control, dark, and cold shock conditions is compared. The chapter concludes with a model of wing development, originating at the wing base and then progressing with the venation, to describe a pattern of cell division and growth exhibiting both morphallactic and epimorphic properties. A number of other species are also examined to help evaluate the model.

The final chapter examines the nature and frequency of wing phenotypic effects in relation to the lineage treatment histories to evaluate any ongoing assimilation. Normal and wide-ranging cold shock phenotypes are modelled in terms of a common morphocline commensurate with progressively larger reaction-diffusion domains; and the general implications of the model are considered. The use of statistical descriptors of shape to study wing morphology is assessed: these descriptors are computed from digitised images. Photographic replicates are used to examine surfaces within a single specimen and simulated damage effects. The amenability of the aegeria breeding structure to assimilation, possible changes in voltinism, and the evolution of subspecies phenotypes are then discussed.

PART ONE

THEORY AND BACKGROUND

CHAPTER ONE

Summary

The current view of evolution is that phenotype is the logical outcome of gene expression, with phenotype evolution explained by genetic mutation and recombination, and selection. This chapter explains how this view arose from Weismann's scheme of Germ Plasm and Soma, introduced as a hereditary scheme to complement Darwin's theory.

Weismann's scheme accounts only for the production and inheritance of *traits*, but not for that of their spatial relationship or *form*. Since the attainment of form involves cellular differentiation, but because all cells derive from a common nucleus, clearly the nucleus *alone* cannot account for development. Indeed development depends on the *relationship* between nucleus and cytoplasm, between which information flows both ways.

Embryonic development is understood as being initiated by introducing an instability to the system, as at fertilisation. Form is attained as the system minimises the resultant free energy through an increase in complexity. Organisation is an adaptive corollary: the more complex a system, the more organised it needs to be to survive. Complexity also tends to increase in evolution as the loss of parts from organised systems are mostly harmful, whereas additions can be beneficial.

Weismann's scheme is elaborated in Goodwin's 'Generative Field' in which what is inherited is the capacity to generate form, through the relationship between inherited particulars (including, but not exclusively, DNA) and environmental influences: it can thus encompass genetic assimilation.

The relational nature of developing systems endows them with the properties of self-organisation and self-correction. Models of patterning and differentiation can help explain *how* things vary; and a number of such models are described. Self-organisation also delimits the possible *effects* of mutation and environmental influences, ie. *what* varies; and can provide insight into *rates* of phenotypic change in populations under changing environments.

CHAPTER ONE

EVOLUTION AND DEVELOPMENT

"We will not articulate a comprehensive theory of evolutionary mechanisms until we can include it in critical reference to an equally complete understanding of (a complex theory of) the causes and control of development." (Thomson, 1985).

1.1 Preamble

The period of the nineteenth century during which Darwin worked was one of economic prosperity. Earlier that century the economist Malthus had proposed his theory of exponential growth and Darwin saw its implications for nature. Organisms produce far more offspring than their environments can support so some must die. The ethos of this period encouraged the striving for betterment, which entered Darwin's thinking as organismal competition with the weeding out of poor performers (Gould, 1980). This weeding out process selected particular individuals from a random variety (hence the term natural selection), leaving the better (fitter) individuals to reproduce and so transmit characteristics to the offspring (Darwin, 1859).

The main problem facing Darwin however was his lack of any theory of stable inheritance. According to Darwin, the propensity to produce characteristics resided in particles or gemmules which circulated in the blood to produce their effects on permeating the appropriate tissues. Their effect depended on their type and number, and when animals bred they returned to the gonads to be mixed in the offspring producing features intermediate to the parents'. Thus was borne the concept of blending inheritance (Bowler, 1984).

Blending inheritance posed a serious threat to Darwin as it could not account for the faithful reproduction of forms on which natural selection could act (*ibid.*). A doctrine which did however account for faithful reproduction was that of August Weismann (1893) which essentially rescued Darwinism by invoking hereditary particles as causative, rather than representative, agents of the entire organism.

These agents or 'determinants' comprised the germ plasm which was centralised in the reproductive cells or germ line. As the body of the organism or soma developed particular determinanats would be reproduced and take effect in the appropriate tissues. But when animals bred they did not return to the gonads, so that the only ones transmitted to the offspring was those of the germ line which was effectively immortal (*ibid.*) (Figure 1.1). With information flow from the soma to the germ line precluded (borne out by a mouse experiment in which tail amputees failed to produce tailess offspring) any notion of Lamarckism could now be safely set aside (Bowler, 1984).

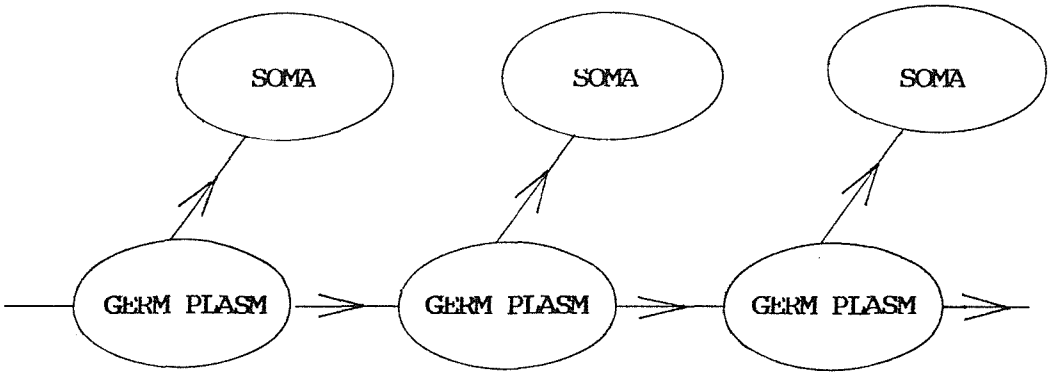


Figure 1.1. Weismann's hereditary scheme: germ plasm and soma. The adult organism is understood to comprise the germ plasm (reproductive tissue) and soma (non-reproductive tissues).

The germ plasm is effectively immortal, being continuous between the generations when it reproduces the soma (heavy arrows). But information can not flow to the germ plasm *from* the soma, which dies after each generation. Hence there is no somatic continuity between the generations nor a means by which changes to the soma (acquired characters) might be transmitted to the germ plasm. Evolution comes about through changes in the germ plasm units or 'determinants'. These are now understood to be the genes and the relevant germ plasm to be the gonadic nuclei.

When the nucleus was discovered and Morgan established that chromosomes were the carriers of hereditary information, the germ plasm became located in the nucleus (Webster & Goodwin, 1981). The further development of chromosome theory provided mechanisms whereby the units of heredity (genes) could be altered and shuffled in subsequent generations.

Natural selection acts on phenotypes (Ho & Saunders, 1979) but was understood to effect evolutionary change by determining which of them would transmit genes to the offspring. It had been tacitly recognised that phenotypes arose during embryonic development, which was now to be understood in terms of the Weismann doctrine. This gave birth to a school of ontogeny which mirrored the causal role of genes in evolution (Webster & Goodwin, 1982). This school was developmental genetics whose basis was largely one whereby genes control development (Webster & Goodwin, 1981, citing Monod, 1972 and Jacob, 1974).

The conclusion was that through mutation and recombination, anything could happen (Webster & Goodwin, 1982; Webster, 1984). The evolution of phenotypes became a theory of genes (Ho & Saunders, 1979); which (following on from Mendel) was expanded by Fisher, Haldane and Wright in the 1940's into mathematical models describing changes in gene frequency *viz.* quantitative genetics (Falconer, 1981).

These ideas were combined as the New Synthesis in which the phenotype and its inheritance were understood in terms of Weismann's doctrine; its variation in terms of random gene mutation and recombination by the rules of chromosome theory; and its differential survival described by the algebra of quantitative genetics (Maynard Smith, 1975). In 1953, Watson and Crick elucidated the structure of DNA, and genes came to be defined as molecular units whose response to selection (and hence the course of evolution) could be predicted with the algebra of quantitative genetics. Crick then proported his Central Dogma that the flow of cellular information was from DNA to mRNA to protein and *one way only*, which endorsed the Weismann doctrine once and for all. The new synthesis was updated, and culminated in a modern version of Darwinism - neo-Darwinism (Maynard Smith, 1975).

However, recent years have seen a renewed interest in the relationship between evolution and development, owing to growing dissatisfaction with neo-Darwinism. One reason for this is that it

endows the genes with 'demon-like' power (Ho & Saunders, 1979) and bestows upon natural selection an omnipotent creative capacity (Ho, 1975; Ho & Saunders, 1976) yet still fails to provide concepts that adequately explain the origin, transformation and inheritance of form (Webster & Goodwin, 1981). Another is that it vehemently resists the exploration of alternative schemes which may do so (Ho *et. al.*, 1986). The reasons for the latter are predominantly psychological (Davenport, 1979), but to understand the former we must return to the Renaissance.

1.2. The blind watchmaker "Our historical analysis however [is] not concerned to understand the past but to liberate ourselves from the present." (Webster & Goodwin, 1981, p40)

The study of celestial bodies in the seventeenth century revealed that they moved in regular trajectories, whose predictability was compared to that of wheel-based machines. With the successful construction of time-keeping devices, the universe came to be viewed as a vast machine, explained in terms of cause and effect or mechanism. Religious explanations were however still prevalent, only influenced now by the philosophy of Newton. God was invoked as a great mechanic, on account of whose humanoid nature the Universe had purpose, this purposefulness being the argument from design (Davenport, 1979).

Darwin threatened the argument from design in transferring the creative power to natural selection, which since it acted on variations that were merely random, removed any purpose from its creative role (*ibid.*). With purposefulness now gone, the watchmaker was blind (cf. Dawkins, 1987), although organisms were still viewed as living machines, a view which permeated the Weismann doctrine with its causal account of biological form. But two further discoveries took mechanism to the extreme and finally dismantled the argument from design. The first was that of the origin and evolution of simple biological molecules (such as amino acids) and procells. The second was that of gene mutation as the ultimate source of all novelty. The former gave vent to the view that organisms were constructed through a process of blow by blow assembly (Davenport, 1979, citing Monod, 1971), whilst the latter asserted that the constituent parts had arisen by chance.

"In other words," quoting Davenport (*loc. cit.*), "biology claimed to have shown not only that the watchmaker was blind, but also that the watches were constructed by accident."

God having been stripped of His all-powerful creative potential, this role became assigned to natural selection and subsequently to the genes (Ho & Saunders, 1979). This reaches its epitome in the concept of the selfish gene (Dawkins, 1976) in which organisms are nothing more than vehicles through which the genes ensure their own replication. This selfishness could be taken as anthropomorphic because it implies intentionality. Another problem is that a *self-directing* genic evolution is incommensurate with one effected by *random* selective forces.

Prebiotic evolution has also proven to be epigenetic and constrained by structural and functional considerations (Davenport, 1979). For example, the formation of simple organic molecules depleted the inorganic species involved in their *de novo* synthesis so precluding their further *de novo* synthesis (Maynard Smith, 1975); and laboratory simulations of primeval conditions have shown that the resultant organic species are not random but tend towards particular sets (Fox, 1984). The lack of silicon-based life forms is explained by its inability to form a sufficient range of polymers (Maynard Smith, 1975). Hence prebiotic evolution can be understood without resorting to random or selective explanations.

1.3. The problem of form

Since all phenotypes arise through development, evolution by whatever means (random or otherwise) is the evolution of developmental processes. Therefore any understanding of evolution must consider development (Ho & Saunders, 1979).

Development can provide insight as to the laws and regularities of phenotypic morphology by looking at *what* things vary (Thomson, 1985). Phenotypes are composed of traits. Traits are properties of the organism such as colour or enzyme polymorphism and can refer to any structural level (Davenport, 1979). Form refers to the deployment of traits and describes the relationship between them rather than the set

of traits itself. It arises through epigenesis which comprises the four-dimensional history of the organism (*ibid.*). Thus, although DNA can account for heritable *differences* in traits (cf. Webster & Goodwin, 1982), being three dimensional it cannot possess an internal description of the *form* of their expression (Davenport, 1979).

Here, developmental studies of cellular and morphogenetic processes - in which there has been a resurgence of interest *in addition* to molecular and genetic ones - can provide insight by looking at *how* things vary (Thomson, 1985). Differentiation refers to the structural and functional changes a cell or tissue undergoes as it assumes its assigned role in the organism. The range of types into which a cell can differentiate is its potential, and the process by which its type is assigned is called determination (*ibid.*).

Cell structure and function reflect its protein composition. Hence determination chiefly concerns *nuclear* differentiation. The nucleus of all uncleaved eggs is totipotent and can differentiate into any cell type of the respective adult. But as all cells derive from a common mitotic nucleus, the information for initial differentiation must come from outside the nucleus. This information source is the cytoplasm. Determination necessarily involves a restriction of potential, but structures are also limited in potential by their functional relationships within the whole which therefore imposes constraint. In this sense, the whole is less than the sum of its parts (*ibid.*).

There is similarity between structural levels in that higher ones are *constructed* out of lower level components. In this sense the lower levels are a *prerequisite* of the higher ones, but they do not *cause* them; as borne out by emergent properties. Emergent properties are those a system possesses over and above its isolated components or a simple collection thereof (eg. those of sodium chloride not present in sodium, chlorine, or sodium *and* chlorine). Individuation (Davenport, 1979, citing Waddington, 1956a) refers to the phenomenon of emergent properties in the context of biological systems *viz.* their ability to attain form and pattern from an initial condition in which they are absent. In this sense, the whole is more than the sum of its parts. For example, a deme is not just a collection of individuals but involves gene flow and breeding systems. Studies of variability in natural populations can provide insight as to the *rate* of variation (Thomson, 1985). Indeed population genetics *must* consider development,

because a prediction of genetic changes in populations is impossible without a specification of the relationship between genotype and phenotype in fluctuating or uncertain environments (Lewontin, 1968).

Thus the causal relations between structural levels cannot be simply mapped to each other one-to-one. This is reflected in their descriptive terminologies so that causal explanations lose information (Davenport, 1979). For instance, some definitions of gene (inherited particular) include higher level concepts (learnt behaviours) not normally viewed as genes; and there are 'genes' (non-expressed DNA) excluded from some higher level definitions (cause of trait) (Ho & Saunders, 1981). Besides if higher levels really were simply caused by the lower ones then the logical conclusion is that biologists would do better studying physics (Ho & Saunders, 1984a).

1.4. The physics of biological systems

The frameworks of thermodynamics and field theory have important implications for biological systems; the former because it can account for their organisation and complexity; the latter because it can account for phenomena not amenable to lower level reduction (Ho & Saunders, 1984b).

There are three laws of thermodynamics: (1) the tendency to increasing disorder or entropy (the driving force of energy transfer); (2) the flow of energy from hot to cold (the direction of transfer) and (3) the conservation of energy. The traditional view is that because organisms become more organised during development and subsequently retain their structure, they contradict the first law, but as these processes take in energy from food or sunlight, there is in fact a net increase in *universal* entropy. However, this still leaves unexplained why there should be local *decreases* in entropy, which endows life with the quality of being *intrinsically* possible despite contradicting the first law. Dreisch attributed this to an *élan vital* extrinsic to the organisms' *material* structure - employing the Aristotlean concept of entelecheia or 'having a soul' (1922, cited in Rotenstreich, 1982). Natural Theology on the other hand had attributed such vitalism to God, whose divine creation supposedly reached its

epitome in Man (Gould, 1980) - a tenet much confused with Darwin's own thinking (Glasson, 1983).

There is organic progression but it is not towards 'higher' or 'better' things. Rather it is towards increasing complexity (Saunders & Ho, 1976). Complexity is defined as the minimum algorithm required to fully describe a system (Saunders & Ho, 1981). In simple terms it is the number of *types* of component (rather than their individual number) that constitute a system and is consistent with Williston's law that nearly identical parts either merge or specialise (Saunders & Ho, 1976). It is comparable to randomness because a greater number of types of component enable a greater number of possible combinations (*ibid.*).

Thermodynamic principles as applied to biological systems are termed talandic after a Greek word meaning oscillation (Goodwin, 1969). Epigenesis is initiated by the introduction of an instability to the system. This increases the Gibbs *free* energy (G) which the system attempts to minimise; and is given by

$$G = H - TS$$

where T is the talandic temperature (organisational potential), H the enthalpy or energy of the system, and S entropy (Saunders & Ho, 1976). This minimisation is achieved through increasing complexity (talandic entropy) which provides the driving force behind epigenesis. The tendency towards organisation simply describes its direction, and comes about because the more complex a system, the more organised it must be to survive (*ibid.*).

Quantum physics has shown that matter does not possess an objective reality but rather is comprised of classes of energy (which *manifest* themselves as the properties of matter) (Davenport, 1979). It is suggested that the constraint imposed by organisation at any given level represents a loss of energy at that level which does not leave the system as a whole but rather manifests itself as a higher level emergent property. This represents a conservation of talandic energy and could explain *why* entropy changes manifest in terms of complexity.

Adaptation can also explain why complexity tends to increase overall (local decreases are possible). The loss of some part must decrease fitness (or the best organisation would be one where that

part is missing) whereas increases can be beneficial (Saunders & Ho, 1976). H decreases with increasing fitness (*ibid.*).

1.5. Primary and secondary determination

Two central problems are how development is initiated and how the ensuing differentiation comes about.

The initial instability is set up on penetration by the fertilisation nucleus, but it would appear to be its general perturbatory effect rather than any structural property that is responsible, since cleavage can also be initiated by artificial evocators such as pricking, calcium ions, electric current and methylene blue. Secondly, cleavage to the 8-cell blastula can proceed in enucleated eggs so is not dependent on the nucleus per se.

Since all cells derive from a common mitotic nucleus, the first stage of differentiation must depend on some initial cytoplasmic asymmetry (Davenport, 1979). This initial differentiation is the ultimate source of form, and as cell type distinctions relate to their potential, the process by which it occurs is known as primary determination. Evidence for the role of cytoplasm comes from the amphibian Dinophilus in which sex is determined by cytoplasmic differences present before meiosis (the male has small eggs whereas those of the female are larger) whilst hormonal sex reversal in other amphibians also shows that germline genotype is not involved (*ibid.*). Nonetheless it is the *relationship* between cytoplasm and genome that is important. For example, if a mouse nucleus is introduced to an enucleated toad egg then the embryo is arrested at the blastula (Webster & Goodwin, 1982).

Nuclear differentiation results in the production of new substances, so changing the cytoplasmic make up. This in turn results in further nuclear differentiation, which again changes the cytoplasm producing yet further differentiation, and so forth. This progressive instability constitutes the cytoplasmic ageing process that characterises epigenesis, and the process by which it leads to new organisational restrictions is known as secondary determination (Davenport, 1979).

The region of cytoplasm that determines germinal function, however, protects the germ plasm nucleus from differentiating influences and endows the germ cells with an affinity to migrate to

the gonads. The germ line provides the information link between the generations, and is suited to this function on account of its stable inheritance - a prequisite for any such information link (*ibid.*). As this link involves a continuous relationship between nucleus and cytoplasm, these constitute the inherited particulars (Goodwin, 1984a) and [insofar as the germ line provides a basis for differentiation,] it is clear that what is inherited is *their capacity to generate form* (*ibid.*). However, the germ cytoplasm also provides a medium for information flow from the environment to the nucleus. For example, Cullis (1981) showed that various culture media could induce heritable changes in flax DNA; this is expanded upon in section 3.12.

1.6. The generative field

Weismann noticed that form was very constant even though traits often showed dramatic hereditary variations. He concluded that the germ plasm was essentially inviolable, *i.e.*, would be conserved by the constraints of developmental and adaptive requirement, so that form would change only gradually by mutation (Davenport, 1979). In this regard, an organism was merely the completed expression of its germ plasm determinants, an 'expressive totality', or in terms of DNA, an 'epiphenomenon of the genome' (Webster & Goodwin, 1981).

However the foregoing discussion reveals that what is inherited is the capacity for the epigenetic attainment of adult form (Goodwin, 1984a). Thus the germ cell constitutes the whole organism at the single cell level, and since the process is self-generating, it is appropriate to regard organisms rather as *self-organising* totalities, or in modern parlance, decentred structures (Webster & Goodwin, 1981).

There are broadly three constraints on form. The first of these concerns materials because in order to produce a given cell type the appropriate constructional components must be present. These derive from the inherited particulars *viz.* nuclei and maternal cytoplasm (Goodwin, 1984a). The second concerns conditions (such as temperature) because these can influence the properties of the constructional materials or apparatus. They may also affect the operation of the third, that is, organisers. These are involved in the deployment of form and largely concern what are collectively known as morphogenetic

fields (Davenport, 1979). They too originate as inherited particulars (Goodwin, 1984a); and are defined as

"an embryonic system or part thereof that contains constituent elements that not only acquire their potential properties in relation to a common source of positional information but can reestablish the informational system, its constituent elements, and their responses, following the disturbance of spatial relationships within the system" (Davenport, 1979).

The ability of a field to re-establish its informational system is called regulation and is possible because the parts acquire their identity relative to the whole (*ibid.*). Field properties are central to the generation of pattern in primary and secondary determination and a number of models will be presented. Davenport (*loc. cit.*) describes twelve general properties concerning field behaviour (Table 1.I).

The germ line re-establishes the capacity to generate adult form at each generation even though the adult itself dies at each generation. This capacity therefore possesses field properties and is termed the generative field, whose form is determined by inherited particulars (materials and organisers) and environmental influences (conditions) (Goodwin, 1984a) (Figure 1.2). It not only allows for hereditary modes additional to DNA, but also provides for the registering and assimilation of environment effects as well as those mediated by changing the inherited particulars (mutation) (*ibid.*).

Table 1.I. Salient properties of morphogenetic fields

Fields are the ultimate source of epigenetic potential. The essential components of embryological systems coincide with field properties.

Fields are anticipatory. Tissues transplanated within a field differentiate according to their *new* positions.

Fields determine gene function. Differentiation depends on positional information being converted to distinct patterns of gene function

Fields can be subdivided without loss of potential. Halved, uncleaved, sea urchin eggs, for example, can produce whole larvae.

Field properties are concentrated in a centre. Organs form near or at the geometric centre of their structurally homogenous organ-forming area but whose *inductive* power decreases away from the centre

Fields possess polarity. Intra-field polarity means that rotation of the whole field reverses the polarity of the resultant organ.

Fields can interact with one another and compete. Abnormality results if one field loses strength and is dominated by another.

Fields can extend their control to strange materials.

Fields possess potential not normally displayed. Redundant properties are always displayed by some other field in the normal embryo.

Fields recognise their spatial relationships such as 'within' or 'next to'. They must be able to do so, especially under abnormal conditions.

Fields regulate growth. The maximum size of an organ being limited by the field boundaries. But the *rate* of cell division is cell-specific.

Embryonic fields control regulation and individuation

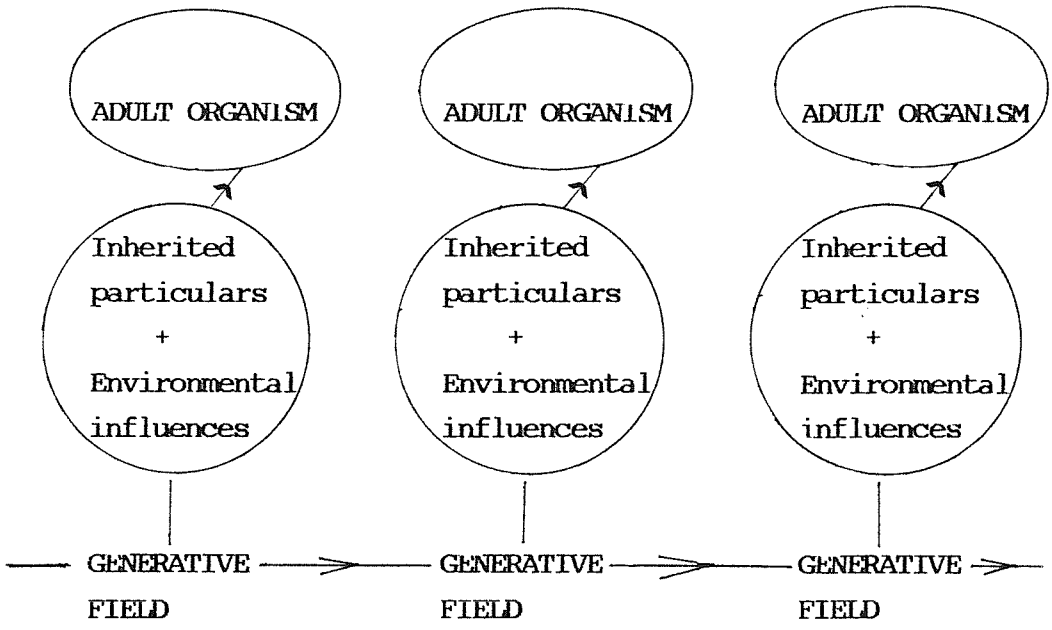


Figure 1.2. Goodwin's hereditary scheme: the generative field. The inherited particulars (which include the genes) together with environmental influences constitute the generative field. The generative field provides the materials and conditions through which the adult organism arises epigenetically; and it is this *property* of a system that is encompassed by generative field, that which is re-established at each generation being generative field *capacity*. Information flows among levels, and, since its information system can be re-established from parts other than just the germ cells, it can also encompass, for example, somatic embryogenesis and propogation by ramets. Evolution comes about through changes in its parameters, whether by mutation (inherited particulars), selection (delimits the initial inherited and environmental conditions as a subset of the possible) or environmental change. Note that the organism can play an active role in establishing the inherited particulars (*eg.* by mate selection) and environmental conditions (*eg.* by habitat choice). For further explanation see text.

1.7. DNA and the generative field

The simplest model of differentiation is where pattern simply reflects differences in an underlying pattern or prepattern (Davenport, 1979) (Fig. 1.3). But as prepatterns depend on other prepatterns they cannot explain their ultimate origin or account for increases in spatial complexity. Moreover, the properties of their parts are acquired intrinsically so their loss cannot be corrected. Hence their applicability is severely limited. The inability of a field to re-establish its informational system is called mosaicism (*ibid.*).

DNA delimits the set of materials (essentially nucleic acids and proteins) available to the generative field. Proteins can serve as material components or functional enzymes; whose own products need not be proteinaceous. DNA delimits the set of possible proteins because it provides the information for sequencing their amino acids which is achieved through transcription and translation. Hence DNA provides the 'information' that positions the nucleotides and amino acids along their linear sequences. In this sense DNA is a source of positional information whose expression and replication involve direct copying and hence prepattern.

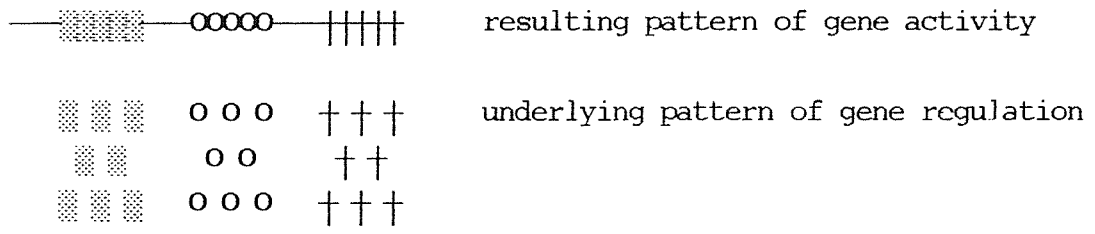


Figure 1.3. Prepattern model of pattern deployment. The pattern of gene activity is understood to map one-to-one to an underlying pattern of gene activity.

But the expression and replication of DNA (RNA in cases such as retroviruses where this constitutes the genetic material) differs from simple prepattern because changes can be introduced during copying as mutation. Conversely, replicative errors or damage can be corrected by copying from the complementary strand (Goodenough, 1978). This is because DNA is not in fact *self*-replicating (Fox, 1984) but depends on nucleotides, enzymes and cofactors for its duplication (mRNA and polypeptide synthesis in ontogeny similarly require factors such as transcriptases and tRNAs) (Goodenough, 1978); and these often bind to specific sites in the nucleic acid. Hence transcription depends on the *relationship* between binding site and enzyme; several of which comprise editing functions able to detect errors. In translation, each tRNA recognises only its particular mRNA triplet and activates only that deaminase able to add its 'corresponding' amino acid to the growing peptide. Thus DNA as a source of the pattern of phenotypic expression (in ontogeny), and of the DNA (as an inherited particular) contributing to the generative field of the next generation, depends on its position within an organised system of functions.

Its higher level organisations also possess field properties. The folding of the duplex into secondary and tertiary structures means that sequences differ in their tendency to undergo strand separation and hence transcription; which may be further controlled by histone proteins (Davenport, 1979). Their mutabilities may therefore also differ so that mutations are *not* all equally probable; CAT-boxes for example are particularly labile (Goodenough, 1978).

Maize cells re-establish their gross chromosomal organisation after X-ray damage in a manner that is always peculiar to the given type of damage; Drosophila cells sense when ribosomal DNA (rDNA) titre is abnormal and make the necessary adjustment; and ciliate nucleii can equalise their DNA content in response to disparity resulting from amitotic division of their parent macronuclei (McClintock, 1984). The genomes of nitrogen-fixing bacteria direct the formation of leguminous root galls; oviposition by wasps induces the development of wholly new plant structures that are geared to the needs of the growing insect; and genomic hybridisation within Nicotiana species is followed by major chromosomal reorganisations with developmental incompatibilities (genomic shock) whose nature is always similar for a given cross (*ibid.*).

Genic dominance shows that gene expression is integrated with the rest of the genome as it is modifiable by selection (Ford, 1940); and modelling genes as binary 'on' or 'off' devices has shown that groups of genes or nets undergo regular cyclic or linear transitions in state (Kauffman, 1969). Since the latter can be switched by metabolites (eg. glucose in the Escherischia coli lac operon), environmental control is also possible (*ibid.*). The induction of specific antibodies by their (often novel) antigens exemplifies an *environmental* stimulus that selectively activates one from a *set* of possible genes.

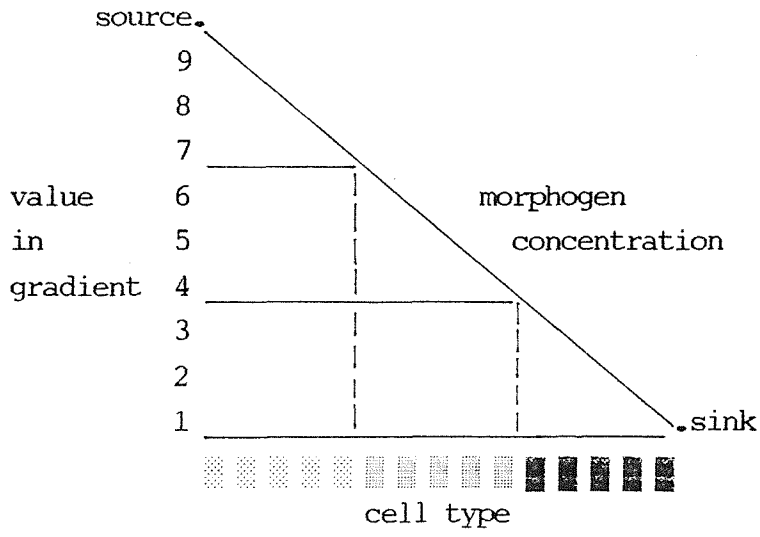


Figure 1.4. The French Flag Model. A concentration gradient is set up between an origin (source) and a sink where it is dissipated. Cells experiencing a particular range of values (vertical axis) differentiate similarly. For example, all cells (horizontal axis) falling within the range of values 4-6 produce cell type '▒'. The result is a pattern reminiscent of the French Flag. For further explanation see text.

1.8. General models of cellular differentiation

In addition to prepattern are models where nuclei respond to *quantitative* differences in some parameter whose values provide positional information. These differences are set up across a gradient which originates at a source and terminates at a sink (the boundary functions). All cells falling within particular ranges of values respond similarly. This produces a pattern like the French flag (Fig. 1.4) whose complexity depends on the number of genetic states that can be activated along the gradient (Davenport, 1979, citing Wolpert, 1972).

The gradient can be re-established following perturbation. Gradients dependent on the *difference* between boundary values change slope when the field dimensions are altered and the *scaling* of the flag changes. This is regulation by morphallaxis (Figure 1.5a). Gradients involving *progressive* diminution in value from source to sink (or to zero value) suffer truncation of the pattern when the field dimensions change. An incomplete flag results but the gradient re-progresses from the damage to regenerate the remainder. This is regulation by epimorphosis (*ibid.*) (Figure 1.5b). Static gradients have been postulated but cannot regulate and are more akin to prepattern.

Two models related to gradients are wave and reaction-diffusion systems (Figure 1.6). In wave functions the nodes demarcate the boundaries between repeating units. Waves can be standing (Goodwin, 1984b) or progressive (Ho *et. al.*, 1983c). In Reaction-diffusion (Murray, 1981) two or more interacting substances diffuse at different rates to yield a profile in the titre of their product (Saunders, 1984). Saunders (*loc. cit.*) refers to such systems as prepatterns, but to avoid confusion this thesis will adopt Davenport's (1979) definition.

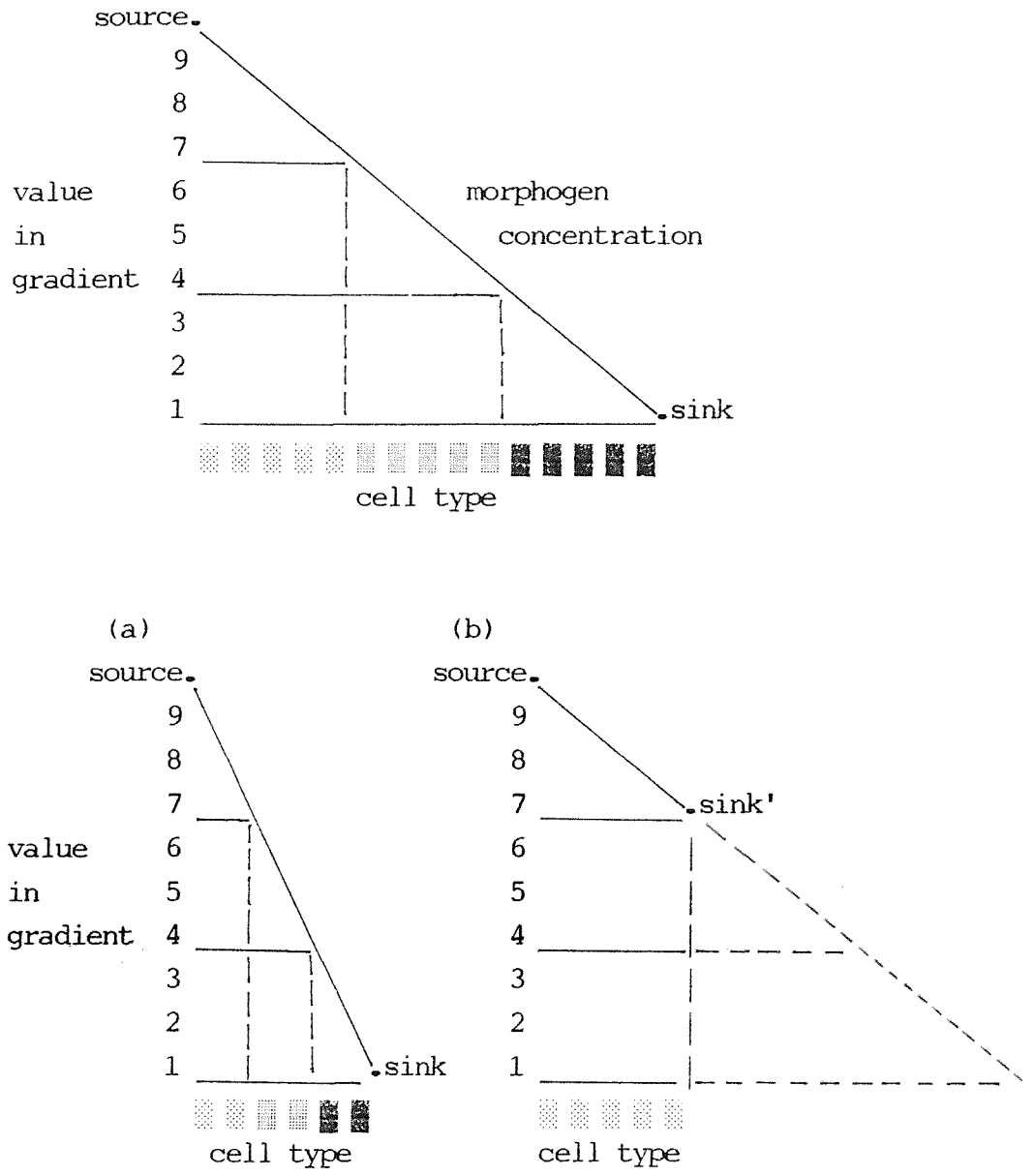
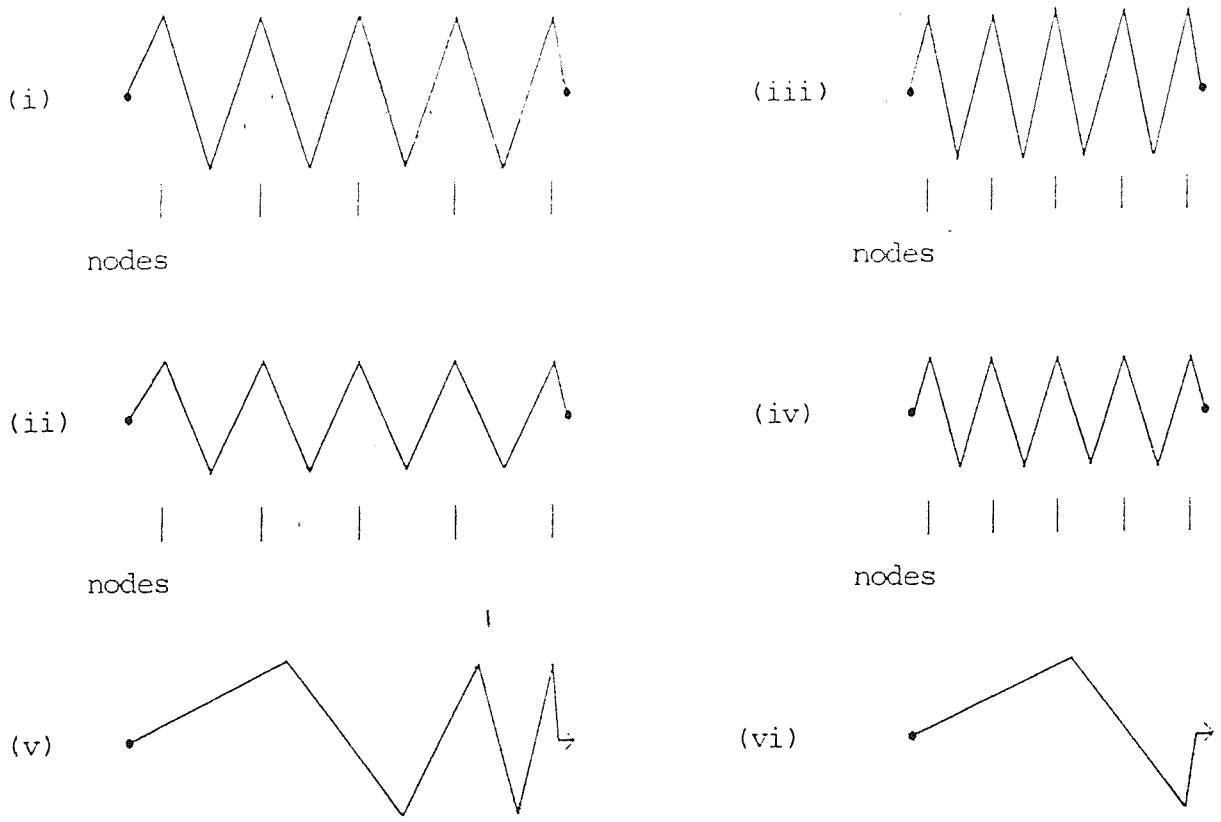
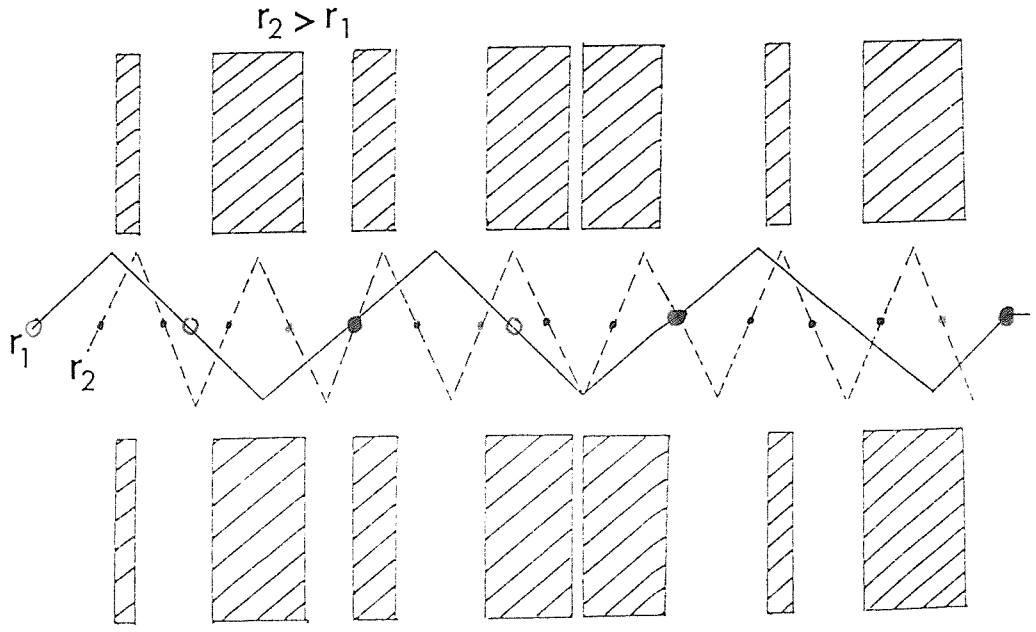


Figure 1.5. Regulation by (a) morphallaxis and (b) epimorphosis. In morphallaxis a change in field dimension changes the slope of the gradient and the *scaling* of the pattern is altered. In epimorphosis the slope of the gradient remains unaltered, but is *truncated* when it encounters a new field boundary (|). The gradient then re-progresses from the damage to re-generate the original field (dotted line) and hence pattern. In other cases, however, the damage or obstruction may in effect become a new sink (sink'), in which case permanent truncation of the pattern results.

(A)



(B)



1.9. Genes and traits

Traits refer to discrete aspects of an organism (Davenport, 1979) such as red hindwing coloration in the tiger moth Arctia caja (L.). The new synthesis holds the cause of such a trait to be a its gene(s) which thus is 'for' the trait. But because the trait results from several processes in relation to the rest of the genome, the gene's 'for red' quality of is *secondary* to its integration within the pathway. Thus it is meaningless to regard traits as existing a priori and *then* being collected into organisms; and there is no sense in which genes can be said to code 'for' any traits (other than just their particular base sequences) apart from their developmental context.

Mutations to genes already integrated in a system are also confined in phenotypic effect by its developmental repertory. Homeotic mutants transform between normal structures and then usually only if

Figure 1.6 (opposite). Wave and reaction-diffusion systems. (A) Wave functions. A standing wave (i) is established between two points (•). Nodes (|) demarcate repeating units. (ii) Amplitude differences may result in qualitative differences in the resulting pattern of cellular differentiation. (iii and iv) Morphallactic regulation of waves (i) and (ii) respectively. The wave is compressed (elongation distends it) but the number of nodes is unaltered. (v) Progressive wave. The wave progresses from its origin until it meets a boundary. Regulation is epimorphic, and truncation of the field (vi) may result in part of the pattern being lost. (B) Reaction-diffusion. In the figure, two substances r_1 and r_2 diffuse at different rates and interact with each other to produce respective concentration profiles (r_1 continuous line; r_2 dashed line); nodes are shown as 'o' and '.' respectively ('•' where they coincide). Wherever r_2 exceeds r_1, cells differentiate into a particular type  . Cells may respond to either the relative concentration of the reactants or to some third product that results when the concentration of r_2 exceeds r_1. Varying the initial concentrations of r_1 and r_2 results in patterns of similar *kind* but whose precise form and dimensions may vary unpredictably.

sharing common types of pathway, such as aristapaedia (in Drosophila) where antennal discs develop as legs (Goodwin, 1984a); whilst heteromorphic ones that produce atypical structures (Sibatani, 1980), on scrutiny show themselves to involve modification of pre-established pathways. For instance, the pale brassy colour that sometimes replaces copper in the butterfly Heodes virgaureae (L.) (*ibid.*) might reflect an altered ridging of the scales.

The notion that genes code 'for' traits originated with Gregor Mendel, whose studies on heritable differences in the garden pea led him to invoke the existence of corresponding causal entities that retain their identity on transmission to the offspring, and implies one-to-one mapping between genes and traits. Whilst this poses few problems with heritable *differences* (Webster & Goodwin, 1981), it cannot explain the generation of form. Firstly genetic content does not necessarily correlate with the form in which the traits are deployed. Humans and chimpanzees are 99% similar in their DNA and possess the same fifty or so kinds of cell, whilst two apparently identical Drosophila species differ vastly in genomic content (King & Wilson, 1975). Secondly, different genotypes can produce similar phenotypes and interact unpredictably. Clarke & Sheppard (1963) found that certain mimetic forms of the African swallowtail Papilio dardanus could be produced by quite different genetic pathways. British and North American examples of the butterfly Boloria euphrosyne L. have identical phenotypes but their hybridisation results in a form quite unlike either parent (Oliver, 1977); and Bowden (1983) reports a similar phenomenon in Artogeia napi when the hybrids are backcrossed to one of the parent races. Thirdly, a given genotype may produce alternative phenotypes under different *environmental* conditions. The European Map butterfly, Araschnia levana sports black flecks on orange in the spring (f. levana), but white bands on black during summer (f. prorsa) (Ford, 1957); the two forms alternate under the influence of photoperiod. Their striking alternation may involve only two kinds of process (and hence minimal complexity change), perhaps inactivation of orange pigment synthesis (unpigmented scales appearing white; cf. Nijhout, 1981) and *unified* expansion of its pattern elements (black components merging as the 'background'). The latter suggests that photoperiod might affect some global process that generates the pattern of differentiation, rather than simply switching an unknown

but determinate number of genes on and off (a more usual sort of interpretation).

1.10. The generative field and environment

"A major gap in our knowledge of internal causes in evolutionary mechanisms is their environmental context." (Thomson, 1985)

Since the inherited particulars can interact with environmental influences, a complete description of the 'generative field' must include such influences (Goodwin, 1984a) which operate in three main ways.

The first is through natural selection (Darwin, 1859; Maynard Smith, 1975) by abiotic and biotic factors that determine *which* genes will be transmitted to the offspring through the differential survival and fecundity of *phenotypes* (Ho, 1984). Since without variation there can be no selection in the strict sense, variation is a priori essential to it (Davenport, 1979). Thus evolutionary theory must also account for the *origin* of variations (Ho, 1984).

Neo-Darwinism asserts that DNA mutation and recombination at meiosis are the source of structural innovation (Maynard Smith, 1975). However phenotype arises through the epigenetic system which *also* includes cytoplasmic processes (Davenport, 1979), and both can interact with the environment during *development*. It is the effects of *this* sort of interaction that are broadly defined as acquired characters (Waddington, 1961). The factors involved are primarily, but not exclusively, abiotic.

The third is through its direct effect on the genetic material itself. For instance, excess exposure to the ultra-violet in sunlight can induce mutation in the DNA. This class might also include induced modification and material input to the genetic material itself (as in immunoglobulin synthesis and human retroviruses respectively) (Pollard, 1984).

1.11. Genetics and heredity

Nuclear DNA is generally regarded as the hereditary ('genetic') material (Maynard Smith, 1975). However other modes of inheritance also occur. Thus it is important to distinguish 'hereditary' from 'genetic'. 'Hereditary' will pertain to any mode by which offspring possess the potential to produce a trait by virtue of its parents having the potential to produce it. 'Genetic' will pertain specifically to DNA and its inheritance; and the *extent* to which a trait results from genes transmitted from the parents is known as heritability in the broad sense or just 'heritability' (Falconer, 1981).

Natural selection and direct modification to hereditary materials do not pose any immediate problem because the former *assumes* variation to be heritable, whilst the latter are by definition so. But heredity is problematic for 'directed' variations that result from the action of environmental influences on development. Firstly, the organism must register these influences (Lewontin, 1982) and accommodate them within its cycle of structures without itself being destroyed (Piaget, 1971). It may or may not assimilate these as a stable change or accommodat within its cycle of structures. Then, if heredity is to be meaningful, there must be lasting assimilation of these temporary accommodats in so far as they can be transmitted to subsequent descendants (*ibid.*).

All characters are to some extent hereditary and to some extent acquired (Waddington, 1982). The latter point is crucial even within the strictly neo-Darwinian framework of quantitative genetics, to understanding *truly* continuous variation (Falconer, 1981); the genetic contribution to phenotype is known as heritability in the narrow sense (*ibid.*).

"No organism can grow and develop without environmental stimuli" (Matsuda, 1982) but this has been a taboo concept for historical reasons (*ibid.*). The notion of acquired characters is largely identified with Lamarck, but the idea had been around since centuries before (Jordanova, 1984). In the mid-nineteenth century Darwin furnished the supplementary theory of natural selection, but his inability to formulate an account of stable inheritance threatened his theory (Bowler, 1984). This had two consequences. Firstly it forced a polarisation of Lamarckism and Darwinism. Lamarckism was set up as a

straw man to be demolished in order to draw attention away from the shortcomings of Darwinism. This was done by exaggerating the acquired characters notion to the exclusion of its other tenets, so undermining its conceptual integrity - facilitated by the mistranslation of his original text from French into English (Jordanova, 1984). Secondly it demanded that a hereditary scheme be provided. This materialised as the Weismann Doctrine that precluded information input to the germ line (Webster & Goodwin, 1982). Weismann amputated tails from laboratory mice, bred the amputees, and examined the offspring for tailess trait. But it did not reappear, and with the need to bolster up Darwinism still up front, this inability to inherit an injury effect was quoted as firmly refuting Lamarck. The reinstatement of Weismann's thesis as

"Crick's central dogma (a dogma dear to those who consider *any* type of Lamarckism-simulating machinery as heresy)" (Wassermann, 1982b)

only confirmed this verdict of certain death.

But Weismann *did* acknowledge the occurence of acquired characters and clearly distinguished between the effects of injury, use and misuse, and environmental influences. His refutations applied only to the first two categories, but this fact is frequently omitted in quotation (Matsuda, 1982). He experimented on the butterfly Lycaena phlaeas L. whose South European representatives are darker than the German ones. He reared the two races at each other's natural temperature, found that there was some interchange of phenotype, and concluded that the environment *could* modify traits:

"Somatogenic character not inherited here, but modifying influence of temperature affects the primary constituents of the wings (part of the soma) as well as the germ-plasm" (1892).

Nonetheless he did go on to say that acquired characters might be transmitted in *other* cases:

"In many animals and plants influences of temperature and environment may very possibly produce hereditary variations" (*ibid.*, p405).

CHAPTER TWO

Summary

Seasonal polyphenism refers to regular changes in phenotypic ratios under environmental control, such as the spring and summer broods of most butterflies which display different phenotypes depending on the temperature or daylength under which the early stages developed. Lepidopteran examples are cited throughout to illustrate the concepts concerned.

Two models for the evolution of seasonal polyphenism are presented. Both require some reliable and biologically meaningful cue which predicts future conditions. The first is Bradshaw's model, where two alleles at a polymorphic locus, each advantageous in a different season, become linked in *individuals*. The other model is genetic assimilation where an environmental stimulus allows an underlying genetic make-up to be expressed and selected. The role of genetic assimilation in the evolution of montane and arctic races is discussed.

The adaptive roles of seasonal polyphenisms are then examined with particular reference to thermoregulation (heat absorption by pigments) and predation (camouflage patterns and behaviour). Seasonal differences in foodplant usage and in migratory behaviour are also discussed. The influence of developmental and adaptive constraints (ie. canalisation and the role of phenotype in courtship) in the establishment of seasonal polyphenism is then considered. For example, not all arctic and montane races resemble their winter 'counterparts'.

The role of environmental cues is reviewed. In temperate regions photoperiod is more reliable than temperature, although their relative importance differs among species. Diapause (dormancy) and non-diapause strategies are compared with special reference to larval and pupal development.

A case for the laboratory study of seasonal polyphenism is presented: most cases have not been examined, and indeed some are still evolving; it also lends itself to the study of canalisation and genetic assimilation.

CHAPTER TWO

SEASONAL POLYPHENISM

2.1. Introduction

Mayr (1963) defined polyphenism as 'the occurrence of several phenotypes in a population, the differences between which are not the result of genetic differences', and Shapiro (1976) specifically defined seasonal polyphenism as 'an annually repeating pattern of changing phenotypic ratios in successive generations under some kind of environmental control'. Examples include the complex seasonal morphological changes (cyclomorphosis) exhibited by Cladocerans and rotifers; the reproductive and migratory cycles of aphids; and wing-length polyphenism in the Gerridae (water striders) (*ibid.*).

The phenomenon is widespread among the Rhopalocera where it is more widely known as 'seasonal variation'; the literature is considerable (see Shapiro, 1976 for a review) and its experimental study dates back at least to the end of the 19th Century (Merrifield, 1893; Weismann, 1896).

Environmental control has the advantage that there is no lag between the presence of an environmental condition and the development (in evolutionary terms) of an adaptive phenotype. Also, since all individuals within a population can respond to the stimulus, the 'load', *i.e.* the degree of selection against any non-adaptive phenotypes, is considerably reduced. These are its main advantages over genetic polymorphism, from which it may have evolved (Bradshaw's model); although the two can occur together in the same species. For example, overwintered pupae of the N. American butterfly Lycaenopsis argiolus pseudargiolus produce four phenotypes, while summer pupae produce but a single form similar to the pale blue vernal f. violacea (*ibid.*).

Two models (Bradshaw's model and genetic assimilation) have been proposed for the evolution of seasonal polyphenism. In both cases, it is essential that there be some suitable (reliable and biologically meaningful) environmental cue which predicts future conditions.

2.2. Bradshaw's model

Short-lived organisms may have polymorphisms whose relative frequencies are adjusted by the selection imposed by seasonal conditions. Yet although the *average* fitness over the year is high, there is always a time lag and genetic load. Given alternating seasonal regimes, and the presence of two functionally related alleles, selection would then favour duplication, recombination and inversion, causing both alleles to lie in a close linkage group. Most individuals would then carry both alleles. If the alleles have independent operators, then each will retain its previous capacity and can respond separately to the appropriate stimulus. Such individuals would possess the same phenotypic potential as an entire population for that locus (Shapiro, 1976, citing Bradshaw, 1973).

It is essential both that the cue be available *before* the time of developmental option (or the organism would not modify its phenotype), and that the cue *and* developmental option precede the environmental selective force (so the organism can respond to the cue in time for the response to be worthwhile). If these conditions are not met, then genetic polymorphism is favoured. For example, variation in diapause *initiation* would be generally polyphenic, since shortening daylength precedes the developmental option (diapause) and both precede the selective agent (winter); diapause *termination* would more likely be polymorphic because larvae awakening on the first thaw might only be frost-bitten by a subsequent refreeze (*ibid.*).

2.3. Genetic assimilation

Selection acts on an adaptive phenotype that arises from an environmental stimulus enabling an underlying genetic constitution to be expressed. A new genetic 'architecture' is built up that expresses the trait in the absence of the original stimulus. The genetic assimilation of butterfly seasonal polyphenisms has been proposed as a general mechanism in the evolution of seasonal polyphenisms (Nijhout, 1984; Shapiro, 1984), and has also been invoked in the evolution of geographical and altitudinal races . For example, arctic Papilio zelicaon are univoltine and ancestral to the bivoltine and polyphenic

low latitude race, believed to have arisen in less than 200 generations (Shapiro, 1976); and the montane subspecies tucemseh Grinnell of Polites sabuleti (Hesperiidae) resembles the vernal (spring) form of lowland ssp. sabuleti Boisduval and retains its phenotype under aestival conditions (Shapiro, 1975a). Genetic assimilation has also been invoked in the origin of genetic aberrants. For example, Cynthia cardui L. (Nymphalidae) regularly produces a dark form 'ab. elymi' similar to cold shock phenotypes (Shapiro, 1975b).

2.4. The adaptive roles of seasonal polyphenisms

Both forms must be adaptive if seasonal polyphenism is to evolve (Weismann, 1896). Most cases are relevant to thermoregulation, with specimens flying in the cooler seasons being generally darker *e.g.*, Pieris occidentalis f. 'calyce' Edwards which also occurs at high altitudes (6000'-12000') (Shapiro, 1973a). This trend is widespread in Lepidoptera (Shapiro, 1976) and probably represents a correlation between phenotype and environment sufficiently general to constitute an 'ecological rule' (Ho, 1984). Watt (1968) demonstrated that heat absorption was greater in the darker vernal forms of Colias butterflies (Pieridae); the species are 'lateral' baskers which perch so that heat radiation impinges on the ventral surface (Shapiro, 1976). Others may bask by absorbing or reflecting radiation from the dorsal surface ('dorsal' and 'reflectance' basking respectively) (Kingsolver, 1975); hence both surfaces can be important. Differences in energy absorption may affect the insects' development rate and hence their exposure to parasites (cf. Porter, 1984).

Crypsis has been implicated in the African Nymphalid Precis octavia L.; the dry season form is sedentary and very cryptic and is believed to protect it from lizards, whilst the wet season form is very active and brightly coloured because its predators, assassin bugs for example, hide in the vegetation rendering crypsis useless (McCleod, 1984). The British holly blue, Celastrina argiolus L. shows seasonal variation in larval food plant, the adults ovipositing on ivy in the summer but holly in the spring. Since the toxicity, nitrogen status and water content of foodplants can deteriorate with plant maturity or vary with the season (Slansky, 1974; Thomas, 1985) this

strategy may be adaptive. The species also displays pattern polyphenism. Temperature may control both traits but the two are independent (Ford, 1957). The migratory behaviour of certain species is also polyphenic but its precise control is unknown (Shapiro, 1976).

The total range of phenotypes comprising the developmental repertoire of a single genotype constitutes Wolterick's (1919) Reactionsnorm. Courtship constraints however may limit the range of phenotypes which become established in nature (Shapiro, 1983). The cold shock form 'ab. schraderi' of Precis coenia Hubner lacks the usual pale dorsal forewing band and suffers reduced courtship success; the form resembles the related species P. nigrosuffusa (Barnes & McDunnough) with which P. coenia can hybridise (*ibid.*). Hence the suppression of ab. schraderi under normal conditions is probably adaptive.

Such buffering of developmental pathways is known as developmental canalisation (Waddington, 1961). The Californian subspecies of the Mourning Cloak (Nymphalis antiopa antiopa L.) produces an aberrant phenotype ('hygiaea') on cold shock whereas the Alaskan one (ssp. hyperborea Seitz) does not (Shapiro, 1981a); and the tendency to produce hygiaea parallels pupal mortality (Shapiro, 1981b) suggesting that such canalisation is adaptive. High altitude Sierran populations however were poorly canalised even though they experience regular night frosts; and it has been suggested that selection has not built up resistance here because the *prolonged* cold (>14 days) required to elicit hygiaea is absent (*ibid.*). Arctic P. zelicaon are buffered against the southern aestival phenotype which would be maladaptive at higher latitudes.

In several Lepidoptera the *pupae* show seasonal polyphenism where it can affect their coloration (Warnecke, 1964). For example, summer pupae of the Speckled Wood are green, whereas overwintering (diapausing) ones are pale brown (Henriksen & Kreutzer, 1982); the former hang from fresh stalks but the latter from wilted straw, and their respective colours are cryptic (*ibid.*). Polyphenism can also affect their structure. In Pieris species diapause pupae have a harder cuticle with a thick wax-like layer which may protect them from the rigours of winter climate (Yata *et. al.*, 1984). Diapause and non-diapause strategies are referred to as heterodynamic and homodynamic respectively (cf. Shapiro, 1979, citing Lorkovic, 1929);

and it is suggested that these themselves be regarded as polyphenisms.

2.5. Geographical and altitudinal variation

Populations exposed to the cool conditions of high altitude or latitude are mostly univoltine and resemble the vernal phenotypes of their lowland or southerly counterparts. In some cases, however, the montane forms, although distinct, do not resemble such counterparts. For example, the montane race montana of Phyciodes campestris is light in colour whereas the vernal phenotype of lowland race campestris is dark (Shapiro, 1975c). But montana will produce a dark vernal phenotype if reared under *lowland* conditions, as well as regaining its potential for multivoltinism and use of lowland foodplant, suggesting that its different temperature response at high altitude may involve some *physiological* adaptation (*ibid.*).

2.6. The role of environmental cues

Although the respective phenotypes are adaptive for particular temperature conditions, temperature may not be as reliable a seasonal predictor as photoperiod, especially in temperate regions (Shapiro, 1976). In Pieris protodice, for example, prolonged nightlength during the spring and autumn determines the production of dark 'vernalis' phenotypes independently of temperature (Shapiro, 1968); whilst in others, such as P. napi venosa Scudder, it may provide some 'backup'; in this species chilling is essential for dark pigmentation (it is lacking in summer) but its greater degree of expression in early rather than late spring is connected with darkness-induced diapause (Shapiro, 1977a). In some groups, however, especially the Hesperiidae and Lycaenidae, temperature is the more important (Shapiro, 1976). For instance, Chrysophanus phloeas (Lycaena phlaeas) displays darker colouration with increasing temperature (Merrifield, 1893) and the greater melanisation may protect it from radiation damage in more southerly latitudes, as with Melanargia galathea L. (Satyridae) (Turner, 1977). In the S. American Pierid genus Tatochila, the

relative importance of the two varies among the species (Shapiro, 1980a).

In tropical regions, photoperiod is an unreliable cue (it does not vary with the season) and temperature is the main cue; in P. octavia it is the larval *temperature* difference between the wet (30°C) and dry season (16°C) that produces the polyphenism (McLeod, 1984). Similarly in the arctic, where the sun is continuously above the horizon and hence photoperiod potentially useless (Shapiro, 1975d), although one such taxon (Pieris occidentalis nelsoni) did produce 'aestival' laboratory phenotypes under 24h daylength (in the wild it expresses only a univoltine 'vernal' form) that was independent of temperature and diapause (*ibid.*).

Particular wavelengths may be important. In Pieris species blue light during pupation induces the formation of brown (versus green) pupae (Harrison, 1928a).

Humidity may be involved in some tropical polyphenisms. The dark pigmentation of Hestina reportedly increased with relative humidity (moisture applied to the pupal wing cases) (Fox & Vevers, 1960) whilst P. octavia is unaffected by humidity (McLeod, 1984). Humidity may serve as a 'back up' in some tropical polyphenisms as temperature does in some temperate cases - for example the dry season form of Melanitis leda Fabricius which can be induced by rearing larvae at 60% r.h. (Owen, 1971) - but closer study is required before any generalisations can be drawn. It should be noted that temperature in tropical regions and photoperiod in temperate zones are *not* the factors for which their phenotypic effects are directly adaptive (Shapiro, 1968, 1973a; McLeod, 1984).

Seasonal trends in food quality (Thomas, 1985) may be secondarily involved in some cases, since colour development in the (non-seasonally polyphenic) garden tiger moth, Arctia caja L. can be modified by feeding the larvae on walnut (White, 1974, citing Kirby, 1882); and desiccated foodplant led to reduced wingspan in Pararge aegeria (pers. obs.).

Temperature and/or photoperiod can also influence larval and pupal diapause (Lees & Tilley, 1980; Shapiro, 1979: P. napi, pupae only). In the latter species the induction of diapause necessarily results in vernal phenotypes, although it is not essential for their production since non-diapause pupae will produce them if chilled (*ibid.*), but

since all diapause pupae must be chilled before they can complete their development (Shapiro, 1977a) the precise role of diapause here remains difficult to disentangle; in P. protodice short photoperiod induces vernal phenotype *independently* of diapause (Shapiro, 1975d).

2.7. The investigation of seasonal polyphenism

The role of the environment in inducing seasonal variation makes seasonal polyphenism especially suitable for examining the action of environmental factors during development. Yet although widespread in the Rhopalocera, the majority of cases have not been given detailed study. They are at various stages of evolution; indeed the Tatochila group is still evolving[1] (Shapiro, 1980a). It lends itself well to the investigation of developmental canalisation and genetic assimilation, as well as Bradshaw's model, but little experimental evidence for these as origins of the phenomenon has been presented, despite their having been implicated in the field.

Lepidoptera are particularly convenient organisms for study, and Shapiro (1976) suggests using a small species with a short life cycle. Pararge aegeria was therefore chosen for the present study (*cf.*, Chapter five) in which pupal cold shock served as the environmental stimulus.

[1]T. sterodice is at least bivoltine but shows no seasonal trend, although ssp. sterodice is poly*morphic*. T. vanvolmexii is not strictly polyphenic though there is a strong seasonal component in males (those from chilled winter pupae show a darkening which varies with genotype) and short days can induce facultative pupal diapause in either sex. The females are poly*morphic* for ground colour (yellow or white). T. mercedis shows strong seasonal polyphenism in both sexes.

CHAPTER THREE

Summary

The sequence of processes through which any trait arises is known as its 'developmental pathway'. Waddington conceptualised this as the path of a ball rolling along 'canals' through a landscape - the epigenetic landscape; and this model is described. The extent to which development is manifestly refractory to perturbation is thus known as 'developmental canalisation'. Conversely, 'plasticity' will refer to the extent to which development can be deflected from the norm (as evidenced by some measurable trait) by potential disturbance.

Such 'noise' can arise from genetic and other intrinsic influences, and from the environment. The particular nature and severity of a stimulus required to modify a pathway is known as its specificity of response. 'Stabilising selection', towards greater canalisation so *precluding* abnormal phenotypes, is here distinguished from 'normalising selection' which *removes* abnormal phenotypes. The implications of canalisation to genetic phenomena at the individual and population level (eg. dominance and genetic drift) are discussed.

Genetic assimilation is introduced with an overview of Waddington's experiments; and the two phases of genetic assimilation are explained.

Some problems with the evolution of seasonal polyphenism are resolved with a model incorporating features of both genetic assimilation and linkage groups. The need to distinguish 'major', 'minor', and 'modifier' genes is emphasised. The requirements for canalising genes to increase in populations are presented in terms of the frequencies of their adaptive environments, and canalisation versus polymorphism in changing environments is examined.

Hereditary modes other than nuclear DNA are described. Examples and mechanisms of genetic assimilation are reviewed; and the sense in which the environment too, as a legacy, is inherited is explained.

Some objections to Waddington's genetic assimilation are met; and his evolutionary scheme is developed. The possible applications of genetic assimilation and their implications are considered.

CHAPTER THREE

CANALISATION AND ASSIMILATION

3.1. Developmental canalisation

Waddington (1957a) conceived development as proceeding along pre-defined pathways or chreods, that are either stable to external perturbation (canalised) or show some degree of plasticity in response to external influences (the degree of canalisation) (Waddington, 1961). These two properties collectively constitute canalisation of development or simply canalisation (*ibid.*).

He stated that chreods are a product of the genome (Waddington, 1942), and that canalisation is also a genetic phenomenon and so can be built up by natural selection (Waddington, 1959) which in this sense exerts stabilising selection (Waddington, 1953). Such stabilising selection prevents developmental variants from arising in the first place. It must *not* be confused with its usage in population genetics in which variants do arise and are only *then* removed; this latter process he terms normalising selection (*ibid.*).

This thesis will take normal to mean typical and stable to mean imperturbable. To illustrate, the white variety alba of Lycaena phlaeas occurs as a rare recessive aberrant (Ford, 1957a); it is *atypical*. It may be maladaptive and removed by selection under conditions favouring the typical red form - *normalising* selection. The development per se of alba (given its aa genotype) (*ibid.*) may be refractory to external perturbation. Yet with respect to both selection and development, the same factor may be imposing the stress, with (say) cold exerting normalising selection on the wild type via thermoregulatory constraint but stabilising selection on its *development*.

Developmental homeostasis or homeorhesis means that organisms retain their structural and functional integrity (accommodate) in the face of fluctuating external conditions. Yet plasticity of development within the limits of canalisation must be possible if temporary accommodats (Waddington's, 1942, exogenous adaptations) are to arise,

or heterorhesis (Ho & Saunders, 1979)

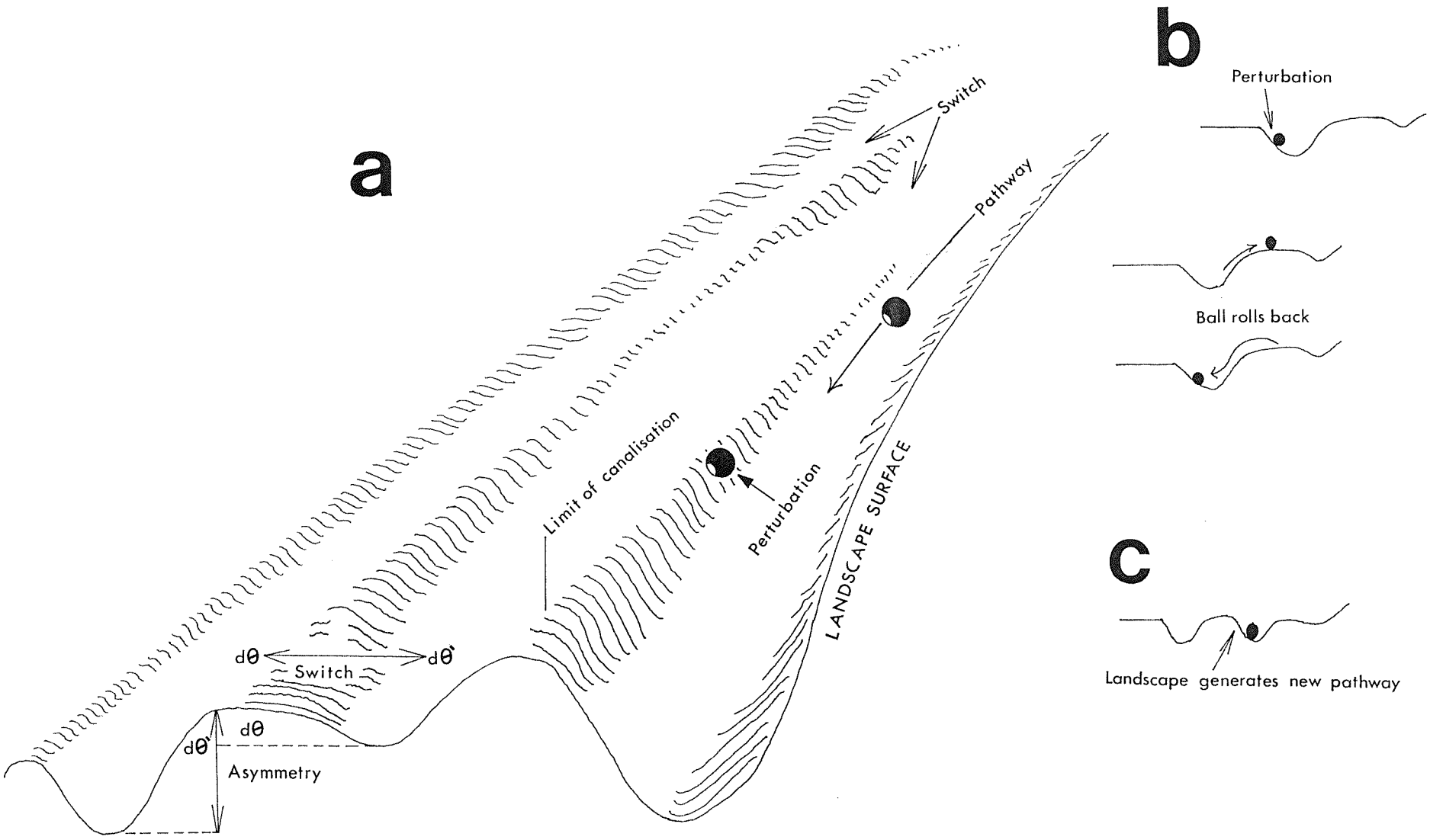
where the organism returns to its initial state on removing the stress.

The capacity to respond is under genetic control (*ibid.*) and modifiable by selection (Waddington, 1959). Developmental plasticity is essential for the concept of acquired characters to be meaningful, and for genetic variability to be manifest in the phenotypes. Thus canalisation must consider extrinsic processes as well as both genic *and* non-genic intrinsic ones.

3.2. The epigenetic landscape

Waddington conceptualised development as a ball rolling over a landscape of hills and valleys - the epigenetic landscape, the path of the ball along the valleys or 'canals' representing the course of development or developmental pathways (Fig. 3.1). Genetic or environmental perturbation (a stimulus) can push development away from the canal, but it will return (the ball roll back) to its original course provided the limits of canalisation (the 'hilltops') are not exceeded. This tendency to return is equivalent to 'stability'; and a pathway is canalised *relative* to the stimulus. Indeed the usual effect of perturbation is to modify some *already* extant pathway whose degree of plasticity depends on the gradient of the slope either side of the canal (Waddington, 1942). The steeper the gradient, the greater the energy (severity of stimulus) required to produce a given deviation from normal development, and the more deeply canalised development is said to be. In that they provide a disturbance, stimuli provide noise. Pathways may branch and a stimulus can determine which branch is followed.

When the limits of canalisation are exceeded the ball (course of development) may leave the landscape (organism) and no longer be accommodated within it. Development ceases; and the organism dies. Lethal alleles and temperature extremes might be conceived as operating in this way. Alternatively the ball may be forced onto another pathway when development is *not* at a branch point (not otherwise undergoing differentiation); but a much greater energy input is needed than for usual switching at a branch point. Each pathway displays a specific degree of stability in relation to particular


a
Switch
Pathway
Limit of canalisation
Perturbation
LANDSCAPE SURFACE
dθ
dθ'
Switch
dθ
dθ'
Asymmetry
b
Perturbation
Ball rolls back
c
Landscape generates new pathway

stimuli at particular times, or specificity of response (Williams, 1982; Ho *et al.*, 1983b).

The 'new' pathway along which development continues will also have its own depth and limits of canalisation which too can be modified by selection (Waddington, 1952; Huxley, 1956) and specificity of response. Asymmetry may be present in that it is easier to switch from one pathway to another than vice versa (*c.f.*, Kauffman, 1969) or a greater tendency to switch in one direction. The tendency to return to an initial pathway or find a viable alternative in the face of stress is, broadly speaking, the essence of the more familiar regulative development; failure to do so essentially constituting mosaic development.

Figure 3.1 (opposite). The epigenetic landscape. (a) Modified somewhat from Waddington's (1957a) model. The landscape is depicted as a surface which itself arises epigenetically. The spatio-temporal deployment (development) of the organism (conceptualised as the path of a ball over the landscape) comes about through the system minimising its potential energy. The excess energy ($+\delta\theta$) is dissipated through increasing complexity, such that

$$\theta_{WHOLE} = \theta_{PARTS} - \delta\theta$$

where: θ is the talandic temperature (reactivity) and $-\delta\theta$ equivalent to the redundancy content (*cf.* Saunders & Ho, 1981); energy some of which may manifest as an emergent property. The energy input can come from mutation or environmental perturbation (initially from fertilisation) and demands the attainment of a new structural configuration: hence a new pathway. Note that there may be asymmetry in the energy exchanges involved in switching between pathways. (b) An accommodat is such that the energy can be dissipated without a new pathway being taken; the ball rolls back down the hill. Alternatively (c), the landscape topology may itself change to re-establish the initial energy condition. This might encompass the production of supernumerary limbs as follows rotational regrafting in insects (French *et al.*, 1976).

3.3. Intrinsic sources of variation

Phenotypic variation can also arise from processes intrinsic to the organism but not *necessarily* involving genomic DNA (although some may do so). These include, amongst others, random developmental perturbations arising from interaction between metabolites and DNA (Kauffman, 1969), or from changes in molecular excitation and stability (Lewontin, 1982a); interactions between morphogenetic processes and their attendant structures, such as the sensitivity of reaction-diffusion waves to fine differences in the surface topology of Drosophila imaginal discs (Bunow *et al.*, 1980) or in the dimension of Lepidopteran wing spaces (Murray, 1981); variations arising from the very nature of constructional components (the size and obligatory unicolorous nature of butterfly wing scales, for example, means they might not accurately resolve an underlying marking boundary, (White, 1974, citing Seilacher, 1973); and mutation in extranuclear DNA (Sonneborn, 1970).

Variability can thus provide a measure of developmental stability (Sokal & Braumann, 1980). Genetic or non-genetic intrinsic factors and extrinsic ones may interact. They can also produce asymmetry (Tebb & Thoday, 1954: genic control of sternopleural chaeta number in Drosophila; Mason *et al.*, 1967: developmental noise; Soulé & Baker, 1968: influence of altitude on wing spotting in the Satyrid Coenonympha tullia) which too may or may not give an indication of developmental stability (*cf.*, Mather, 1953; Reeve, 1961: Drosophila chaeta number).

3.4. The evolutionary implications of canalisation

Natural selection can deepen the canalisation of established adaptive responses (Ho & Saunders, 1979). This could mask any underlying genetic variability that might be available for genetic assimilation (Nijhout, 1984). Such masking also has other important consequences.

Genic dominance buffers against undesireable genetic effects that may result from segregation (recombination) within the gene-complex; and it can be modified by selection (Ford, 1940). The Magpie moth,

Abraxas grossulariata (L.) has a unifactorial yellow form lutea whose homozygote replaces the normal white pigment to a varying degree; heterozygotes tend to be closer in colour to the typical. By selecting heterozygotes closest to either extreme, Ford (*loc. cit.*) was able to render lutea dominant or recessive to grossulariata. This modification results from an alteration in the response of the organism to the gene but *not* in the gene itself (*ibid*); and as such reflects the genetic property of canalisation in general (Waddington, 1959). Such canalisation is relevant to the lack of phenotypic difference between species (King & Wilson, 1975) and subspecies as with the British and French races of Pararge megera L. (Oliver, 1972). The masking of their effects could explain why certain alleles are functionally silent and so account for their neutral phenotypic and adaptive status (Ho & Saunders, 1982a); and how the underlying variability available for assimilation is allowed to get established in the first place. In that genes take part in developmental processes, pathways require to be stable in order that such genetic control be possible (Waddington, 1942; Matsuda, 1982).

One expects wild-type forms to be less variable than their homozygous or heterozygous aberrants; since the wild-type form will have been tuned by selection to produce the optimum expression for the conditions under which it normally occurs, genetic variation in the effects of wild-type genes are especially liable to be disadvantageous (Ford, 1940).

Random genetic drift may fix deleterious genes in the homozygous state (Lewontin, 1982b) (an allele is said to be fixed in a population when it occurs to the exclusion of its alternative allelic states which are lost, Falconer, 1981); here dominance as such would be irrelevant, but genetic epistasis could serve a similar function. Substantial random drift may occur in the first generation of a new colony established by only a few individuals - the founder principle (Falconer, 1981), and indeed in some colonising species, mechanisms have arisen to limit its effects (Matsuda, 1982). That alleles can be lost under random drift itself suggests that it is better for development *not* to be totally dependent upon a specific genetic constitution.

3.5. Pupal cold shock

Subjecting pupae of C. cardui to -2°C at 3-5h post-pupation induced a darkening of the wing and a reduction in the extent of the pattern elements. The phenotypes varied in their *degree* of expression but all belonged to a common trend or morphocline (Nijhout, 1984). Similar phenotypes have been recorded as wild aberrants in this species (Shapiro, 1975b) and in others of the genus (Shapiro, 1973b), where nocturnal frost exposure during pupation has been suggested as the cause (Shapiro, 1975b) and is supported by meteorological evidence (Shapiro, 1981c). These aberrants too showed variable expression of a common tendency (Shapiro, 1973b). The most extreme laboratory phenocopy of C. cardui had a ventral hindwing pattern reminiscent of *typical* C. virginiensis Drury; and genetic assimilation of cold shock phenocopies might be responsible for the cardui-virginiensis morphocline (Nijhout, 1984). The latter species requires a more severe shock to induce elymi phenotypes than does cardui, suggesting that virgiensis has also become more canalised (Shapiro, 1981c). It is likely that the wild elymi forms of C. cardui themselves have a genetic basis resulting from such assimilation (Shapiro, 1975b); and the recurrence of the form in C. annabella Field means that if produced by a recessive gene, the gene would be present in the population at sufficient frequency (6%) to qualify as a polymorphism (Shapiro, 1973b).

Disruptive selection (the splitting of a sympatric population into reproductively isolated units) might facilitate the genetic assimilation of species differences; such as that between Precis coenia and P. nigrosuffusa under courtship constraint (Shapiro, 1983) and whose hybrid viability would suggest that it results from genetic differences at but a few loci only (*ibid.*). Disruptive selection (albeit without genetic assimilation) has been implicated in the origin of a grass-feeding race of Lethe eurydice Johansson that has become adapted to dry habitat in only twelve years (Shapiro, 1974), and in producing sub-species of the univoltine Maniola jurtina (L.) that differ in flight season (Thomson, 1971). It is therefore conceivable that disruptive selection could isolate cold shock phenocopies through a possible difference in their development rate.

3.6. Specificity of response

Pupal cold shock can induce phenotypic modification in several other species (Merrifield, 1893; Høegh-Guldberg & Hansen, 1977) although they differ in the timing of the sensitive phase and the precise nature of the eliciting stimulus. Specificity is probably adaptive in ensuring that phenocopies arise only under conditions in which they are appropriate (Shapiro, 1981b).

Høegh-Guldberg & Hansen (1977) review the effects of cold on phenotype in the Lycaenidae. Lysandra coridon Poda requires several 6h periods at -14°C starting not earlier than 5h post-pupation; whilst Polyommatus icarus needs four weeks at 2-5°C starting within 24h post-pupation. Aricia artaxerxes, on the other hand, was originally thought refractory to pseudo-natural cold exposure, since it interacts with a genetic aspect (citing Høegh-Guldberg, 1968; 1969), although it does yield phenocopies given at least one 9h stint at 2-5°C starting within 15min pre- or post-pupation.

Phenotypic expression can also differ with the severity of the stimulus within a species. Pupae of C. cardui, for example, produced more frequent and extreme phenocopies (where the environment simulates genetic mutants, Waddington, 1952, here those presumed responsible for elymi aberrants) when subjected to -2°C for 72h than for 48h (Nijhout, 1984).

In the moth Panaxia dominula L., the sensitive period is the *late* pupa (when the wings would normally be taking up pigment) which requires *heat* (>22°C) rather than cold to modify pattern (Kettlewell, 1944a). Indeed cold actually inhibits the expression of one gene (medionigra) (*ibid.*).

3.7. Genetic assimilation

Waddington (1953) demonstrated that early Drosophila pupae exposed to 4h at 40°C produced flies with a variable reduction (including total loss) of the wing posterior crossvein, and that by selectively breeding those flies showing the greatest expression of this acquired character, and repeating the process at each generation, it would become an inherited one.

He proposed that the degree of expression of crossveinless (cv) trait depended on the number of modifier genes present. Under standard conditions, their number in any gene-complex is insufficient to bring the trait to expression, that is, sub-threshold. Selecting cv flies of greatest expressivity (most modifiers) built up a gene-complex in which the number of such modifiers (cv genes) exceeded the threshold for expression under standard conditions, and in which the expressivity of cv genes was increased. The trait no longer required heat shock for expression, and had become an inherited characteristic. This constituted phase 1 which depended on there being genetic variation in the base population. Continued selection for cv, with or without heat shock, further increased the proportion (penetrance) of cv flies; and this constituted phase 2 (*ibid.*).

Waddington (1953) termed the phenomenon genetic assimilation, which he later (1961) defined as 'a shift (towards a greater importance of heredity) in the extent to which a character is acquired or inherited'. His definition will be retained here.

The mechanism is essentially the same as that described by Ford (1940) for the evolution of dominance, only that the expression of traits is modified (by the rest of the gene complex) in relation to environmental rather than genetic (dominant allele) parameters. Again the wild types were less variable than the cv flies (Waddington, 1953).

Waddington performed several other such experiments (1952, 1956b, 1960; 1961 for a review), including one (1959) for increased anal papilla size in Drosophila larvae on exposure to salt. This case is especially interesting because it involves a *temporary* physiological response (exogenous trait) in individual larvae, is adaptive for the *eliciting* stimulus, and is non-threshold in that it shows a continuous range of expression in both exposed *and* unexposed larvae.

Characteristics that resemble exogenous traits but which are normally inherited he defined as pseudoexogenous (Waddington, 1957a), describing, for example, the hardened skin patches (callosities) on the underbelly of the ostrich that support it at rest but which appear in the embryo long before exposure to hatching (*loc. cit.*). He attributing pseudoexogenous traits to subsequent chance mutations that supercede the environment (the Baldwin effect) (*ibid.*; *cf.* Waddington, 1961).

3.8. Dominance relationships and genetic architecture

Waddington (1953) divided his heat shocked flies into two lines: the UP line selected for maximum cv expression; and the DOWN line selected for normal flies only.

At each generation the UP line was split into two: a HIGH subline in for which were selected flies of maximum cv expression; and a LOW subline for which were selected flies of minimum (lacking) cv trait.

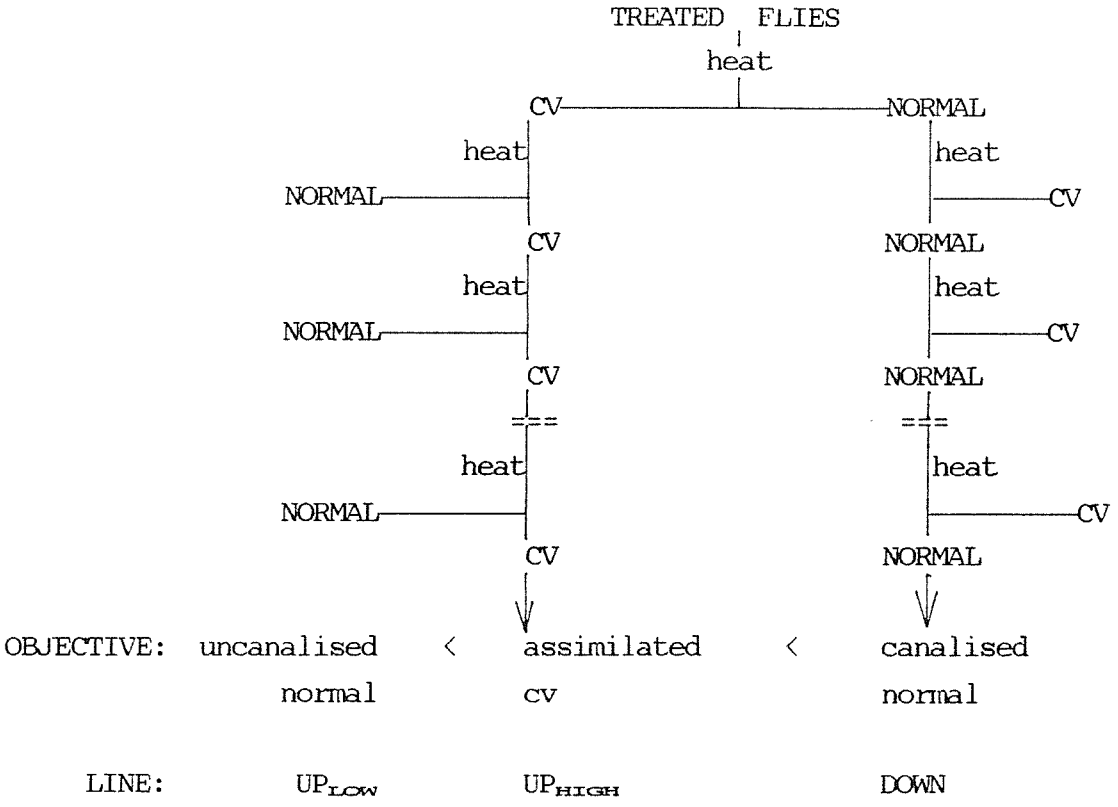


Figure 3.2. Waddington's (1953) crossvein-less (CV) protocol. DOWN line flies were selectively bred to develop normally even under heat shock. The UP$_{HIGH}$ line was selectively bred from flies producing CV trait under heat shock. UP$_{LOW}$ line flies developed normally under heat shock but were not selected to do so. Increasing dominance reading left to right is indicated (<). For further explanation see text.

The DOWN line was essentially canalised against cv under standard conditions. The LOW subline was essentially canalised against cv under heat. The HIGH subline was essentially canalised for cv under standard conditions. It was the high line that formed the basis of the genetic assimilation process (Fig. 3.2).

It would be expected that cv be adaptive and so increasingly canalised under heat conditions (HIGH) for which heat is 'normal'; and that non-cv be adaptive and so increasingly canalised under standard conditions (DOWN) for which standard conditions are 'normal'. But flies canalised for non-cv under heat conditions (LOW) for which heat conditions are 'abnormal' are expected to be maladaptive.

Thus, under standard conditions, HIGH cv might be expected to be dominant to the maladaptive LOW non-cv, but recessive to the DOWN non-cv which is more adaptive than either of the others; and indeed this was borne out (*ibid.*). Hence canalisation and dominance are dialectically related and depend on co-adaptation within the gene complex.

DOWN and LOW show that a common phenotype can be produced by different gene-complexes. The assimilated cv had several genetic differences from the foundation stock, so arguing against an induced mutation therein, or the Baldwin effect. Nor could a chance mutation account for the large number of flies affected at each generation. But this does not rule out mutation (induced or otherwise) occuring in addition (Waddington, 1953).

Waddington (1961) found in all the cases of genetic assimilation he studied that all the chromosomes were involved, although the *relative* importance of particular genes varied. For example (citing Bateman, 1959), the relative importance of a 'dumpy' dp^{+P}-type allele varied between 1.57 and 4.38 times that of the rest of the gene complex and was so designated a major gene to distinguish it from the remaining modifiers or <u>minor</u> genes.

3.9. Canalisation and assimilation

Seasonal polyphenism is problematic because it is difficult to see how *alternating* phenotypes could arise from a *cumulative* genetic assimilation of phenocopies. But the paradox can be resolved by distinguishing between the construction of a pathway, the canalisation of that pathway, and the choice between that pathway and some other, the latter being effected by a switch. The switch may be environmental or genetic (or involve interaction of the two) but not constitute a *component* of the pathway itself.

Seasonal polyphenism might evolve as follows. The switch can choose one pathway, those individuals with optimal expression being selected, whose offspring will possess a greater *propensity* for expression under its eliciting stimulus. This stimulus will be absent at the next season, when the process occurs with the alternative pathway, and so on. But individuals selected for optimal expression of one pathway might not be those carrying the (unexpressed) propensity for optimal expression of the other - which may even be selected against. However, the average propensity for optimal expression of the other would still be higher than in its previous *corresponding* season - because it will have then already been increased by selection. In other words, the level of minimal propensity for expression of each pathway will increase at each respective season. Thus, both phenisms could become assimilated by a kind of selective ratchet, which, since both are adaptive, would build up a gene complex in which each is optimally expressed under its appropriate stimulus. Shapiro (1973b) suggests that the similarity between the elymi phenotypes of three Cynthia species might involve reversion to a primitive trait derived from a common ancestor (atavism), so that if elymi is *dominant* in C. annabella, then, because dominance is an evolved characteristic, it reflects the persistence of *previously* selected dominance modifiers (a paleogenic condition). Moreover, an assimilated Drosophila bithorax (Bxl) phenocopy involved a Bxl gene dominant for haltere enlargement but recessive for lethal effect (Waddington, 1957b), showing that selection can operate simultaneously on associated yet opposing traits, and build up an integrated gene complex.

Of course, a pathway might become canalised against becoming switched on per se so that the pathway and switch become redundant.

Here its propensity for realisation is retained but no longer expressed under once inductive conditions (*cf.* Shapiro, 1979) so that it in effect becomes jammed in position. Severe stress may simply unjam the switch so once again allowing the pathway to be expressed. This could also come about through the restructuring of some *associated* developmental process. The modern lynx has the second lower molar (M_2) which is otherwise unknown in Felidae since the Miocene, and modification of its associated dental complex may have carried the M_2 part of the molarisation field above its realisation threshold (Kurten, 1963). This is an exception to Dollo's Law that structures cannot be totally lost and then regained in a similar form (*ibid.*); although when borne out, it might now be understood as modification to a field *overall*, which, because its *relational* nature imposes constraint on contingent positions within it, means that reapparent structures must necessarily comply. Redundant must not be confused with latent which will be used here to mean 'unexpressed' (for example the latent aestival form of the univoltine Pieris virginiensis Edwards that is not expressed in wild populations but which readily appears given continuous light) (Shapiro, 1971).

The choice between each of the four vernal pathways of L. argiolus pseudargiolus (including f. violacaea) might be understood as a genetic switch whose overall vernal state is itself switched by short daylength from the aestival pathway (which may involve processes in common with that of violacaea). Some polymorphisms are sufficiently variable to require statistical analysis to diagnose their presence versus a single continuum, for example that of forewing crossband fusion in the moth Diachrysia chrysitis (Jarvinen & Vepsäläinen, 1979). These might be conceived as a switching between pathways themselves not yet canalised for optimal expression under each of two *spatial* conditions; and underlines the need for 'major', 'minor' and 'modifier' genes to be explicitly defined according to the context of their useage.

3.10. Evolutionary requirements for assimilation

Since genetic assimilation is essentially just the canalisation of a new (environment-induced) pathway, the considerations are the same as for canalisation in general.

The main requirement is that the stimulus be a reliable predictor of the environment for which its *effect* is adaptive (or at least not maladaptive). An acquired trait may enable an organism to exploit alternative or additional environments, so that any model must allow for its evolution in more than one or in a changing environment (Waddington, 1968). Waddington (*loc. cit.*) gives the conditions for canalisation to occur, *i.e.* for a canalising gene to increase in frequency, where

K_C = selection coefficient for a canalising gene (c)
K_N = selection coefficient for a non-canalising gene (n)
P = frequency of the type of environment in which the respective gene is adaptive

as follows:

Clonally reproducing $haploid_C$: if $K_C < 2PK_N$
$Recessive_C$ versus adaptive $dominant_N$: if $K_C < PK_N$
$Dominant_C$ versus adaptive $recessive_N$: if $2PK_C < K_N$

Thus a canalising recessive can make its way against an adaptive dominant more easily than an adaptive recessive against a canalising dominant. Hence, when $P > 0.5$, it is advantageous to be canalising, even more so if also dominant (*ibid.*).

A gene complex or mutation (Baldwin effect) that replaces an environmental switch renders switching more reliable (Waddington, 1942); but must act before the time at which the environmental stimulus would act or else its job would have already been done for it (*ibid.*).

Maynard Smith (1982[b]) defines a strategy as 'a specification of what an individual will do in any situation in which it may find itself' and as evolutionarily stable when a common strategy is not

invaded by another. In a spatially variable environment that is coarse-grained (where any individual experiences only one patch type) there is no single optimum strategy, so polymorphism is favoured (although there can be a single optimum *provided* that the environmental extremes do not exceed its limits of tolerance) (Jarvinen & Vepsäläinen, 1979).

In genetic polymorphism the strategies are unconditional on environmental cues. But when the cues in each patch are *reliable*, environmental control is favoured even more, the particular strategy now being conditional upon which patch is entered (Lively, 1986). However, two conditions must be satisfied. The first is that the stress-tolerant form do better in the stressful patch (where fitness = k_s) than the non-tolerant form with the non-tolerant form doing better in the benign patch (where fitness = k_b) than the stress-tolerant form *ie. a cost to the stress tolerant form in the benign patch* (although the morphs need *not* affect each other). The second is that where benign patches occupy a proportion p of the total habitat and stressful patches a proportion 1-p, *the probability of the appropriate form (with respect to fitness) arising in each patch type be > 0.5.* It can be seen that as these parameters increase the range of patch frequencies over which environmental control is stable increases (*ibid.*).

In a narrow range of patch frequencies, the two morphs might be maintained by genetic polymorphism (in which case they *do* compete). Where environmental and genetic controls are mixed (as in L. argiolus) the probability of making the right choice must again be > 0.5. Under mixed control, some of the population shows canalised development while the rest remain polyphenic (*ibid.*). Lively (*loc. cit.*) suggests that different species can show various degrees of genetic and environmental control. This may explain the evolution of the Tatochila group (cf. Shapiro, 1980a; 1984). Genetic variation, however, is not essential for assimilation. Ho *et al.* (1983b) demonstrated assimilation of a bithorax phenocopy in a highly inbred Drosophila strain, and under opposing selection at that.

3.11. Further modes of inheritance

In addition to chromosomal DNA are mitochondrial genes (in eukaryotes) and plasmids (in prokaryotes). Eukaryotes, however, may also have extrachromosomal elements. Lysogenic bacteriophages and RNA retroviruses can be transmitted by entering and replicating with the host DNA.

Nucleic acids are not the only hereditary vehicle. Others include templating on the cell surface (Sonneborn, 1970) and transmission via the egg cytoplasm or maternal inheritance. An example of the latter is the melanic form of the butterfly Papilio glaucus L. (Burns, 1966). It is also involved in seasonal polyphenism in Polygonia c-aureum, which at 20°C produces its dark autumn form only if daylength is <13h (Hidaka & Takahashi, 1967). Under these conditions, larvae derived from *summer* females produced the autumn form *only*, but larvae derived from *autumn* females always produced *some* summer forms *as well*; implicating maternal effect in the induction of the latter (*ibid.*).

Several groups of organisms can regenerate a new organism from certain tissues throughout their lives ie. reproduce by somatic embryogenesis; and these include *all* plants, fungi, multicellular monerans and protists, certain sponges, coelenterates, and worms (Buss, 1983). In groups that reproduce by means of ramets a somatic variant incorporated into a ramet will be present in the new organism; and in cases where the gametes are sequestered from somatic tissues it will be present in the sex cells (*ibid.*).

Social skills can be acquired by learning, such as the use of leaves as drinking vessels by monkeys (Plotkin, 1982) and human personality traits which the offspring pick up by modelling their behaviour on that of the parents (Skynner & Cleese, 1983). Such cultural traits are termed *memes* (Dawkins, 1976). Another possibility is causative formation in which the mere *occurence* of a pattern (physiological or psychological) facilitates the formation of a like pattern elsewhere (Sheldrake, 1981). To illustrate, naïve rats in New York learned to solve a maze more quickly when presented with it after rats in London had already learned it, than when presented with it beforehand (*ibid.*). Finally, the use of say books to record and pass on human knowledge represents the creation of a heredity mode by the organism itself. In this case, the legacy of information left to

future generations might be regarded an example of Ho's (1986) inheritance of the environment.

Odling-Smee & Plotkin (1984) conceive heredity as involving a sensor which gains and registers information; a store or memory; replicator entities which pass on their structure directly in replication; and an interactor function that interacts as a cohesive whole with its environment in such a way that replication can be differential. Thus,

"Heredity is to be understood not primarily as the transmission of DNA but as a process involving feedback inter-relationships between organism and environment at all levels" (Ho, 1986).

3.12. Exemplars and mechanisms in genetic assimilation

Haploid wheat pollen grains became diploid and fertile on exposure to cold but lacked the usual stratified cytoplasm, and represents a direct effect on the gametes (Rives & Picard, 1977). The resultant plants produced gametes which also lacked stratification. Rives & Picard (*loc. cit.*) suggest that palindromic hair-pin extensions of DNA away from its main axis regulate the activity of proximal structural genes. Cold causes these regions to cross over and recombine so changing their regulator activity. The natural occurence (and hence reversal) of such change is very rare. In haploids reversal is even less likely so response to the first cycle of selection is quick and strong. In Capsicum unstratified gametes increased frequency from 10^{-4} to 10^{-2} in a few generations (*ibid.*, citing Pochard, 1971, 1974).

Various culture media induced a plastic variety of flax plants to develop as smaller genotrophs which had a reduced complement of rRNA and 5S genes distributed among a number of chromosomes (Cullis, 1983). They also differed in their isozyme banding pattern on gels and in the hairiness of the seed capsules. When sections of induced stem were cultured on non-inductive medium, the resultant plants also had less rDNA. Crossing over within tandem arrays is the most likely mechanism. (*ibid.*). The barley stripe mosaic virus (RNA) of maize activates transposable genetic elements that may enter gene loci and modify their expression. These genetic changes (but *not* the virus itself) are

often transmitted (McClintock, 1984).

LSD prevented larvae of the butterfly Pieris brassicae L. from developing into diapause pupae under an otherwise inductive <9h daylength (Vuillaume & Berkaloff, 1974). But the inhibitory effect of LSD in the resultant F_1 larvae was much *weaker*, thus implicating a detoxification mechanism transmitted to the offspring; this effect was also observed even if only one grandparent was treated (*ibid.*). However, the diminution in the effect of LSD was most effective when only the male parent had been treated. Vuillaume & Berkaloff suggest that LSD targets the parental (male) germ cell DNA, since LSD can damage chromosomes in vertebrates; and because successive backcrosses to untreated butterflies reverted towards the original condition, concordant with progressive dilution of affected genetic material at each generation. In the above cases, genetic assimilation involves a direct effect to the DNA, which in *P. brassicae* appears to be inherited *paternally*.

On the other hand, reciprocal crosses (control x treated flies) between an inbred and a massbred line of Drosophila after six generations of ether treatment showed that the penetrance of bithorax phenocopy (with ether) depended on the line from which the *female* parent was taken; and the difference between lines was even more pronounced in the F_2 (with respect to reciprocal cross) (Ho *et al.*, 1983b). But the latter did *not* occur in the control-female x treated-male reciprocal, suggesting some interaction between control-maternal cytoplasm and treated-paternal nuclear genes (where previous treatments may have induced segregation and recombination) (*ibid.*). The inbred line showed an assimilated prolongation of the ether-sensitive phase (*ibid.*). A hierarchy of switch genes is ruled out as whole compartment transformations were rare. But the distribution of metathoracic transformations is still non-random (Ho *et al.*, 1983a). Phenocopy maps suggest that two waves progress over the egg blastoderm at 90° to one another (Ho *et al.*, 1983c), the first rendering the area sensitive to ether and competent to respond to the second; which commits competent areas to metathorax and so no longer sensitive to ether. The patterning affects mainly the cytoplasm and then proceeds centrewards. The attendant nuclear differentiation is *secondary* (*ibid.*). It should be noted that it is the *perturbatory* effect of ether (not its precise nature) that

specifies the change (Ho *et. al.*, 1985). Ho *et al.* (1983b) suggest that assimilation involves a faster propogation of the competence wave with a later and slower determination wave.

Harrison (1928a) found that the green pupal colour formed in Pieris when deprived of blue light (using orange filters), appeared in their F_1 pupae formed under *ordinary* light conditions; in P. napi, *all* such pupae were green. Since blue wavelengths are essential for the deposition of pigments in the integument (without which the green haemolymph shows through) (*ibid.*), it may involve the induction and inheritance of some melanin-inhibiting factor (cf. Vuillaume & Berkaloff, 1974, above).

The microfilaments that emanate from the cell walls of Salmonella comprise flagellin which can occur in either a wavy or curly conformation. If a wavy or curly fragment or 'seed' is added to solubilised flagellin, then the latter (irrespective of origin) organises itself like the seed which thus serves as a 'nucleation' centre in a process akin to crystallisation. Such molecular templating is known as unit copying, and has also been demonstrated in unicells such as Paramecium, Stentor and Tetrahymena (Sonneborn, 1970). When cilial orientation on part of the cortex of Paramecium was reversed, the reversed area increased in size at each cell division until it covered the entire cortex. The reversal was stable over 800 generations, and such cortical inheritance is adaptive in ensuring uniform cilial deployment without which the animal could not feed or respond to stimuli (*ibid.*). Existing structure can also play a comparable role in multicellular organisms such as the turbellarian worm Stenostomum. In the above cases, the genome controls the topographic relations between existing and developing structures but *cannot* restore the original condition if the existing ones are altered (*ibid.*).

A process related to unit copying is Template Induced Molecular Assembly (TIMA) (Wasserman, 1982a) which may effect genetic assimilation as follows. An environmental stress on the tissue or cell surface induces changes the cell surface structure that could act as templates for TIMA (Wasserman, 1982b). New types of protein then appear in the cell surface that increase its permeability to mitosis inhibitors or chalones. These in turn may prevent new cells arising by mitosis, or, alternatively, they may induce mitosis so resulting in

extra cells. New membrane proteins might similarly lead to new genes arising in the germ line cells. And if several genes arose by multiplication, they might retain a common control element enabling the gene 'battery' to be controlled *en masse* during development (*ibid.*).

The pond snail Limnaea stagnalis is normally elongated, but in some Scandinavian lakes occurs as a very short variety, bodamica Class., whose shortened form is the result of muscle contraction during growth and protects it from strong currents (Piaget, 1971). Bodamica is hereditary, and most likely arose through genetic assimilation as its contractile form is beyond the range of normal variation in the species (*ibid.*). Piaget suggests that natural selection acts *with* genetic assimilation but is not essential since strong currents do not actually eliminate the long ones. Besides, bodamica *chooses* the harsher environment, for it could avoid the currents by burying; indeed, in the sublittoral occurs an elongated form bollingeri Piag. (*ibid.*).

Terrestrial landhoppers may have evolved from supralittoral ones by genetic assimilation (Matsuda, 1982). The former are smaller and have fewer moults, and since they reach sexual maturity at an earlier age are neotenous. They inhabit dark forest leaf litters, where the reduced light intensity decreases the production of androgenic hormone by the X-organ (hence diminished moult frequency) and induces a hormone that stops the Y-organ releasing ecdysone (hence slower growth). Matsuda proposes that each terrestrial form arose independently, but that their respective populations might have moved onto land *en masse* and showed adaptive responses *before* establishing themselves as new taxa, so contradicting the founder principle.

Neoteny has also been implicated in the evolution of the salamander Ambystoma tigrinum which in montane lakes retains its juvenile aquatic habits (*ibid.*). This is adaptive at high altitude where aquatic environments provide more food and shelter than terrestrial ones. The neoteny is believed to result from decreased tissue sensitivity to thyroid hormones T_3 and T_4, or from a loss of pituitary sensitivity to thyrotropin-releasing hormone so that the relative amount of prolactin is too high for metamorphosis. In the related A. gracile - which also shows a greater frequency of neotenous

forms at high altitude, laboratory experiments confirmed not only that in some individuals neoteny could be induced by cold, but also that in others it was genetically determined (Matsuda, 1982, citing Sprules, 1974).

Hormonal mediators have also been implicated in the influence of temperature on wing length in Heteroptera, where it decreases with cold (Southwood, 1961). In protothely, there is a deficiency of juvenile hormone in the larva, and the animal undergoes fewer than normal moults with a precocious development of adult structures (paedogenesis). In metathely, there is an excess of juvenile hormone in the adult, but the animal undergoes the normal number of moults and is akin to neoteny. Matsuda (1982) suggests that apterous insects could have arisen through the genetic assimilation of metathely, and that photoperiod might also have been involved.

Denenberg & Rosenberg (1967) found that an increase in the activity of female rats in response to being placed in a can of wood shavings was present in untreated F_1 and F_2 animals (but not in the F_2 if the F_1 females had been reared as pups in confined conditions - which may have restricted their movement). Although possibly transmitted by learning, its expression in the F_2 was stronger in females than males, and the importance of their *mother's* rearing conditions suggests that their increased activity might have induced physiological changes in the developing foetus or milk supply (*ibid.*).

Indeed canalisation is implicated in the evolution of appropriate attachment behaviour in the Japanese quail, Coturnix coturnix japonica (Kovach, 1984). It was found that newly-hatched chicks imprinted with a blue screen tended to then choose blue over red more than either those imprinted with red or controls (unimprinted); and that the strength of the preference increased over the generations (*ibid.*). Young chicks normally imprint to the hen, which is adaptive since attachment to inappropriate objects can mar their social development, and Kovach (*loc. cit.*) suggests that their preference for the hen as an imprinting stimulus has become an inherent one through canalisation. There are however occasions when it is better for development *not* to be canalised, such as in the (sessile) dry-season form of Melanitis leda where the variability of its wing-spotting may prevent predators from forming a search-image (Brakefield & Larsen, 1984).

Genetic assimilation can also involve changes in development *rate*. The N. American cicada genus Magicicada includes three coexisting species which undergo synchronous 17 year life-cycles (the species within a brood are held together by introgressive hybridisation). Several woodlands, however, have more than one brood so that adults appear more than once in every 17 years; and it was found that such broods are separated by four years with a number of cicadas emerging 'ahead of schedule' (Lloyd & White, 1976). The most likely stimulus is a stereotyped fighting behaviour among the nymphs that cues overcrowding (and hence impending food shortage). It is probably effective only when severe - so indicating that *several* nymphs might benefit from (and hence be likely to undergo) acceleration. This ensures that enough go through to maintain a population; which may then retain its normal 17 year periodicity (*ibid.*). In the south of their range the species undergo synchronised 13 year cycles as the *norm*. Lloyd & White suggest that this arose through genetic assimilation of the four-year acceleration; indeed they assert (*loc. cit.*) that it is the only hypothesis yet proposed for explaining how almost perfect 13-year periodicity could have evolved from 17-year periodicity without passing through intermediate stages (which would have destroyed periodicity), although it is also possible that the number of years required for completing the cycle might be quantised, since 13 and 17 are prime numbers (Richard J. White, pers. comm.). The origin of the acceleration response is unclear, but it may involve *reversal* of a 4-year *inhibition* in their early history which freed them from a synchronous parasitoid, since 17-year cicadas differ from the 13-year ones in that their growth is very inhibited during the first four years (Lloyd & White, 1976).

In somatic embryogenesis, mutations can mount up much more quickly than in cases where germ cells are sequestered - in humans the frequencies of viable germline and somatic mutations are 10^{-12} and 10^{2} per generation respectively (an artificial example but it illustrates the point) (Buss, 1983). Although such a somatic mutation must compete with other cells in the ramet or germline, or may suffer immediate environmental selection, its high frequency means that if *favoured* by environmental demands then such organisms may evolve very quickly (*ibid.*).

In humans, it has been suggested that the Negroid skin type might

have arisen as a direct result of hot climate (Bowler, 1984, citing Maupertuis, 1968). However, McKinney (1973) argues that such assimilation is unlikely since Negros exposed to sunlight actually become *paler* (*ibid.*), but the latter is just fading as the pigment is destroyed by ultra-violet (R.J. White, pers. comm.). Indeed McKinney (*loc. cit.*) had cited the case of a Caucasian woman with negroid skin on the left arm, attributed to her mother having received a fright while pregnant by stepping on a live lobster - although, of course, this might have been just a somatic mutation (R.J. White, pers. comm.).

Human *personality* traits, however, *are* transmitted. These arise through matching ones behaviour to the family environment during pre-adult emotional development, whilst trauma too can inhibit the development of appropriate emotional responses which may therefore retain their childhood level of maturity (Skynner & Cleese, 1983). Since emotions are expressed outwardly, the person's family history is also reflected in their physical demeanour; which is recognised by others and on whose basis partners choose each other assortatively. The result is that they maintain the original family environment, from which their own offspring effectively acquire their social repertoire. This is also, therefore, an example of Ho's (1986) inheritance of the environment.

3.13. Inheritance of the environment

Since environmental factors can influence form, an organism is not uniquely defined unless its environment is specified (Ho, 1984a). However, the environment *itself* can change during the life of an individual organism, so that (as in the last example) it is better to consider potential *life histories* (Ho, 1986). Furthermore, traits are not simply the *outcomes* of an organism's development but change *throughout* its life (Dobzhansky, 1956); for instance, butterfly wings may fade or suffer damage *after* their ontogeny has been completed. Insofar as an environment is still present during the next generation, it is effectively left as a *heritage*; and in this *general* sense environments too are inherited (Ho, 1986).

The environmental parameters may be effectively constant from one

generation to the next. For example, several British butterflies have undergone major changes in their geographical distribution over time periods (less than 2000 years) much shorter than those of geological epochs (as marked by the onset and retreat of glaciations) (Downes, 1948). However, environmental factors may differ *within* or *between* life-cycles; and such difference can be *spatial* or *temporal*. In these cases an organism's developmental and hereditary fate can be determined by its *choice* of environment (Lewontin, 1983); and this choice can be to *remain* (as with Piaget's snails), or to *move*. For example, Speckled Wood butterflies need a high body temperature for oviposition (Shreeve, 1984). In spring and autumn, when the ambient temperature is low, they bask in woodland rides to absorb solar heat, but in summer the warmer air temperature enables them to lay in the shade as well (*ibid.*); and their choice influences the offspring's development (Shreeve, 1986). In autumn the pupae may 'choose' to develop *before* the winter if conditions are suitable, so producing a false brood (Shapiro, 1977a; Winokur, 1988); whilst in spring the adults may 'wait' for suitable conditions in their pupal cases and then eclose synchronously (Goddard, 1962).

Hence it is the *organism* that defines its environment and there are no pre-defined niches (Lewontin, 1983). It is therefore meaningless to say, for instance, that there are no vertebrates which slide along the ground, climb trees and eat grass, because such a 'niche' would only become such by *virtue* of a vertebrate sliding along the ground, climbing trees and eating grass (*ibid.*). Rather, adaptation describes a *functional* relationship between organism and environment (Ho, 1984a); and it is this *relationship* that develops and evolves (Costall, 1986).

An acquired trait may enable an organism to exploit alternative or additional environments; and there may be more than one solution to an adaptive problem. For example, migratory moths often develop a darker wing pattern under cold so enabling more effective thermoregulation in the autumn (Kettlewell, 1963). But this could also enable them to remain active and so *seek out* a warmer habitat by *migration* (*ibid.*). On the other hand, this darkening could *enable* them to invade cooler habitats and hence further expose themselves to the very conditions that brought about their darkening in the first place. The latter involves positive feedback in that it stabilises heredity (cf.

canalisation); and since it can occur between nucleus and cytoplasm, it can involve any structural level (Ho, 1986). It also reveals that heredity not only involves material continuity, but is also a process phenomenon (*ibid.*).

Environmental effects may be reinforced. In Japanese Quails, the initial imprinting means that a chick is more likely to further choose its attachment stimulus (Kovach, 1984); while an initial cohort of accelerated cicadas would support subsequent incomers (regarding predator satiation and breeding structure) (Lloyd & White, 1976). Alternatively, organisms may *construct* a domain in which conditions remain suitable by using some provision from the niche (such as caterpillars that build a 'tent' by joining leaves together) or by taking advantage of their own physiology (as in caterpillars that spin cocoons).

Organisms also act *on* the environment (Ho, 1986). A good example is ecological succession, in which the invasion of coastal dunes by beach grasses converts the sand substratum to soil when the grasses die back and decompose (Odum, 1975). Here, the formation of humus renders the soil unsuitable for the very grasses that formed it in the first place. But it also enables invasion by hardy shrubs that can only *then* become established. The process continues until eventually mixed woodland predominates (Odum, 1975). This principle is also relevant to early evolution, where the oxygen released by photosynthetic cells would have poisoned their anaerobic progenitors (Schopf, 1978); and rendered the atmosphere too reactive for the de novo formation of the molecular species from which life had originally arisen, but now suitable for the development of aerobic forms (Dickerson, 1978).

3.14. Genetic assimilation : a critique

Waddington's early experiments might be criticised on the grounds that the stimuli applied were artificial and the criteria by virtue of which phenocopies were selected for further breeding not biologically meaningful. However, the modifying capacity of stimuli and the nature of their effects would still be delimited by the pathways available to the organism (cf. Goodwin, 1984a). Moreover, developmental effects resembling those produced by *artificial* manipulations are sometimes found in *nature*. French (1984, citing Bateson, 1894) reports natural occurences of insects bearing supernumerary limbs, a phenomenon otherwise known to result only from rotational regrafting (French *et al.*, 1976). Thirdly, Waddington's (1959) demonstration that salt tolerance could be increased through genetic assimilation in Drosophila larvae *did* involve an adaptive response, and one selected by the very agent for which it was adaptive.

Williams (1982) asserts that if an organism's response to a stimulus is not adaptive, then the organism should be regarded as 'susceptible' rather than 'responsive'; and that because most organisms are 'susceptible', genetic assimilation could not effect evolutionary change. Yet one might argue similarly against the role of gene mutations, most of which are deleterious or at best neutral. Maynard Smith (1975) expresses dissatisfaction with Waddington's experiments in that the assimilated lines were not 100% penetrant and showed variable expressivities. But he seems to miss the point, namely that of genetic assimilation as *an* evolutionary process, for genetic mutants too often fail to reach fixation (Falconer, 1981) or vary in expression (Ford, 1940). Yet interest in genetic assimilation has been maintained and over the last two decades has led to many novel discoveries both in the laboratory and in the field (see section 3.12.). Of these, only Matsuda's (1982) appears to have been at all called into question, and even here, only on the grounds of faulty assumptions pertaining to landhopper physiology and ecology (Duncan, 1985) and *not* to genetic assimilation as such. Polikoff (1981) does, however, unfairly criticise Waddington for his failure to *fully* explore the evolutionary potential of his ideas.

3.15. Waddington's evolutionary scheme

Waddington (1982) viewed the overall evolutionary system as comprising four components: the exploitative, insofar as the organism can choose its niche and modify its environment; the epigenetic, in that abiotic or biotic stresses *reveal* certain developmental potentialities; the natural selective, through which adaptive efficacy determines the *fate* of such potentialities; and the genetic, where mutation and recombination modify the *nature* of these potentialities.

Begg (1952) felt Waddington's (1952) notion of phenocopy (cf. genocopy where a mutation mimics the environment: Matsuda, 1982) to be misleading on the grounds that such 'acquired characters' are no more 'acquired' than those arising from any other aspect of the genotype-environment interaction. However, the latter is now understood in terms of the generative field; in which the genetic is an aspect of the inherited (Goodwin, 1984a). Notwithstanding, Polikoff (1981) had criticised Waddington for de-emphasising physiological genetics, which, when the genetic system is taken as the primary level of integration, has important evolutionary implications. Still, *this* gap has closed considerably with dynamic models of gene expression (Kaufmann, 1969), nuclear differentiation (Nijhout *et al.*, 1986) and pattern formation (Wolpert, 1962; French *et al.*, 1976; Murray, 1981); and since the generation of form is itself epigenetic, it is more accurate to describe biological systems as manifesting epigenetic properties than as possessing an 'epigenetic *system*'. Here, the relation between structural levels presents a number of epistemological problems, and these are examined in chapter four.

Now the essence of heredity is continuity (Ho, 1986); which it is suggested be partitioned into the material and the thermodynamic. Indeed structure can only exist *vis-a-vis* thermodynamic possibility whether it is replicated or *not*. The probability of structural change (P) is greater the smaller the increase in complexity (δC), where:

$$\delta C = \log_2 (1/P)$$

(Saunders & Ho, 1976) and this constrains the possible adaptive solutions. For example, frequency-dependent Batesian mimics would do far better by evolving a foul taste, but do not, because it would

require a greater increase in complexity than does a change to the wing-pattern (Saunders, 1984). As Waddington (1957a) remarked, organisms lose out in selection, not so much for doing the wrong thing, but for not doing the 'right' thing well enough. In this regard, adaptation refers to 'fittingness' to the environment (Darwin's actual contention, cf. Pribram, 1982), or in other words, structural stability *vis-a-vis* external relations.

The principle of minimum incease in complexity would suggest that replication is easily evolved because it simply involves a step from generation to *re*-generation. Hence, in heredity, the notion of adaptation simply extends to also include that of *replicative* stability. Here, natural selection *describes* the notion that fittingness to the environment selects from the set of structural possibilities the subset of replicative possibilities. Indeed Wright (1931, cited in Saunders & Ho, 1976) had introduced the concept of 'selective valley' to describe positions of maximum fitness, mirroring the epigenetic landscape and Waddington's (1957a) view of organisms as 'positions of organic stability'. Waddington's failure to explain the *origin* of physiological (pseudo-exogenous) responses (Polikoff, 1981), can now be understood in terms of epigenetic possibilities ('phenotypic space') which through *happening* to be adaptive might become assimilated as exogenous ones (cf. Shishkin, 1984).

Matsuda (1982) has criticised Waddington for his objection to the Baldwin effect. Baldwin (1896, cited in Matsuda, 1982) had spoken of organic selection, which mid-twentieth century geneticists took to mean that organisms adapt to new environments using non-genetic means until a genocopy turns up whose frequency is then increased by natural selection. Waddington's (1961) objection was that it deemed the genotype irrelevant to phenotypic plasticity, and natural selection as having no effect on the system *until* the appropriate allele turned up. But Matsuda (1982) saw genetic assimilation itself as an attempt to explain organic selection in terms of mid-twentieth century genetics. Indeed some neo-Darwinians have in turn mistaken genetic assimilation to mean directed mutation (Smallwood, 1986).

However, organic selection has been seriously misrepresented even by those who are not neo-Darwinians (Costall, 1985). Rather, acquired characters are not selected as such, but influence the fate of subsequent heritable variations (which may *or* may not coincide with

the initial accommodat). Behaviour can also influence the course of evolution, whence organisms take on the role of agents rather than puppets (*ibid.*) (so mirroring Waddington's (1982) 'exploitative' component). For example, an animal might respond to flooding by swimming or by burying themselves, after which a given variation may be good for one but not the other (*ibid.*).

3.16. The applications of genetic assimilation

Perhaps the most famous is that by the Russian T. D. Lysenko during the 1930's in an attempt to end the country's chronic wheat shortages. He claimed that the effect of vernalisation (in which the seeds are frozen to accelerate their germination in spring) was inherited; once the wheat had been processed it would germinate earlier in all future generations (Bowler, 1984). Genome shock, which induces major chromosomal restructuring, *has* however been used to improve the reproductive stability of wheat (Triticum), by hybridising it with Rye (Secale) to generate the commercial food crop Triticale (McClintock, 1984). Indeed it has been suggested that Triticum itself may have arisen in this way (*ibid.*); whilst Kieser (1987) asserts that major advances in plant breeding may be achieved using stress environments to allow the expression of underlying genetic variability.

That human personality is moulded by one's family environment and life events, and influences the upbringing of the children, is now well grounded in the psychoanalytic school as a structured set of concepts that provides the basis for its methods of individual and family therapy (Skynner & Cleese, 1983) and which therefore qualifies it as a paradigm. The ability of modern medicine to treat genetic diseases, notably inborn errors of metabolism, means that their incidence is likely to increase in the population (Goodenough, 1978). The respective genes are not selected as such, nor are their *immediate* effects adaptive (although they might be *used* to confer some *other* advantage such as exemption from hazardous occupations), but their treatment does keep them 'afloat' in the population and so echo organic selection.

In human heroin addicts, the drug in a mother's bloodstream may

enter that of the developing foetus with the result that the baby is born dependent on heroin. If the baby is not desensitised by medical treatment, then it too will grow up an addict, who, if female, may in turn pass on the dependency to her offspring. Certainly the risks to the foetus from smoking while pregnant have been well publicised.

Ho (1984, citing Fujii, 1978) reports serum calcium disorders in parathyroidectomised rats being inherited up to the F_4 generation. It is thus conceivable that the surgical ablation of endocrine organs to treat medical disorders such as hyperthyroidism, might affect the offspring, who, unlike the parent(s), would not be expected to undertake any necessary hormone replacement therapy.

CHAPTER FOUR

Summary

The view that the 'inheritance of acquired characters' has long been refuted is based on only the scantiest of evidence, it having come about through the inability of Weismann's scheme - once vital were Darwinism not to flounder - to encompass environmental effects on *conceptual* grounds.

Yet modern evolutionary theory has come up against phenomena which challenge some of its most basic assumptions. It is therefore necessary to re-examine our metaphysical understanding of evolutionary theory. This chapter essentially aims to provide a broader conceptual stance.

This chapter reconsiders 'cause' and 'effect' and the way in which experience is described and explained. Grounds are presented for preferring the view that the genome, organism and environment evolve as an integrated *system* rather through stepwise changes.

The relationship between form and function and how these are delimited by constructional considerations is explained. Adaptation is understood as the replicative stability of structural possibilities and illustrated by early cell evolution and mimicry. Development and evolution are viewed as differing in their adaptive processes occuring within or across generations, and a case for considering construction *and* genealogy to delineate species is presented. The principle of 'competitive exclusion' is re-examined.

The relationship between theory and observation, the structure of scientific theories, and how they change, are explained. The theories of Lamarck, Darwin, Weismann and Waddington are fully expounded: they were never as crude as is popularly believed.

Neo-Darwinism, however, still views natural selection as the sole evolutionary force, and the current status of the debate is assessed.

The role of scientists as people is compared with the popular high regard for institutionalised science, and their bearings on scientific practice are discussed. Darwin's theological position is clarified and a possible unity of science and theology is presented.

CHAPTER FOUR

EVOLUTIONARY EPISTEMOLOGY

"sociobiologists are just running scared of ferocious philosphers" (Waddington, 1975)

although perhaps more generally the case with the *socio* cut out...

...and yet,

"there is nothing contradictory between evolutionary epistemology as a philosophy, psychology or biology, though a demonstration that they are indeed complementary would be more to the point" (Costall, 1983)

4.1. Overview

The problems pertaining to the relationship between structural levels are necessarily epigenetic - otherwise higher levels are mere collections (whether random or regular) of their lower level components, when no problem arises.

The first problem is that of our *perception* of that we call 'reality', because perception is limited to what can be resolved by the senses (and hence is reductive), and because it distinguishes between classes of experience that may not reflect 'real' differences (a matter of essence) or which may sever lower level connections so dissipating higher level emergent properties (a matter of analysis) (Davenport, 1979). The problem carries over into our description of reality because its terms but symbolise classes of experience (a matter of misplaced concreteness) (*ibid.*). The second is that of explanation, where, because 'to explain' is to describe higher level phenomena in terms of lower level ones, the problem of analysis resurfaces as information loss between structural levels (Davenport,

1979). These problems are pertinent to the construction of rational taxonomies (Webster, 1984) and to what constitutes a 'unit of phenotype' (Kemp, 1985).

The latter are also afflicted by the problem of causality. The notion that lower levels give rise a priori to higher ones *without* recourse to emergent properties or the downward flow of constraint distinguishes mechanism from individuation. 'Machine thinking' is central to the relationship between genes and traits as understood by such authors as Monod (1971) and Dawkins (1976); and its implications *vis-a-vis* the construction of organised systems are also considered.

Models of organisation fall into two main categories, namely random (and stochastic) and structuralist schemes. Randomness is often assumed a feature of molecular evolution (Monod, 1971) and phenotypic variation (Dawkins, 1976), but has been criticised on grounds that the range of extant forms is rather limited (Gould & Lewontin, 1979) and that the time-scales required to have narrowed these down from unrestricted possibilities are unrealistic (Wasserman, 1982b; Fox, 1984). Structuralism asserts that life is subject to the same considerations as physical systems (Ho & Saunders, 1984b) (for example the upper limit imposed on insect size by gaseous diffusion rates given the structure of their respiratory physiology: Ford, 1957a) and is restricted by 'laws of form' (Webster & Goodwin, 1982) (for example that no organism has active muscles *and* photosynthesis: Schubert, 1985 *re* Waddington). Units of structure are defined by virtue of their (component attributes) sharing a common *generative* process (Goodwin, 1984b) which could also aid in designating phenotypic characters as 'relevant' in phylogenetic reconstruction (Webster, 1984). The *relatedness* of structural units delimits the possible systemic changes (through which derive a *subset* of forms) and serves as 'information' that enables their self-correction following perturbation (Webster & Goodwin, 1981). The main criticisms of structuralism are that it has yet to *specify* 'laws of form' (Kemp, 1985), that its relatedness is confined to the domain of the spatially instantaneous (Reed, 1982a) and that it ignores historical considerations on grounds that history cannot be tested (Kemp, 1985).

History is however a centrepin of Darwinism, in which forms are related via genealogy to a common ancestor, and transformed by natural selection. The main problems are whether a *particular* form arising on

more than one *independent* occasion would still constitute a single species, and how distinct species arise *vis-a-vis* the phenotypic continuum of gradualism (Webster, 1984). The paradox is resolved by defining the common ancestor as a type and the essence of species in terms of reproductive isolation *and* indivisible generative field capacity within whose bounds natural selection can operate. Punctuated changes involve the field conditions breaching a threshold (Kieser, 1987).

A formal scheme of adaptation is presented with consideration to its content and nature. The former is exemplified by the evolution of eukaryotic cells. The traditional view is that they originated from symbiosis between simple organisms (Margulis, 1970); and is essentially *atomistic*. A novel scheme is sketched in which they arise epigenetically; and is essentially *structuralist*. Here alternative solutions to the problem of osmolysis fall into a single 'logical type' that allows for *de novo* organelle formation. The latter is exemplified by mimicry whose definitions have hitherto been fraught with semantic difficulty (cf. papers (1981) in Biological Journal of the Linnean Society, 16: 5-54. A hierarchical taxonomy of mimetic *relationships* is suggested to resolve the difficulty.

A key figure in understanding hierarchical organisation was the mathematician and philosopher A.N. Whitehead, whose ideas complement those of Piaget (1971). The distinction between teleology ('aimed at') and teleonomy ('ends up at') is clarified. Whitehead was a major influence in Waddington's thinking (Schubert, 1985) and in the inception of process theology (Hick, 1983) (below). A corollary of these is that process is *recursive*. Recursiveness features in heredity (Odling-Smee & Plotkin, 1984), and through levels of cyclical change as exemplified by cell division, physiological homeostasis and ontogeny (Davenport, 1979). It is also impicit in Haeckel's famous dictum: 'ontogeny recapitulates phylogeny'.

The origin and early history of the evolutionary idea are sketched. That the 18th century preformationists saw homunculi because they expected to do so (Davenport, 1979) bears on the relation between theory and observation (Chalmers, 1978). The nature of scientific theories is outlined, and Darwin, Lamarck and Waddington are given a retrospective look. Neo-Darwinism is currently undergoing a Kuhnian crisis (Earthey, 1988; Collingridge & Earthey, in press). Kuhn viewed

science in terms of paradigms that reach crisis point when their explanatory power is exhausted (1970, cited in Chalmers, 1978). The status of natural selection, which looms large in the dissatisfaction (Ho, 1985, 1987; Ho & Saunders, 1984a; Ho *et al.*, 1986, 1987; Saunders & Ho, 1981), is re-assessed.

Such rivalry is reminiscent of that between political ideologies (Kemp, 1985). Indeed biology (and evolutionary theory in particular) and politics have had important bearings on each other (*ibid.*). Darwin, for example, was inspired by the ethos of his day, whilst Lysenko exiled Mendelians to Siberia on account of his allegiance to Lamarckism (Bowler, 1984). It will be argued that the complementarity of biology and ideology results, not a priori from their similarity, but from the a priori similarity of a given *type* of thinking to *each* of them. This brings home the fact that science is a human endeavour. The emerging paradigm also appeals for pluralism (Ho & Saunders, 1984b), but in doing so stands to put its concepts in atomistic relation to one another, rather than in *unified* transcendent relation to their predecessors.

Yet unity is at the very root of holism. Such *holos*, completeness, is also the root of *holy*, which is often mistaken in theology to mean perfection. Eastern Taoism, however, and the more recent western Process Theology, both view God as constrained by the nature of reality (Smullyan, 1981; Hick, 1983); and, whilst they have been instrumental in shaping modern physics (Davies, 1983), their metaphysical bases have yet to be assimilated by biology (Davenport, 1979), although a possible pointer in this direction is the Anthropic Principle (Gale, 1981). Man's place in Nature is then considered.

This discourse is intended to spark enthusiasm and highlight the dialectic between biology and philosophy, thus allaying Costall's (1983) fear that evolutionary epistemology will be seen, as he cites Kierkegaard as having described philosophy, like the shop with a sign in the window saying 'Trousers pressed here' which you enter only to discover it's the *sign* that's for sale.

4.2. Experimental embryology and genetics

In the late nineteenth century, the German biologists Hans Dreisch and Wilhelm Roux pioneered the study of development, so founding the school of experimental embryology. However, it soon emerged that its phenomena would not be reducible to mechanism. Dreisch had found that the bisected sea urchin embryo could regrow as two whole but smaller larvae, and being unable to find a mechanistic explanation, ascribed it to non-material force that guided development towards its end point (Davenport, 1979, citing Dreisch, 1891). This mirrored Maxwell's discovery of electromagnetism which, as far as Newtonian mechanics was concerned,

> "proved to be the monkey wrench that ruined the machine" (Davenport, 1979).

Field concepts figure largely in modern biology (Wolpert, 1962; French *et al.*, 1976; Goodwin, 1984a). Yet several biologists remain convinced that the study of genetics will ultimately be able to explain development, and they represent the school of developmental genetics (Davenport, 1979).

4.3. The problem of analysis

An aspect is distinguished through the experience of some difference whose recognition depends on perception (Davenport, 1979). Experimentation extends the limits of resolution, enabling lower levels to be observed eg. cells by microscopy, or to infer higher level distinctions eg. species differences by genetic marker. But since experience is ultimately restricted to that which can be perceived (or thereby inferred) it is reductive.

The various levels at which structure can be perceived constitute a perceptual hierarchy (Davenport, 1979), a hierarchy of nominal essences (see below). For example, the difference between Pararge aegeria and the related P. xiphioides might be percieved as the presence of a given eyespot, the number of spots (irrespective of which *particular* spots are present) or in their spatial deployment

(irrespective of number) (Higgins & Riley, 1975). But descriptions of form in terms of lower level components may overlook their higher level relationships. For instance, spot size asymmetry might differ between species although neither side alone does so; and this is the problem of analysis (literally, to loosen or untie) (Davenport, 1979).

These problems are pertinent because morphometric characters are often used to compare geographic races, to examine genetic variability, or to discern ecological differences (White *et al.*, 1988). Moreover, various descriptor systems differ in their ability to detect subtle gradients (clinal variation) or to discriminate between groups (*ibid.*). For this reason, the present study will be examining the P. aegeria wing pattern at a number of levels; namely manual measurement of individual wing markings under travelling microscope; the perusal of photographs to isolate more visually obvious and overall pattern differences; and the digital analysis of moment invariants, which provide a statistical description of the wing in terms of the distribution of light and dark without reference to individual markings.

4.4. Description and reductionism

Nonetheless, entities at each level do have autonomous properties (Webster & Goodwin, 1981) which derive from their underlying structure and processes by virtue of which an entity *is* of a particular kind, its real essence (Webster, 1984). The success of chemistry is due largely to its concepts (pi orbital, valence, methyl bond and the like) being based on real essences, for it too involves taxonomies (the periodic table for one) and processes that might be regarded as competitive (such as the displacement of sodium ions by potassium from an aqueous solution of its hydroxide). Each entity also has observable qualities by virtue of which it might be correctly *identified* as being of a particular kind, its nominal essence (*ibid.*). The trouble with nominal essences however, is that they are unreliable. Had chemistry used nominal essences for classification (say white for phosphorus and red for arsenic) or explanation (say water the only group six hydride to be liquid because oxygen is the only group six element to be a gas), then chemists might still be struggling with Mendeleev's

periodic table (in fact red and white solids are both phosphorus isotopes and water is a liquid because it forms hydrogen bonds). But biology still bases its taxonomies on traits (nominal essences) that are chosen from an individual and *then* used to gather in other members. But the set of, say, speckled white objects does not present *itself* for classification (Chalmers, 1981). In other words, its members do not appear similar because of some underlying relationship, but are related by *virtue* of their *apparent* similarity. And, since the range of possible criteria is in principle unlimited, the choice of traits is arbitrary, and even when presupposed by some theory as to which are relevant, the selection is still partial (*ibid.*).

4.5. Explanation and reductionism

"Apples can be described in quantum mechanical terms but not very usefully" (Lewontin, 1968)

High-to-low level mapping involves their *correspondence* but does not reflect low-to-high causation. For instance, the difference between normal and sickle cell haemoglobin can be mapped to the substitution of a glutamine for a valine. But this change is not the a priori cause of the anaemia; if it was then a similar change to insulin should also affect *its* function (it does not) (Goodenough, 1978). The reason is that differences in function (oxygen transport or glucose uptake) emerge from changes in the *form* of the amino acid sequence whereas mutations change only its *content*. Hence a complete description of each haemoglobin will include that of glutamine and valine, but not *vice versa*. Thus, higher level descriptions are more inclusive, whereas lower ones are more precise, literally, 'cut out', that which is cut out from them being distinctions arising from their connectedness in the higher levels (Davenport, 1979). For instance, the *difference* between base triplets AAT and ATT and between GAT and GTT will be identical at the transcription ('AAT and ATT' and 'GAT and GTT' both have an A-T middle base difference) but not translation level (AAT and ATT and GAT and GTT *each* code a different amino acid).

Terminologies such as 'gene' are but linguistic symbols *assigned* to perceived levels of repetitiveness. They neither cause them nor

mirror them in their inherent structure, a misconception that A.N. Whitehead termed 'misplaced concreteness' (Whitehead, 1926, cited in Ho & Saunders, 1981, and portrayed through Magritte's painting 'The Key to Dreams'). Hence descriptions of higher level phenomena in terms of lower ones *viz.* explanation will necessarily suffer the same reductive limitation as the levels they represent, and so fail to adequately encompass individuative and formative processes (Davenport, 1979).

But determinism remains popular with the modern synthesis because taxonomies based on traits can then be safely held as valid reconstructions of 'real' genealogical connections via the DNA (Webster, 1984), as in Cladism where traits on whose basis taxa are assigned are regarded as Aristotelean 'atomistic' entities (Kemp, 1985); and because development can be regarded as mere gene to trait *connection* (rather than trait *generation*). This endows gene and trait with synonomy *vis-a-vis* the mechanics of phenotype evolution (selection of 'causative' genes), and so neo-Darwinism may rightly be accused of applying misplaced concreteness (Ho, 1985). Neo-Darwinism has asserted that an understanding of development is of no consequence to evolution (Ho, 1985). Although this *is* a valid assertion within the language of *neo-Darwinism*, because the language within which the development that is talked about is framed (or object language, Chalmers, 1981) is the *same* as that of the statement that refers to it (or metalanguage, *ibid.*), it is not valid within that of experimental embryology where development is understood in terms of concepts whose language *transcends* that of the modern synthesis. Conversely, the new paradigm accuses neo-Darwinism of reductionism (Ho *et al.*, 1987), but in so doing fails to recognise its own reductive nature (Davenport, 1979). The problem of reductionism is largely one of description, that of mechanism one of causation; namely that it is limited to denoting temporal priority because (by definition) an effect cannot precede its cause (*ibid.*).

4.6 Genes, memes, and machines

Sydney Brenner has been cited as saying that if we knew its entire DNA sequence then we could compute the entire beast (Lewontin, 1982b), but this is clearly wrong because not even the organism computes itself from the DNA (*ibid.*). The view that genes determine traits remains, because, unlike the notion of design, the notion of purpose did not die away with the advent of Darwinism but was simply relegated from that of divine fiat to that of adaptive efficacy. With the formulation of the new synthesis, came an ontology whereby constraint flows from the lower levels in which such 'purpose' is held to reside (Davenport, 1979). This view reaches its epitome with Dawkins' (1976) notion of the selfish gene, which, because 'selfishness' is a product of conscious thought, is necessarily anthropomorphic in implying that genes possess some sort of a priori 'knowledge' as to their phenotypic effect and adaptiveness. And, insofar as adaptiveness assumes competitive success, it implies their possession of 'wills' (A.P. Costall, pers. comm.). But consciousness is an emergent property that arises out of only much higher structural levels, so it cannot possibly be prior or inherent in the genes (Hofstadter, 1981a,b). Thus, it is also meaningless to describe evolution as proceeding through genes behaving *as if* they were consciously selfish, since such a kind of behaviour, by definition, implies that they possess a property (selfish-like behaviour) attendent only with consciousness itself. Moreover, even *were* genes to map one-to-one to traits, their adaptiveness would still be only secondary to their integration within the organism and in turn the organism-environment interaction (Costall, 1986).

Dawkins (1976) did however suggest that units of cultural learning, or memes, might provide an additional hereditary mode, an important contribution, but unfortunately viewed these too as atomistic and 'selfish'. Memes could change more quickly than genes (Baerends, 1984), although Baerends (*loc. cit.*) asserts that the changes are more likely to be inconsequential to survival. Yet the meme which determines the desire to seek nuclear disarmament, for example, is hardly inconsequential to survival (Richard J. White, pers. comm.). Besides, similar criticism could equally be levelled against genic mutation. The only sort of knowledge genes might be said

to possess is the sort Bateson (1979) alludes to in asking, for example, how a starfish might 'know' to produce five limbs, namely that *vis-a-vis* their real essence, they *delimit* and *not* determine subsequent organisation.

The New Synthesis view of prebiotic evolution is that the earliest biomolecules arose amidst a milieu in which all were possible and the building blocks came together by chance (Davenport, 1979, citing Monod, 1971). This conception is quite plausible *given the proviso of sufficient time* and is attractive to modern biology because Darwin's (1859) 'struggle for life' is inherent in it. Although complicated by its notion that protein reproduction depends on particular nucleic acids, the new synthesis does not see this as problematic because holds that nucleic acids too arose through chance and serial condensations whose probability space was unconstrained. Thus, it was only a matter of time before the appropriate nucleotide sequences turned up (Maynard Smith, 1975). Hence both kinds of molecule came together as organised systems whose components were stabilised through their association (Davenport, 1979).

The main difficulty is that the probability of any polymer arising is extremely small. Given twenty amino acid species, the number of possible polypeptides of length one hundred units is 20^{100} or 10^{130}, so that each would appear only once every 10^{130} sequences; and the likelihood of any combination of polymers arising is the product of their individual probabilities. Thus, prebiotic evolution would proceed imperceptibly slowly. Indeed,

> "random guessing is no way to go about constructing...intricate molecules. (Cook, 1977, cited in Wasserman, 1982b)

The above kind of argument is often used by Creationists opposed to evolutionism, when it is known as the specious probability argument, which, it may be asserted, does not apply to evolving sequences on grounds that, rather their units coming together 'at once', their lengths increase with time (R.J. White, pers. comm.). But the latter introduces only sequential aspect and does not, *per se*, reduce the probability space. But even with unlimited time, each species would have to be stable for long enough to overcome

dissipative forces that might counter their build up in number.

The modern synthesis meets this difficulty by arguing that nucleic acids were much more limited in possible form than proteins (Fox, 1984), but it is hard to imagine, given that both arose in similar fashion, why this should be: it is hard to imagine why those nucleic acids that were more prevalent a priori should be those involved in the production of proteins that would then *happen* to be more efficacious; or conversely, why given the efficacy of certain peptides, those nucleic acids that would then effect their production should happen to have been more prevalent a priori - unless they in some sense 'knew' the function and stability of their resultant peptides. Such a scheme is therefore untenable and presents a problem of origin. Moreover, simulations of prebiotic conditions have revealed that similar ranges of organic species tend to result (Dickerson, 1978). Some never form, whilst others predominate amongst those that do, and artificial polymerisation of nucleic and amino acids has shown the various condensations to differ in prevalence and permanence (Fox, 1984). Hence molecular probability space is limited by their reactivity and structural stability (*ibid.*).

Furthermore, DNA (and RNA) replication and protein synthesis are interdependent so that replication and transcription (even in their simplest conception) become meaningful only *once integrated* as a system, and this presents a problem of organisation. The earliest systems were most likely akin to coacervates that effect a few simple functions within the confines of a membrane through which metabolites are exchanged with the milieu (Schopf, 1978). The complexity principle has shown that removing components from organised systems (say a tRNA from a coacervate) tends to lose with it some essential function (Saunders & Ho, 1976) such as replicative capability. This renders the construction of coordinated systems from preformed components problematic; for if it occurs through their piecemeal acquisition, then until their completion such systems will be continually awaiting and so missing some vital capacity; whilst the notion that preformed bits all come together 'at once' implies some notion of a priori 'conception'.

Yet even *machines* do not arise from parts that randomly assemble de novo (Davenport, 1979). When one considers the construction of say lexicographical devices such as quills, pens, stencil writers or the

Caxton printing press, it is evident that they are not elaborations of earlier models, but were built for a common purpose - putting alphabetical characters on paper - whose modi operandi imposed constraint on their design; quills and biros must both fit the hand and allow the free flow of ink; it is hardly surprising therefore that both are of similar form and never made of wood, yet no-one would suppose that quills were ever used in the construction of the latter; dot-matrix printers and the Caxton press both employ pounding actions so durable materials must be used for the hammering parts. Another difficulty with so-called 'machine thinking' (Davenport, 1979) is that machines have only *motive* power, whereas biological systems have inherent *formative* power that can impart form to material initially devoid of it and involves not just motion but also *time* (Rotenstreich, 1982).

The notion of a priori biological ordering is deemed to have died with the argument from design, so there has been generally no call to question the view of lower levels as causes of higher ones. The problem of missing parts (say a DNA replicase) is met by arguing that some other function will stand in for them (say direct hydrogen bonding of nucleotides). But it must be remembered that regulation is *secondary* to relatedness (which constrains the possible kinds of lower level changes); whilst the problem becomes more serious with a blow by blow assembly dependent on replication, as it will reach an impasse (best highlighted by analogy with the development of a printer whose manufacture requires referral to design drafts that it itself must print) in that the process will halt at any omission, unless, of course, reference is made to some *external* ordering source. Thus formative constraint flows from higher to lower levels (Ho & Saunders, 1979).

The problem is resolved by viewing prebiotic and subsequent molecular (genic) evolution as involving an increase in the complexity of molecular *interactions* (Wasserman, 1982a,b). Increases in molecular complexity call for reorganisation of the whole system (generative field), which itself delimits the kinds of change possible in the first place and can correct imbalances. Environmental factors such as UV radiation may facilitate this reordering, whilst reactive molecules may destabilise it from without. Hence, factors normally regarded as strictly 'selective' become formative and interactive when understood

as constraints on structural (and replicative) stability.

4.7. Structure and form

Any object from the set of which a trait is an aspect will have other qualities which if chosen as criteria might generate a different set, and this is why traits are unreliable classificatory criteria, unless the aim is to compile purely *descriptive* classes. For example, no problem arises in classing butterflies as 'browns' (Ford, 1957a) by virtue of their brown colour, as brown butterflies are, by definition, brown; but problems do arise in using brown to assign them to the Satyridae (vernacular 'The Browns') because there are Satyrids that are not brown, whilst the Brown Argus (Aricia agestis L.) is a Lycaenid (*ibid.*).

The paradox arises because the similarity between members of a class lies in the *relationship* between their traits or form rather than in the traits themselves. To illustrate, butterflies have narrow bodies and clubbed antennae, are colourful and diurnal, and rest with their wings raised vertically; moths on the other hand are fuller bodied with fine antennae, are drab and nocturnal, and rest with wings held horizontally (Novák, 1980). The difficulty comes with say the Crimson Speckled, Utetheisa pulchella (L.), a gaudy day-flying species with a narrow body, but which has filamentous antennae and perches wings akimbo (Skinner, 1984). It is in fact classed as a moth owing to its unclubbed antennae, the most *consistent* distinction between moth and butterfly (Warnecke, 1964). Consistent features are convenient because although form is *recogniseable* it is difficult to *describe*, and a 'reliable' distinguishing feature enables its assignment to a class whilst sidestepping the need to describe form. But the recognition of form must be the prior, otherwise mothness would come to be defined as 'lack antennal clubs', rendering 'mothness' synonomous with 'lack antennal clubs' and hence redundant, and so overlook moths' other aspects of general similarity (cf. Webster, 1984). Conversely, the recognition of 'mothness' cannot depend on all its 'component' aspects being present, otherwise it would come to refer only to that organism which possessed all 'mothness' traits so rendering the concept redundant; which could be avoided only by

becoming a total description of all moths when it would describe a collection rather than define a class; and bypass their aspects of difference (cf. Webster, 1984).

Rather, form describes an archetype or type that comprises a *set* of (descriptive) elements and their (spatial) relationship (*ibid.*). An example is the Nymphalid Groundplan (Schwanwitsch, 1929) which describes the archetypal butterfly wing pattern, and any species exemplifiying the type can be described in terms of some or other alteration to its components. Here, eighty percent of all Lepidopteran patterns can be derived by removal or transformation of selected groundplan elements (Nijhout, 1981). Elements are defined by their *relative* positions within the set which therefore delimits the kinds of change they may undergo (Webster & Goodwin, 1981). Yet within this constraint they do have some autonomy. For instance, groundplan Discalis II can never cross the Media but *can* shift within the area between them (Schwanwitsch, 1935). The groundplan is depicted in Figure 9.2. It must be emphasised, however, that the groundplan does *not* represent any supposed ancestral form, nor are all its elements fully expressed in any actual species (Schwanwitsch, 1924).

That parts derive their identity from their relation to a whole; that the whole delimits their individual properties yet within this constraint leaves them with still some autonomy; and that their relatedness within the whole enables self-correction for loss or damage, constitute the three main tenets of Structuralism (Piaget, 1971; Webster & Goodwin, 1981). The components of types are sometimes called 'forms' (Webster, 1984), but the term element will be used to avoid confusion with form whose more familiar meaning will be retained.

4.8. Form and transformation

Types also feature in other disciplines, for example the generative grammars of linguistics (Webster, 1984, citing Chomsky, 1968) and the collective unconscious of psychoanalysis (Jung, 1961). The parametric distributions of statistics might also be regarded as types whose elements are the parameters. For example the curves of the binomial and poisson distributions share a common topology (type) whose respective bell and tongue shapes describe their form. This is important, because the moment invariants used in the present thesis to describe wing pattern and shape are statistical parameters which, because differences between them can be tested for statistical significance, render form (pattern and shape) amenable to explicit and repeatable description - and hence formal comparison. Indeed the carapace shapes of various crab species are transformations of one another (Bateson, 1979, citing D'Arcy Thomson, 1961).

The notion that organisms are transformations of one another dates back to before Aristotle, and has certainly been applied at least as the underlying assumption in the use of laboratory animals as surrogate humans (Rosen, 1982), which, for some reason, are held particularly interconvertible with Guinea pigs. Yet caution must be taken, for the drug LSD has quite different effects on house fly, elephant and human (Gould, 1980).

Structuralism has been criticised for emphasising types comprised of topological and geometric relations (pattern) almost to the exclusion of those comprised of temporal relationships (process). Forms whose occurrence is independent of the earlier existence of one another are said to be time-independent or synchronic (Waddington, 1962). Those that depend on the occurrence of some earlier form are time dependent and diachronic (*ibid.*). An example of a diachronic type is Darwin's common ancestor, whose forms are related via genealogy and transformed by natural selection (Webster, 1984). Adaptiveness essentially concerns stability; and the stability of forms as *occurrent* structures is now considered.

4.9. Structure and construction

Since the *ontogeny* of each synchronic form is a diachronic process, the spatial and temporal domains become unified in biological process. However, the ontogenetic production of a form (and hence its occurrence) requires that it be structurally possible. It is often forgotten that organisms are physical structures and hence subject to the same considerations as non-biological matter. For instance, a level of intelligence comparable to Mankind's necessitates that brain size be massive to accomodate the large number of neurons required before conscious thought can emerge (Gould, 1980). Viewing consciousness as emergent (Hofstadter, 1981b) ties in with nature being continuous from the inorganic to the biological and cultural domains (Ho, 1987). Indeed, organisms have a formal kinship with the physico-chemical realm (Ho, 1984b). D'arcy Thompson found that fusel oil dropped into water, for example, took on shapes reminiscent of jellyfish (Ho & Saunders, 1984b).

Although traditional biology has recognised this kinship (Gould & Lewontin, 1979; Gould, 1980), it has been criticised for restricting its link with physics to the Cartesian and Newtonian (Ho, 1985) when rather the flow of causation is *among* levels, although still simplistic (Ho, 1987). Structuralism , on the other hand, has yet to fully articulate laws of form (Kemp, 1985), although it *has* identified fundamental features of holoblastic cleavage, namely binary cell division, that subsequent cleavage planes are always perpendicular to foregoing ones, and that animal-vegetal cleavage is preferred when a choice exists (Goodwin, 1984a); as well as six essential features common to ontogeny and phylogeny, these being (1) an increase in the size and complexity of gross form; (2) conservation of basic symmetry; (3) progression from unicellular systems to cell aggregates through to three primary tissues and finally many tissues; when post-gastrulation one or more areas become localised into a major organ forming region, organ areas, then distinct organs; (4) that as cell number and diversity increases they become spatially delimited with respect to function; (5) that organelles become structurally modified in a pattern of diversity that corresponds to the pattern of cell function; and (6) that proteins increase in number and diversity and become spatially restricted with respect to the pattern of cell

differentiation (Davenport, 1979). However, the occurrence of spherical field geometries, and the attainment of bilateral symmetry especially, still "presents a problem of enormous conceptual difficulty" (*ibid.*). In the following section, pointers as to how these might be resolved are presented.

4.10. Fields and conformation

Models whereby fields are established by two boundary conditions are held unable to account for spherical field geometries such as typify the egg and early embryo. But this is resolved by allowing the field geometry to extend beyond the animal-vegetal axis, just as magnetic fields have a spherical geometry that extends beyond their north-south dipole. The organic material then falls into accord with the geometry, just as do iron filings in a magentic field. And since physical systems exhibit such 'action at a distance', there is no a priori reason why more complex organic matter should not. Indeed, Sheldrake's (1981) formative causation recently entered the public eye with an experiment conducted on television, in which the abilities of American viewers to interpret an impression of light and dark before and after British viewers had been given the 'solution', were compared. Although inconclusive *either* way, it did not inspire much fuss, and little effort has since been given over to furthering such investigation. The most likely reason that such effects remain largely undetected and are not amenable to reduction, is simply because they require biological matter for their resolution and cannot be described in terms of lower levels, no more than magnetism would have been discovered using wooden apparatus or its field geometries understood by hunting for their 'units' or generative 'mechanisms'.

The problem of bilateral symmetry arises because 'leftness' and 'rightness' are *relational* concepts that *cannot*, in fact, be intrinsically defined but whose individual identities derive from some *extrinsic* difference imposed on them from without (Bateson, 1979). But this provides a clue as to its origin. It is suggested that the direction of sperm entry provides an axis which with the animal-vegetal defines a plane that bisects the embryo. Symmetry results because the spherical contours of field strength cross this

plane twice and equidistant from the A-V axis (Fig. 4.1). Thus the symmetrical pattern of gross cell behaviour arises, not a priori from the cells behaving in relation to one another (although they may do so), but through *each* cell behaving in accord with its contingent position in a *field* such that any plane through it happens to be symmetrical. In other words, cells do not *need* to know on which side of the symmetry axis they lies, for each behaves in a contingent manner but whose net manifestation is a pattern of gross *cell behaviour* that *happens* to be symmetrical. No, bilateral symmetry is a matter of enormous conceptual simplicity. After all, no physicist ever lost sleep over the symmetrical deployment of iron filings on a sheet above a magnetic bar.

The above do *not*, however, rule out fields whose domains *are* established by molecular morphogens or electrochemical charges; calcium ions, for instance, stimulate contraction waves in gastrulation (Goodwin, 1984a). And since certain field behaviours tend to characterise particular species groups - supernumerary limbs for example being most easily generated in hemimetabolous insects though they can also occur in moth larvae, beetles and the axolotl (French, 1984), field properties could contribute to formulating a taxonomy of real essences ie. generative parameters. Indeed, structuralist models can help phylogenetic reconstruction by assessing whether or not a pattern is likely, and so preclude various 'morphospaces', *independently* of evolutionary process (Kemp, 1985).

Organisms can certainly respond to and generate more familiar kinds of field. Birds navigate by exploiting the earth's magnetism; and sharks hunt for prey by tracking down the electrical discharges associated with muscular activity, while electric eels stun theirs by delivering a bolt of several hundred volts. These involve interactions with the environment, and the ways in which organisms overcome the problems it presents constitutes *the problem of adaptation*.

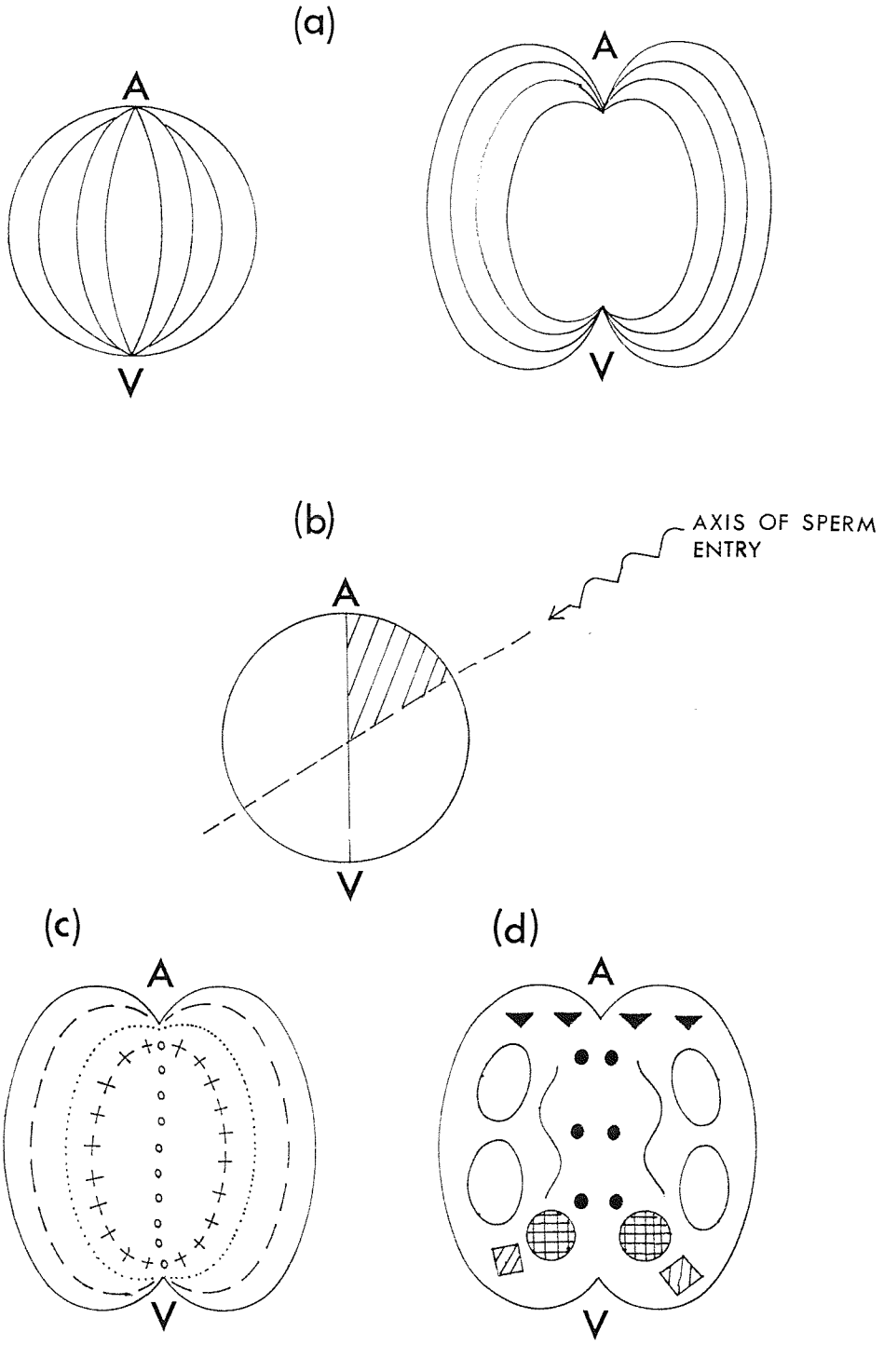

(a)
A
V
A
V
(b)
AXIS OF SPERM ENTRY
A
V
(c)
A
V
(d)
A
V

Figure 4.1 (opposite). The attainment of biateral symmetry in a simple field. (i) The spherical field geometry associated with the animal-vegetal (A-V) axis; alternatively (ii) the geometry at each pole may more resemble a torus. Note that it is these conceptualised lines of equal field strength that confer relatedness on the system. (b) The axis of sperm entry (dashed line) together with the A-V axis defines a plane (line shading) in an otherwise homogeneous egg. (Nodes of surface free energy, *cf.* Goodwin, 1984a, may similarly define such planes). (c) Planar section through the field as defined in (b): the values at positions equidistant from the A-V axis *happen* to be symmetrical on the plane because the system it transects is rotationally homogeneous about the A-V axis. (d) Cells within the system simply differentiate in accord with the field value at each of their contingent positions; information concerning the side of the axis on which they lie or as to the state of their neighbouring cells is irrelevant since, prior to differentiation, the system is structurally homogeneous (although the A-V polarity may, of course, result in *A-V* distinctions).

4.11. The problem of adaptation

Adaptive efficacy is usually taken for 'fitness' or the capacity to leave as many offspring (or gene copies) as possible (*eg.* Dawkins, 1976). But as long as *any* of its offspring survive to reproduce, then a species would be no worse off producing but a few offspring at each generation than many. Another reductio ad adsurdum is that r-strategists (rapid reproduction of many individuals, Odum, 1979) should, by definition, displace k-strategists (slower but more resourceful reproduction and fewer individuals, *ibid.*). This leads on to the notion of competition, but the notion is misleading. For example, the fungus Penicillium produces the antibiotic penicillin which kills bacteria in its immediate proximity that might reduce the availability of nutrients. But the problem that Penicillium is solving is not, a priori, one of bacteria, but one of nutrient availability: nutrient stress similarly confronts spores settling on igneous granite substrata, when competition is *not* invoked. To invoke the notion of competition because the threat to structural or replicative stability comes about, directly or indirectly, from living (as opposed to dead or abiotic) matter, results in erroneous conceptions as to what adaptation is really about and is anthropomorphic.

Let us imagine a species with lifespan 100 years (100 year form). After 100 years it dies and so is no longer present as an *occurrent* entity. But were it to reproduce, then its offspring would be extant for the next 100 years, so that the species (but not any individual) would be occurrent for 200 years. Thus, reproduction can be conceived as a solution to the problem instability arising from the (natural) mortality of *individual* organisms. Furthermore, it is postulated that since reproduction is simply re-*production*, it involves but an increase in number and so a simple change (versus a complex one, *sensu* Saunders & Ho, 1976) and hence is an easily achieved innovation.

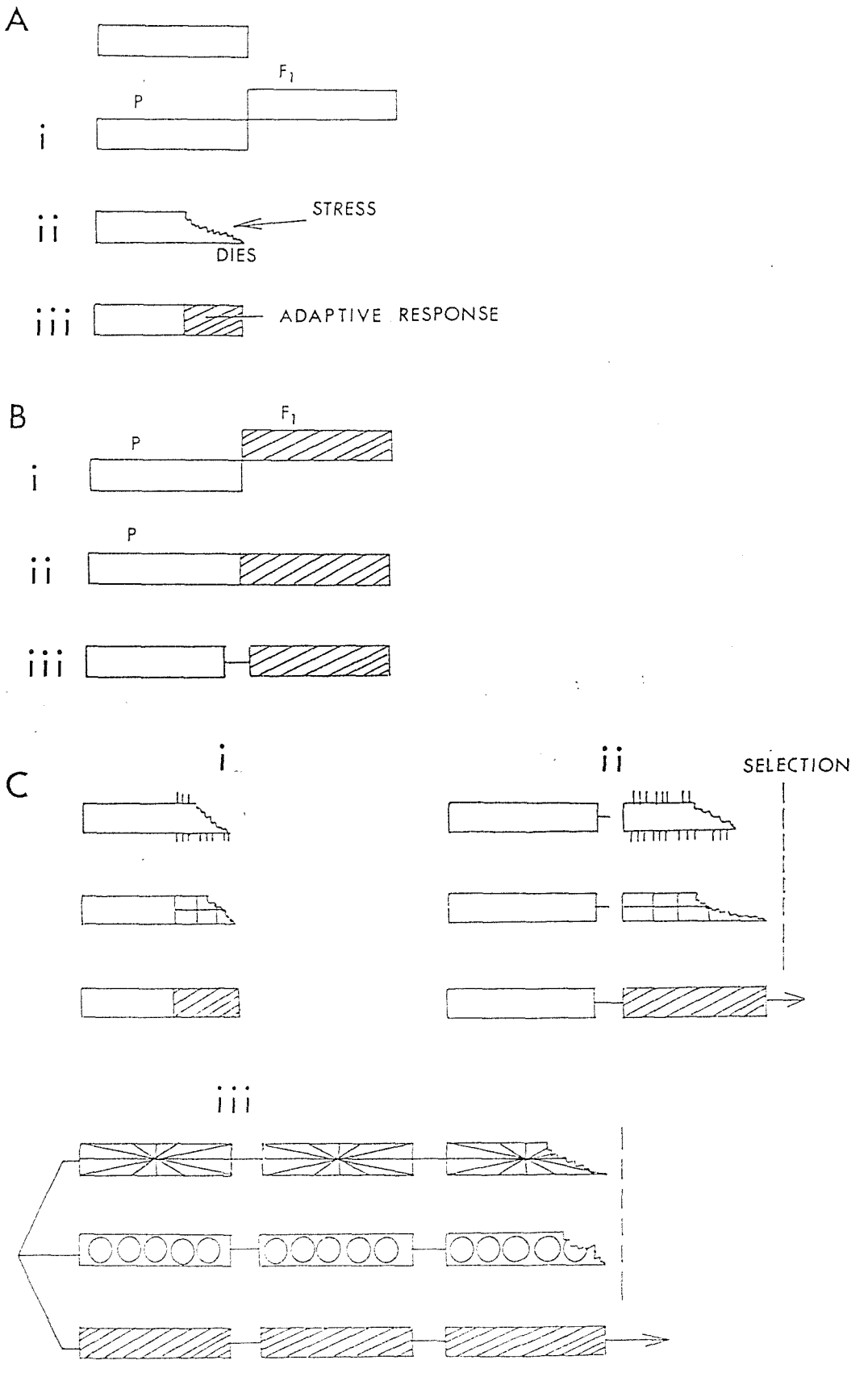
Let us now suppose that after 50 years the 100-year species experiences a potentially lethal stress, but one which it might overcome through physiological (ontogenetic) adaptation. The set of structures available to it will, of course, be delimited by ontogenetic possibility, but should one of these, say darkening, enable such individuals to survive, then, from among all the

individuals, the dark ones (only) will remain occurrent. Thus, adaptation refers to the stability of a species as an occurent entity *vis-a-vis* its interface with the environment (Figure 4.2a).

Now let us imagine a reproductive species identical to the above, only of 50 years natural lifespan (50 year species), which similarly experiences a potentially lethal stress after 50 years but one which it might overcome through physiological adaptation. Again, the set of possible structures is delimited by ontogeny but one of them (darkening) enables such individuals to survive. The *only* difference now is that, since the species is of 50 rather than 100 years lifespan, this ontogeny would be that of new *individuals*. Again, too, from among all the individuals, only the dark ones remain occurent (Figure 4.2b). But because in the case of the 50 year species the ontogenetic state has changed *between* rather than *within* a generation, its form will have been said to have *evolved*. Yet, without knowing the lifespan of individuals or their reproductive history, it would not be possible to distinguish them as belonging to the 50 or 100 year species. Put another way, development and evolution differ only in the level of resolution (in terms of filial generation of the individuals) of change within the continuity of the generative field.

4.12. The problem of species. I. Genealogy

Insofar as continuity of the generative field is maintained by the reproduction of individuals within it, succeeding generations represent a genealogy. Webster (1984) cites Hull (1959) as asserting that if a form arose today identical to some extinct species of pteradactyl, then it would not constitute the same species - although most biologists would still find this rather strange given species as defined by the ability to function as an interbreeding population (Webster, 1984). However, this paradox might be resolved by regarding related species not as each form *derived* through genealogy, but rather each form *deriveable* from a common generative field *whether via genealogy or not* (Figure 4.2c). Nonetheless, when derived via genealogy, the initial state of the generative field can rightly be regarded as their common ancestor. To give an analogy, all the chemical elements can be derived from a high temperature plasma of


A
P
F1
i
ii
STRESS
DIES
iii
ADAPTIVE RESPONSE
B
P
F1
i
P
ii
iii
C
i
ii
SELECTION
iii

neutrons, protons and electrons. In this sense the plasma can be regarded the 'type' from which each element (form) is deriveable, and, insofar as each element is derived from a common type they are related to one another. Now certain elements can also form through nuclear fusion, such as helium from two hydrogen atoms, when the sequence plasma-hydrogen-helium can be regarded as a genealogy. However, helium can also form directly from plasma without the genealogical connection via hydrogen. In both cases, hydrogen and helium share a common generative type (plasma), which when helium does arise via genealogy can be regarded as 'ancestral', but with respect to which the genealogical or non-genealogical nature of its connection to helium has neither a bearing on its capacity to generate the element nor on the properties of the helium itself.

FIGURE 4.2. Adaptation and selection in the generative field. (A) Reproduction enables a species to survive as an occurent form beyond the lifespan of its individual organisms (i). Individuals suffering a potentially lethal stress (ii), might overcome the stress through physiological adaptation (iii). (B) Such a stress arising only in the F_1 results in the change in form occuring between generations (i) rather than within a generation (ii); the change to the *species*, that is evolution, however, is the same in both cases (iii). (C) The possibilities available to the generative field are the same within and between generations. Ontogenetic efficacy (i) determines which forms survive as individuals within a generation, while reproductive efficacy (ii) determines which forms survive as replicative entities. (iii) Each form is a subset of the potentialities of a common generative field, at which level they might thus be conceived as being of the same 'species'. Reproductive isolation further defines each form as a species in terms of its potential subset of inherited particulars. Natural selection delimits which subset of these are present as extant forms. For further explanation see text.

The Russian biologist Sergei Chetverikov defined species in terms of reproductive isolation (as opposed to differences in form), a definition central to 'population thinking' and the 'biological concept of species' (Babkoff, 1977). It is suggested that this definition, *together* with the notion of species in terms of like kind of generative field capacity, provide an improved conception of species. For example, the moths Laothoe populi (L.) and Smerinthus ocellata (L.) (Sphingidae) can hybridise, although the F_1 moths are infertile and so would not maintain (via genealogy) the generative field as an occurrent entity. But that they can produce F_1 hybrids might qualify them as a single species in terms of the lack of reproductive isolation (though not if defined by the ability to produce fertile *offspring*). However, coexistent forms of the periodic cicada genus Magicicada *are* highly interfertile, yet classified as *distinct* species (Lloyd & White, 1976), and here, their distinction must involve some criterion other than simply reproductive isolation. Conversely, it is conceivable that forms might be identical in essence except for the particular karyotype or genital structures that prevent them from interbreeding. Indeed the Lepidopteran genus Pyrgus (Rhopalocera: Hesperiidae) compises several species reliably distinguished only by differences in their genitalia (Higgins, 1975; Higgins & Riley, 1975). Of course, if two (or more) forms are identical in essence *and* can breed, then no problem arises: by this improved definition, then, Hull's pteradactyls are the same species.

A further kind of difficulty arises, however, with phenomena such as lysogeny. The lysogenic bacteriophage lambda, for example, infects the bacterium Escherischia coli within which it can reproduce as an autonomous genome, producing new phage particles which kill the infected cell (Goodenough, 1978). Alternatively, the phage DNA can insert into the E. coli chromosome when it is stably reproduced as part of the host genome (*ibid.*). The problem here, therefore, is by what bacterium and phage might be regarded as distinct species. It is suggested, therefore, that a species be defined as an indivisible unit of generative field capacity. Thus phage and bacterium are distinct (but not necessarily independent) species; phage head particles and bacterial cell walls are not. Reproductive isolation is a necessary consideration since, because the finite longevity of its individuals renders the generative field dependent on their reproduction for its

maintenance as an occurent entity, it is essential that each reproductively isolated form be able to continue the generative field through reproduction. Thus adaptation must encompass the stability of species as *replicative* entities. And so on to natural selection.

4.13. The problem of species. II. Natural selection

It is therefore clear that for the maintenance of a given species beyond the lifespan of its individuals, there *must* be *reproductive* stability. (Of course ontogenetic possibility is a priori because it is meaningless to talk of the reproduction of forms that cannot or did not exist in the first place.) For instance, the moth, Arctia caja can occur as either red or yellow hindwing forms (Skinner, 1984), but were, say, the yellow form to succumb to a lethal virus, then it would be eliminated and so no longer *available* for reproduction. Of course, insofar as the yellow pigment may be an ontogenetic precursor of the red one (Ford, 1957a), the yellow form would then still be just as possible *in principle* (in the same sense in which copper is an electrical conductor even if a current is never passed through it: Webster, 1984), but it would no longer be occurent owing to its instability as a *replicative* entity. It is therefore suggested that [natural] selection be understood as effecting a change in the kinds of extant, stably replicated, forms as a subset of the *possibilities*.

The reason for this is that any individual of a species will manifest only one of a possible number of forms available to the generative field: no individual A. caja for instance can be both the red and yellow form. Now, insofar as the range of forms manifest by a species depends on the subset of inherited particulars (for example genes from the total gene pool) available to each of its individuals (*ie.* the generative field at the level of each individual), it is clear then that the differential inheritance of these particulars can change the range of manifest forms. Moreover, were the inherited particulars as generative *parameters* of form to exceed some threshold in such a parameter, then the generative field *could* manifest a form never previously *extant* (although previously possible *in principle*). Hence, in stark contrast to the assertion of Ho (1987) (section 4.22), natural selection *can* effect a creative role in evolution. Indeed, it

is essentially through shifts in the distributions of developmental controlling parameters that evolution is effected (Keiser, 1987).

Thus, mutation to the inherited particulars (*including* genes) can be conceived a potential source of novel parameters of form, with exchange of inherited particulars (*including* genetic recombination) a means by which generative field potentialities can be brought to manifestation; the potentialities of a species being constrained at the outside by the *essence* of its generative field (including potentially formative environmental influences), potentialities of which those that become manifest are constrained at the outside by reproductive isolation (and the subset of potentially formative environmental influences that its individuals actually *encounter*).

Selection simply refers to any process which, *through effecting the differential reproduction of individuals*, evokes the ontogeny of structures that stabilise the species as a *replicative* (so necessarily also occurent) entity *vis-a-vis* environmental stress. For example, the black and white forms of the peppered moth both result from the interaction of their respective inherited particulars with the generative field. If the trees on which they roost become blackened with soot, then the white form, no longer being camouflaged, may become more heavily predated upon and so unstable as a replicative form. This leaves a greater proportion of the black form in the population, so that when the moths breed, it is more likely that the generative field in their offspring will encounter those parameters [genes] that evoke ontogeny of the black form. Here the environmental stress is predatory constraint, which is not meaningful as a formative influence on *individuals* (although it *is* at the population level), only because at the individual level the generative field (as an occurent entity) is destroyed. After all, were an animal to survive a predation attempt and so modify its behaviour to avoid future attack, then it is easy to envisage that if still able to reproduce and that its offspring modelled their behaviour on the parent, then predation would serve as a formative environmental influence within the lineage of an individual (sensu Waddington, 1961, and Goodwin, 1984a). Indeed, the similarities between Wright's (1931, cited in Saunders & Ho, 1976) selective valley and Waddington's (1957a) epigenetic landscape are worthy of reflection.

Maynard Smith (1978) suggests that sex may have arisen as a

solution to the problem of adaptation sensu replicative stability; the concomitant redistribution of [inherited particulars] making it more probable that an adaptive potentiality would become *manifest*.

4.14 The problem of competition

The principle of competitive exclusion states that no two (or more) species can occupy exactly the same niche. When examined more closely, however, the principle reveals a number of conceptual and empirical difficulties. The first reason usually put forward in support of the claim is that any niche has only just enough resources to support the number of individuals of a species occupying it - its carrying capacity. But this *assumes* that the number of individuals occupying the niche is necessarily maximal, yet there is no a priori reason why this should be. Moreover, it is hard to see why, a priori, if *x* number of individuals of one species can occupy a common niche, *x/n* individuals each of *n* number of species should not be able to do so. Thus, the limitation imposed by carrying capacity is not a matter of the number of kinds of species, but of the total number of individuals or, more precisely, biomass.

Of course, insofar as organisms *define* their niches (and *vice versa*: Lewontin, 1982, 1983; Ho, 1984; Odling-Smee, 1988), it might be argued that by definition, no two or more species can occupy a common niche. However, a number of species separated from one another solely by reproductive isolation or by some intrinsic structural quality, can - and *do*, occupy common niches, for instance the three coexistent and interfertile cicadas Magicicada cassini, M. septendecim and M. septendecula (Lloyd & White, 1974; *cf.* section 3.12). At a number of sites on Madeira, Pararge aegeria is sympatric with P. xiphia and both do spiral flights (*cf.* section 5.6), the two species separated apparently only by reproductive isolation. For although P. xiphia is less tolerant of dense shade (Swash & Askew, 1982), this difference in niche would not *per se* prevent them interbreeding where they do fly together. However, their coexistence will require future study before its result can be ascertained (*ibid.*).

The second reason put forward in support of the principle is its

inherent assumption that any one of two or more species either directly or indirectly displaces the other. For example, Shapiro & Cardé (1969) argue that the N. American Satyrid butterflies Lethe eurydice and L. appalachia use the same larval foodplant but are kept out of competition by their different adult behaviours, while L. appalachia and L. portlandia anthedon share the same adult habitat but are kept out of competition by differing in larval foodplant. It is therefore difficult to see why, given the a priori compatibility of species appalachia and eurydice on the same foodplant, and of species appalachia and portlandia in the same habitat, two or more of the species should *not* be compatible using the same foodplant *and* habitat. Moreover, in the case of the British Pierid species Pieris napi and Anthocaris cardamines which occupy a very similar habitat (Lees & Archer, 1974), the A. cardamines larvae, being cannibalistic but not otherwise carnivorous (Cribb, 1983), are in fact much more likely to harm themselves than those of P. napi. Besides, individual plants often *do* serve as food for more than one [related] species, as in the case of a Brassica on which were feeding a larva of P. rapae and several of P. brassicae (pers. obs.). Admittedly, here, species brassicae far outnumbered species rapae, but it must be remembered that the essence of fitness is not to maximise the number of individuals of a species, but the stable maintenance of the species as an occurent *kind* of entity (section 4.11-12).

It is argued, therefore, that sympatry occurs because of the very fact that the respective species *are* compatible within a niche. But, since each will experience environmental stresses, they might become more stable were they to function as one (in the sense in which males and females can be thought of as two forms that function as a unit *viz.* the respective sexually reproducing species), since this (as with the evolution of sex, Maynard Smith, 1978) would increase the likelihood of a reproductively stable field condition (=solution). In the case of Magicicada then, it is conceivable that the species might merge still further in their reproductive compatibility. On this count, it is worth speculating that sympatric Pararge aegeria and P. xiphia, rather than diverging, too might merge.

It is suggested that such merging need not only be reproductive (as in Magicicada) but can refer to *any* context within which merging to form a fuctional unit renders each component reproductively more

stable than when alone. Such a merging of components I shall call the coalition field, which, like the generative field, too effects an increase in [replicative] stability through increasing complexity. It features in mimicry (phenotypic convergence), symbiosis (spatio-temporal convergence) and socialisation (pooling of resources).

That closely related species often behave *as though* competing, might now be understood as follows. The more that species resemble one another (in terms of structure and behaviour and so niche utilisation), the more they function (*re* resources) as though a single species *but of greater population size*. But the more similar each species, the more similar would also be their resource utilisation. Thus, the effectively greater number of individuals is *not* matched by an effective increase in the complexity (and hence carrying capacity) of the niche itself. The result is that the stress on any individual (*irrespective of species*) within a group of sibling species is greater the more similar the species are. Since differences in the ability or means (eg. migration) by which individuals cope with the stress are reflected in their replicative stability and respective niche construction, the dynamics of such individuals is *as though* they 'compete' for resources or 'displace' one another. Species may be said to 'compete' only because the *individuals* so partitioned *happen* to be of different species - for this is not necessarily the case. In Magicicada, for instance, the distinction between accelerating and non-accelerating individuals (in response to overcrowding) does not resolve their species differences with the result that accelerated (and lagging) cohorts comprise all three (Lloyd & White, 1974).

In support of the above postulate is that species involved in symbiotic relationships tend to be different rather than similar in their singular forms and requirements (and so involve less competition between individuals of one species and any those of the other). Perhaps the reason why cases such as Magicicada are exceptional is *not* because such close species would compete (in which case one wonders how they might have come together in the first place) but rather because of the asymmetry in their gain from merging into one: when loss of a [species] from the coalition field would almost certainly be detrimental whereas the formation of a coalition field can be beneficial; in other words because coexistent species tend to merge.

In this regard, it is worth speculating that, say, the European Pyrgus butterflies are prevented from merging only because of their reproductive isolation. Indeed, the species may have arisen independently, having come to occupy similar niches secondarily to their similar structures (and hence similar nutritional and climatic constraints). Such a notion resolves two paradoxes, the first being the occurence of species of very similar form but very different genetic constitution (cf. King & Wilson, 1975), the second being how [one] species whose individuals would be similar in virtually every respect (including niche) could have diverged into species still similar in virtually every respect (including niche) but so different only in their genital structures (or, as a corollary of the first paradox, in karyotype). After all, a moth looking 10% like a bird dropping might be better off than one unlike a dropping at all (cf. Gould, 1980), but an individual with even slightly defunct genitalia would be worse than useless. Besides, such a [mutation] would have to affect not only more than one individual but also both sexes *and* in a way which left them still reproductively compatible.

The foregoing conception of speciation might help clarify taxonomic distinctions - say within the genus Pararge. The Madeiran Speckled Wood P. xiphia is phenotypically like Tenerife examples of P. aegeria subspecies xiphioides (Higgins & Riley, 1975) but differs in genital structure (Higgins, 1975). It is suggested that the species arose independently on each island, the geographical isolation preventing them from merging during their evolution. Since the only constraint on genital structure is that members of each species be able to breed among themselves, *ie.* that genital structure does not *per se* contribute to any *species* difference in fitness, the species could still differ in genital structure.

Southern French P. aegeria (subspecies aegeria) has similar genitalia to its Tenerife subspecies xiphioides (Swash & Askew, 1982) but a different wing phenotype (Higgins & Riley, 1975). It is therefore suggested that P. xiphia and P. aegeria first originated independently in Madeira and S. France repectively. Indeed, these differ in both genital structure and phenotype (Higgins, 1975; Higgins & Riley, 1975). P. aegeria then spread to Madeira where, because the environment is similar to that in Tenerife, its *form* tended towards that of P. xiphia to become P. a. xiphioides while there would be no

call for a change in genital structure. If anything, one would expect genital structure to be conserved by interbreeding constraint.

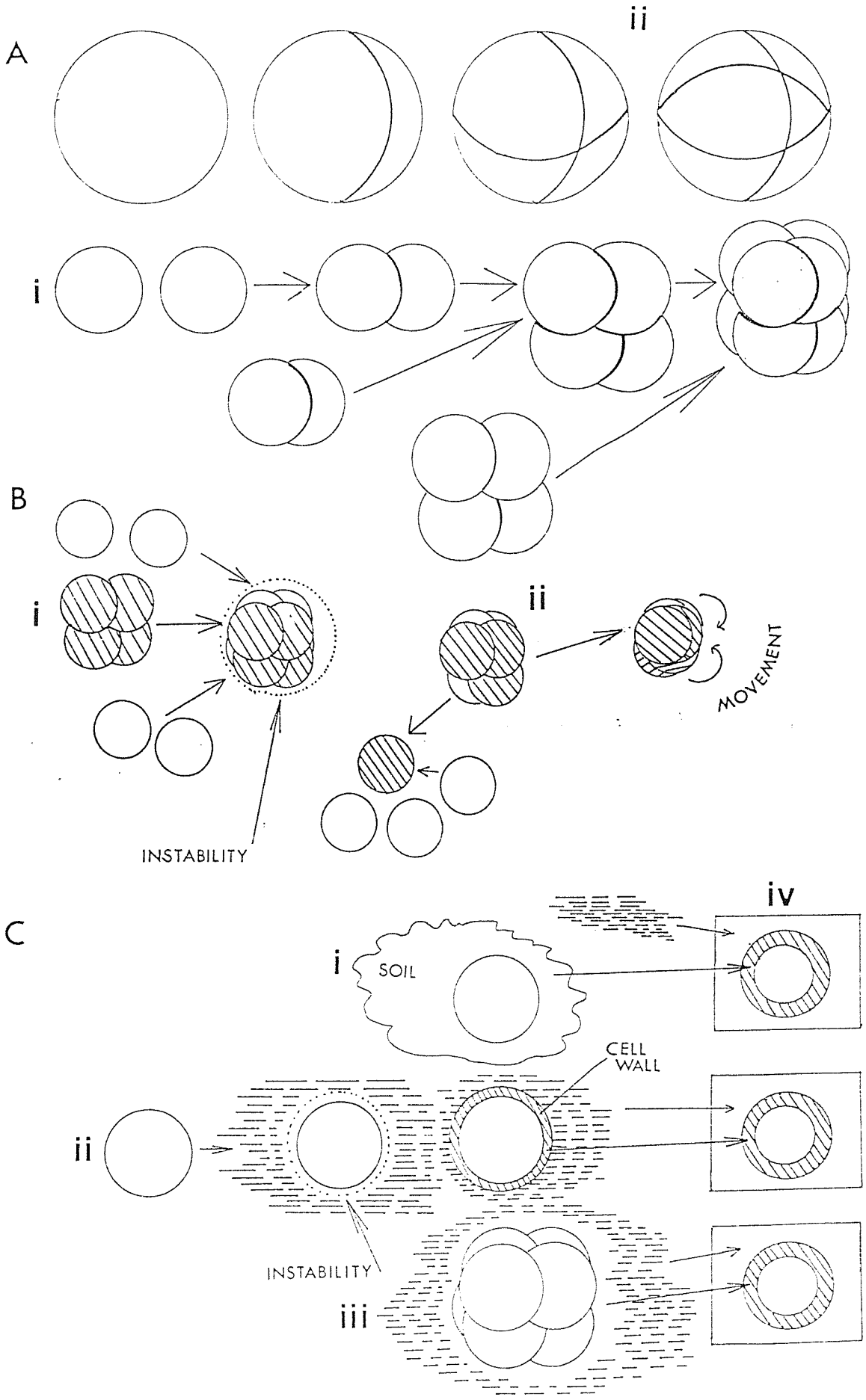
P. a. aegeria has recently spread [again] from continental Europe to Madeira (Swash & Askew, 1982) where, because the two species beforehand occupied similar habitats, they now coexist but are prevented from merging by (structural) reproductive isolation.

Nonetheless, it does not rule out competition in the strict *behavioural* or *intentional* sense - as when male Pararge aegeria contest the ownership of sunlit woodland patches (Davies, 1978, 1979). However, it is suggested that the notion of competition be limited to its behavioural sense (from which the analogy is originally derived).

4.15. Structuralism in cellular evolution

The paradigm for the origin of eukaryotic cells is symbiosis between various anuclear forms, on grounds that mitochondria and chloroplasts have a unique genetic code (chloroplasts also having ribosomes and tRNAs), that the plastids of eukaryote algae resemble free-living algae, and that eukaryote cilia and flagella are similar in structure to spirochete bacteria (Margulis, 1970).

But the scheme presents several problems. The first is that mitochondria cannot perform glycolysis although it occurs in *all* prokaryotes including anaerobes. Secondly, the infecting organism would have needed major readjustment in giving up its ATP to the host. And thirdly, organelles are very homogeneous in form and function given that more than one infecting species might be expected (Tribe & Whittaker, 1980) (although they could have converged). Animals, plants, fungi and slime moulds all have 18S cytoplasmic rRNAs which characterises them as Urkaryotes (Woese & Fox, 1977), and a fourth problem is that it is uncertain what "engulfing species" this rRNA represents (*ibid.*, citing Stanier, 1970). Woese & Fox (*loc. cit.*) propose two further kingdoms, the Archaebacteria which includes methogenic forms, and the Eubacteria, which includes all typical bacteria and chloroplasts. They also suggest that the three were anteceded by simple progenotes.


A
ii
i
B
i
ii
MOVEMENT
INSTABILITY
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iv
i
SOIL
CELL
WALL
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INSTABILITY
iii

The earliest progenotes may have used a metabolism like modern chemolithotrophs where CO_2 is the carbon source and where energy comes from redox reactions in which H_2S is the electron donor (Lehninger, 1981). Both these chemicals were abundant in the early atmosphere (*ibid.*). It is suggested that the depletion of inorganic energy sources from their milieu (to form more complex molecules: Dickerson, 1978) imposed constraint as osmotic stress, since membrane-bound spheroplasts (which develop when bacterial cell wall synthesis is blocked by penicillin) lyse under normal osmotic conditions (Spratt, 1975), and that there were three possible solutions.

The first was to stay where osmolarity remained high, or retreat, with no increase in structural complexity, this solution still with us as the Archaebacteria. Indeed these inhabit anaerobic conditions where they reduce CO_2 to CH_4, so reflecting conditions on earth 3-4 billion years ago (Woese & Fox, 1977). They are also distinct in their coenzyme requirement and nucleic acid chemistry (*ibid.*), possibly

FIGURE 4.3. **Internalised constraint and the coalition field.** (A) The binary and progressive aggregation of prebiotic cells (i) which it is hypothesised might have reduced osmotic stress. The solution of a field condition through aggregation constitutes a coalition field. Note the similarity of the symmetry axes to those of holoblastic cleavage (ii). (B) When comprising a critical number of cells the aggregate may have become unstable (i). Loss of a cell would render it more stable while the cell itself would be available for aggregation (ii). Alternatively, the aggregate might overcome the instability through cell movement. Note the similarity of the above to the sequestration of gametes and gastrulation respectively. (C) Internalised constraint. Under osmotic stress a simple progenote could have (i) remained under hypertonic conditions such as mud ie. retreat, (ii) developed a cell wall ie. shield, or (iii) formed an aggregate ie. coalesce. In all three cases the progenote component of structure is maintained and effectively protected from osmotic stress; and the topological relationship (iv) between progenote structure (unshaded), the particular kind of solution (retreat, shield or coalesce) and the stress is identical. For further explanation see text.

unchanged since the Lower Precambrian when life forms originated (Schopf, 1978).

The second was to develop a cell wall, or shield the structure from osmotic stress. Eubacterial cell walls contain peptidoglycan (Woese & Fox, 1977) and it is suggested that the potential for its specific synthesis *enabled* this solution to be invoked. Archaebacteria have cell walls but no peptidoglycan (*ibid.*) and may have been precluded the shield solution. Indeed halophiles whose cell walls too lack peptidoglycan (*ibid.*) inhabit strongly hypertonic milieux. This, far from being an adaptive specialisation, may be a restriction to an only available solution.

The third was for the progenotes to enlarge. This would have reduced their relative surface area at which the lytic force would be exerted. Such forms could have represented the earliest Urkaryotes. It might be argued that such cells could not have evolved without oxygen because it is essential for mitosis (Schopf, 1978), but it must be remembed that it is *energy*, not oxygen *per se*, that is required.

The predisposition of some enzyme to photoactivation may have enabled certain *progenotes* to photosynthesise with H_2S and it is suggested that under ensuing osmotic constraint *these* gave rise to the photolithotrophs. The depletion of inorganic molecules would have also imposed constraint as shortage of the electron source, whose possibly only 'solution' was to switch to water instead, easily achieved since both are group VI dihydrides, and so involving minimal complexity change. It should be noted that until this time all respiration was anaerobic (Schopf, 1978). The oxygen released now introduced further constraint as oxygen toxicity. Indeed excess oxygen inhibits respiration and photosynthesis in cyanobacteria (*ibid.*).

Toxicity constraint would be irrelevant to archaebacteria and to eubacteria living under anaerobic conditions. In exposed eubacteria and urkaryotes an end product of metabolism could have solved the problem of oxidation damage by 'absorbing' it (possibly pyruvate as it is not too important in glycolysis). Indeed modern plants perform photorespiration where glycollic acid (a by-product of photosynthesis) is oxidised by molecular oxygen to glyoxyllate for no apparent purpose, which in the peroxisomes (organelles similar in size and form to progenotes) occurs *non-enzymatically* (Clowes & Juniper, 1980), whilst in bacterial cell membranes, an electron transport system

reduces oxygen to water (Hinkle & McCarty, 1978). Now anaerobic glycolysis yields two ATP per glucose (Lehninger, 1981). But the ability of by-products to undergo further oxidation could have predisposed them to further metabolism. It is therefore suggested that aerobic glycolysis arose *not* as a means of obtaining energy but as a result of *dissipating* it into chemical form (and ultimately structural manifestation). This is in accord with notions of talandic entropy (Saunders & Ho, 1976) and conservation of energy (section 1.4.). It also shows how the solution (specific chemical reaction) to a problem (oxidation constraint) can enable ie. form the basis of an innovation (aerobic metabolism) itself precluded *until* the very source (oxygen) of constraint appears.

Urkaryotes would still have been at risk from osmosis stress. It is speculated that as the milieu became further diluted the cellular enthalpy reached the upper limit compatible with structure. In cells of a size similar to modern urkaryotes this constraint may have been solved by the *spontaneous* formation of mitochondria-like structures. This idea is not far fetched. Centrifugation of the sea urchin egg yields four fractions, including a clear quarter with *no* discernable mitochondria. When fertilised, it develops as a normal larva in which, although the early embryo is completely *devoid* of mitochondria, mitochondria *do* appear at some time pre-gastrulation (Davenport, 1979, citing Harvey, 1956). Eubacterial and mitochonrial structure are most likely similar because their a priori available materials and energy conditions were similar. The latter too reduce oxygen in an electron transport chain. Its further reduction would most likely involve a molecule not too important in aerobic glycolysis, say an end product. Indeed in urkaryotes, pyruvate is further oxidised to Acetyl-CoA. Moreover, this occurs in the cytosol, not the mitochondria (Hinkle & McCarty, 1978); and it too probably arose to dissipate (rather than provide) energy. Chloroplasts probably arose in comparable fashion in large photosynthetic progenotes. Their gross resemblance to mitochondria is attributed to their similar a priori constraints. But their different fine structure is attributed to the need for stability given their much greater exposure to free oxygen as a result of photosynthesis. Of course, such progenotes would have developed mitochondria as well.

In smaller progenotes unable to make peptidoglycan the solution may have been to coalesce. Indeed fossil cell clusters have been found where each cell diameter is only 10 microns when most urkaryote cells (including fossils) are about 100 microns across (Schopf, 1978). Further dilution of the milieu may have evoked a similar strategy in urkaryotes. Amoeba must continually and actively expel water to prevent the cell bursting. Such a 'coalition field' may have solved a surface energy condition similar to that in holoblastic cleavage (cf. Goodwin, 1984a) where the egg in effect becomes an aggregate of cells (Fig. 4.3a). If so, then Haeckel's dictum, namely that 'ontogeny recapitulates phylogeny', would apply right back to the single cell stage; and hence globally. The same solution could have been achieved by the formation of an intervening membrane (cell division) as osmosis threatened to force cell size beyond its stable limit. Insofar as binary division can be conceptualised as 'internalised' binary coalition, it represents an assimilative phenomenon. Some eggs are larger, but are also surrounded by water-impermeable membranes (Davenport, 1979); and since fertilisation changes their permeability (*ibid.*), it may be osmotic stress that evokes cleavage.

That holoblastic cleavage occurs without an increase in embryonic size (Goodwin, 1984a) suggests that the aggregate may have had an upper limit to stable size. It is possible, therefore, that cell movement (as in gastrulation) and differentiation may have been the next 'solution'. Alternatively, were one of the cells to come away, it would 'allow' the aggregate a little more time before *requiring* this new solution, while *itself* repeating the process of coalition or division as above. This may have originated the first cellular reproductive scheme, where reproduction itself arose simply as one of a number of 'solutions' to a problem (Fig. 4.3b). The nucleus probably arose to ensure *coordinated* cell division and not to direct aggregate *form* as such. The next stages in phylogeny were probably colonial forms, which themselves reiterate several embryonic processes (cf. Davenport, 1979). The attainment of form might simply involve materials falling into 'accord' with the field geometry, in much the same way as iron filings in a magnetic field, with *field property* representing the energy condition in a stabilised condition.

Plant cell walls arose later in evolution. These solve the problem of structural support. Their formation would be precluded in non-photosynthetic forms which would be limited to two nutritional solutions. The first is to obtain nutrients from the ground, like plants, as do certain fungi. But their lack of cell walls constrains their size and their stalks are relatively much thicker than those of plants. The other is to search for food. The need for movement precludes total rigidity, but any need for local rigidity is solved by an endo- or exoskeleton. No animal has muscle motility and photosynthesis (cf. Schubert, 1985, re Waddington) because with photosynthesis, no need to hunt for food would have arisen; whilst in *unicellular* photosynthetic animals their motile physiology must be non-muscular by definition.

In the retreat response (Archaebacteria), the mud in which the progenote remains protects it by keeping the constraint irrelevant. In the shield reponse (Eubacteria), the cell wall 'component' protects the progenote 'component' by containing it and preventing the influx of water. Hence the mud and cell wall each stand in identical functional relationship to the progenote. In the coalesce response (Urkaryotes), each progenote is protected by the reduction in the *effect* of osmosis. Now retreating and shielding also reduce the effect(iveness) of osmosis. Hence the three responses are reducible to a common type regarding the relation between structure and destabilising influence. Its salient feature is that initial conditions in effect become internalised in structure. But in so doing, the most structurally complex response (coalesce) also generates a new level of organisation (the multicellular) which in turn can form the basis for solutions to subsequent problems.

4.16. A hierarchical description of mimicry

One such problem is that of mimicry, which

"combines paradigms for evolution and of epistemology" (Vane-Wright, 1981).

However,

"As a knowledge of mimicry increases, mimicry becomes harder to define" (Rothschild, 1981).

The concept of mimicry dates back to Henry Walter Bates' field studies last century on South American Heliconiid butterflies (Stearn, 1981). Thus mimicry as it is commonly known is called Batesian mimicry (Edmunds, 1981). It typically involves the relationship between a predator and two potential prey items, one of which is actually unsuitable as food (the model), the other suitable but gaining protection from its resemblance to the model (the mimic) (Wickler, 1968). The predator is often called the dupe (Turner, 1977). Yet mimicry also occurs in other contexts, for instance parasitic birds which lay their eggs in other species' nests, the cuckoo of course being one such parasite (Wickler, 1968). The following general definition is suggested:

> "A resemblance between an organism and another structure such that they are not resolved as different by another organism *vis-a-vis* some real difference between them that would be relevant to one or more of the three were it to be resolved."

A model and its mimics collectively comprise a mimicry ring (Wickler, 1968), although the term might more appropriately also include the dupe. Yet there are different *kinds* of mimetic relationship, and innumerable examples; which may comprise more than ten percent of all species (Rothschild, 1981, citing Wiens, 1978). The different kinds of mimicry concern primarily the nature of the relationship between mimic

and model *vis-a-vis* the dupe.

Endler (1981) groups mimetic relationships into six kinds. In crypsis (camouflage) the mimic is not perceived against the background; in masquerade the mimic resembles some inanimate object; in Batesism the mimic is mistaken for the model; in Mullerism both members of the pair are *models* but resemble one another and so also afford each other protection; in polymorphism the mimic has various forms or 'morphs' each dependent on a different background; and in convergence the mimics are similar because they are selected for resemblance to a common background. The last two categories too can include the others (below).

But a problem arises with cases such as stick insects which can be either cryptic or stick-mimics (Edmunds, 1981); and which previously had been put aside with 'special resemblances' (*ibid.*, citing Cott, 1940). The problem is that classing the mimic as either *one* overlooks those properties that would assign it to the other (ie. incurs information loss). And a definition trying to encompass *all* its attributes would become a description, *not* a generalisation, and hence cumbersome. The problem arises because concepts like 'cryptic' and 'masquerade' are taken as referring to the mimic itself, when they actually refer to its properties as acquired by virtue of its mimetic *position* (an *element*) within a mimicry ring (the *type*) of the *form* to which 'cryptic' or 'masquerade' and so on refer. Here, it is suggested that masquerade be defined as the model being irrelevant to the dupe, rather than inanimate. This would resolve the sort of problem that crops ups with, for instance, fulgorid bugs that look like alligators; namely, whether they exemplify disguise (masquerade) or mimicry (Batesism) (Cloudsley-Thompson, 1981). In both cases the dupe's 'conception' of the bug is that of an alligator. If alligators *per se* have a bearing on the dupe, then the mimicry is Batesian; if not, then it is masquerade. But whether alligators are inanimate or not is contingent. Sticks, for instance, are inanimate. But they have a bearing on avian predators - after all, they use them in their nests. It is a rather unfortunate thing, then, that a stick-insect might too be woven into so elaborate a home. The issue as to whether the mimic benefits is also, therefore, contingent. A stick might not be worth eating (Robinson, 1981). But it is very good for building one's home with.

This particular problem was resolved by extracting the underlying natures of the mimetic forms. This was achieved through attempting to reduce the forms to their simplest conception. An outcome of this is that the forms increase in complexity with two corollaries. The first is that the form of mimicry cannot be assigned a priori; the specifics of each element must be known, for example the mimic's habits and what signals are relevant to the dupe (Robinson, 1981). The second is that the more complex ones encompass the simpler ones. Stick insects can masquerade on the forest floor *and* be cryptic among sticks in the shrubbery. Batesians can also be masquerades if they influence the dupe as though they were a model when none is present. The peacock butterfly, Inachis io startles birds by flashing its eyespots (Ford, 1957a) and rubbing its wings to sound like a snake, but the dupe need never meet either eagle or adder. The ventral wing surface of I. io is black and renders it inconspicuous while roosting (Ford, 1957a). Batesians, however, are also cryptic in a semantic sense, in that they are *not perceived as different* from the 'background' of the model (Endler, 1981). Mullerian mimics, through one another's similarity, necessarily also exhibit Batesism. Some Mullerians may be difficult to detect and so cryptic under the lighting within the canopy level at which they fly (Turner, 1977). With Mullerism, however, the mimic, because it is inherently protected, is no longer *dependent* on its mimetic position (Batesian or otherwise). This frees it from the constraint (stable population structure *vis-a-vis* elimination of individuals) attendant with the simpler forms so enabling it to enter into new environments and *kinds* of relationship thereto. Polymorphism can include Batesism (the various mimetic morphs of P. dardanus and their respective 'background' models) and Mullerism (the various 'covarying' morphs of Heliconius melpomene and H. erato) (Wickler, 1968). And in that selection would increase the resemblance between Mullerians (to each other) and between Batesians (with a common model), convergence too can take in lower level forms. Their hierarchical relationship is shown in Figure 4.4.

Hence an organism may be involved in more than one form of mimicry. Moreover, mimicry involves dynamic interactions, so that no group of organisms should be viewed as a 'physical constant' (Erlich & Raven, 1964). Nonetheless, the kinds of transformation that can occur will be delimited by a priori structural constraint. A mimetic pattern

may not be available to ontogeny (Saunders, 1984); the evolution of a bad taste will depend on its physiology being able to cope with a toxin, for instance the Monarch butterfly Danaus plexippus L., which derives its toxicity from cardiac glycosides in the larval foodplant (Brower *et. al.*, 1978). Indeed, the *kinds* of change of foodplant that Lepidoptera can undergo might be limited. Nymphalid and Lycaenid groups for instance have switched from dicotyledons to monocotyledons on at least eighteen *independent* occasions (Erlich & Raven, 1964). And the animals can impose *active* constraint on themselves. For example, female choice of mate in Hypolimnas misippus L. precludes the development (*sensu* stable inheritance) of mimetic patterns in the males (Stride, 1958).

Organisms that hide from predators too involve a 'retreat' response; cryptic, masquerade, and Batesian mimics 'shield' themselves against undesirable backgrounds; whilst those that become inherently protected (Mullerians for instance) are now free to 'coalesce' or enter into new environments or population structures. It appears that coalesce solutions can form bases for solving further problems because the original constraint has been *contained*. For this reason I will therefore call this type the Internalised Constraint Model.

The relevance of development to mimetic phenotypes *per se* is taken up in section 10.2.

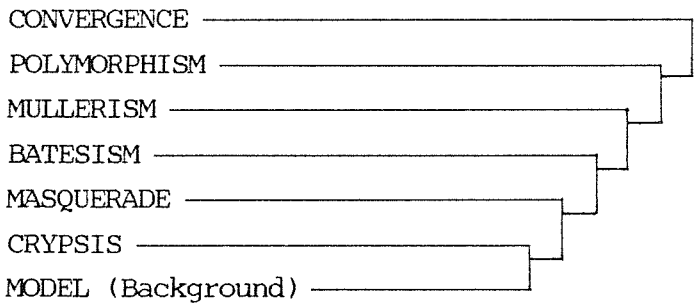


FIGURE 4.4. A hierarchical ordering of mimetic relationships. Each progressive level includes the lower ones. For further explanation see text.

4.17. Causality and constraint in epigenetic process

Lower levels are deterministic only in that they *delimit* higher level organisation. They do *not* prescribe it. For example, the synthesis of cysteine requires the a priori availability of sulphur, carbon, nitrogen, hydrogen and oxygen. If any of these elements are missing then the amino acid cannot form. And the five elements cannot form nucleic acids because these require phosphorus. Thus any set of lower level structures will necessarily have precluded from it certain higher level structurings. And given higher level structurings, the relationship of the lower level structures to them is that of prerequisite and *not* cause. And within the higher level structurings they stand as components. Of course, from such structurings may *arise* emergent properties.

The formation of methionine is also possible in the context of available elements. But their relative amounts may favour their structuring into cysteine over methione. But sulphur does not have any contained description or information (literally 'to form within', Davenport, 1979) of its structuring into cysteine or methionine; information such that when sulphur is in greater proportion the 'contained description' becomes in some sense 'completed'. Rather, the relative amounts represent a *condition* that precludes the formation of one of a set of structurings *possible with respect to components*. Nor do the cysteine and methionine in some (non-anthropomorphic) sense 'compete'. That two structures A and B 'compete' implies A and B are already present. Thus it is meaningless to talk of the *formation* of either A and B over the other in terms of competition between them. Temperature may mean the cysteine then condensing into cystine. Insofar as the resultant cysteine, but not methionine, can then dimerise, past events delimit further interaction; and it is in *this* sense, and *in this sense only*, that lower levels 'provide' direction. It is thus suggested that the diachronic realm should be more appropriately regarded as time-*delimited* rather than time-dependent.

The notion that epigenesis *ends up* in some position is known as teleonomy, which must be distinguished from the notion that it is *aimed at* some position or teleology. It is easy to assume the latter because of the asymmetry inherent in the fact that lower levels are

prerequisite for the existence of higher ones but not *vice versa*, for example that genes can occur without traits but not traits without genes, or neurons without minds but not minds without neurons. But the confusion arises through confusing prerequisite with cause.

A similar sort of reasoning has been presented by Piaget (Webster & Goodwin, 1982). These principles apply across all structural levels with any level 'given' only in the sense that it is the starting point in the analysis (*ibid.*), and hierarchical levels recursive although each has a different time base (Odling-Smee & Plotkin, 1984). Thus epigenesis does not itself recognise distinctions such as the genetic or the cellular, nor partition process into realms such as the chemical or the biological (Rosen, 1982), but is impartial (Davenport, 1979). Cyclical processes occur at all levels from the biochemical (citric acid cycle), through the genetic (cell cycle) and physiological (homeostasis), to the ontogenetic (reproduction). Thus recursive *process* itself recurs through the structural levels (Davenport, 1979). This continues on to the relationship between development and evolution, which is most famously attributed to Haeckel.

Haeckel believed that modifications were 'packed down' into development, so that phylogeny became the 'cause' of ontogeny with ontogeny a record of evolution (Costall, 1986). Haeckel distinguished existing or coenonogenetic features from palinogenetic derived from previously adaptive ones, only phylogeny did not provide a perfect record as it was obscured by natural selection (*ibid.*). In that established variations provide a basis for new variety, the range of phenotypes recorded by selection gets extended; while once-successful phenotypes are incorporated into developmental process and buried by the accumulation of further changes; and in this sense, ontogeny recapitulates phylogeny (Davenport, 1979). But these assimilated changes may then be modified in development by the downward constraint of new modifications; which also frees them from immediate phenotypic selection; and in this sense, ontogeny does not recapitulate phylogeny (*ibid.*). This notion of his, that 'ontogeny recapitulates phylogeny', although not deducible from Darwinism, was certainly compatible with it, and was perhaps the first move towards a unity of development and evolution.

4.18. The development of evolution

The concept of evolution dates back at least to Aristotle (Bowler, 1984), but neither Darwin, Lamarck or Haeckel used the term 'evolution' as such. The word had previously been used to describe an *embryological* process *viz.* preformation, where it meant 'unrolling', and Darwin used it at the very end of his 'The Origin' only to emphasise the fluidity, as opposed to rigidity, of organic development (Gould, 1980). Darwin's original critics were dissatisfied mainly with the lack of direction of his theory.

The preformationists believed that the adult organism was preformed in miniature in the gametes, where in humans it was known as a homunculus (Davenport, 1979). But the theory was severely strained by the problem as to how the offspring for all furture generations might be contained and by the ability of certain organisms to produce inumerable offspring (*ibid.*). Yet the preformationists were not going to be thwarted, and many of them imposed imaginary projections onto the blank features of the egg and early embryo. Indeed,

> "This tendency was so strong that even after relatively sophisticated lens systems were used, some observers actually made drawings of the tiny homunculus they thought they observed huddled in the gamates" (Davenport, 1979, p36).

The preformationist school was divided into spermists who believed the miniature to be huddled in the sperm, and ovists who believed it to be huddled in the egg and took parthenogenesis in aphids as conclusive of their theory. These points have important implications for the nature of observation and its relationship to theory.

4.19. Science and its application

> "In fact, no valid distinction exists between description and experimentation since, realistically, the latter constitutes a means of observing beyond the senses and, hence, becomes a tool of description" (Davenport, 1979, p36).

Indeed, all observation is necessarily made in the light of some theory, since the data which presents itself to the senses can not be interpreted without some prior basis upon which it may be structured and so become meaningful (Chalmers, 1978). And, as the case of the preformationists demonstrates, theory can exert a powerful influence on the nature of observation itself. Moreover, had ovists performed statistical test on the frequency of male and female parthenogens, they would have obtained a significant result, so confirming their theory. The subsequent demise of preformationism clearly shows the fallability of their assertions.

Karl Popper (1969, cited by Chalmers, 1978) held science to progress by means of conjectures, which if borne out by experimentation were upheld, but if not were then refuted. But as the observational bases of proof and refutation are themselves theory-laden and hence suspect, falsification is itself fallible (Chalmers, 1978).

> "After all," as Maynard Smith (1982) remarked (in defence of neo-Darwinism), "one case of the Indian rope trick should be sufficient to disprove Newton."

Proof too is fallible, as Chalmers (*loc. cit.*) amusingly cites Russell's (1912) inductivist turkey, who having been rewarded by an early morning breakfast on the 364 days preceeding Christmas, expected to be so rewarded on Christmas day only to itself end up on the platter the night before.

Thomas Kuhn (1970, cited by Chalmers, 1978) on the other hand, viewed science as comprising paradigms; which constitute structured conceptual frameworks, their laws, and methodologies. The neo-Darwinian paradigm, for example, comprises concepts such as gene

mutation, recombination, and natural selection; the mathematical principles of quantitative genetics; and an empirical directive centred largely on the role of selection in natural populations (Manly *et al.*, 1972; Manly, 1985). The structuralist paradigm, on the other hand, comprises concepts of heredity additional to DNA, developmental constraint, and dynamic interaction between organism and environment; the organisational principles of types and Bauplans (Schwanwitsch, 1929; Gould & Lewontin, 1979; Webster, 1984; Wiklund & Karlsson, 1984); and experimentation along the lines of artificial manipulations and controlled environmental stresses (Sonneborn, 1970; Nijhout, 1980a; Ho *et al.*, 1983b; French, 1984).

Kuhn (1970, cited by Chalmers, 1978) viewed scientific progress as follows. The disorganised and diverse activity that precedes the formation of a science eventually becomes structured and directed when a single paradigm becomes adhered to by the scientific community. Workers within a paradigm practice what Kuhn calls normal science, whose practitioners elaborate the paradigm in their attempt to understand the behaviour of some relevant aspects of the real world as revealed by experimentation (Chalmers, 1978). In doing so, they inevitably experience difficulties and encounter apparent falsifications. If these get out of hand, then a crisis state develops, which is resolved when an entirely new paradigm emerges and attracts the allegiance of more and more scientists until the original paradigm is abandoned. Such discontinuous change constitutes a scientific revolution, after which the new paradigm directs the new normal science until it too reaches crisis point. Kuhn's scheme can be summarised thus:

> *pre-science — normal science — crisis — revolution — new normal science — new crisis (ibid.).*

Ho & Saunders (1984a,b) argue that evolutionary biology is in a state of Kuhnian crisis, their main contention being the power with which natural selection has been endowed as an explanatory mechanism for evolution. Since this crisis has been critically assessed by Earthey (1988), the discussion will focus primarily on the status of natural selection. However, it will prove more fruitful if the stances taken up by Darwin, Lamarck, Weismann and Waddington are reviewed

beforehand.

4.20. Darwin and Dr. Pangloss

> "Neo-Darwinism is not simply Darwinism minus Lamarckism - it is hardly Darwinian at all." (Costall, 1985)

Charles Robert Darwin, the grandson of Erasmus Darwin, was borne in 1809, his father a successful physician, his mother coming from the Wedgwood family of pottery fame. He originally trained as a physician at Edinburgh, but, finding surgery distasteful, then moved to Christ's college Cambridge to train for the ministry. Nonetheless, Edinburgh had steeped him in a more radical intellectual tradition than he was to find at Cambridge. Darwin was initially a literal follower of the Bible, but soon turned Paley's argument - that adaptation evidenced divine creation - on its head, asserting that adaptation was a *process*. He became interested in studying tropical natural history, and his opportunity arose in 1831 with the British navy's despatch of H.M.S. Beagle to chart the South American seas, when Robert Fitzroy, the captain, wanted a gentleman-companion to enliven the voyage (Bowler, 1984).

The Beagle spent some time in the Galápagos archipelago, where Darwin was told the natives could identify to which island a giant turtle belonged from its shell, and this inspired him to ponder on the more general significance of the fact. Darwin's clue to evolution came, however, not from the finches which were so distinct that he did not realise their relatedness, but from mockingbirds, whose similarity to South American forms were more obvious. Darwin found it hard to believe why God should put a distinct species on each island, and came to the opinion that some form had migrated from S. America, some hundreds of miles from the Galápagos, and then diverged (*ibid.*).

Darwin is traditionally quoted as having coined the catchphrase "survival of the fittest", but it was actually Herbert Spencer - who was not even a convinced Darwinist - who coined the phrase (Gould, 1980). Moreover, Darwin's thesis was concerned primarily with descent with modification (*ibid.*) given the constraint of fittingness

(stability *vis-a-vis* the environment), although this soon became corrupted to fitness (most able in the 'struggle for life', Darwin, 1859) in the hands of his Victorian successors (Pribram, 1982). Thus, Darwin's notion of random variations, random with respect to *fittingness* (in fact, all the theory actually requires, Webster & Goodwin, 1982), came to be mistaken for randomness with respect to their *production*:

> "Then Darwinism, which explained how by throwing stones one could build houses of a typical style" (Dreisch, 1914, cited by Webster & Goodwin, 1981).

The view that, without the primacy of structural constraint, "God being dead, anything could happen" (Webster, 1984), came to be known as the panglossian paradigm, after Dr. Pangloss who was ridiculed by Voltaire for asserting, for example, that legs were clearly intended for breeches (Gould & Lewontin, 1979), in holding that

> "our world is the very best we could have, each trait must be as it is" (*ibid.*).

However, such a Panglossian view was not held by Darwin, although it was that of Wallace and Weismann in which the modern adaptationist programme is steeped (*ibid.*).

Darwin accepted variations as givens, as he was unable to account for their a priori causes (Bowler, 1984), although he did acknowledge that they might have several (Ho, 1985). He believed that organisms underwent gradual transmutations (the tenet of gradualism) from a common ancestor, with natural selection acting on *available* variations (Costall, 1986). Species were understood to arise by isolation, with their genealogies describing a branching process (*ibid.*).

The main criticism of Darwin came from Creationism, though *not* for any blasphemous or heretical claim, but for its materialism - in which mental and spiritual phenomena are just a by-product of matter - since Darwin did not see progress as an *inherent* quality of organisms (Gould, 1980). Indeed it was for this reason that, although he developed his theory in 1838, he did not publish it in his The Origin Of Species till 1859 (*ibid.*).

Gradualism, however, was essential to Darwin, since gaps in the continuity would have given space for 'Divine Intervention' to creep in as an explanation (Costall, 1986), and it was probably for a similar reason that he turned to vestigial organs as evidence for evolution (Scadding, 1981). Darwin argued that these provided evidence for descent because they resembled useful organs, their vestigial nature being said to have come about through natural selection or by decay through their disuse; and he probably used the argument to preclude some forms of Creationism for which vestiges were an embarrassment. In fact, it can not be proven whether such organs are useless or unadaptive, as they might might have some (as yet unascertained) structural role in *ontogeny* (*ibid.*).

Darwin saw natural selection as better *vis-a-vis* creationism, not *vis-a-vis* Lamarckism, and it was with this that he launched into natural selection in his Origin. Indeed, Darwin's paradigm *did* encompass intentionalism, and he frequently appealed to Lamarckism, although the latter was attacked by *others* as a scapegoat for excusing Darwin's talk of 'will' and 'intention' which were often dismissed as vestiges of natural Theology (Costall, 1983). For instance, his observations on the choice of male partners by female [Peahens, for example] were hardly ever discussed (*ibid.*).

It was largely the geologist **Charles Lyell** who rejected Lamarck, on grounds that inducible changes were limited in effect (Bowler, 1984), in response to which Darwin asserted that natural process may operate too slowly to be observable in a human lifetime (*ibid.*). Darwin saw heredity as the *conservation* of form and variation as a *disturbance*, and, whilst he stressed that natural selection was the *most* important mechanism (Bowler, 1984), he *never* asserted that it was the exclusive one (Costall, 1986). Similarly, whilst it is true that he did *de-emphasise* Lamarckian-type inheritance for a more direct link, he *never* abandoned the possible influence of the environment on the reproductive system (Bowler, 1984). Indeed, it was by virtue of his appeal to embryology that he rejected saltation (discontinuous change) for, since embryonic development describes a continuum, he thought that to admit to saltation was to admit a 'miracle' (Costall, 1986). Conversely, most protagonists of saltation ignored embryology (*ibid.*).

In fact, it was *neo*-Darwinism that based Darwinism on natural

selection alone (Costall, 1986). Moreover, it even asserted that certain views in fact *similar* to Darwin's own were actually *anti*-Darwinian; and it relegated consciousness to an epiphenomenon with no causal power (*ibid.*). Yet natural selection and the laws of nature are independent of any genetic details (Reed, 1982a).

Darwin's main shortcomings were his lack of a theory of stable inheritance and laws of form. His gradualism, togther with the Panglossian conception of variation, gave vent to an ontology, Pangenesis, whereby any form could, in principle, blend into any other, which

> "at once did away with any deeper meaning for zoological classification ... the totality of living forms appeared as meaningless as, say, the forms of the clouds in their accidental peculiarity" (Dreisch, 1914, cited by Webster & Goodwin, 1981).

In pangenesis, Darwin

> "intuitively recognised the need for alternative descriptions of genotype and phenotype but floundered on the logistics of their interactions" (Davenport, 1979, p402);

and so, in a move towards providing the logistics, we shall now examine Lamarck.

4.21. Lamarck

> "Since it is regarded as proper etiquette to deride crude Lamarckism, it has become accepted strategy to dismiss summarily all ways in which subtle types of Lamarckism-simulating procedures could have operated" (Wasserman, 1982b)

Jean-Baptiste Pierre Antoine de Monet de Lamarck was born on the 1st of August, 1744. As a young man he had trained as a soldier when he was renowned for his tenacity, but he was injured and so turned to his academic interests, when he collected plants as a hobby (Jordanova, 1984). In 1794, Lamarck was elected by Buffon to the Muséeum d'histoire naturelle to classify invertebrates, and became one of the founders of invertebrate taxonomy (Bowler, 1984). Lamarck was essentially a 'Romantic thinker' but was much influenced by the materialism of the enlightenment, which stressed the creative power of nature (*ibid.*). Lamarck belonged to many learned societies and was active as a biologist for over 40 years, during which time his ideas changed (Jordanova, 1984). It was only because he did not develop his theory of transmutation until his fifties, when the age of enlightenment was already coming to an end, that it tended to be dismissed by his contemporaries (Bowler, 1984).

Lamarck's epistemology was that all knowledge comes ultimately from the senses and that linguistic symbolism is necessary for thought to be possible. He argued that the natural sciences could only progress if they employed the correct language, and wished to see a biological language that was stable yet sufficiently flexible to comfortably incorporate new terminologies. He realised that this was problematic in classification, but that the naming of natural forms was at least a step towards understanding them and communicating such insights to others. He was also aware that an understanding of the way in which the mind works was important in understanding the practice of science. He maintained that where empirically evidence is unavailable, science must aim for a *plausible* account, theoretically cohesive and contradicting no known facts. Lamarck was essentially a late

enlightenment thinker, interested in chemistry and meteorology (indeed he unified the sciences), who searched for a conceptual basis of life.

Within 18th century biology had prevailed the notion of the Great chain of being - a single hierarchy from crude matter to God. But Lamarck rejected the idea of a single series, because animals, plants and minerals were quite distinct and because it included abstract beings such as angels that were not empirically observable (Jordanova, 1984). He thus separated nature from theology, although he was a deist (*ibid*), and rejected life forms as the 'miraculous' product of the Creator so endorsing them as suitable subjects for study (Ho, 1984). Indeed he introduced the term Biology in his Hydrogéologie of 1801 (Jordanova, 1984). Lamarck held life to be a discrete structural level, *comprised* of lower material levels but manifesting emergent properties, while rejecting vitalism on grounds that it was deistic and not rationally analysable. Life was distinguished by virtue of it being able to create complexity out of simpler levels, having had a finite origin with its spontaneous generation out of pre-existing inert matter - *not* ex nihilo (Jordanova, 1984). He opposed Lavoisier's new chemistry of fixed simple compounds, believing rather that matter comprised an unlimited number of chemical elements able to combine into a myriad of compounds. But matter had no *inherent* power to build compounds which, since the main cause of chemical reactions was fire - which tended to *break down* compounds, he contended were only built up by the non-material force of life. He argued that the molecular organisation of life was driven by a tension, the vital orgasm, which he regarded as a subtle 'fluid' (as were magnetism, heat and electricity) that came ultimately from atmospheric fluids. In simple organisms it penetrated the body directly, while in higher ones it came from food - from which were derived, for example, nervous fluid which flowed along the nerves to act on the solid body parts. The nervous fluid, like the electrical, nonetheless, were recognised as modifications of but one fluid - the caloric. He noted that life involves an increase in complexity - a pedagogic progression - with irritability as the starting point. 'Fecondation' (fertilisation) gave the egg the 'life' property - *with the proviso* that the egg was predisposed to do so, and he thus rejected preformationism.

He recognised that the theoretical biology would require laws and axioms. Since the mid-17th Century, Descartes mechanism had prevailed where organisms were conceived as machines, their organs being 'where' a function took place, but for Lamarck, organ and function had developed *together*. He recognised that higher animals were more differentiated than lower ones, the former having less procreative and regenerative power but being better able to adjust physiologically and behaviourally to their milieu.

Lamarck started out as a plant taxonomist, adopting Carl Linné's binomial nomenclature and his use of reproductive organs in classification but adding that arbitrary criteria were unsatisfactory and that organisms change. He placed emphasis on their levels of complexity and their internal *relations*, considering plants as structural wholes - on which grounds he criticised Linnée for emphasising single parts. He argued that the 'real' taxonomic unit was the species but at the same time that classes, genera *et seq.* should be based on criteria corresponding to their respective levels of generality - the genus Sagittaria, for instance, denoting all plants with pointed leaves, and for levels of complexity based on, for instance, the number of cotyledons.

He observed that both the plant and animal kingdoms have communal life forms - for instance that trees are made up of leaves - and that simple plants and animals resemble one another more than do complex ones. He held that changes in complexity also occured over time, asserting that simple forms were an abstract of two simultaneous historical processes with polyps and mosses the rough drafts of animals and plants respectively. He regarded these as distinct kingdoms, with plants based on carbon, but animals on nitrogen and possessing sensitivity and irritability - which the swiss von Haller later explained as nervous and muscular activity. He regarded animals too as structural wholes. Organisms possessed faculties in that they were 'able to do things', constant faculties being functional and unalterable eg. respiration, alterable ones owing as much to environmental changes as to the power of life itself eg. locomotion.

Lamarck's classification emphasised physiological systems such as the respiratory, reproductive and nervous systems (in animals stressing the latter as central to their behaviour) to assign large general groups (eg. animals as intellegent (vertebrates) or apathetic

and dependent on the environment) that showed the *overall* progression of life, relegating the use of outward characters to the species level only. His classification began with the *most* complex, simpler ones being described in terms of what they *lacked* compared to higher ones.

He asserted that proper accounts would have to consider *both the nature and history* of living things. Life comprised a *dynamic* interaction between the responsive organism and its environment, which was *not* seen as hostile but rather as a 'contrary' ie. *contrasting* 'inert' force with a dialectic between them. Organic deterioration (ageing) was seen as a change in an *inherent* balance between nutrition and excretion. He recognised both short-term (physiological) changes in individuals as well as long-term historical changes but he was unitarian and *did not* distinguish the two. In his Hydrogéologie he held that meteorology could provide insight into the process of change, emphasising the action of water on account of analogies with erosion, the carving out of channels, and sedimentation, although he did overlook heat as an agent of geological change. He did not view the environment as becoming more complex, although he was aware of the effects of atmospheric conditions on plants (drought) and animals (thermoregulatory stresses). That plants were more dependent than animals on the environment was seen as highlighting life's dependence on the environment and plasticity in accommodating environmental changes - which could also result from human action such as the cultivation of new crop plants, and hybridisation.

Elucidating the 'March of Nature' would be a formidable task, not being inferrable from direct observation since organisms were often the irregular results of the continuous interaction between the 'power of life' and the random environment, but a task he stated could be assisted by recognising *the progression towards higher forms* and that there were a *number* of *branching* series depicting the actual relationships within the animal kingdom. Cuvier had believed that punctuations in the fossil record resulted from geological cataclysms and that biological series had been created more than once, whereas Lamarck believed that changes were gradual and due to the same forces then as now.

Lamarck held such evolution to encompass four main tenets:

(1) Nature is *not* constant;
(2) Organic forms develop gradually from one another;
(3) The natural sciences must recognise that nature has a history;
(4) The laws of life have produced increasing complexity over a long time.

This theory, transformisme, was further elaborated in his Philosophie Zoologique of 1809, which married classification, the *nature* of life - especially in simple animals, and the behavioural capacities of higher animals:

Firstly, a classification aimed at establishing 'real' relationships, with transformism and taxonomy *not* mutually exclusive, with a theory of differences (due to the environment) and one of similarities (due to the life-force), and encompassing an increase in structural complexity - though the latter not necessarily evident on a contingent (within genera) level.

Secondly, that life be defined as the power of nature to produce increasingly elaborate, integrated and active organic beings - environmentally-induced deviations from the logical progression being important in defining the family level. Also, that life is a *physical* phenomenon, *not* reducible to 'cause' and 'effect' but rather *relational and emergent*, and that the power of life is *itself* a mechanism of transformation. Lamarck recognised that there was an intrinsic tendency for size to increase and that its upper limit was constrained by the properties of life itself. In simple animals, the source of vital stimulation came mainly from environmental sources, while in complex ones it came from within (ie. the nervous system).

Thirdly, that biological systems possessed sentiment, ie. *sentience*, in that they had the capacity to receive sensations and to *react* to these. Organs were understood to arise from needs which persisted and from the movements that these needs gave rise to. By 'needs' he meant simply the impulses to survive and the immediate reactions to stimuli in the particular way they related to biological necessities such as food, drink, procreation and death-avoidance: Lamarck *never* claimed that animals wilfully choose to 'build up' organs. The development of organs and faculties related to their

usage, such organs being built up (habituated) through the *physiological* effects of the environment and animals' active *response* to it. He *did not* attribute active response or behaviour ('will' and 'consciousness') to all animals so psychologising biology as he is often accused of doing (Jordanova, 1984) and caricatured by the giraffe and palm tree in the famous cartoon by Caran d'Ache (reproduced in Bateson, 1979, p168).

Fourthly, that everything that has been *so* acquired or *changed* in the organisation of an individual during its lifetime is preserved in the reproductive *process* and passed on to the next generation by those who experienced the changes (Jordanova, 1984). Lamarck also acknowledged that organisms could act *on* the environment, indeed asserting that all compounds in the Earth's crust had been formed through the action of living things - not an absurd idea, for chalk and limestone are composed of the shells of minute sea creatures (Bowler, 1984) and the formation of soils depends on decomposed plant material as humus (Odum, 1975).

Lamarck argued that we see all levels of complexity of life today because forms are *not* all derived from a common ancestry but rather [each] level corresponds to a different *temporal* origin of the *simplest* forms. He could not abide extinction and Cuvier's renewed creation which he felt was incommensurate with transformism, arguing rather that new species were still being discovered. Nor did he realise that palaeontology could support his idea of evolutionary progression.

Man he saw as but one of many species that nature had produced, humans being simply of the greatest psychological *complexity* - of which moral qualities were an aspect. For Lamarck there was no mind-body duality and he eschewed notions such as soul or spirit. He conceived God as having unlimited power, but, Lamarck not being a teleologist, as having no bearing on natural science. He defined the Universe as the totality of physical matter, and nature as a system of laws which with motion, used space and time to produce bodies amenable to perception, production implying a time scale as opposed to an instantaneous 'Creation'. Nature, he saw as harmonious - emphasising adaptation as opposed to conflict, and he saw cooperation and consensus as the ultimate goals of society. He believed in simplicity and economy of explanation, with Nature a *unified* system and science

emphasising the relationships, analogies and affinities between her constituents, although he was also an empiricist. Lamarck was interested in the watch, not the watchmaker, and it is not really possible to label his metaphysics which is probably best described as 'Naturalistic'.

His theory was the most detailed of those worked out in the enlightenment, but was not met with great acclaim, mainly because, in Britain, Natural Theology was undergoing a revival, while the ethos in France was too conservative. The vehement attack on his inheritance of acquired characteristics, however, came from his rival, Cuvier, who detested Lamarck on scientific and religious grounds and used his political and scientific status to ensure that Lamarck did not get a fair hearing. Cuvier had reconstructed extinct species from fossil vertebrates, and divided animals into four 'types' which could *not* be ranked *hierarchically*, arguing rather that each 'type' was infinitely flexible in the ways its external modifications could be adapted to the demands of the environment, but their internal structures far too complex to have arisen by any natural process...

4.22. Weismann and Waddington

.....but,

> "Lamarck was basically not as completely wrong as is widely believed, and neo-Darwinians are not as thoroughly right as is equally widely assumed. The best of both worlds may be somewhat intermediate between the two extremes."
> (Wasserman, 1982b)

Lamarck's transformism clearly *was* compatible with Darwin, only it lacked a mechanism of heredity (which was nevertheless still *problematic* for Darwin), although Lamarck had furnished an account of the *origin* of life, which Darwin did not. In fact, Lamarck's inheritance of acquired characteristics was describing a *process*, not

a mechanism, while Darwin proposed natural selection as a *mechanism* of change (Ho, 1984b). But, owing to the de-emphasis of Lamarckism instigated by Cuvier's singular assault, the need for an adequate account of heredity came to be seen exlusively in the context of Darwinism, being furnished by August Weismann (1893) who, as Davenport (1979) put it,

> "clearly recognised the direct inheritance of formal constraints via the primordial germ cells but was misled into assigning the primary role in this transmission to the nucleus." (p402)

Notwithstanding, the inheritance of acquired characteristics was exhumed by Waddington (1942), who successfully formulated not only Lamarckian-type inheritance, but also a number of Lamarck's other assertions, within a conceptual scheme framed largely within the paradigm of the new synthesis. Since the empirical and theoretical bases of Weismann's and Waddington's biology have already been reviewed in chapters one and three, the remaining discussion will be reserved to that of Weismann *vis-a-vis* crude Lamarckism and to Waddington's epistemology in the context of its historical setting.

Weismann's alleged refutation of Lamarckism with his experiment in which he amputated mice's tails and showed this not to be inherited, has been criticised by Lamarckians as unfair on grounds that Lamarckism was based on *purposeful* response (Matsuda, 1982). In fact, its purpose had been to demonstrate 'hard' heredity, for Lamarckism and, to an extent, Darwinism, had been based on 'soft' heredity (*ibid.*). Bateson (1979) criticises Weismann on grounds that his reasoning was deductive, namely, that there was no *imagineable* way in which the soma could communicate with the germ plasm. However, as Matsuda (1982) points out, Weismann was not a Weismannist but rather a 'neo-Lamarckist', as was Darwin. In fact, as we have seen (section 1.13), Weismann (1893) *did* acknowledge the possible role of environmental influences on heredity. The germ-plasm idea was actually first introduced by Nägeli (1884) who referred to it as the ideoplasm (Matsuda, 1982), and in fact both Darwin *and* Lamarck saw the germ plasm and soma as freely communicating (Buss, 1983). Weismann did, however, believe in the 'all sufficiency' of natural selection,

arguing also for 'germinal selection' as each determinant 'competed' for nourishment within the germ plasm which, if it produced a new trait, could be subject to natural selection (Bowler, 1984).

The second criticism of Lamarckism is that it is too tied up with determinism in that were it the rule, or even just common, then all the interconnected stochastic processes would grind to a halt (Bateson, 1979). However, since this asserts that stochastic process is a priori essential for evolution, it is not, on its *purely* deductive grounds, a valid criticism.

The third criticism of Lamarckian is its lack of empirical evidence, but, as we have seen (sections 3.11 and 3.12), there is a very considerable body of data in support of Lamarckian-type inheritance. Besides, returning to Weismann's mice, non-events too suffer from the limitations of inductive reasoning (the non-event here being the non-inheritance of taillessness), and, as Earthey (pers. comm.) has pointed out, it is doubtful whether any philosopher of science would be at all happy with the notion of any idea as 'dead'. That Lamarck's transformism was describing a process, not a mechanism, should, if anything, leave the phenomenon *open* to further conceptual development (eg. Waddington, 1953; Sonneborn, 1970; Sheldrake, 1981; Wasserman, 1982a,b; Ho *et al.*, 1983b), and in fact it was T.H. Morgan who first separated heredity from development (Ho, 1984b). However, it was not long before a move was made towards putting heredity and development back together again, and the first move in this direction came with Waddington.

As an undergraduate, Conrad Hal Waddington or 'Wad' as he was known to his friends, held a studentship in philosophy at Cambridge and was a good friend of Wittgenstein. He wrote his thesis on 'The vitalist-mechanist controversy' at Edinburgh, and his philosphy of science was staunchly anti-Popperian, supporting rather Kuhn's theory of paradigmatic change as well as Piaget's structuralism. Wad started out as a geologist, moving into palaeontology when he studied the evolution of certain groups of fossils, in particular the cephalopods (which lay down spiral shells) - a group "which forces one's attention on the Whiteheadian point that organisms undergoing the process of evolution are themselves processes". He moved on, through genetics, to experimental embryology, and in the 1930's developed the notion that genes interact to form a 'unified concrescence' or 'creode' in the

process of becoming, say, a nerve cell, speaking of 'epigenetics' in 1940, and introducing 'canalisation' and 'genetic assimilation' in 1942. He was elected a Fellow of The Royal Society in 1947.

As an experimentalist, Waddington was strong on embryonic induction in birds and mammals and on genetic assimilation, and as a theoretician perhaps his most important contribution was his concept of *time* as a parameter of change in biological systems. Waddington was essentially a follower of Whitehead - whom he spent more time studying than his exam subjects - although Wad himself did not produce many mathematical descriptions, resorting rather to analogies, stating in The Evolution of an Evolutionist (Waddington, 1947) that

> "I tried to put the Whiteheadian outlook to use in particular experimental situations" (p11, cited by Schubert, 1985).

The scheme that Waddington outlined in The Evolution of an Evolutionist was essentially that evolution affects *phenotypes*, that characters can be acquired at the population level, and that the influence of genotypes on behaviour can influence the selective pressures on the phenotypes to which these genotypes give rise. He also introduced the notion of indeterminism in that the same phenotype can arise from more than one genotype and *vice-versa*. He was struck by the fact that the number of *types* was restricted, for example that no vertebrate is fully hermaphrodite, and the need for a theory of phenotypes. He regarded neo-Darwinism as inadequate in ignoring the effect of behaviour on selective pressures and that selective values belong to the *phenotype* and only *secondarily* to the genotype.

Waddington was the earliest dissenter from the orthodoxy of the new synthesis, only Wright, Dobzhansky and Lewontin in the USA taking equally heretical and independent positions - as did the American Valerius Geist whose views were most similar to Wad's in emphasising the aesthetics of biological organisation and the importance of development, and whose punctuationalism too could be accommodated within the scheme, it resulting from drastic environmental change without resorting to gene mutations.

Waddington held a very pragmatic stance towards Lysenko, but not towards his own Soviet counterpart, Schmalhausen. Schmalhausen had used the term labile morphosis to describe structural changes to the

system (Shishkin, 1984) and, in 1949 (cited by Matsuda, 1982), put forward an idea similar to Waddington's genetic assimilation involving three main stages. The first is primitive or dependent morphogenesis where the environment influences the *degree* to which a trait is expressed, the second being autoregulatory development where the environment triggers an adaptive response of definite intensity [equivalent to phase 1 of genetic assimilation, section 3.10], followed by autonomous development when the norm of reaction had been changed. Schmalhausen (*ibid.*) also recognised that the number of kinds of solutions to problems were restricted and that these could come about in alternative ways. One example was material compensation in plants facing drought, namely that tropical plants shed their leaves to reduce water loss, whereas in xerophytes the leaf loss is more permanent although young plants may develop temporary ones, while more specialised xerophytes never have leaves but the shoots sometimes form leaf-*like* branches (Matsuda, 1982, citing Schmalhausen, 1949).

Waddington, although not politically active, had started out as 'leftish' in the 1940s but by the 1960s had become rather conservative. Waddington's hierarchy encompassed a Great Ladder of Being ranging from bacteria to humans (not God) whom he wrote about in The Ethical Animal (1960). He regarded Man as being nearest the perfection to which all living beings are pointed, relegating other species to the realm of 'sub-human' on the grounds that they have no ethics. He was, however, cynical about non-verbal communication in humans. He contended that Man's role as a social being developed from childhood and involved the internalisation of authority, going as far as to say that neonates accept authority because of an 'innate need to obey' (*ibid.*, p205, cited by Schubert, 1985). He overlooked the role of play in other primates and carnivores, and was very late in recognising ethology, although he did anticipate behavioural ecology.

Neo-Darwinism and Mendelism, however, have tended to 'stonewall' the heresies of apostates such as Waddington (Schubert, 1985). For example, Abercrombie *et al.* (1980, p106) define 'genetic assimilation' as being 'like Lamarckism but depending on mutation', so missing Waddington's point entirely, namely, that evolution encapsulates mutual feedback for both phenotype and genotype within, between, and among individual organisms of both the same and diverse species - so putting life in a theoretical frame of reference with the momentary

'present' for any individual related by development to both its historical past and potential future, and by reproduction to both the historical past and potential future for the species (Schubert, 1985).

Such 'stonewalling' has afflicted not only Waddingtonian thought, but also Lamarckism (=only inheritance of acquired characteristics), Darwinism (=only random variation and survival of the fittest) and Weismannism (=only that there is no communication from soma to germ plasm). Only recently, neo-Darwinism was accused of paying lip-service to development - by a structuralist who dismissed natural selection as 'irrelevant'. It is time to have a look at this issue.

4.23. Kuhnian crisis and natural selection

> "It is an extraordinary feature of systematic biology that the arguments between apologists for the various schools are often conducted at the most passionate level" (Kemp, 1985).

At present, many non-biological as well as biological disciplines are aiming towards evolutionary puzzle-solving (Collingridge & Earthey, in press). Indeed, pluralism has been suggested by Ho & Saunders (1984a) but who appeal

> "to natural selection only at the end of their study, rather than at the beginning" (*ibid.*).

However, they appear to confuse the role of natural selection *per se* with the neo-Darwinian assertion that natural selection is *the* all-powerful creative force, since they had previously maintained (Ho & Saunders, 1981) that the laws of natural selection and of increasing complexity have a non-derivative relation to one another. The place of natural selection in evolution is probably more realistically

reflected in their statement that:

> "Natural selection still gives a distinctive flavour to evolutionary studies, but loses its place as the all-powerful creative force" (Ho *et al.*, 1986)

But, in 1987, Ho contended that natural selection is irrelevant, that it has no role, quite a different matter from contending, as previously (Ho & Saunders, 1984a), that it has no *creative* role on grounds that it can act only on forms *already* present. Moreover, we saw in section 4.13 not only how natural selection might be incorporated into the structuralist (or rather generative paradigm, Goodwin, 1984b), but also how it could lead to the *manifestation* of potentialities never before *expressed* - in which sense it *is* creative. Waddington himself acknowledged both random *and* directed change, while we have already seen too that Darwinism and Lamarckism are compatible. Ho (1985) asserts the *maintenance* of a form depends solely on heredity, not on natural selection, but clearly if all forms with a given potentiality are eliminated by, say, preferential predation, then they will no longer be *available* for its hereditary maintenance.

However, neo-Darwinism may rightly be criticised (Ho *et al.*, 1987) for *equating* evolutionary studies with those of natural selection, and (Ho & Saunders, 1982b) for its closure at the level of the gene, such 'genism' (Ho, 1985) reminiscent of preformationism only that the homunculi are composed of DNA (Bowler, 1984). Even Dawkin's (1976) memes have been invoked in support of genism, on grounds that memes have an ultimate effect on the genes (Baerends, 1984). Indeed, Baerends (*loc. cit.*) argues that the term 'evolution' be reserved for processes by which genes (genotypes) differentiate. But while it is true that

> "For far too long, a silent majority have been dissatisfied with over-simplified views of evolution" (Pribram, 1982),

the Structuralists' *vehemence* is perhaps unfortunate, in being overly opposed to Darwinism from which they might have drawn some inspiration (Reed, 1982a).

Indeed,

> "The structuralists' ill-advised antipathy to Darwinian thinking (which is a consequence of their *understandable* aversion to *neo*-Darwinian *dogmatising*) is extremely harmful to their *own* views" (Reed, 1982a, italics added).

In fact, from the neo-Darwinian camp, Maynard Smith (1982) - whom Webster & Goodwin (1982) themselves assert as neo-Darwinism's 'most able and forceful proponent' - *agrees* with the structuralists for criticising Crick's (1953) scheme as a *dogma*, but fears that they are planning to abandon Weismann who did at least provide a hereditary scheme. In fact, it is Weismann's scheme that Buss (1983) and Goodwin (1984a) have elaborated as the basis for *their* conception of heredity.

There is certainly no reason why Structuralism should contradict Darwinism (Buss, 1983). Einstein's relativity was never seen as so starkly contradictory to Newton's mechanics: in fact it offered an explanation as to why Newtonian mechanics appeared to work in the first place (Chalmers, 1978). It is hoped that the arguments expounded in sections 4.11 to 4.13 will do similarly for neo-Darwinism. For example, Keiser's (1987) minimax model of development and Goodwin's (1984a) view of inherited particulars as parameters of form can explain why punctuated equilibria can occur: the limits of canalisation must be exceeded. In fact it simplifies the explanation, because the notion of a threshold allows for sudden change given *constant* rates of mutational (or otherwise mediated) change to the genes (and other inherited particulars) (cf. Arthur, 1981). Besides which, Maynard Smith (1982) *likes* Webster & Goodwin's (1982) idea that genes 'evoke' from a set of possibilities. Moreover, some authors, especially within psychology, have been criticised for giving undue weight *to* Haeckel's dictum (almost to the *exclusion* of natural selection) because it *strengthened* Darwin's arguments (Charlesworth, 1986). And Haeckel himself, as long ago as 1876 (cited by Bowler, 1984), had openly proclaimed his intention to formulate a synthesis of the evolutionary theories of Darwin, Lamarck and Goethe (the latter whose evolution was anti-materialistic, involved an archetypal plant form, and progressive over time) (Bowler, 1984).

Ho (1985) asserts that neo-Darwinism lacks a theory of form and

pays but lip-service to the need to understand development. But this omission does not render all the assertions that it *does* make necessarily and utterly *wrong*. Again, Maynard Smith (1982) agrees that neo-Darwinism lacks 'laws of form' (although he prefers to think of it as lacking laws of development) and that field theories are needed. He asserts that the neo-Darwinian conception of genes as 'developmental programme' is a good *analogy*, only not the *full* explanation as genes require some *context* within which to operate. This context has since been furnished as the generative field (Goodwin, 1984a). In 1982, Webster & Goodwin put forward a theory of form, although Reed (1982a) preferred to think of it as a theory of *transformation*.

Dobzhansky (1956) pointed out that much modern evolutionary theory requires that traits be adaptive. Yet there are other explanations as to why a trait may be present - developmental constraint for example (Gould & Lewontin, 1979). But on these grounds, the structuralists have come to dismiss adaptation all out (Ho, 1987). In 1982 already, Maynard Smith feared that Webster & Goodwin (1982) found adaptation 'boring'. Conversely, the neo-Darwinians have in the main become less dogmatic over the last decade or two - after all, in 1966 Maynard Smith had claimed that

> "The combined effects of adaptation during development to environmental stimuli, canalisation of development and genetic assimilation are to mimic Lamarckian inheritance without involving any process not known to occur" (cited by Ho & Saunders, 1982a).

Yet neo-Darwinism has never been *wholly* monolithic. For example, LLoyd Morgan's firm adherence to Darwin *included* Darwin's acknowledgement of acquired characters (Bowler 1984); the stances of Waddington (post-1940), Dobzhansky (post-1950) and Gould (post-1970) we have already seen; and Lewontin's (post-1980) view is that organisms shape the environment and are responsive to it at *all* levels *including* the genes.

Ho *et al.* (1987) argue that they (Ho *et al.*, 1986) had been describing a new paradigm, a paradigm first articulated as such two years previously (Ho & Saunders, 1984) when they claimed pluralism (regarding levels of explanation) would be a central issue. Yet,

ironically, the structuralists have virtually ignored adaptation, dismissed natural selection as irrelevant or, as a conceptual tool, to be invoked 'only as a last resort' (Ho & Saunders, 1984a), and remain to indicate how their paradigm might be extended to encompass the population level.

Of course, realistically, any new paradigm *will* render certain of its predesessor's tenets obsolete. Nevertheless, a few neo-Darwinians are equally vehement in their rejection of novel conceptions, for example Richard Dawkins who adheres to Panglossianism (1987, penultimate paragraph, quoted by Gilson, 1989), and his contemporary Mark Ridley who regards Lamarckian inheritance to have been 'disproved' outright (1989, in Gilson, 1989). Of course, as Kemp (1985) maintains,

> "There is nothing wrong with making assumptions nor seeking to justify them..."

but,

> "...What is unforgiveable is to forget that they *are* assumptions and to behave as thought they are known certainties when there are no such things" (*ibid.*).

For instance, neo-Darwinian accounts of phylogenetic reconstruction have several empirical justifications, but these are equally compatible with other theories including Lamarckism (Kemp, 1985). Ho (1987) dismisses natural selection on grounds that supporting evidence is scanty (cf. Ridley's above dismissal of Lamarckism notwithstanding the considerable body of evidence in *its* favour: chapter three), and, while it is true that there was almost no field evidence for natural selection until Kettlewell studied pale and melanic peppered moths in the 1930's (Bateson, 1979), as Davenport (1979) points out,

> "It is not the method of observation, but the quality of inference, that confers importance on the results... The inferences of Mendel and Darwin were based on the merest of visual observations, but the quality and validity of these inferences have rarely been matched... We must be careful not to confuse procedural pyrotechnics with the quality of theoretical insight" (p36).

Besides, empirical evidence in support of environmentally-induced changes can *complement* natural selection. For instance, Harrison (1928b) found that manganese and lead salts on the larval foodplant of the Early Thorn moth Selenia bilunaria (Esp.) (Geometridae) induced melanism in the adult, while 1% manganese chloride resulted in pupae of the F_4 (relative to the first generation of treatment) eclosing ahead of schedule - in December rather than in March as usual. Thus, pollution could induce the form and so render it available for predatory selection, the latter perhaps effecting its increase in frequency *also* through genetic assimilation.

Thus, vehement stances, for or against either paradigm, run the risk of dismissing individual tenets which, when combined into a synthesis, could give rise to emergent concepts that might facilitate the development of the new paradigm or the elaboration of the science under stress.

Perhaps Dawkins' (1976, 1987) tenacious adherence to neo-Darwinism stems from the "pronounced professional insecurity" that sets in when anomalies come to be seen as posing serious problems for the (existing) paradigm (cf. Chalmers, 1981). And, perhaps Ho's (1987) relentless assault on natural selection simply reflects the fact that, as with political ideologies, the views held by workers within a school tend to reflect their individual personalities (Skynner & Cleese, 1983). Here, the structuralists, as dissenters from neo-Darwinian orthodoxy, are non-conformists. But adaptation implies conformity (to the environment), a conformity held to come about through natural selection. It is hardly surprising, therefore, that dissenters from neo-Darwinism should attack a neo-Darwinian tenet whose central theme is to bring about conformity and that they not then replace it with such a structuralist one.

4.24. Biology and Ideology

One reason for opinions being held so strongly may be

> "because 'belief' or 'non-belief' in evolution has spilled out of the scientific framework into politics and religion" (Kemp, 1985).

Yet the various schools appear to abhor labels indicative of their own politico-type nature. Neo-Darwinists, for instance, much prefer to be regarded as neo-Darwin*ians* (M.F. Earthey, pers. comm.), and the structuralists appear similarly shy, in wishing to regard their school as just 'the new evolutionary paradigm' (Ho & Saunders, 1984; Ho *et al.*, 1986, 1987). Yet, as Ho (1987) points out, it is not labels such as 'holist' or 'vitalist' that are the real issue, but rather that their proponents had a passionate commitment to vital process.

Analysis probably arose during the evolution of consciousness because the reduction of experience to levels of regularity allows prediction and control over subsequent experience (Davenport, 1979). Thus, experiences that support a sense of well-being are promoted. The expectation that distress might be so alleviated was originally vested in magic and then religion, but these have been largely replaced by science whose power lies in that it can distinguish causality from coincidence and so enable *intervention* (for example the prevention of disease). This has given rise to a condition in which

> "the value of knowledge is judged largely by its efficacy in promoting successful actions that bring future experience into harmony with our expectations" (*ibid.*, p343).

Biology, however, adheres to mechanism as biology deals with phenomena in a range of magnitude where Newtonian mechanics is not obviously contradicted (Davenport, 1979). Thus, mechanistic intervention appears effective regarding our expectations which have taken as given the hierarchical functioning of biological systems. Moreover, cultural expectations preclude the serious development of alternative

approaches that do not offer obvious and quickly realised advantages. Hence, the transcendence of present limitations will be slow and meet with great resistance. Physics was *forced* to relinquish mechanism by the stubborn irreducibility of the experiences that confronted it, so it is unlikely that biology will readily give up assumptions that till now appeared to serve it so well (*ibid.*).

This would account for the "pronounced professional insecurity" attendent with crisis states, when attempts to solve the problem become increasingly radical, the rules set by the existing paradigm are loosened, and when its normal scientists resort to philosophy and metaphysics to defend their innovations (Chalmers, 1978, citing Kuhn, 1970). For new fields in statu nascendi, the national scientific traditions, cultural environment and the personalities of their practitioners can all influence their development (Babkoff, 1977),

> "only grown up science [being] universal, international and individual" (*ibid.*).

Yet it was not only Lamarckism that was overshadowed by the uprising of Mendelian genetics that followed the dogmatising of the Weismann doctrine. A symbiotic theory of chloroplast evolution had been proposed in 1893 by the German Andreas Schimper (then expanded in 1905 by K.C. Mereschevsky in Russia) with a comparable account of mitochondrial evolution being first reported around 1925, but faded with the growth of modern genetics when

> "non-Mendelian inheritance seemed aberrant, inexplicable, and not worth studying" (Keller, 1986).

The scepticism facing Lynn Margulis's subsequent articulation of the theory exemplifies biology's reticence in the face of change. When she commenced her PhD in 1960, her professors tried to change her direction of research, but in 1962 Hans Ris discovered chloroplast DNA which provided her 'alibi' (*ibid.*). Keller's phraseology (*loc. cit.*) in discussing Margulis's Origin of Eukaryotic Cells (1970, Yale University Press) highlights the power with which the scientific

community is endowed:

> "...It has to be a young scientist and a woman who *dared* to challenge the scientific establishment by writing such a book" (italics added),

one who

> "takes special pleasure in defying established scientific dogma" (Keller, 1986, p47).

Margulis is fully aware that she is seen as heretical. She teamed up with James Lovelock in 1975 when he propounded his Gaia hypothesis ie. that life and the atmosphere had *co*-evolved from the outset. This view too was seen as retrogressive although Margulis went on to defend it as creative and productive. In the 1980's Margulis started to become respectable in the scientific community, being elected to membership of the National Academy of Sciences (US) in 1983, and is currently professor of biology at Boston University (*ibid.*). At this point it is worth noting that the new evolutionary paradigm has already been acclaimed one Nobel prizewinner, namely **Sonneborn** for his work on cortical inheritance (Earthey, 1988).

The esteem with which science is endowed has come about largely on account of its technological developments, a view enhanced by the economic and political power scientists gained from their prominence during and subsequent to World War II. The public was very receptive over this time when, since science was also concerned with the quality of entrants to its profession, science was put on a social and educational pedestal (Kelly, 1977).

In the 1960's, however, came the innovation of teaching science *by enquiry*, which it was seen would allow for the feel of research, provide motivation, develop creativity and give insight into the nature of scientific progress - although the latter has barely entered secondary school curricula (*ibid.*). But this approach to biological education is criticised on three accounts (Kelly, 1977), namely, its presentation of scientific progress as based solely on deduction, its inadequate portrayal of scientists *as people*, and its failure to recognise that human biology encompasses sociological phenomena.

Firstly, it presents science as developing sequentially through deductions that follow logically from earlier deductions, when all experimentation actually *starts out* with some expectation as to the outcome of the enquiry.

Secondly, it presents science as élitist and exclusive rather than as adding to common knowledge, in that it portrays science in terms of qualities - emotional neutrality, organised scepticism, rational procedures and reserved judgement - that typify the institutionalised norms of science or its ethos. In fact, even those with no formal education have some intuitive conception of heredity, indeed Mendel's own insight was attributable to his 'common sense' approach (*ibid.*). Besides, scientists are only human in that they make mistakes, have personal prejudices and are influenced by social pressures and cultural demands - qualities that Kelly (*loc. cit.*) cites R.K. Merton as defining the cudos (sic) of science. For example, the Russian population geneticist Sergei Chetverikov held symposia or soors (sovmestnoje oranije, literally 'communal screaming') in which everyone shouted out their ideas spontaneously but with their members cooperative and the leader able to hold the keynote of the discussion and direct it in the desireable way (Babkoff, 1977). One positive outcome of the 'new biology', however, is that it has tried to emphasise research methods, levels of functioning (eg. cellular), general principles (eg. ecological), and the *relationships* between structure and function *as well as* simply different *kinds* of organism (as was previously the tradition), although Kelly (1977) would prefer it to talk of similarity rather 'unity' among forms.

Thirdly, it fails to recognise that sociology is essentially the sociobiology of Man as one of many life forms, so maintaining the divergence of social and biological science that began in the early twentieth century. After all, it must be remembered that it was a social scientist, T.R. Malthus, who provided the final clue to Darwin's and Wallace's theory. And current issues such as class stratification, demography and whether IQ is inherited or acquired involve both social and biological considerations (*ibid.*), as do the ethical considerations concerning the applications of, for instance, recombinant DNA technology.

Moreover,

> "If we are to understand ourselves and our own species we have to understand the links within our biological and cultural natures" (*ibid.*, p13).

Waddington's sociopolitical ideas, however, were not based on observation, but were non-empirical and logically derived from his scientific theory (Schubert, 1985). In his Ethical Animal he projected his own middle-class scholastic and patriotic values - his chauvinism extending also to age (he was pro-youth) and a certain sexism. The role of Ethical Animal had been to *justify* the technology responsible for the good life he had, although it was 25 years out of date when he, ironically, depicts Nazism, hydrogen bombs and the like as *evil* appliers or appliances of technology (*ibid.*). He asserted that biological wisdom consists of encouraging the forward progress (anagenesis) both of the mechanism of the socio-genetic evolutionary system and of the changes in the grade of human organisation which that system brings about. He welcomed a period of 'co-existence' between capitalist and communist states, but argued that too *much* aid from better to more poorly developed countries would impede biological wisdom because it would result in too great a social and economic equality. He contended that the major problems concerning individual to individual behaviour were to be sought in those types of attitude and activity that facilitate or hinder the development of a healthy authority structure. It is possible that Ethical Animal may be more popular today given the new conservatism sweeping America in the 1980's (*ibid.*).

Schubert (1985), however, offers an alternative epigenetic interpretation of socio-cultural change. Schubert argues that *individual* differences within any society can be *preserved* by the goals of egalitarianism and the need for socio-political policies, and that the constitutional fitness of nations depends on maintaining the diversity and individuality of their people and *not* on their increasing conformity and organisation into some super-organismic holistic entity. There might be a number of ways in which any political society may further develop the particular environment that the population chooses as its niche. While political theories based on

the slow change of phyletic gradualism or individual ontogeny are set in premises that make drastic or sudden transformation undesirable, revolution *can* be constructive (just as can punctuated equilibrium) in resulting in a modified *population* that is better adapted, and such revolution can be on a limited scale (*ibid.*).

Waddingtonian epigenetic theory models faster biological change than gradualism (Schubert, 1985). If gradualism models socio-political stasism, and punctuated equilibrium revolution, then epigenesis suggests a model for transactional and recursive social change. Here, cultural change accelerates changes in constitution and regime, to bring human political societies into better adaptation with dynamic environmental change and *vice-versa*. Indeed, modern research in cultural evolution is moving in this direction (*ibid.*).

4.25. Biology and Theology

The idea of the church being bitterly opposed to evolution dates back to the famous meeting in Oxford in 1860, when Bishop Wilberforce asked T.H. Huxley on which side of his family he claimed to be an ape, and Huxley, "Darwin's Bulldog", replied ferociously that he would rather be descended from an ape than from a man of high position who misused his talents to attack a theory he did not understand. Robert Fitzroy, Darwin's old captain on the Beagle, then stalked round the room waving a Bible (Bowler, 1984). Yet, regarding Biblical inspiration,

> "Any realistic approach to the subject must begin by accepting that the Bible does contain some factual error. It is simply not the nature of the Bible that all its statements are correct. To insist that they must be correct is to impose a false character upon the Bible" (Barr, 1984),

and in fact even the early church accepted Genesis as allegorical (Glasson, 1983). Indeed, Canon Kingsley spoke favourably of Darwin's

Origin, quoted in its second edition thus:

> "I see no good reason why the views given in this volume should shock the religious feelings of anyone",

and most church leaders felt that a study of life would give greater insight into the method of creation. It was mostly the *scientific* community that opposed Darwinism, on grounds that it countered the immutability of species. Darwin was actually very pro-church and, after the speech, recommended Wilberforce's review, which had been based on *logical* and *scientific* arguments, to his local vicar. What had happened was that the rhetoric was blown out of all proportion by the media (*ibid.*).

The notion of God as a sentient a priori creator presents two problems. The first is that it does not explain God's *own* origin. This issue is usually avoided by taking God as being outside time, or given. But it only

> "tells us that the earth rests upon the tortoise : it does not tell us on what the tortoise rests" (Reade, 1887, p180).

The second is that the notion of the Creator as a mind, that the Universe is an expression of God's will, puts God into the role of central directing agency with respect to which Nature is but an epiphenomenon. This view has been the basis for a number of attempts to reconcile evolutionism with, in particular, modern Christianity. Houston (1981) for example, criticises a number of evolutionary ontologies (in particular that of Monod, 1971) in favour of others, but takes as moral and theological grounds for his justification, albeit valid, what are actually logical and scientific ones. Such a view is strongly upheld on account of its teleology: that Man is made in God's image gives him a special place in Creation.

The notion of God originated with apparitions of deceased tribal leaders that haunted the dreams of primitive men. They believed their chiefs were still alive (so ameliorating the pain of bereavement), who, since they were never to be seen in waking life, now inhabited an underworld (Reade, 1887). That God originated as an illusion of an

authority figure suggests that the idea of God as central directing agency is deeply rooted in Man's culture. His authority bestowed him the right to endow reward or punishment, in accord with which Man fitted his behaviour. And with 'good' and 'evil' juxtaposed in Mankind's conscience, so the underworld became polarised into 'heaven' and 'hell' (*ibid.*). Hence religion provided for the same needs as the science that succeeded it, namely prediction, pre-emption of disaster, and amelioration of distress (Davenport, 1979).

As Man's description of nature developed, so did his Deistic notions. For example, it was once believed that there were separate Gods for each tree and animal (Reade, 1887) or (as in Nordic mythology) weather condition, whereas by Biblical times, in the Middle East, there was accepted to be but one. Thus religious metaphysics too became more complex. So did its morality, for instance, the succession of Leviticus's demand for strict adherence to the six hundred Judaistic laws by Christ's singular assertion that they need only hold in principle on grounds of unconditional forgiveness. Hence,

> "It is incorrect to say 'theology is not a progressive science'." (*ibid.* p541).

But its metaphysical and moral bases remained (and still are) essentially of the same *kind*, for forgiveness implies the *notion* of punishment; by a God whose moral stance is one of authority, and whose metaphysical position is that of director. But the latter, as in biology, suffers an inherent duality, namely the distinction between creator and created; which is closely bound up with a second kind of distinction, namely that between the moral and metaphysical. Moreover, religious sentiments have been criticised for being out of step with Man's intellect (Reade, 1887).

Yet religious thinking is moving into accord with modern science as Process Theology, which has adopted as its metaphysical framework that of A.N. Whitehead (Hick, 1983). It mirrors eastern Taoism where God is understood not as having a *role* in the scheme of things nor as *aiding* the enlightenment process, but as *being* the scheme and the process; in other words, God does not do things but through God all things get done (Smullyan, 1981). According to process theology, God is neither omnipotent nor prior to creation, but

"is subject to the limitations imposed by the basic laws of the universe, for God has not created the universe *ex nihilo*, thereby establishing its structure, but rather the universe is an uncreated process that includes the deity" (Hicks, 1983, p50).

In other words, the universe was not created; it arose; and modern physics argues that space-time, matter-energy and the deity cannot exist apart from one another (Davies, 1983). Its process is not caused; it happens; and, since every 'actually' is inherently creative by way of organising and receiving data of the previous moment, and by virtue of the way *it* is then prehended (Hicks, 1983), it is epigenetic.

As with Taoism, God is not anthropoid; and hence evil must be defined ostensively, ie., as that which causes suffering (Smullyan, 1981). This is closer to reality *viz.*, to define it ostensively, experience; and so more attractive than *ad hoc* explanations in terms of Man's fall, God's discipline (albeit benevolent in purpose), or the like, which, as Reade (1887) has pointed out, are based on sentiment and should be abandoned. Rather,

"in constrast to the notion of divine self-limitation, process theology holds that God's exercise of persuasive rather than controlling power is necessitated by the ultimate metaphysical structure of reality" (Hicks, 1983, p50).

Notions of heaven can be rejected because they preclude coherent structuring. For instance, a murderer's bullet would turn to thin air, whilst psychological and social structures such as altruism would be meaningless were everything provided (Hick, 1983).

Nonetheless, God does have a personal aspect because natural process includes persons (Smullyan, 1981). The anthropic principle (Gale, 1981) holds human existence as sufficient to explain the history of the universe, in as much as the way it was being the *only* way that could have lead to Man's appearance. Although often misrepresented as teleological (*ibid.*), it still is, nonetheless,

invoked a posteriori and contains a certain tautology.

Rather, Man is simply the logical *outcome* of universal epigenetic process, whose bodily and psychological structures are emergent properties. These would include human ideologies; religious beliefs, into which would fit their notions, such as 'heaven' or 'hell', their material representations such as biblical scriptures; sentiments such as self-preservation and altruism; human social structure and morality; human creativity as with the arts and science, its paradigms, and its implementations. In a very real sense, then, technology and, in the final analysis, even that we call 'artificial', is an outcome of natural process. As Smullyan (1981) pointed out, in trying to contradict the laws of Nature one obeys them. Man's position is not the artifact or reflection of some anthropoid God. Nor is it the 'highest' in any moral sense. Most complex, perhaps, but special, no, within the unity of process we call Nature.

Schrodinger proposed his universal wave equation and went on to ask 'What is Life?' (1943). But nature does not partition itself into structural levels or disciplines (Rosen, 1982) other than in our description of them (Davenport, 1979). And since this itself has arisen through the process, our explanatory power, the nature of our existence, will too be so constrained. After all, *ex sistere* is to 'stand apart from' - and observe (Davenport, 1979). In answer to Schrodinger, then, life describes all levels of complexity above that where a particular kind of emergent property appears, say, generative field capacity. How readily such notions will become embodied in biology remains to be seen. The notion of emergent properties means that some lower level properties be left behind, which, since the latter include ourselves and our knowledge, may pose as a threatening prospect. As Davenport reminds us with a pun by Heraclitus,

> "The name of the bow (bios) is life (bios), but its work is death" (p364).

Hence biology and epistemology should have a happy marriage. After all, *scientia* is 'to know', and *philosophia* is to enjoy doing so. And if this should sound ridiculous then perhaps one only need reminding, that the most ridiculed life scientist of all, Jean-Baptiste de Lamarck, was the one who coined the very word Biology.

CHAPTER FIVE

Summary

This chapter reviews the natural history of the Speckled Wood butterfly, and opens by explaining why it is especially suitable for studying environmental and genetic influences on development: it exhibits seasonal polyphenism and sexual dimorphism; it can overwinter as larva or pupa; its subspecies differ in phenotype and voltinism; and it is a common species which can be continuously reared in captivity. It has already been much studied in many contexts, yet environmental control of the wing phenotype and life cycle, and the origins of its various races, remain rather poorly understood. It is towards closing these gaps that the thesis is aimed.

The life cycle is described. The main British and European subspecies are then compared, and their postulated origins and courses of divergence are discussed. The implications of environmental changes in ecologically marginal populations are also considered: genetic load in the species concerns more the genic balance of individuals than it does population structure.

The species is famous for its 'spiral contests' where males compete for sunspots in which to raise body temperature: this enables courtship activity. The payoffs of the univoltine and bivoltine strategies are compared. Univoltine adults are larger: the males are more successful in courtship and the females lay more eggs. Bivoltinism, on the other hand, enables a rapid build up of numbers.

The chapter closes by considering the ecological genetics of the wing spot pattern; in Satyrids such patterns are polygenic. 'Spring' forms have fewer hindwing spots than 'Summer' forms. Both forms are present in each generation; but the 'Spring' form, which causes larvae to pupate in time for winter, is selected against in summer. Finally, the possibility that the sharp change in spotting frequencies between Ireland and S.W. England reflects a sharp environmental change is considered.

CHAPTER FIVE

THE BIOLOGY OF PARARGE AEGERIA

Introduction

5.1. History as an experimental animal

The Speckled Wood was first described in Edward Topsel's English translation of the Latin Theatrum Insectorum in 1658, the first English entomological publication, and was referred to as 'Albin's Hampstead Eye' in James Petiver's Papilionium Britanniae of 1717 (Ford, 1957). It has also passed under the picturesque names of 'Enfield Eye' and 'Wood Argus' (Plant, 1987). It was classified as Papilio aegeria by Linnaeus in 1758.

Amongst the first investigative work on the species was that of Weismann (1896), who showed that the southern variety meione (now ssp. aegeria) developed a paler facies when reared under temperatures typical for the German race egeria (now ssp. tircis); which did not, converesely, produce a darkening when reared at Mediterranean temperatures. The species has since been the focus of considerable investigation concerning the evolution and development of the wing pattern (Schwanwitsch, 1935, 1948; Robertson, 1980a); territoriality (Austad *et al.*, 1979; Davies, 1978, 1979; Shreeves, 1980; Wickman & Wiklund, 1983); life cycle strategies and diapause options (Lees, 1962; Lees & Tilley, 1980; Wiklund *et al.*, 1983); ecology (Shreeve, 1984, 1986; Wiklund & Karlsson, 1984); ecological genetics (Packer, 1984) and population biology (Shreeve, 1985); and it has been suggested that a data bank be established for the species (Winokur, 1988).

5.2. Use as an experimental animal

The species is one of the easiest to rear and breed in captivity (Cribb, 1983); and it can be cultured continuously at 18°C given adequate cycles of light and dark (Stone & Midwinter, 1975), producing up to five (possibly six) annual generations, making it very suitable for continuous breeding programmes (Winokur, 1988). Stocks can readily be obtained from pairings of wild-caught adults (Robertson, 1980b), and would probably be amenable to hand pairing as the male has been observed to continue copulation *post mortem* (Lipscomb, 1971). The larvae feed on a wide variety of common grasses (Cribb, 1983) and can also be reared on artificial diet (Morton, 1981); although the latter is not used in the current investigation because of possible mortality in the first generation (*ibid.*).

The wing pattern is relatively simple and exhibits sexual dimorphism (Plates 1 and 2), as well as a seasonal trend in marking size (Robertson, 1980a) and eye spot number which also differs between localities (Packer, 1984). Weismann's (1896) results suggest that the pattern has a genetic component which can interact with environmental factors. The precise mechanism of pattern control, however, is poorly understood (Robertson, 1959), and Ford (1957) had suggested experiments involving its two main subspecies and genetic analysis. Studies on wing pattern have included photographic analyses of seasonal variation (Robertson, 1980a), quantitative analyses of seasonal and geographic variation in eyespot number (Packer, 1984) and metrical analysis of seasonal variation using digital techniques (Shreeve, 1985). Pattern variations have been induced by forcing pupal development with high temperatures (Robertson, 1959).

Plate 3 (opposite). Deciduous woodland (in late April) at Glen Eyre Hall, Southampton, where the Speckled Wood flies.

5.3. Life cycle and general ecology

The Speckled Wood inhabits deciduous (and coniferous) woodlands, clearings, copses and hedgerows, and often invades nearby open spaces, gardens and orchards (Dennis, 1977). Its choice of habitats is rather diverse throughout its range (Hillis, 1973; Panchen & Panchen, 1973a; Refseth, 1973a,b; Coutsis, 1985) but usually prefers moist, warm situations.

The eggs are laid singly (Davies, 1979) on various grasses in damp, shaded, woodland patches (Shreeve, 1984), and are pale green and spherical. The larvae feed both day and night (Ford, 1957), although Lees (1962) had reported only nocturnal activity. They feed on a wide variety of grasses, the favoured ones being Common Cock's-Foot grass, Dactylis glomerata (L.), and Common Couch or 'Twitch' grass, Agropyron repens (L.). Known foodplants are listed in Table 5.I. The larvae are green with a darker longitudinal stripe, which may afford crypsis by disrupting their outline (Plate 4a); passerine birds and small mammals probably represent the main visually hunting predators of larvae and pupae (Vane-Wright *et al.*, 1984). The fully grown larvae pupate at the base of grass stems or straw (Henriksen & Kreutzer, 1982). Pupal colour varies from greenish yellow to reddish brown depending on location (*ibid.*) and too is probably cryptic. Termination of pupal diapause results in a more or less synchronous emergence of the adults in spring (Goddard, 1962). The species is very tolerant of shade and dislikes direct sunlight, and is one of the last species to be shaded out by dense conifer succession (Thomas & Webb, 1984). The adults feed mainly in the tree canopy (Davies, 1978) especially on aphid honeydew (Thomas & Webb, 1984), although they occasionally visit flowers, in particular Cruciferae (West, 1980), as well as ergot infestations of grasses; and the males also obtain mineral salts from damp earth (Shreeve, 1984). The adults live for one to three weeks (Goddard, 1962). The males are protandrous by about a week (Wickman & Wiklund, 1983) and are famous for their territorial 'spiral contests' (Davies,1978, 1979). P. aegeria is the only British species to overwinter as either larva and pupa (Cribb, 1983), the rather complex developmental options having been well documented (Lees & Tilley,

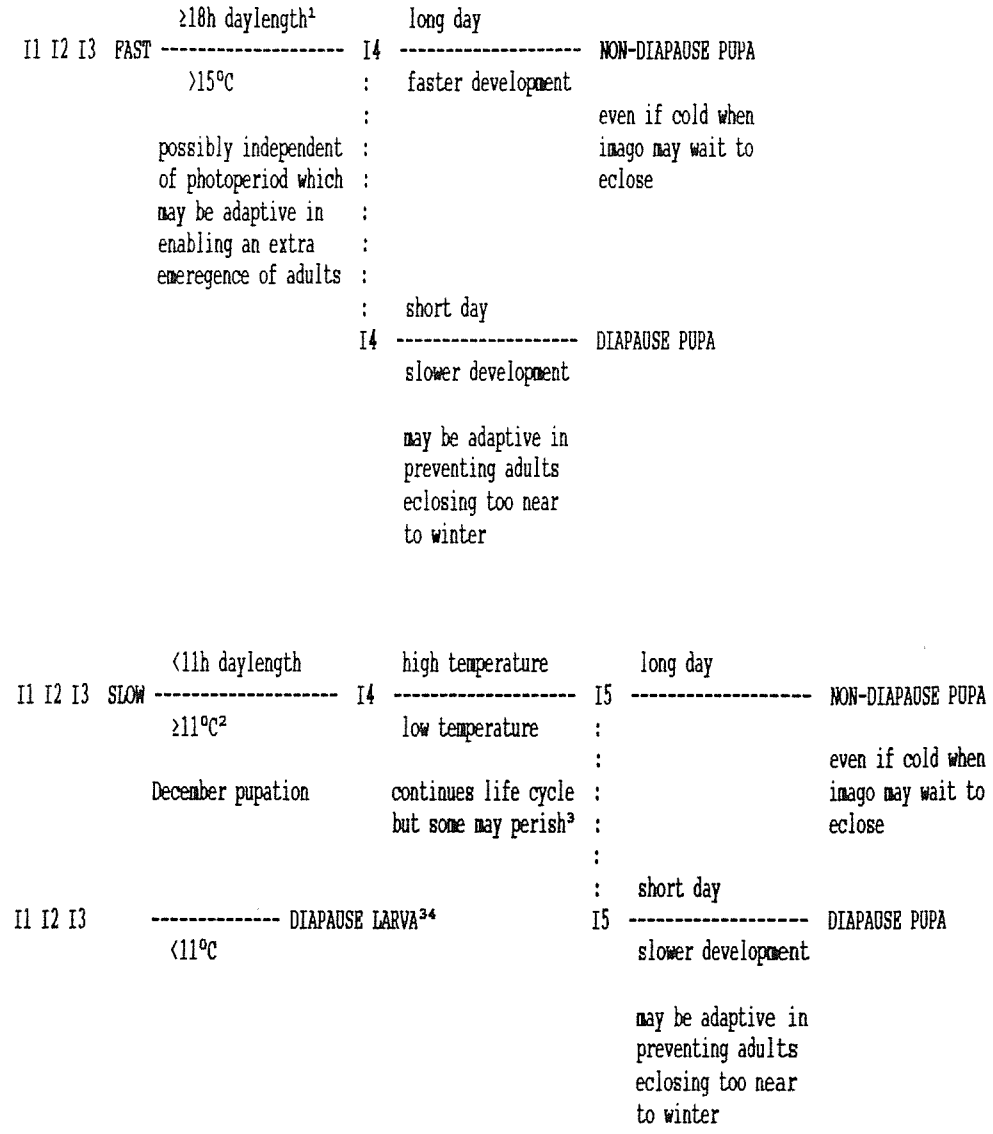
1980; Wiklund *et al.*, 1983; Shreeve, 1985).

Table 5.I. Known larval foodplants of Pararge aegeria.

Agropyron spp.[1]	
Agropyron (=Triticum) repens[2]	(also on Permean limestone)[5]
A. canina[3]	
A. tenuis[3]	
A. stolonifera[3]	
Bamboo spp.	(only in captivity)[4]
Brachypodium pinnatum[3]	(also on Permean limestone)[5]
B. sylvaticum[3]	
Bromus ramosus[3]	
Dactylis spp.[1]	(also in captivity)[6]
D. ashersoniana	(in Scandinavia)[6]
D. glomerata[7]	(also on permean limestone)[5]
Festuca spp.	(also in captivity)[8]
F. ovina[3]	
F. pratensis[3]	
F. rubra[3]	
Holcus lanatus[3]	
H. mollis[3]	
Lolium perenne[3]	
Melica uniflora[3]	
Phleum pratense[3]	
Poa spp.	(also in captivity)[8]
P. annua	(on Permean limestone)[5]
P. nemoralis[3]	(also on Permean limestone)[5]
P. trivialis[3]	(also on Permean limestone)[5]
Triticum spp.	(in captivity)[8]
Young wheat shoots	(in captivity)[8]

[1]Dennis, 1977; [2]Heath *et al.*, 1984; [3]Shreeve, 1986;
[4]Stone & Midwinter, 1975; [5]Garland, 1977; [6]Henriksen & Kreutzer, 1982;
[7]Thomas & Webb; 1984; [8]Cribb, 1983.

Figure 5.1. The developmental strategies of pararge aegeria



[1]In central Sweden 3rd and 4th instar larvae aestivate and their development is slowed and females have a 5th instar (Wiklund *et al.*, 1983)

[2]The entire life cycle can ensue at as low as 11°C
Short daylength can induce diapause even at 22°C
>22°C photoperiod may be irrelevant (Lees & Tilley, 1980)

[3]1st instar larvae do not feed below 8.0°C
4th instar larvae do not feed below 6.5°C
5th instar occurs only where vernal and aestival nocturnal temperatures are relatively high (Lees, 1962)

[4]In Sweden wild larvae continue to pupate at as low as 3° - 4°C (Wiklund & Persson, 1983)

Legend: I1 = 1st larval instar; I2 = 2nd instar; I3 = 3rd instar; I4 = 4th instar; I5 = 5th instar.
FAST = fast development; SLOW = slow development. For further explanation see text.

5.4. Voltinism and phenology

In Britain the species is bivoltine, each brood consisting of two emergences on account of overwintering as either larva or pupa:

Generation 1	part i	(1.i)	April
Generation 1	part ii	(1.ii)	late May - early June
Generation 2	part i	(2.i)	July (week 3)
Generation 2	part ii	(2.ii)	August (and September).

The timing of *eclosion* is unaffected by weather conditions, only the adults' *flight* activity (Goddard, 1962). The species develops too slowly to produce a third brood (Lees, 1962), although an autumnal 'false brood' (Shapiro, 1977) sometimes appears in October (Winokur, 1988). The latter comprise late 2.ii or premature 1.i emergers (Luckens, 1983). Flights 1.i and 2.i are continuous with overwintering pupae; 1.ii and 2.ii with overwintering larvae. Parts i and ii of each generation (especially the second) usually overlap (Goddard, 1962) although their precise timing varies between populations and years; British larvae may aestivate (Heath *et. al.*, 1984). Larval and pupal development is influenced mainly by temperature and photoperiod (Lees & Tilley, 1980); although there is probably also a genetic aspect as 1.ii larvae can include 'fast' and 'slow' developers (Robertson, 1980b) and 2.ii animals have been known to yield overwinter pupae (Goddard, 1962). The main interactions with photoperiod and temperature are shown in Fig. 5.1.

5.5. Britain

The British distribution is shown in Fig. 5.2 and the predominant subspecies is tircis (Butler, 1867) = egerides (Staudinger, 1872) (Dennis, 1977), is the nominate subspecies of continental Europe. It is characterised by its cream coloured markings; which tend to be smaller in the summer (Robertson, 1980a), ie. form aestivalis Fruhstorfer (Dennis, 1977).

In Snowdonia, south west of the river Conway, occurs a univoltine race, drumensis Thompson, that flies above the tree line and has pale prominent markings (Dennis, 1977).

In the Isles of Scilly, flies the bivoltine subspecies insula (Howarth, 1971), typified by its rather orange colour, larger markings (especially on the hindwings), and its brighter underside with a grey-violet submarginal hindwing band (Beavis, 1975).

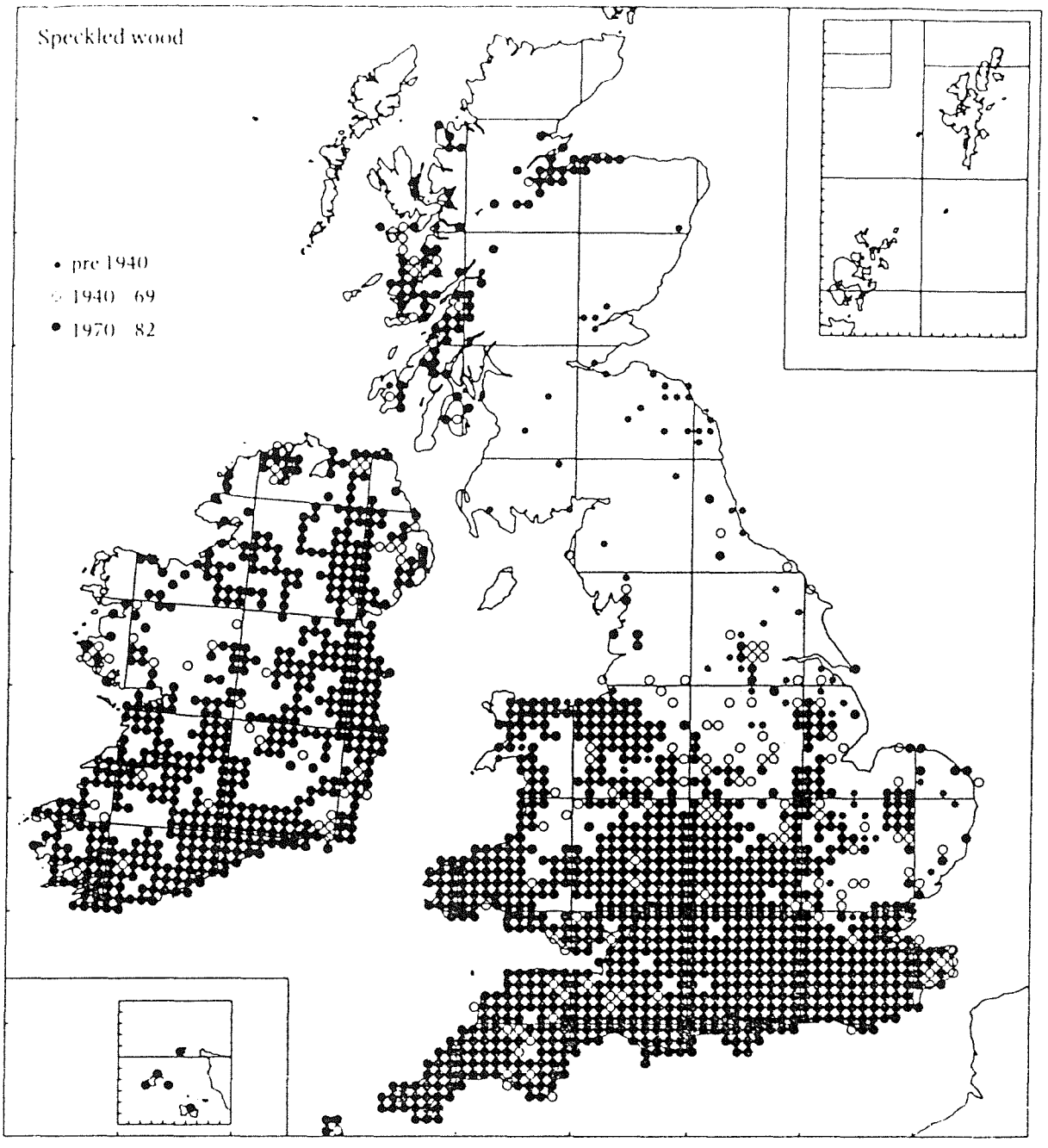
On the Scottish Isle of Rhum, especially near Kinloch Castle and to the south of Loch Scresort, flies subspecies oblita Heslop-Harrison 1949 (Dobson, 1973), characterised by its paler markings on both surfaces and larger hindwing spots, and a marked seasonal polyphenism (Dennis, 1977) It may also include specimens from the Hebridean Isle of Canna (Campbell, 1978) and possibly those from Oban and North Argyll, which are distinct from colonies from South Argyll, Islay (Dennis, 1977) and East Ross-shire (Hulme, 1969).

The Irish race, aegerides, differs only in its larger size (Dennis, 1977).

5.6. Continental Europe

Subspecies tircis also occurs in north, central, and eastern Europe including the Balkan countries (Higgins & Riley, 1975), extending its range through to the western Urals, the Caucasus and south Russia, although it is absent from boreal regions (Higgins, 1975). In continental Europe it occurs at up to 4000' above sea level (Higgins & Riley, 1975). In Sweden and south Norway, however, its pattern is more greyish and less well defined on the ventral surface

Figure 5.2. Distribution of Pararge aegeria in Britain and Ireland



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(Henriksen & Kreutzer, 1982).

In northern Norway occurs the univoltine (June-July) subspecies pallida (Verity), which too is rather greyish with a less well defined ventral pattern (Henriksen & Kreutzer, 1982). It reaches its arctic limit of 63° 30' N at Stadsbygd, Norway, where it inhabits sheltered deciduous woodland slopes (Refseth, 1973b); not being simply stray specimens as had been previously thought (Refseth, 1973a).

The nominate subspecies for southern Europe is aegeria (L.) which occurs in south and central France, southern Switzerland, (especially peninsular) Italy, Spain, Portugal and the Mediterranean islands (Higgins & Riley, 1975), and has been recorded at up to 2700' in the eastern Pyrenees (Ducrot, 1976). It also occurs in Morocco, Algeria and Tunisia at up to 5,500' (*ibid.*), and has recently extended its range to Madeira (Swash & Askew, 1982). Indeed, the distributions of tircis and aegeria are not clearly defined; a bivoltine island population from Samos, Greece (area 486 km^2) appears intermediate between the two subspecies. However, subspecies aegeria *is* constant from the western Mediterranean to Sicily (Higgins & Riley, 1975), although, like ssp. tircis, it does exhibit *local* phenotypic differentiation. Corsican specimens, for example, are similar to southern French ones only rather more heavily marked (form sardoa Verity) (Panchen & Panchen, 1973b).

There are two further subspecies. The first is xiphioides (Staudinger, 1871) from the Canary Islands including Tenerife, with tawny fulvous markings; the second is xiphia (Fabricius, 1775) from Madeira, which is similar except for its characteristic convex forwing termen (Higgins, 1975; Higgins & Riley, 1975). The latter, however, has very distinct genitalia from aegeria, and it may represent a distinct species although their ecologies are remarkably similar (Swash & Askew, 1982). The aegeria-xiphia-xiphioides complex may represent a polytypic 'superspecies' with very well defined subspecies (Higgins, 1975). Indeed, subspecies tircis has a haploid chromosome number of 28 (Federley, 1938), whereas in subspecies aegeria it is 27 (Bigger, 1960).

5.7. Origin and establishment

Ford (1957) postulates the species as having reached Britain in the 3rd Interglacial of the Pleistocene, then largely lost from north England and Scotland in the last Ice Age. Dennis (1977) however, suggests the early and middle pre-Boreal zone IV as its period of arrival. Yet past records and its strong presence in Scotland today, suggest that it was established there continuously until the 1850s and then retracted its range till the early 1900s, although remnant colonies may survive (*ibid.*). The bivoltinism of British P. aegeria probably derived from genetic variation in the larval growth rate of univoltines (as occurs in Maniola jurtina (L.): Brakefield, 1982 b) leading to a bimodal emergence (Packer, 1984). Here, the later pairings would given rise to larvae that would not have had time to complete the life cycle before winter. Instead, these would overwinter as pupae and eclose the following spring. Disruptive selection (as in M. jurtina: Thomson, 1971) may then have established a regular bivoltine phenology (Packer, 1984).

Central Swedish univoltines probably originated from Finnish univoltine coniferous woodland populations (Wiklund *et. al.*, 1983); and subspecies pallida was probably the first to be established in Scandinavia (Henriksen & Kreutzer, 1982). Scandinavian bivoltines probably originated from Danish mixed woodland populations (Wiklund *et. al.*, 1983).

5.8. Divergence

In Scotland, P. aegeria is extremely colonial with marked breaks between local populations. This may have led to the divergence of ssp. oblita, which then formed a steep cline with south Argyll populations during periods of contact. The species probably colonised Ireland following a glacial period. Race drumensis probably gained access during the warmer Boreal and Atlantic periods when the tree line was higher than today; having adapted to the cold, humid regime

and retreat of the tree line over the last 3000 years. Colonies at the edge of the species' range are often sparse and narrowly confined (Dennis, 1977), for example those on Magnesian limestone near Selby, Yorkshire. These inhabit woodlands but are absent from other locally suitable habitats, and may represent ecologically marginal populations under isolation stress (Packer, 1984). Such populations would be subject to homoselection, which favours increased homozygosity, random drift, free recombination and adaptive specialisation to particular habitats; when shifts in environmental conditions could be more harmful (Brakefield, 1979). Inbreeding seems to affect mainly the pre-hatch larva although genetic load, which varies between populations, is probably more inportant to the genic balance of the individual rather than to the population structure as a whole (Oliver, 1981). Subspecies insula is uniformly distinct (Dennis, 1977) but is probably geographically peripheral rather than ecologically marginal (Dennis, 1977).

The species can be supported by very small areas of habitat (Thomas & Webb, 1984) and the sexes differ in niche utilisation. Males remain within 50m of their eclosion site, and compete for 'sunspots' from which they pursue females in courtship. The sex ratio is roughly equal, but the protandrous eclosion (Davies, 1978) gives the males time to attain sexual maturity (Cribb, 1983). Females wander over 600m (Davies, 1978) and often traverse intervening open spaces between suitable habitats (Vane-Wright *et al.*, 1984). Since the eggs are also laid singly, competing males are probably *not* close relatives (Davies, 1979). The species mates randomly, although the 'resident' male in a sunspot has a rather greater opportunity for *courtship* than the 'intruder' (Austad *et al.*, 1979), and sometimes gives a horizontal rather than a spiralling chase (Wickman & Wiklund, 1983). Sunspots are most likely used for thermoregulation enabling males to maintain (courtship) for longer, since flight itself reduces the body temperature (Shreeve, 1984). Females can mate more than once (males often do) although single pairings are probably the norm for them in the field (Wiklund & Persson, 1983).

Egg size decreases over the oviposition period and this is most likely related to the adult's nutritional status, but females sacrifice the total number of eggs laid for the gain in egg weight (*ibid.*) since larger eggs give rise to larger pupae (Wiklund &

Karlsson, 1984) which may increase the fecundity of the resulting adults (Wiklund *et al.*, 1983). There is no advantage in females producing more eggs but of smaller size (with concomitant prolongation of the oviposition period) because they may not be able to increase their own survival by redistributing their energy resources (Wiklund & Persson, 1983).

5.9. Voltinism and fecundity

Voltinism strategies may serve a similar role to the above. Bivoltines inhabiting southern Swedish deciduous forests produce smaller but more numerous pupae which increases the number of adults the following spring; univoltines inhabiting central Swedish coniferous plantations, however, aestivate as larvae, producing fewer but larger pupae, the females from which carry more eggs and the males from which are better at territory defense (Wiklund *et al.*, 1983). A similar principle may apply to British race drumensis.

Bivoltinism enables the species to recover rapidly from losses due to (for instance) summer heat and drought, which can impose heavy losses on 1st instar larvae (Thomas & Webb, 1984) or decrease fecundity by shortening adult life span through their increased activity (Goddard, 1962; Brakefield, 1982b). Overwintering larvae may be more exposed than pupae to parasitism (Packer, 1984); and female larvae having a fifth instar may be further exposed (Wiklund *et al.*, 1983). Indeed in the Nymphalid Euphydryas aurinea (L.) the influence of temperature on larval growth rate interacts with parasite abundance to determine the sex ratio (Porter, 1984).

5.10. Ecological genetics of the wing spot pattern

Satyrid eye-spot patterns are largely polygenic (Brakefield, 1979a) and this is assumed for P. aegeria (Packer, 1984). Packer (1984) proposed that seasonal difference in hindwing eye-spot number reflects a difference in the *relative* frequency of *both* phenotypes under cyclic selection. This could be compared with Bradshaw's model (section 2.2) and might represent a transitory stage in the evolution

of *strict* seasonal polyphenism in the species. And even when in a coarse grained environment a single optimum *is* possible (Jarvinen & Vepsalainen, 1979), variation might *still* be maintained by mixing of populations, counteracting selective pressures in the same (or alternate) seasons, or by heterosis (*ibid.*).

Selection may act on the pleiotropic effects of genes. In Maniola jurtina, larvae that give rise to males with '2 splay' ventral hindwing spotting develop slower than those producing '4 costal' males (Brakefield, 1979b, 1984). In the moth Panaxia dominula (L.), the medionigra gene (Kettlewell, 1944b) exhibits several pleiotropic effects including decreased fertility in both sexes, its particular selective value depending on its relative frequency (Sheppard & Cook, 1962) (although unlike P. aegeria the mating strategy is disassortative choice by the females: Sheppard, 1952).

In P. aegeria (Irish examples excluded), paler ('spring' form) specimens tend to have three dorsal and five ventral hindwing spots, whilst darker ('summer' form) specimens tend to have one more hindwing spot on each surface. Both 'spring' and 'summer' forms are present year round except for a *'spring' form paucity* in *summer*. This is due to 'spring' form increasing larval growth rate in the *winter* to produce overwinter pupae, whilst 'summer form' genes confer an advantage on overwinter larvae, with selection only acting against the 'spring form' in summer (Packer, 1984). Packer found no spotting difference, however, between the sexes.

In Ireland, 'spring'-type spotting predominates[1], but on the English south-west peninsular, 'summer'-type spotting predominates. The latter may result from the warmer peninsular winters not conferring an advantage on overwinter pupae; and it may be an edge of range effect (Packer, 1984). In M. jurtina at least, such a boundary phenomenon does occur between Devon and Cornwall, where it may represent the boundary between two co-adapted genotypes at a critical point in an environmental gradient (Brakefield, 1984).

[1]The large size of Irish P. aegeria is consistent with their paler facies, as winter larvae produce the largest adults (Robertson, 1980a), as do Swedish aestivators (Wiklund *et al.*, 1983).

We have discussed largely the influence of genetic aspects in the evolution and ecology of the butterfly. Now let us look at what the influence of *environmental* factors means for the species.

PART TWO

THE EMPIRICAL INVESTIGATIONS

"It is one thing to conclude that a phenomenon is possible, even likely, and another to show that it actually happened in the past or is operating now" (Thomson, 1985).

CHAPTER SIX

Summary

This chapter examines linear growth from egg to adult and the relationships among the life cycle stages to elucidate the possible controls of growth and cues for metamorphosis. Only animals succesfully pupating were analysed; the sample of sixty animals derived from two pairs of spring brood, P. a. tircis from Hampshire.

The growth curve is sigmoid, with a superimposed deviation towards maximum length shortly before pupation; the larva then contracts to form the prepupa. It is proposed that at this elastic limit, ingestion is prevented so imposing nutritional stress, which destabilises development and initiates the differentiation of adult structures. Lepidopteran larvae produce two pulses of ecdysone before pupation: hormones alter physiological equilibria, and the already destabilised development may explain the redundancy of the second pulse. It might also render early pupae more susceptible to external perturbations.

Comparison of four- and five-instar larvae reveals their growth curves to be of similar form, although individual moult positions do not correspond directly: they differ in their *relative* positions along the growth curve. Theoretical manipulation of moult positions reveals holo- and hemi-metabolous metamorphic strategies to be transformations of one another: shifting the 'pupal' moult prior to the deviation eliminates pupation. Holo-metabolous metamorphosis differs essentially in that sclerotisation of the cuticle occurs after, rather than before, larval growth: the two strategies otherwise share virtually identical structural and physiological processes.

The stages within holometabolous development differ in the deployment, rather than nature, of structural components, suggesting that the strategy would have easily evolved. Similarly with the visible pupal polyphenism of aegeria. Larval/pupal intermediates can occur but cannot survive as replicative forms. The latter necessarily undergo distinct metamorphic stages, and demonstrates how continuous change in an underlying parameter can generate discontinuity in its outward manifestation. The broader implications for punctuated equilibria are outlined.

CHAPTER SIX

THE DYNAMICS OF METAMORPHOSIS

"Few, if any, generalisations can be made concerning metamorphosis, and every organism must be dealt with separately" (Davenport, 1979)

Introduction

Hormonal regulation of pupation in Lepidoptera is now generally understood, and the following account is based on Manduca sexta (L.) which has been much studied in this regard (Nijhout, 1974, 1975, 1976; Nijhout & Wheeler, 1982). The corpora allata (CA) of late final instar larvae stop producing juvenile hormone (JH), which had hitherto kept the imaginal discs undifferentiated (Nijhout, 1974). At the next photophase a pulse of prothoracicotrophic hormone (PTTH) and ecdysone (EC) promotes pupal preparatory behaviour (Nijhout, 1976). A second pulse two days later induces apolysis and the formation of pupal cuticle. Four days later the pupal moult occurs (Nijhout, 1974).

Yet there are anomalies. The second pulse is not a physiological necessity although it may be eight times the titre of the first, and the development of pupal cuticle on the clypeus and anterior ocelli requires much less EC than does such differentiation elsewhere (Nijhout, 1976). EC secretion succeeds JH-induced changes at most moults and it appears to be the timing (rather than concentration) of JH secretion which is important (Nijhout & Wheeler, 1982).

Mechanical controls too have been implicated in pupation. M.sexta(L.) larvae exceeding a critical head width always pupated at the next molt and it was suggested that stretch receptors inactivate the CA neuronally (Nijhout, 1975), although the precise cause of change in the *nature* of the moult remains unclear (*ibid.*). JH-sensitive periods appear to follow a pre-determined allatotropic programme which is poorly understood but known to have a genetic basis, and which can be modified by mechanical and maternal factors (Nijhout & Wheeler, 1982).

Holo- and hemimetabolous insect larvae can at any time remain as larvae/nymphs, develop into pupae/solitary adults, or develop into imagines/migratory adults, which option is realised (ie. most stable) depending on JH concentration (Nijhout & Wheeler, 1982). The present consideration examines the growth dynamics of P. aegeria including linear dimensional and temporal relationships between the principal life-cycle stages. The possible role of cuticular mechanical properties in pupation is outlined. A model of pupation involving homeorhesis and developmental canalisation is presented that can account for the general effects of hormones and which allows for the action of developmental shock. The dynamic is conceived as a diachronic type. The evolution of holo- and hemimetabolous strategies is then understood as changes in the complexity of insect growth dynamics.

Materials and methods

6.1. Samples and culture

Details of culture up to the pupal stage have been given by Winokur (1988) from which most of the following account is taken. The animals under study were derived from two fertile pairings between wild caught adults of generation 1 part i from Glen Eyre Hall, Southampton (Plate 3). The adults were paired in "Clear view breeding cages" (R.E. Stockley) indoors under mixed sunlight. The adults were fed from cotton wool pads on the inside cage supports: 10% sucrose was used to stimulate egg production (Cribb, 1983). Pads were replaced daily to stop the solution becoming too concentrated which can inhibit oviposition (Stone & Midwinter, 1975) or be fatal (Cribb, 1983). Nectar was also provided by various yellow flowers as P. aegeria favours this colour (West, 1980). Oviposition was on the cage bases and netting. One pairing yielded 84 ova (family 002), the other 57 ova (family 004) and these were reared indoors throughout the life-cycle at 18.9°C $\pm$ 2.08°C $\pm$ under natural daylength but out of direct sunlight. The males were sacrificed once they had paired but the females were left to complete the life cycle.

Ova from each pairing were transferred daily with a camel hair brush to individually labelled 3" x 2" x 1" transparent plastic boxes. These were humidified twice daily by exhaling on the inside of the box lids (Stone & Midwinter, 1975). Newly hatched larvae were transferred to fresh boxes (not more than ten in each) lined with tissue paper to prevent condensation. It was ensured that they had eaten their egg shells which is necessary for their survival (Ford, 1957), or were transferred with them.

Larvae were fed cut Cock's-Foot Grass, Dactylis glomerata (L.), obtained as seed and grown in trays on "John Innes Compost No. 2". Seeds were mist propogated under a 16 hours light : 8 hours dark regime at 15°C in a greenhouse which resulted in germination within a week. Standard seed and compost ensured a uniform composition and quality of foodplant as nutritional factors can influence development rates (Slansky, 1974).

Excess food was provided to ensure adequate nutrition and to prevent the desiccation of 1st instar larvae. Boxes were cleaned and replenished daily. With older larvae this was done every other day (but more often if needed). At each ecdysis, the larvae were segregated according to the dates of these and earlier changes (a maximum of five pre-fourth instar larvae or four older larvae per box). The durations and dimensions of each life-cycle stage, and the number of instars, were recorded. Length was scored by letting the larva (or the leaf on which it perched) rest on the box lid through which it was measured by ruler.

In preparation for pupation the larvae hang from a silk pad, and this stage will be referred to as the prepupa. Just prior to this the larvae stop feeding and appear to contract. Such larvae and pre-pupae were transferred to individual tissue-lined boxes, but when prepupae formed on the box sides or bases (if they fell), any remaining larvae were transferred to new boxes and the prepupal box relined. Boxes were inspected early morning and late evening for pupae. Pupae were left *in situ* for at least 48h to allow the cuticle to harden and *then* their length measured as for larvae. They were then transferred, by their attachment pads, to grooves of corrugated card in "clear view breeding cages" (R.E. Stockley), and an identification key recorded. Pupae were sprayed twice daily through the netting with tepid water to prevent dessication.

A few days before eclosion, the pupal wing cases begin to appear to yellow as the developing adult becomes visible through the cuticle. When two or more pupae coincided they were placed in separate cages for accurate identification. Pupal development up to the first appearance of such yellowing will be referred to as the prepharate stage, the remaining pupal development being the pharate stage. The sex of each animal was ascertained on eclosion and the empty pupal cases were discarded. Adults were maintained as above except that only sucrose was provided; and that animals not used for breeding were sacrificed once the wings had hardened. All non-living specimens were stored in greaseproof envelopes for subsequent examination.

6.2. Data compilation and analysis

In all, forty nine life cycle parameters were recorded for the species (Appendix I) of which nineteen are examined here (Table 6.I). Pupal data were obtained during an investigation into the effects of cold shock (see Chapter four) and affected characters are analysed here using data from untreated pupae only. The characters DOVIP NOV and ILONG refer to their resultant adults.

Data from families 002 and 004 were pooled. Untransformed data was used. Linear correlations among the life cycle characters were computed as product moment coefficients (Sokal & Rohlf, 1981). Only individuals reaching at least the prepupa were included in the analysis to exclude extraneous data that might have distorted the results. Individuals with missing data were similarly excluded.

TABLE 6.I

Life cycle characters used
in the investigation of Pararge aegeria growth dynamics.

Character	Description
OVDUR	Oval duration.
OHLEN	Larval length on hatching.
I1DUR	First instar duration.
E1LEN	Larval length at the first ecdysis.
I2DUR	Second instar duration.
E2LEN	Larval length at the second ecdysis.
I3DUR	Third instar duration.
E3LEN	Larval length at the third ecdysis.
I4DUR	Fourth instar duration.
E4LEN	Larval length at the fourth ecdysis.
I5DUR	Fifth instar duration.
TLDUR	Entire larval duration.
PPDUR	Pre-pupal duration.
PLEN	Pupal length.
PPHDUR	Pre-pharate pupal duration.
PHDUR	Pharate pupal duration.
DOVIP	Duration of the oviposition period.
NOV	The number of ova laid.
ILONG	Adult longevity.

Durations are measured in days. Lengths are measured in millimetres.
For further explanation see text.

6.3. Developmental correlations

The direction and significance of character correlations are summarised in Table 6.II. The product moment coefficients are given in Appendix II. 1st instar duration correlated negatively with oval duration. Yet oval duration *plus* larval development to 1st ecdysis correlated more strongly with total larval duration ($R_{(55)}$ = 0.60212, $0.001 < P < 0.01$) than did solely oval duration (negative correlation) or 1st instar duration suggesting that the former is really one stage interrupted by hatching; *i.e.* hatching is not a process similar to ecdysis.

Oval duration correlated positively with all other larval stages, but negatively with all post-larval stages, implicating a change in growth dynamics at pupation.

Length increased with instar duration, and each instar (except the 2nd) contributed additively to entire larval duration.

Pre-3rd instar durations correlated negatively with post-3rd ones, although correlations *within* pre-3rd (inclusive) and *within* post-3rd (inclusive) instar durations were positive, implicating some change in larval growth dynamics during the 3rd instar.

Cuticular elasticity may impose a maximum mature length (Warnecke, 1964) (reported as 27mm by Henriksen & Kreutzer, 1982) so that the more growth is completed earlier on, the less is possible later. This would also account for the inverse relationship between 4th and 5th instar durations. Individuals spending longer as larvae probably grow more slowly, rather than attain greater mature lengths.

Pupal length was similar to that at 3rd ecdysis and correlated positively with it, but was shorter than that at 4th ecdysis and correlated negatively with it. It appears to be mainly *length* that increases during instars 1-3, followed by a predominant increase in tissue *mass*. This could account for sex differences in larval size (Shreeve, 1985) when there is no sex difference in any of the larval lengths (Table 6.III).

Pupal length correlated negatively with all previous stage durations. It is suggested that larger larvae experience more

cuticular stress (1) causing greater relative contraction at pupation and (2) promoting a more rapid moult. Indeed prepupal duration correlated negatively with all larval lengths.

Entire larval duration showed significant negative correlations with the prepharate and entire pupal durations.

Adult longevity increased significantly with entire larval duration (although only females were used in this analysis) as it did with prepharate and pupal durations. The timing of the life cycle may be constrained by the pattern of growth, and the pupal stage appears particularly labile.

6.4. The pattern of growth

The number of instars is not determinate (four or five being possible) and there is no heteromorphosis (change of pattern) in the final instar.

The dimension at completion of each stage was plotted against stage duration (Figure 6.1a-d). The overall growth curve appears as a sigmoid form with a pre-pupational deviation (PPD). Growth returns to the sigmoid curve at pupation.

The size and form of the curve are similar for four- and five-instar larvae and the two sexes. But there is not necessarily a one-to-one mapping between particular moults where these differ in number between insects. The form of the curve will be termed the

TABLE 6.II

The correlations (i) among the durations; (ii) between the lengths and durations; and (iii) among the lengths of the various life cycle characters.

(i)	I1	I2	I3	I4	I5	TL	PP	PPH	PH	PDUR	NOV	ILONG
OV	(-)	(+)	(+)	(+)	++	(+)	(-)	(-)	(-)	(-)		(-)
I1		(+)	(+)	(-)	++	++	(+)					
I2			(+)	(-)	+	(+)	(+)					
I3				+	++	++	(+)					
I4					--	++	(+)					
I5						++	+++					
TL							++	--		--		++
PPH									-	++		
PH												+
DOVIP											++	

(ii)	OV	I1	I2	I3	I4	I5	TL	PP	PPH
E1		++	-	+	(+)	+++	++	(+)	
E2		(-)	(+)	-	+	--	(-)	(-)	
E3		(-)	(-)	(+)	(+)	--	(-)	(-)	
E4		--	(-)	--	(+)	-	--	-	++
PLEN	(-)	(-)	(-)	(-)	--		--	(-)	

(iii)	E2	E3	E4	PLEN
E1	(+)	(-)	--	(-)
E2		(+)	+++	(+)
E3			+++	+
E4				-

Suffices omitted for brevity. + or - show direction of correlation. Non-significant correlations () shown only when supporting a trend. Significance levels: - or + $0.01<P<0.05$; ++ or -- $0.001<P<0.01$; +++ or --- $P<0.001$.

Linear Growth Dynamic (LGD) and it appears to be very constant. It may be species specific.

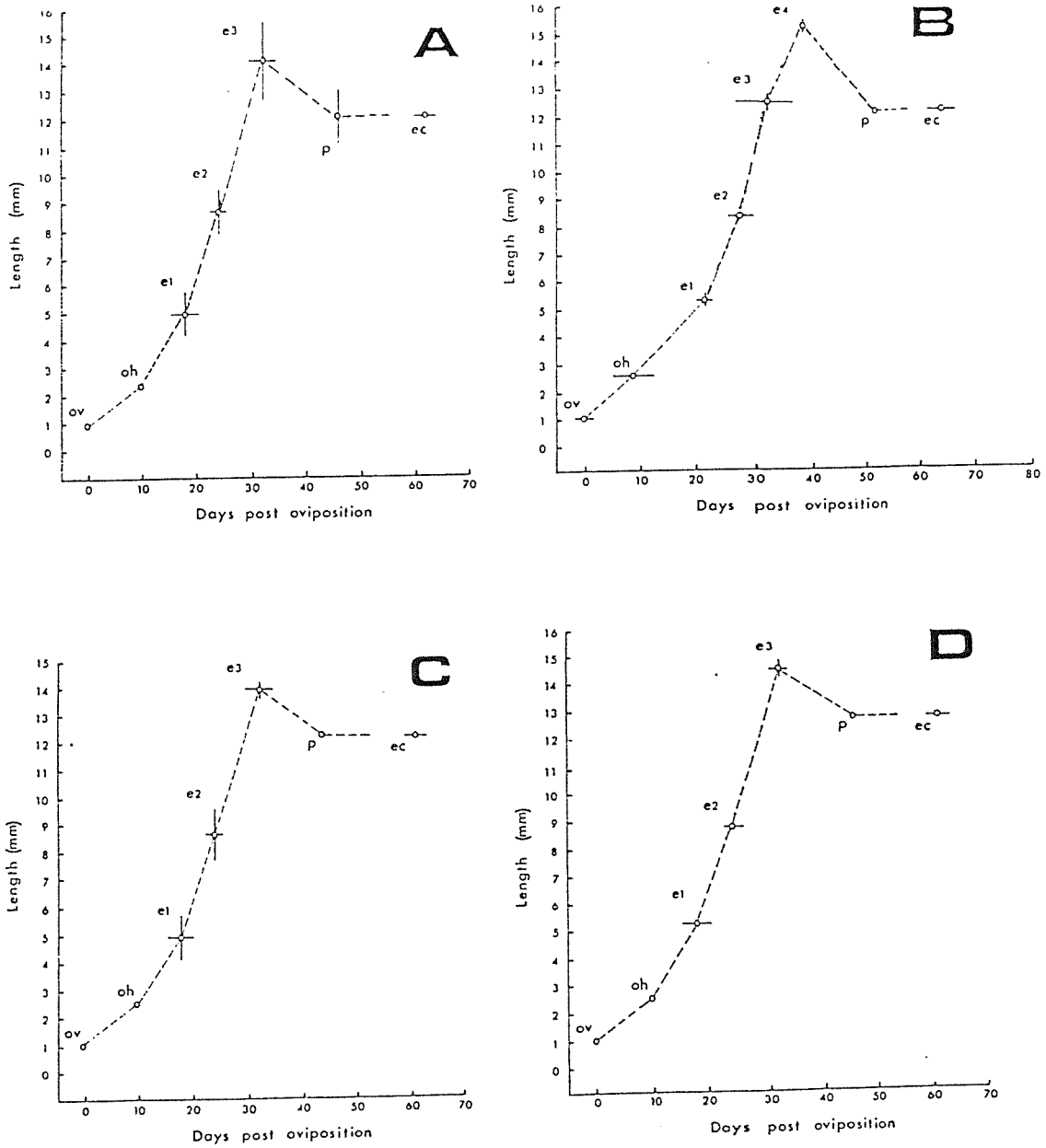
The following model is proposed. Moults are superimposed on the LGD. The nature of a moult depends only on its position along the LGD. The total number of moults may be changed, but the constancy of the LGD precludes rearrangement in the sequence of *kind* of moult.

TABLE 6.III

Lengths of male and female larvae at each ecdysis

Character	Sex	Mean ± SE	Df	F-value
E1LEN	Male	4.9 ± 0.18mm	1,39	1.9720ns
	Female	5.2 ± 0.17mm		
E2LEN	Male	8.6 + 0.18mm	1,42	0.3434ns
	Female	8.7 ± 0.16mm		
E3LEN	Male	13.8 + 0.26mm	1,45	0.8305ns
	Female	14.2 + 0.36mm		
E4LEN	Male	17.0[1] mm	1,1	4.4815ns
	Female	14.2 ± 0.75mm		

Data taken only from insects eclosing. [1]No standard error available as sample size = 1


A
B
C
D
Length (mm)
Days post oviposition
ov
oh
e1
e2
e3
e4
p
ec

6.5. Oval development

The ovum represents a nutrient source for *larval* development until post-hatch consumption of the shell (Ford, 1957). Hatching occurs when larval bulk exceeds shell capacity, and since all the shelss were similar in size this would explain the constant hatch-length of 2.5mm (Winokur, 1988). The 1st instar therefore comprises intra-oval ($I1_{IN}$) and extra-oval ($I1_{EX}$) nutritional phases; demarcated by hatching.

6.6. Larval growth

Larvae of longer $I1_{IN}$ have an initially shallow LGD. Larvae with an initially steeper LGD reach hatch length sooner (Fig. 6.2a-c). As $I1_{IN}$ increases it represents proportionately more of 1st instar development, hence the negative relationship between OVDUR and I1DUR; the (linear) correlation is weak because growth is non-linear. Regular ecdyses are required during the remainder of larval growth, because the integument does not grow and it is of limited elasticity only (Warnecke, 1964). The larva is shown on Plate 4.

FIGURE 6.1. The relationship between linear dimension and age (days post-oviposition) at the completion of each of the main metamorphic stages and larval instars of (a) animals undergoing four instars; (b) animals undergoing five instars; (c) males; and (d) females. Vertical and horizontal bars give $\pm$ one standard deviation of each correlate. Life-cycle stages: ov = oviposition; oh = hatching; e1 to e4 = 1st to 4th larval ecdyses; p = pupation; ec = eclosion. The overall form of the growth curve describes the Linear Growth Dynamic (LGD). Note that there is no difference in overall size or form of the LGD betwen 4- and 5-instar animals or between the sexes. For further explanation see text.

6.7. Pupation

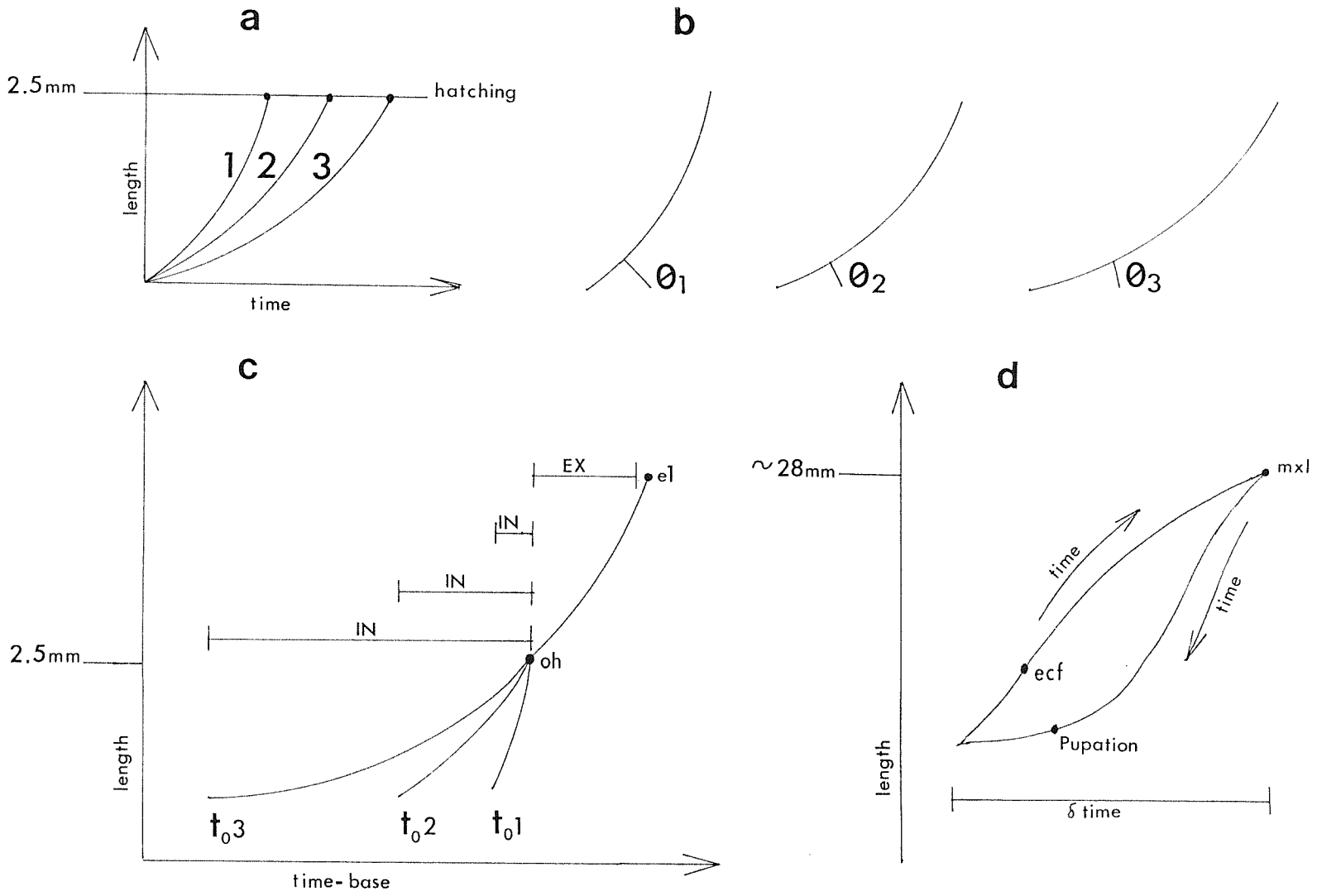
The model proposes that the integument reaches its elastic limit at pupation. Its tautness renders the larva translucent, hence the visibility of the internal organs (cf. Nijhout, 1976), and physically prevents the ingestion of food so that it can no longer provide the energy to overcome cuticular forces. The larva starts to contract. Histolysis ensues as an alternative energy source. This metabolic stress may be the trigger for hormonal changes, since starving final instar larvae for just twenty minutes can induce premature pupation in several species; and the larvae are very sensitive (Stone & Midwinter, 1975).

The suspended prepupa is curled (Plate 5a). This might result from dorso-ventral differences in elasticity, possibly facilitated by muscle contraction. It may help mould the underlying pupa; and it disappears at pupation. Pupae may be shorter than penultimate ecdysis larvae if the elastic property of the cuticle exhibits hysteresis (Fig. 6.2d). Contractile forces may be best delocalised by tending towards sphericity; and female pupae (from bulkier larvae) were visibly fatter, in accord with the general trend for Lepidoptera (McFeely, 1986).

6.8. Pupational stability

It is suggested that growth and differentiation hormones work by pushing meta-stable (precarious) systems from stable to unstable states. It is suggested that the nutritional stress will have already destabilised development somewhat, so that the amount of hormone required to shift it into instability is reduced. This might explain the redundancy and apparent excess of the second EC pulse (Nijhout, 1976). Although JH may be present at a constant level throughout (Nijhout & Wheeler, 1982), the pulse increases the *relative* amount of EC. It is the relative amount that is important (*ibid.*).

PLATE 4 (opposite). Penultimate instar larvae of Pararge aegeria.


a
2.5mm
hatching
length
1
2
3
time
b
θ1
θ2
θ3
c
EX
el
IN
IN
IN
2.5mm
oh
length
t0 3
t0 2
t0 1
time- base
d
~28mm
mxl
time
time
ecf
Pupation
length
δ time

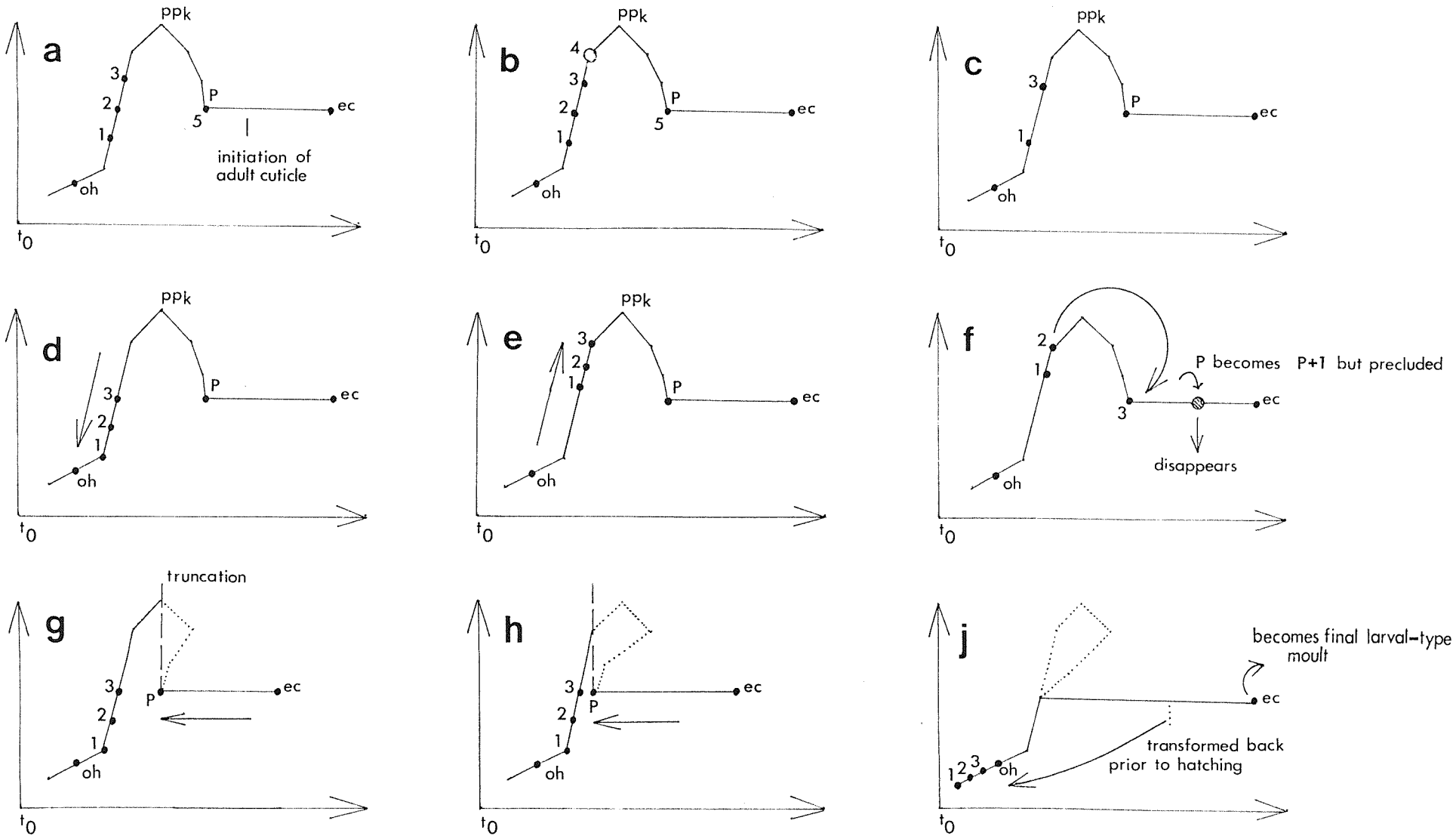
6.9. Canalisation and complexity

The pre-pupational deviation represents a perturbation to the LGD. This increases the talandic temperature which the system attempts to reduce. But because the LGD-delimited sequence precludes reversion to a previous state, an alternative pathway (sequence of structural changes) ensues *viz.* imaginal development manifests. Incomplete pupation may fail to provide sufficient destabilisation for imaginal development; and indeed, abnormal pupae died before completing this stage. Hence pupation is in effect a solution to a problem of structural integrity *vis-a-vis* nutritional (metabolic) constraint. And since the pupal moult effects the return of the LGD to sigmoid form, it can be conceived a canalisation process.

Figure 6.2 (opposite). The relationships between age at oval hatching and the slope of pre-hatch linear growth dynamic given the constant larval hatch length, where curves 1, 2 and 3 depict pre-hatch slopes of decreasing initial gradient; and t^{o} denotes time of oviposition (a) to show increasing oval duration with decreasing initial slope, where (b) 0 indicates the angle of slope with $0_1 > 0_2 > 0_3$; with (c) curves 1 - 3 superimposed so as to synchronise hatching to a common reference time, to highlight the increasing proportion of 1st instar development within the ovum (IN) relative to that without (EX) with decreasing pre-hatch slope (and so greater age at hatching). (d) The possible elastic properties of larval integument showing hysteresis. The greater contraction between maximum larval length (mxl) and pupation than between final ecdysis (ec_F) may result from metabolic energy being stored as additional contractile energy on the integument reaching its elastic limit. oh = hatching; e1 = 1st ecdysis.

PLATE 5 (overleaf). Prepupa, and larva showing post-traumatic lateral extensions to the longitudinal stripe.


#2


a
ppk
oh
1
2
3
P
5
ec
initiation of
adult cuticle
t_0
b
ppk
oh
1
2
3
4
P
5
ec
t_0
c
ppk
oh
1
3
P
ec
t_0
d
ppk
oh
1
2
3
P
ec
t_0
e
ppk
oh
1
2
3
P
ec
t_0
f
oh
1
2
3
P becomes P+1 but precluded
ec
disappears
t_0
g
truncation
oh
1
2
3
P
ec
t_0
h
oh
1
2
3
P
ec
t_0
j
oh
1
2
3
becomes final larval-type
moult
ec
transformed back
prior to hatching
t_0

6.10 The evolution of holo/hemimetabolous strategies

The LGD comprises linear and temporal, ie. *two*, dimensions. The superposition of moults linearly along the LGD comprises but *one* dimension. Transformations *along* the LGD are therefore less *complex* and more likely than changes in the LGD overall. Salient features of the LGD are summarised in Fig. 6.3a.

Additional or deleted moults are possible (Fig. 6.3b-c). A metamorphic moult may be missed (Nijhout & Wheeler, 1982) but its hemi/holometabolous nature remains unaltered. Similarly for linear transformations not breaching the PPD (Fig. 6.3d-e). Transformations breaching the PPD towards the initiation of embryogenesis (t_o) pull the LGD in this direction. The prepupational deviation becomes truncated and eventually disappears (Figs. 6.3g-j). Indeed, an absence

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Figure 6.3. Salient features of the linear growth dynamic and the results of transformations with respect to its axis. (a) The Linear Growth Dynamic (LGD). Moult numeration is shown only to denote temporal ordering and to clarify the transformations; hatching (oh), pupation (p) and eclosion (ec) are also shown. The prepupational deviation from sigmoid growth (PPD) is depicted. The form of the LGD can be conceived as sigmoid with the PPD superimposed. Open circles show additional moults; (b) Additional moult. It must be emphasised that positions 1-3 do not correspond *individually* to 1-3 in (a), only that the larval stage as a whole is a unit; (c) Removal of moult 'position' 2; (d) Shift in larval moults towards t_o not breaching PPD; (e) Shift in larval moults towards PPD not breaching PPD; (f) shift in moult 'position' 3 breaching the PPD. This introduces an additional putative moult between p and ec but whose occurrence is precluded by the continuum of adult development within the pupa; (g) Shift in moult p (pupation) breaching PPD towards t_o. The realised PPD becomes truncated (dotted line shows its putative location) until it meets the final larval moult to (j) regenerate a purely sigmoid growth curve with loss of the PPD. The shift of larval moults towards t_o breaching oh regenerates the hemi-metabolous condition. The LGD is therefore a type. For further explanation see text.

of JH (by ligation) in larvae of the moth Galleria mellonella, disc derived structures as well as abdominal epidermis bypass the pupal stage (Nijhout & Wheeler, 1982).

Of course, it is the pupal moult that distinguishes holometabolous metamorphosis; indeed the hemimetabolous strategy is otherwise similar. In both, the corpora allata cease production of juvenile hormone (JH) in the final instar (Nijhout, 1975); larvae have three metamorphic options dependent on JH concentration (Nijhout & Wheeler, 1982); cuticular stretching promotes moulting (Nijhout, 1979) and final instar larvae have fat body reserves for adult development (*ibid.*). If all moults are transformed towards t_o, then the initiation of the development of adult integument within the pupa can be traced back prior to hatching from the *ovum* (Fig. 6.3j). Indeed in hemimetabolous insects the nymphs hatch with sclerotised cuticle already formed. This suggests that the holometabolous larval stage involved an extension, to the pupal stage, of early within-egg (nymph). The attainment of adult size would now be achieved as a caterpillar rather than as a nymph; when ecdysis provides the solution to the problem of limited integumental elasticity. The hypothesis is supported by the consideration in section 6.1; that the first instar of P. aegeria is distinct from the other larval stages in having a relatively small, black, head capsule; and that in both hemi- *and* holometabolous insects, the wings appear only after the imaginal moult.

The pupal integument of P. aegeria hardens into sclerotised cuticle within 24-48h post-pupation. The local sclerotisation of integument around the exuvial glands in Manduca sexta larvae might be a response to injury (Nijhout, 1976); whilst final instar larvae treated with JH and beta-ecdysone formed constantly sized patches of pupal cuticle on the clypeus and anterior ocelli (Nijhout & Wheeler, 1982). Moreover, individual epidermal cells in many hemi- and holometabolous insects can secrete 'composite' cuticle, which has the sculpture of one stage but the pigment of another, after exposure to (JH) at a brief critical period in the final instar. Furthermore, in ligated G. mellonella, bits of pupae that are still larval cuticle become pupal when the rest of the animal develops into the adult (*ibid.*). Hence it appears that the transformation of part of the cuticle from larva to pupa facilitates the transformation of adjacent

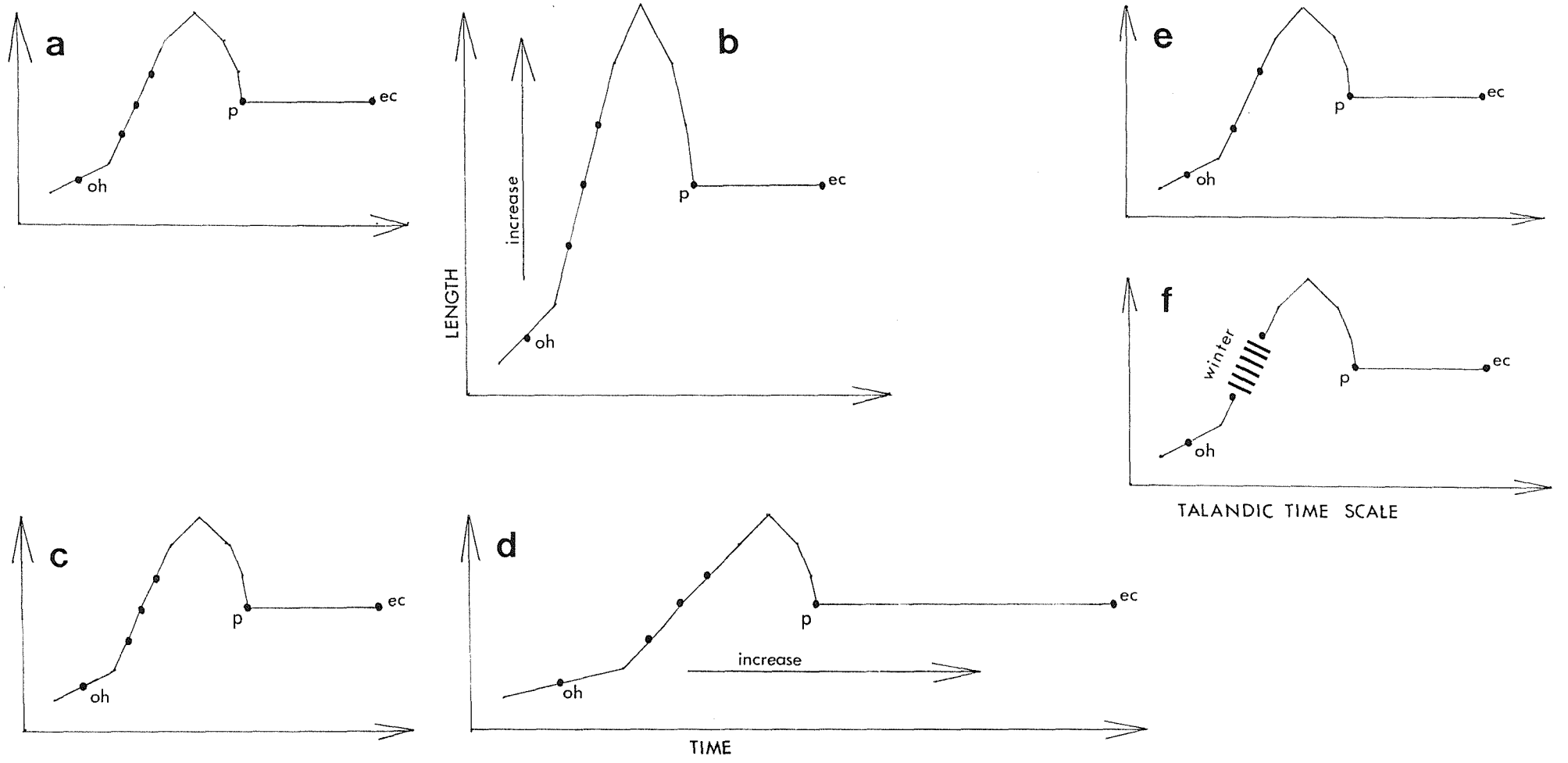
areas. Thus pupal cuticle formation might have arisen from a unit copying response to local integumental tearing that became assimilated into the life cycle. The efficacy of the response would be delimited by cuticular composition and hence certain structural genes (material prerequisite). These would be available a priori as a heritage from their evolutionary earlier pre-hatch nymph development. It is also worth noting that pupation can arise without recourse to any *de novo* hereditary *mutation*.

Changes in the life cycle duration or in mature size, typical differences between Lepidopteran taxa, are less likely as they involve the curve of the LGD *itself* (rather than just *along* the curve) (Figure 6.4a-d). Hence the holo/hemimetabolous dichotomy would be expected to be prior to distinctions *within* these two groups. It is also suggested that pre-pupational hypersensitivity to ecdysone (Nijhout, 1976) might represent the superposition of the PPD upon an already established (hence more primitive) hemimetabolous pattern of hormone secretion.

6.11 Relativity and quantisation

That the final moult breaches the PPK ought to imply that hemimetabolous life-cycles be of shorter duration than their holometabolous counterparts. Yet the two groups exhibit comparable life-cycle durations. This paradox is overcome by invoking talandic time scales, which are marked by stability and complexity changes (just as increasing entropy marks time in the familiar sense), being matched to a common *reference* time-base. The notion of relative time scales is thus introduced.

The deployment of moults along the LGD need not respect the distinct metamorphic stages, hence the occurrence of larval-pupal intermediates. But such intermediates fail to continue their development into adults. Hence there is discontinuity in the *kinds* of transformation (larval/larval (=ecdysis) and larval/pupal) that remain viable *ie.* solve the problem. In that larval-pupal intermediates are possible as *occurrent* structures but not as *replicative* ones exemplifies the sense in which natural selection can be said to act.


a
oh
p
ec
b
LENGTH
increase
oh
p
ec
e
oh
p
ec
f
winter
oh
p
ec
TALANDIC TIME SCALE
c
oh
p
ec
d
oh
increase
p
ec
TIME

Hence a continuous change in an *underlying* generative parameter (the LGD) can lead to discontinuities (larva/pupa) in the *manifestation* of this parameter.

6.12. Polyphenism and complexity

If newly formed pupae of *P. aegeria* were damaged they exuded green haemolymph. Since the cuticle of eclosed pupae is colourless, their green colour is due to the haemolymph showing through, as in Pieris napi (Harrison, 1928a). Just as vernal Pieris pupae have a thicker cuticle and wax layer (Yata *et al.*, 1984) it is suggested that vernal aegeria pupae too have a thicker cuticle and wax layer. The former might prevent light reaching the haemolymph and obscure any light reflected from it, while the latter might reflect more incident light. Hence the difference between the reddish brown vernal pupae and the green vernal ones is quantitative only; the illusion might be enhanced were they to deposit more melanin in the cuticle. It is suggested that it is easy for the species to produce this seasonal polyphenism because it involves a difference in *degree* and not in *kind*.

FIGURE 6.4. Changes in overall size and form of the linear growth dynamic: (a) and (b) to show increase in the adult size attained; (c) and (d) to show prolongation of the life cycle with respect to the talandic time scale. (e) and (f) show prolongation of the life cycle with respect to a reference (*eg.* seasonal) time sale *without* a change in talandic development time (as marked by overall change in structural complexity) as might occur during larval diapause. Only hatching (oh), pupational (p) and eclosal (ec) moults are labelled. For further explanation see text.

6.13. The myth of metamorphosis exploded

Nijhout & Wheeler (1982) had suggested that there might be times at which development is refractory to JH, or developmental 'windows', whose presence is not dependent on the presence or absence of JH itself. They also suggested that something might cause the 'potential' to go along a particular pathway. These can now be understood as the destabilising effect of the pre-pupational deviation (from sigmoid growth) enabling EC to render it unstable.

In early pupal development, all tissues except the digestive, nervous and respiratory systems (and discs) are broken down; this stage constitutes the passiphase (Kettlewell, 1944a). These systems are already developed in the larva, and continue, with growth the only change, to the adult (Warnecke, 1964). However, it appears that the integument (or at least its pattern determinants), despite the gross change at pupation in *visible* morphology, is also continuous throughout the life cycle. An abdominal asymmetry in a larva from a cross between the Nymphalids Phyciodes tharos and P. batesii persisted to the adult (Oliver, 1979); and spiral segmentation on the 9th segment from the head in a larva of Acherontia atropos (Sphingiidae) persisted not only on the pupal cuticle, but also on the that of the adult developing inside the pupa (Aindow, 1988). One larva of P. aegeria was accidentally 'clipped' by a box lid during a food change in its second instar, and the integument healed with two supernumary projections of the longitudinal dorsal stripe; which remained through the final instar (Plate 5b; a normal larva is shown on Plate 4a-b). This suggests that integumental pattern is established by circumferential values, which has a number of interesting implications for lepidopteran pattern development (Chapter nine).

Thus holometabolous development, despite popularly being endowed as 'miraculous', is a continuum where changes are mainly in degree rather than kind. Indeed the relationships between egg and larva are essentially (*sensu* Webster, 1984) the same as those between pupa and adult: the initiation of larval development by the instability introduced on fertilisation *vis-a-vis* the initiation of adult development by the instability introduced by EC during the

prepupational deviation; the store of yolk for larval development *vis-a-vis* the fat bodies for imaginal development; that the former are sequestered by active feeding of the adult in which the egg is formed *vis-a-vis* the fat body store sequestered by active feeding by the larva in which (on pupation) the adult structures are formed; the eggshell protecting the developing larva *vis-a-vis* the pupal cuticle protecting the developing imago; the change in nutritional status on hatching *vis-a-vis* the change in nutritional status on eclosion. The only difference as such is that the PPD with ecdysone initiates secondary (rather than primary) determination, although with regard to the formation of adult structures, it is in a sense primary.

6.14. Metamorphosis as a 'type'

Hence the cycle egg-larva-pupa-imago exhibits recursiveness, and it does not actually have an *inherent* 'start' or 'finish' (only reproductive ones on account that it is the adult that *reproduces* the cycle). Metamorphosis can be regarded as a <u>type</u> of which the hemi- and holometabolous strategies are <u>forms</u>. The type involves temporal dimension and thus occupies the <u>diachronic</u> realm (Waddington, 1962). It is suggested that each moult (including hatching and eclosion) be regarded as <u>elements</u>, which can be grouped into four main kinds: oval hatching; ecdysal; pupational; and eclosal. Hatching and eclosal both involve a behavioural solution (active feeding) to a nutritional problem; the initiation of larval and adult ontogeny solving problem of instability. The sequence of *kinds* of moult is constrained by the cycle as a whole, and, indeed, the sequence ova-larva-pupa is very hard to alter in most insects (Nijhout & Wheeler, 1982). This may be because a new kind of moult can only occur if the limit of canalisation of the *preceding* kind is exceeded; in other words, because it is a minimax phenomenon (cf. Keiser, 1987). In this sense, the moults are simply logical *outcomes* of a progression that occur when the system meets an instability; the nature of the moult delimited by the constraints of growth, integumental elasticity and nutritional stress. The sequence of *kinds* of moults is therefore a diachronic counterpart of epimorphosis. However, the kind of moult is not inherent to the moult itself, which probably does not *per se*

recognise the distinction in kind. Thus, to paraphrase Goodwin (1984b), it is meaningless to ask which moult in a five instar larva is missing in a four instar one. Rather, the *kind* of moult depends on where the moult *happens* to be *vis-a-vis* the a priori constraints above. But because the possible kinds are 'quantised', the superposition of moults on the LGD gives the *illusion* that their kinds are mapped to particular moults.

Holo- and hemimetabolous metamorphosis become unified within a common scheme (the type) and each becomes a process of remarkable underlying unity. And thus, in reply to Davenport, with metamorphosis a unity - a oneness, then what, by definition (Saunders & Ho, 1981), could be simpler.

6.15. The implications

The instability imposed by the pre-pupational deviation may predispose this stage of pupal development to environmental influences. Indeed, the next chapter examines the possibility that the early P. aegeria pupa is susceptible to cold shock. And pupation as a recursion of pre-hatch development means that it may also be amenable to genetic assimilation: juvenile hormone administered to queen ants of Pheidole before oviposition determined the development of queens in their offspring (Nijhout & Wheeler, 1982).

A talandic time base allows for the origin of diapause, since the total complexity change between its bounding moults is unaltered (Fig. 6.4e-f). The quantisation of kind of moult shows how a gradual change in an underlying parameter (stability) can lead to sudden changes in *outward* form as it breaches thresholds (at hatching, pupation and eclosion). This too is in accord with Kieser's (1987) minimax model. Thus, it is possible that holometabolous development could have arisen quite suddenly from hemimetabolous development in response to some environmental stress; and through transformation within the bounds of available materials and an already established growth dynamic, hence without recourse to gradualism or genetic mutation, although the latter are not, of course, ruled out *stat.*

CHAPTER SEVEN

Summary

The effects of early pupal cold shock on ten post-larval life cycle parameters were investigated; the effects of darkness alone were also examined. Controls were altogether untreated. The experimental sample comprised all pupae from the same two families described in Chapter Six. Sex and family differences were examined using untreated pupae. The only genetic difference found was between families in pupal duration prior to 'colouring up'.

Darkness prolonged overall pupal duration. Cold prolonged pupal duration through its effect on the pre-colouring phase. The combined effect of cold and dark was greater than that of either alone. Four timings of treatment application were tested to examine specificity of response. No *particular* timing of darkness was found most effective, although the <12h onset with 96h duration was found most effective with cold: this application is therefore chosen for all subsequent investigations. None of the treatments nor timings significantly affected fecundity or survival; while cold-derived adults lived at least as long as in nature. Cold shock was therefore deemed suitable for studying genetic assimilation.

Darkness effect was greater with the earlier than later onset, but little influenced by duration: it is proposed that the first post-pupal photophase initiates ecdysone (EC) production after which light is not required. Cold effect increased with duration: it is proposed that cold diminishes *sensitivity* to EC, but *not* its production, which therfore accumulates at the tissue interface, 'shock' effect resulting from the sudden surge in EC *turnover* on removal from cold. This would agree with its effect on wing phenotype, also under EC control, and that the extent to which pupal duration was prolonged (one to two days) was less than the duration of the treatment itself. The increase in cold effect with duration, however, was greater with the *later* treatment onset: ecdysone production will have already begun and so accumulate more rapidly.

The circumstantial evidence is considered and suggests that wild pupae experience winter frosts in nature.

CHAPTER SEVEN

THE EFFECTS OF PUPAL COLD SHOCK ON THE LIFE CYCLE

Introduction

Temperature studies in Lepidoptera include those that simulate natural conditions (Weismann, 1896; Kettlewell, 1944) and those that involving temperature 'shocks' (Merrifield, 1893; Hoegh-Guldberg & Hansen, 1977; Nijhout, 1984). Shock studies have used heat and cold (see Hoegh-Guldberg & Hansen, 1977), but have focussed exclusively on adult morphology. The effects on the pupal and subsequent development (except mortality: Nijhout, 1984)), however, have been largely ignored. Cold shock has been given recent attention (Nijhout, 1984) and is of interest because it mirrors conditions experienced by some species in nature (Shapiro, 1975, 1981a,b). Cold shock effects have been suggested as a basis for genetic assimilation (section 2.3) but again only with regard to pattern effects.

This chapter examines the effects of early pupal cold shock on the pupal and subsequent life cycle stages in P. aegeria. Temperature is known to affect the species' development rate directly (Shreeve, 1985). Robertson (1959) performed heat shocks but only adult phenotypic effects were outlined. Lepidopterans show considerable interspecific variation in the timing and degree of sensitivity to shocks (Hoegh-Guldberg & Hansen, 1977) and therefore a number of timings of shock application are tested. The effects of darkness during the early pupa are also considered. Shock treatments can cause severe mortality (Kettlewell, 1944) so pupal survival is also assessed. The possible mode(s) of action of dark and/or cold are assessed in view of known hormonal controls in pupal development (eg. Nijhout & Wheeler, 1982).

7.1. Experimental material

The experimental material comprised pupae of STOCK 01 families 002 and 004, reared as described in section 6.1. The larval foodplant, Dactylis glomerata (L.), was grown from seed as described by Winokur (1988) to ensure that any cryoprotectant content (Baust & Edwards, 1979) was as uniform as possible. Pupae were left in their plastic boxes in preparation for experimental treatment.

7.2. Experimental treatment

Three types of treatment were applied:

(i) *Cold shock (experimental class 'E')*

Two timings of treatment onset were used: <12h post-pupation and 12-24h post-pupation. Pupae under 5 hours old were assigned to the subsequent 12-24h batch since pupae less than 5h old can suffer severe mortality under cold shock (Nijhout, 1984). Boxes were wrapped in aluminium foil (to protect pupae from spurious light whilst transferring others to and from the refrigerator) and labelled, then kept at -1°C ± 0.8°C and oriented so the pupae hung downwards. Each onset class was divided into two treatment duration classes: 48h and 96h.

(ii) *Controls with foil (control class 'C')*

These controlled for the influence of darkness on cold shocked pupae. Pupal boxes were wrapped in aluminium foil only and kept at room temperature. Each onset and duration was applied as above.

(iii) *No-foil controls (control class 'N')*

These were untreated and reared as described in section 3.2.1. These controlled against class 'C' for darkness-induced effects and against class 'E' for effects of cold *and* darkness. Following treatment, pupae were returned to room temperature and their lengths measured (after 48h with class 'N'), then maintained as described in Chapter three. However, only class 'N' and 'E' adults were kept for pairing.

TABLE 7.I

Life cycle characters used
in the investigation of the effects of pupal cold shock

Character	Description
PLEN	Pupal length.
PPHDUR	Prepharate pupal duration.
PHDUR	Pharate pupal duration
PDUR	Entite pupal duration
PECOP	Pre-pharate pupal duration.
PROV	Pharate pupal duration.
DOVIP	Duration of the oviposition period.
NOV	The number of ova laid.
ILONG	Adult longevity.
LFCY	Entire life cycle duration

Durations are measured in days. Pupal length is measured in milimetres. PECOP was transformed to the power 0.6751 to render the data additive. For further explanation see text.

7.3. Data analysis

Ten life cycle characters (Table 7.I) were examined. Characters with a non-normal distribution or showing interaction between family, sex or treatment were transformed before comparing means (Sokal & Rohlf, 1981). NOV was meristic and so raised to the power 0.5 to render the analysis of variance more reliable. The data was tested for heteroscedasticity with Bartlett's test for heterogeneity (Sokal & Rohlf, 1981), and if significant, the Mann-Whitney U-test was applied in preference to ANOVA. Not more than one transformation was applied per variable. Results were back transformed before reporting (*ibid.*). Individuals with missing data were excluded from analysis.

Coefficients of variation (V*) were estimated for post-treatment characters and compared after correcting for bias (Sokal & Rohlf, 1981). V* always employed *transformed* data.

Correlations were investigated with principal coordinate analysis. Survival was compared using the G-test with Williams' correction (Sokal & Rohlf, 1981) and selection was examined with the cross product ratio (Manly, 1985).

Results

7.4. Survival

Pupal survival was estimated as *number eclosing/number pupating*. Except for foil controls in family 004 (survival = 67%) survival was at least 75%. There were no significant differences between treatments, although the families differed slightly in pupal mortality (number failing to eclose/number pupating) (Family 002: mortality = 22.6%; Family 004: mortality = 10.4%; G_{adj} = 5.461, d.f.=1, $0.001 < P < 0.01$).

7.5. Interactions

The time interval between eclosion and copulation showed interaction between treatment and family ($F_{(1,12)} = 14.214$, $0.001 < P < 0.01$) although this may be due the way in which adults were chosen for pairing.

7.6. Family differences

Mean prepharate duration was 0.7 days longer and the entire life cycle almost a week longer in family 002 (Table 7.II). Family differences are probably controlled genetically. There were no such difference between the sexes.

TABLE 7.II

Family differences in pupal and post-pupal life cycle characters

Character	Family	Mean	95% confidence	Df	Z-value
PHDUR	002	2.7d	2.29 - 3.15d	26,21	-2.6800**
	004	1.1d	0.99 - 1.22d		
LFCY	002	64.2d	62.08 - 66.36d	21,20	-3.9348****
	004	57.9d	56.14 - 59.67d		

Families 002 and 004 as defined in section 6.1. 95% confidence intervals and degrees of freedom are indicated.
Significances: ** $0.001 < P < 0.01$; **** $P < 0.0001$.

TABLE 7.III

Treatment differences in
pupal and post-pupal life cycle characters

Character	Treat	Mean	95% confidence	Df	F-/Z-value
PPHDUR	N	11.7d	10.61 - 12.82d	$1,37_F$	15.2426***
	E	14.2d	13.42 - 14.93d		
PPHDUR	C	12.5d	11.67 - 13.27d	$1,41_F$	9.5697*
	E	14.2d	13.42 - 14.93d		
PDUR	N	13.8d	12.57 - 15.01d	$1,22_F$	6.8321*
	C	15.4d	14.69 - 16.06d		
PDUR	N	13.8d	12.57 - 15.01d	$1,32_F$	20.5230****
	E	16.7d	15.93 - 17.47d		
PDUR	C	15.4d	14.96 - 16.06d	$1,34_F$	6.0122*
	E	16.7d	15.93 - 17.47d		
LFCY	N	60.1d	57.71 - 62.46d	$10,9_Z$	-2.3793*
	C	56.0d	53.36 - 58.62d		
LFCY	C	56.0d	53.36 - 58.62d	$9,22_Z$	-3.5140***
	E	63.7d	61.52 - 65.96d		

95% confidence intervals are indicated. The statistic used is denoted by the subscript following the degrees of freedom: F = one-way ANOVA; Z = Mann-Whitney U-test. Significances: * $0.01 < P < 0.05$;
** $0.001 < P < 0.01$;
*** $0.0001 < P < 0.001$; **** $P < 0.0001$.
Treatments: N = No foil control; C = Foil control; E = Cold shock.

Classes 'C' and 'E' differed significantly in prepharate and pupal duration but only when given <12h onset with a 96h duration, when prepharate duration was 1.9 days longer and pupal duration 2.1 days longer in class 'E' (Fig. 7.2b).

7.7. Treatment effects

These are shown in Table 7.III. Only pupal duration was significantly longer (by 1.5 days) in class 'C' than in class 'N'.

Prepharate duration was 1.7 days longer in class 'E' than class 'C'. Pupal duration was also longer in class 'E' than in 'C'. The entire life cycle was a week longer in class 'E' than in 'C'; but this probably results from cumulative, cold-independent sample differences at earlier stages which alone were not significant.

The effect of cold with dark on pupal duration was much greater than either cold or dark alone, class 'E' spending nearly three days longer as pupae than class 'N'. These effects probably act at the prepharate stage because the difference in prepharate duration between these classes (about 2.5 days) was similar to that for entire pupal duration.

Adult longevity (females) was three times as variable in class 'E' ($V^* = 18.2$, $s_{v*} = 5.02$, $n = 7$) than in class 'N' ($V^* = 5.1$, $s_{v*} = 2.12$, $n = 3$; $F_{(1,9)} = 12.7350$, $0.001 < P < 0.01$).

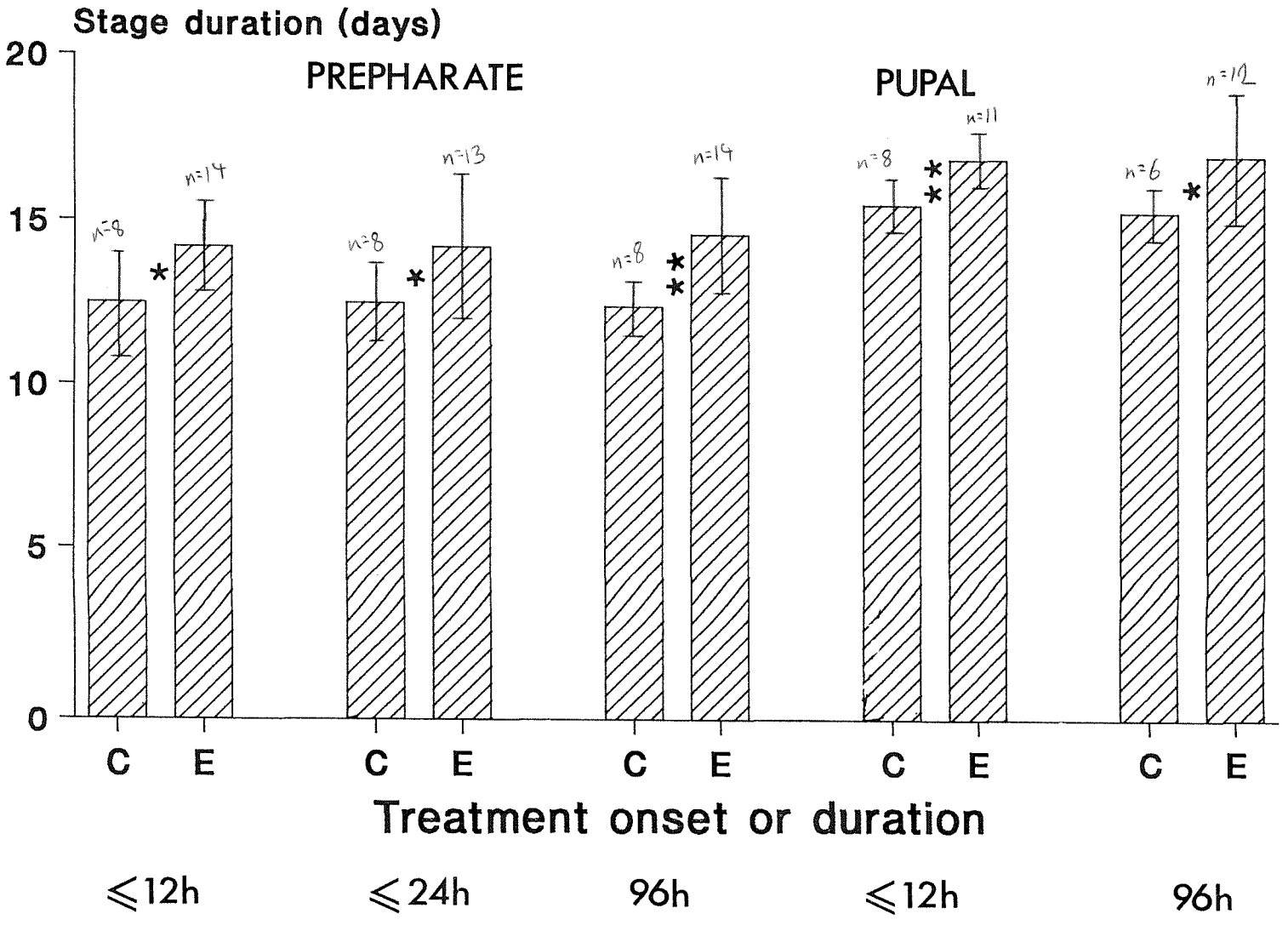
7.8. Specificity of response

Each onset and duration class was examined to determine whether any in particular produced differences *between* the treatment classes. No differences were found between classes 'N' and 'C' for either of the onsets or durations.

However, the <12h post-pupation onset and the 96h duration each produced significant differences in prepharate and pupal duration between classes 'C' and 'E'. Prepharate duration also differed between classes 'C' and 'E' in the 12-24h onset class (Fig. 7.1).

Differences between the onsets and between the durations *within* treatments were explored; but none were found. Nor did onset and

Figure 7.1


Stage duration (days)
20
15
10
5
0
PREPHARATE
PUPAL
n=8
n=14
n=8
n=13
n=8
n=14
n=8
n=11
n=6
n=12
*
*
**
**
*
C
E
C
E
C
E
C
E
C
E
Treatment onset or duration
⩽12h
⩽24h
96h
⩽12h
96h

duration interact. Each *combination* of onset and duration was then examined for treatment differences which may have been masked in pooling them.

Classes 'N' and 'C' differed significantly only in pupal duration given a <12h onset with 48h duration, which was 2.0 days longer in class 'C' (Fig. 7.2a).

7.9. Developmental Correlations

Prepharate (but *not* pharate) duration correlated significantly with pupal duration within each treatment class.

Prepharate and pharate duration correlated negatively within each treatment class, being significant within classes 'C' ($R_{(12)}$ = -0.66001, $P < 0.01$) and 'E' ($R_{(23)}$ = -0.33655, $P < 0.05$). There may be a subsequent developmental correction for the immediate effects of darkness or cold. Indeed coefficients of variation were lower for pupal than for prepharate duration, and these differences were greatest in classes 'C' and 'E'.

The number of eggs laid increased with increasing duration of the oviposition period ($R_{(7)}$ = 0.85495, $P < 0.001$), although both were independent of adult longevity. The greater variability of adult longevity under cold-shock would therefore *not* be expected to affect the number of eggs laid.

FIGURE 7.1 (opposite). Mean durations of the prepharate and entire pupal stages with each onset (<12h or 12-24h post-pupation) and with the 96h duration of foil ('C') and cold shock ('E') treatments. Error bars are given to ± one standard deviation. Only significant treatment differences are shown: * $0.01 < P < 0.05$; ** $0.001 < P < 0.05$.

FIGURE 7.2 (overleaf). Mean durations ±SD of the (a) prepharate and (b) entire pupal stages for each combination of onset (<12h or 12-24h post-pupation) with duration (48h or 96h) of foil ('C') and cold shock ('E') treatments and for untreated samples ('N'). Significance level: * $0.01 < P < 0.05$.

Figure 7.2

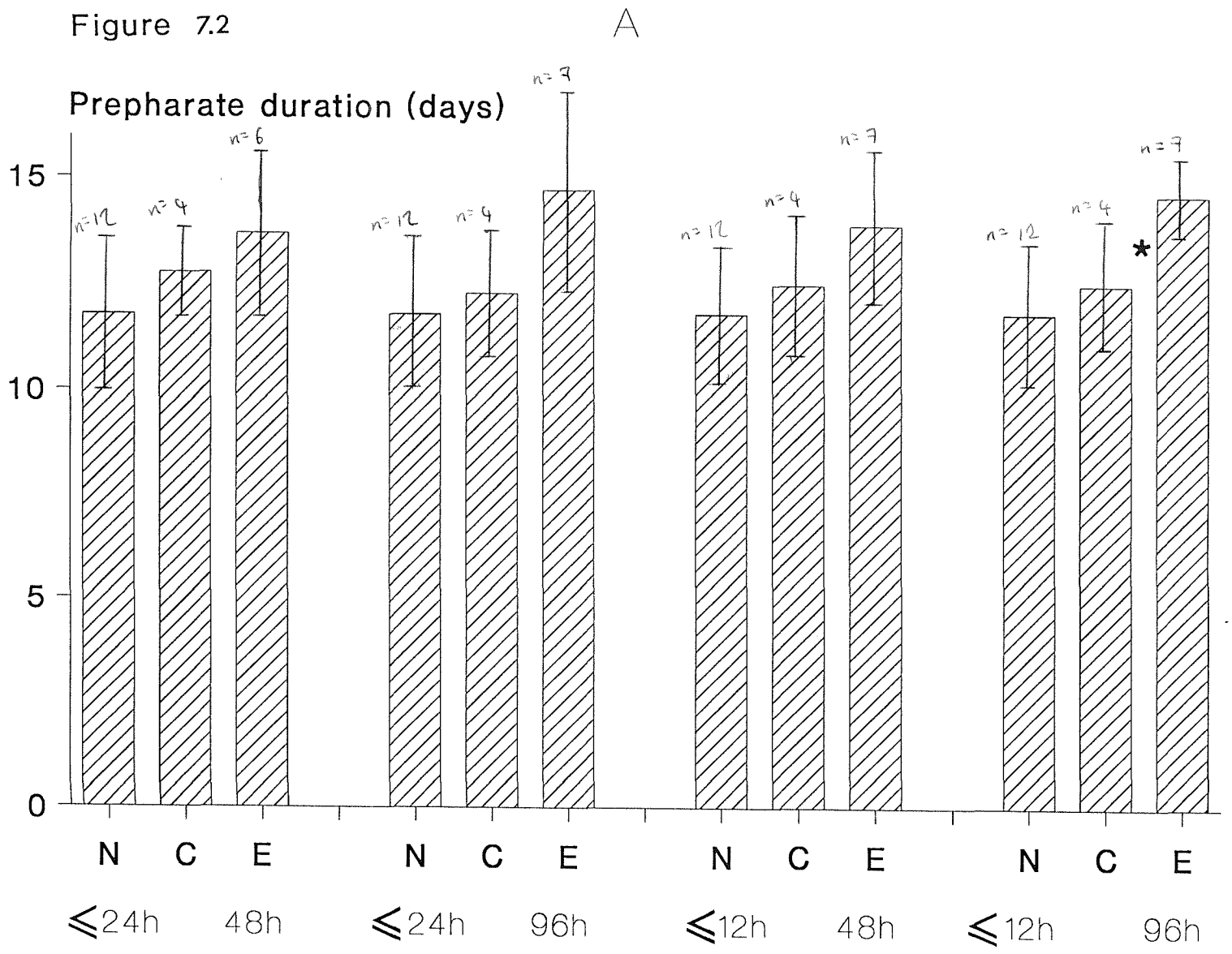
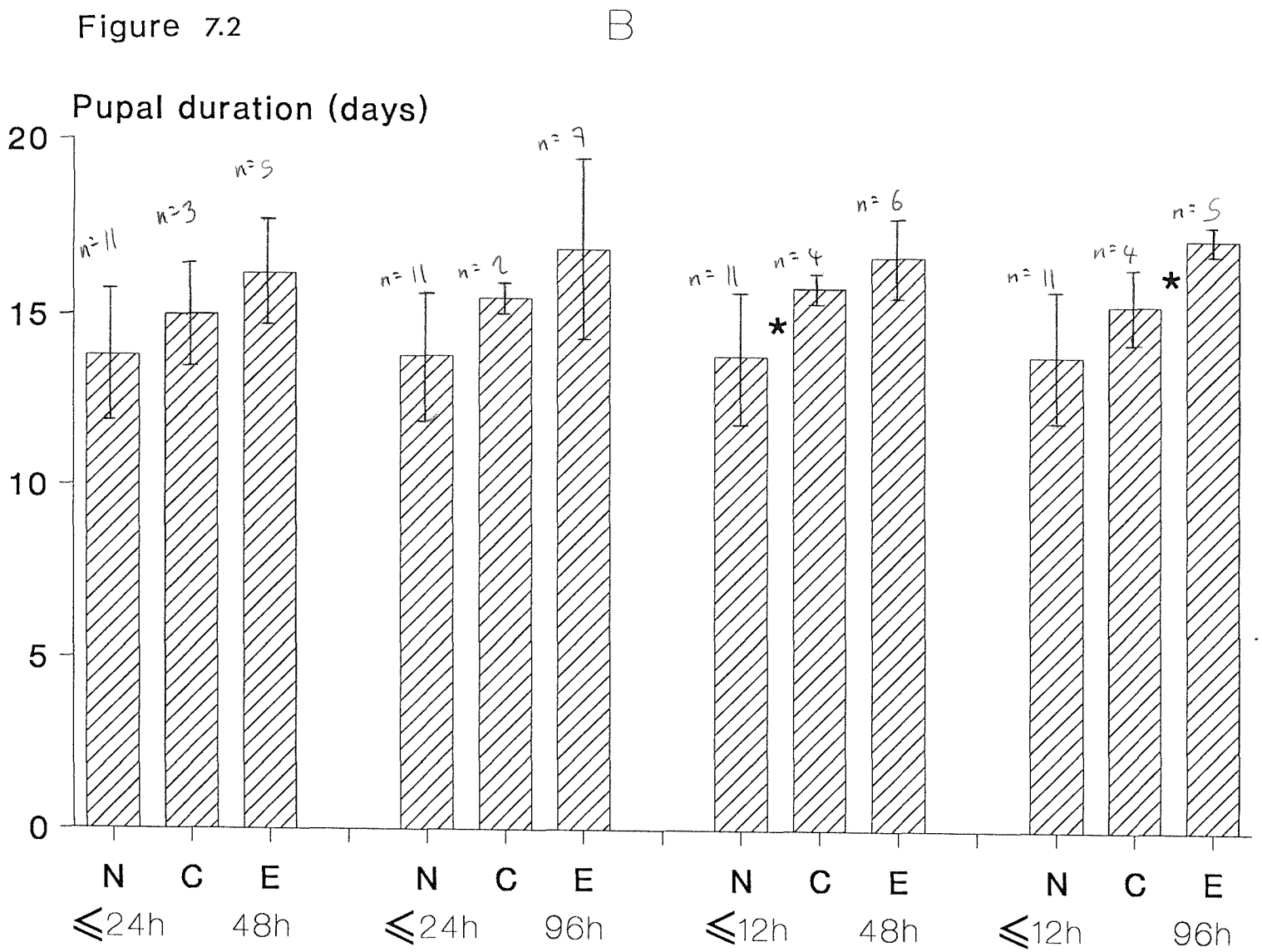

A
Prepharate duration (days)
15
10
5
0
n=12
n=4
n=6
n=12
n=4
n=7
n=12
n=4
n=7
n=12
n=4
n=7
*
N C E
N C E
N C E
N C E
≤24h
48h
≤24h
96h
≤12h
48h
≤12h
96h

Figure 7.2


B
Pupal duration (days)
20
15
10
5
0
n=11
n=3
n=5
n=11
n=2
n=9
n=11
n=4
n=6
n=11
n=4
n=5
*
*
N C E
≤24h 48h
N C E
≤24h 96h
N C E
≤12h 48h
N C E
≤12h 96h

The term 'shock' usually refers to gross disturbances to development which mimic the effects of artifical factors such as X-rays, while 'normal' usually refers to conditions similar to those in nature (Kettlewell, 1944). Shock as used in the present thesis however, indicates only that treatment involved a rapid change in temperature applied for a short time.

It might be argued that pupal cold shock does not mirror any natural environmental stimulus; or that its effect on development time cannot provide a basis for genetic assimilation. Yet several Lepidoptera *do* experience short exposures to cold in nature (Hoegh-Guldberg & Hansen, 1977; Shapiro, 1975, 1981c) and show species-specific reactions to cold shock (Hoegh-Guldberg & Hansen, 1977). And in periodic cicadas, overcrowding, an environmental influence, not only increases their development rate but this effect is genetically assimilated (Lloyd & White, 1974).

7.10. Survival

Cold shock did not significantly affect mortality; if anything, cold shocked pupae fared better than foil controls. Most deaths occurred before the pupae would have commenced treatment; and their brown discoloration suggests bacterial disease contracted at an earlier stage (cf. Winokur, 1988). Adult longevity was a week shorter in cold shocked animals but still similar to that in nature - about three weeks (Goddard, 1962). Thus cold shock is suitable for viable breeding programmes.

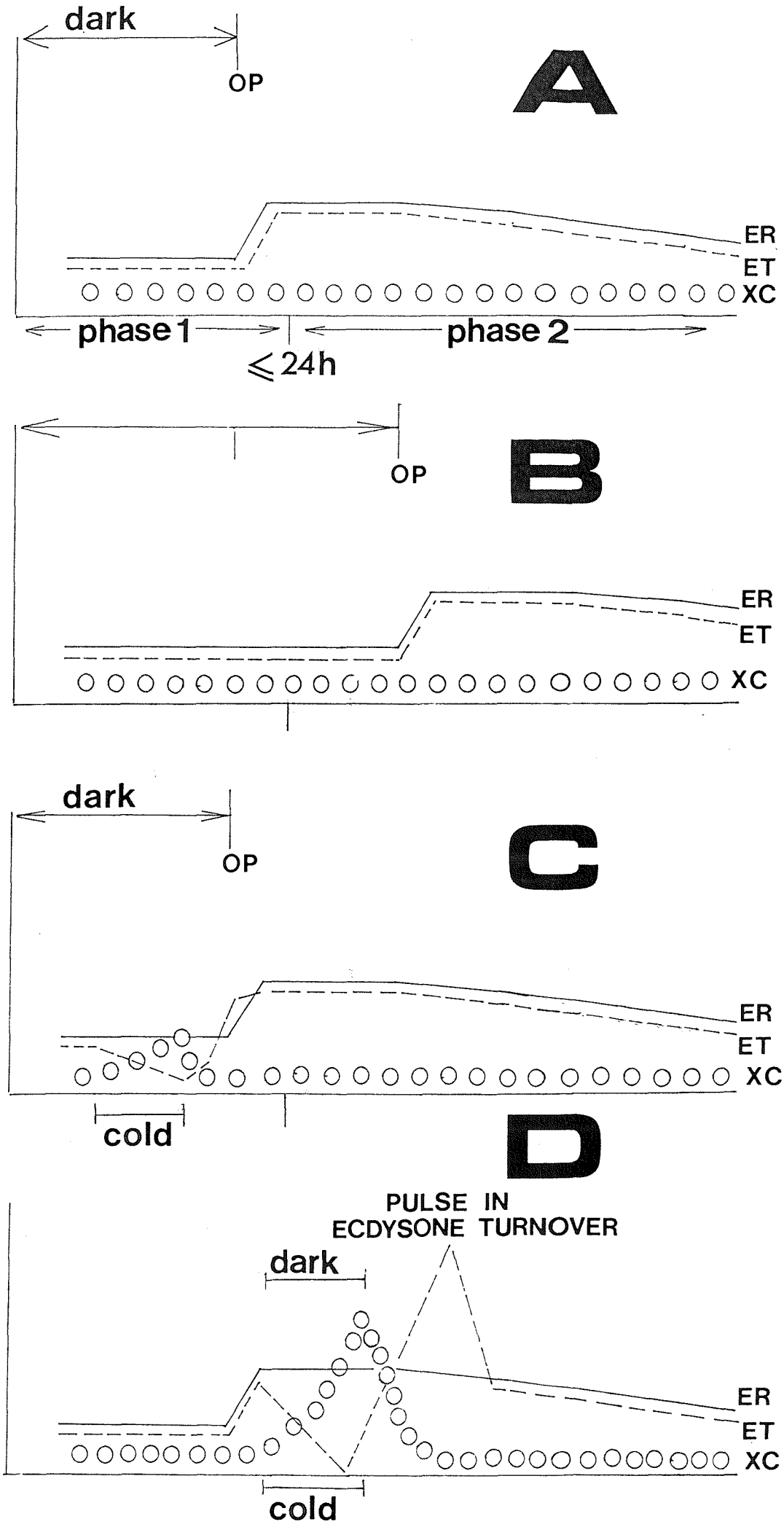
7.11. Darkness effects

No-foil controls eclosed within 10-14 days post-pupation, which is typical of non-diapause pupae in the species (Lees & Tilley, 1980). Thus the rearing regime did not induce diapause.

Foil controls show that darkness during the early pupa prolongs development without inducing diapause. This effect was greatest with 48h darkness commencing within 12h post-pupation. Development time was prolonged more by advancing the onset of darkness than by prolonging its duration. Thus sensitivity to darkness appears to cease or fall markedly between 12-24h post-pupation. This reduction in sensitivity may continue for some time after, since those entering treatment after 12h post-pupation still showed a *slight* increase in pupal duration.

Non-diapause Lepidopteran pupae secrete ecdysone shortly post-pupation (Nijhout, 1980b). The neurosecretory initiation of ovarian maturation in Polygonia c-aureum (L.) depends upon exposure to summer photoperiod within 28h post-pupation. Once initiated, maturation no longer requires neurosecretory activity (Shapiro, 1976). In Pararge aegeria darkness appears to act immediately since it had no affect on pharate duration. The following hypothesis is proposed.

Pupal development is initiated by a pulse of ecdysone which is gated by the onset of the first post-pupational photophase; usually within 24h post-pupation (Fig. 7.3a). Darkness prevents its release so development is delayed (Fig. 7.3b). Now ecdysone normally has a very rapid turnover (Nijhout, 1976); which might explain the apparent post-pulse fall. Once commited to development, a high level of ecdysone may no longer be needed, although lower titres may be necessary for continued development (*ibid.*). Since development can be influenced by photoperiod *after* the pupal moult, it is important to control for darkness during cold shock.


dark
OP
A
ER
ET
XC
phase 1
phase 2
≤ 24h
OP
B
ER
ET
XC
dark
OP
C
ER
ET
XC
cold
D
PULSE IN
ECDYSONE TURNOVER
dark
ER
ET
XC
cold

7.12. Cold shock effects

Kettlewell (1944a) describes a pupal hibernating factor in the moth Heliothis peltigera (Schiff.) that is satisfied by cold. Hence cold might be expected to reduce the prolonging effect of darkness. But in P. aegeria, cold shock augmented its prolongation of pupal duration. Thus darkness does not induce such a hibernating factor here.

Cold shock was most effective when applied within 12h post-pupation for 96h. That prepharate (and pupal duration indirectly) but not pharate durtion was significantly prolonged, would suggest that cold exerts an immediate effect.

This effect of cold period was increased more by extending its application than by advancing its onset. And when classes 'N' and 'E' were compared, this increase in cold effect with prolonged application was greater when in conjuction with the *later* onset than when with the earlier one. It is possible that cold might prolong development by reducing the sensitivity of pupal tissues to ecdysone (perhaps by denaturing a protein receptor on the cell surface), just as it reduces tissue sensitivity to thyroid hormones in the salamander genus Ambystoma (Matsuda, 1982). The following scheme is suggested:

Darkness applied within 12h post-pupation prevents the initiation of ecdysone production. Therefore any reduction in tissue sensitivity as a result of cold applied during this time will have little further effect because the hormone is lacking anyway (Fig. 7.3c).

Darkness applied 12h-24h post-pupation does not stop ecdysone production because it is already initiated (Fig.7 3d).

Figure 7.3. Hypothetical relationships between ecdysone production (ER), ecdysone turnover (ET) and extracellular ecdysone titre (XC) under (A) normal photophase and temperature; (B) darkness during the phase of light-dependent ecdysone production (phase 1); (C) dark and cold during phase 1; and (D) during the phase of light-independent ecdysone production (phase 2). OP denotes the onset of the first post-pupational photophase. For further explanation see text.

But cold-induced tissue desensitisation means the hormone cannot take effect until the cold is removed; prolonging cold application simply extends the duration of tissue insensitivity.

It is suggested that pupae still produce some ecdysone under cold but that its *turnover* is prevented so that the titre of ecdysone at the tissue (cell) interfaces increases. Then, on removal from cold, there is a sudden surge in ecdysone *activity*, and it is likely that it is *this* which destabilises development (rather than cold *per se* (Fig. 7.3d). This might also explain the effects of shock treatments in general, including those on wing pattern (Nijhout, 1984), since ecdysone also controls pigment synthesis (Needham, 1974). Prolonging the cold produces a more extreme effect because it allows more ecdysone to accumulate.

7.13. Cold Shock in nature

In the wild, a number of larvae pupate in late November or early December (Shreeve, 1985). The mean January temperature in Britain is 2°C (Dennis, 1979), but the actual temperatures experienced by British pupae could be considerably cooler: Cole (1962) found a number of pupae amongst short grass under a gap in a damp woodland canopy, and one pupa was fully exposed. In winter, such habitats experience frequent frosts at night (Geiger, 1950) when larvae are active (Lees, 1962), yet larvae showed no inclination to move to more sheltered locations (Cole, 1962). Pupae of P. aegeria can survive severe winter cold in the wild (Shreeve, 1985); and it is postulated that the larvae might sequester cryoprotectants from the foodplant as in the midge Belgica antarctica (L.) (Baust & Edwards, 1979). Thus pupal cold shock simulates an environmental stimulus that the species is likely to encounter in nature.

A reduced level of ecdysone under darkness may actually help pupae to withstand cold shock by reducing metabolic rate (Masaki, 1980). Of course, cold shock could reduce metabolic rate directly. And the apparent correction of development rate following cold shock provides an example of developmental canalisation (Waddington, 1942).

CHAPTER EIGHT

Summary

The influence of parental cold shock on the life cycle of the offspring, and the genetic assimilation of these effects, are explored. The offspring of cold-treated and control animals were themselves exposed to cold and control treatments, and the procedure repeated at each generation.

Hampshire stock initiated the protocol, although hybridisation with Lincolnshire stock and then subspecies aegeria proved necessary to continue the lineage. Pairings were between like-treated animals. An 'assimilation coefficient' (A_i) was developed to indicate the extent to which lineages had previously experienced cold shock, but as no lineage survived the F_4, it was not ascertained whether assimilation or a only a temporary trend had occured; the term 'lineage exposure index' is thus felt more appropriate.

The protocol was first examined overall. When a life cycle character was influenced by more than one independent variable, the relationships between such variables were themselves examined to eliminate artifacts. Before considering detailed analyses, however, it is advised to read the discussion on p288.

The prolonging effect of cold shock increased with A_i as did pupal duration *per se*. Several life cycle characters not directly affected by cold also showed trends with A_i; these may follow its influence on egg maturation at least. The results were not artifacts of selection, rearing regime, the order in which stocks were used, or inbreeding; though the latter showed similar trends to A_i and may act syngergistically with it.

Other species were also examined to explore interaction between A_i and rearing temperature, increases which both led to faster larval growth but smaller resultant pupae. These findings were modelled thus. At higher thermal temperature the system has a greater entropy content and so is more unstable: it has a higher 'talandic temperature'. Thus the rate at which 'talandic temperature' is minimised through increasing complexity (ie. at which the system develops) is greater. Increasing A_i has a similar effect to higher temperature as it

increases sensitivity to ecdysone which also destabilises development. Larval integumental growth involves constant-rate breakage and rejoining of structural cross links of limited elasticity. Faster larval growth increases the strain on the elastic component and, since bulk is disproportionately greater than linear dimension, the sooner the elastic limit and hence pupation ensue. Such disproportionate growth may also explain why the pupal (versus ecdysal) moult only occurs above a critical larval mass.

Individual stocks were then considered. Hampshire animals of high A_i could undergo pupal summer diapause, a strategy new to the species. Heterosis masked underlying assimilation so freeing its effects from selection, when recurrent inbreeding might facilitate their canalisation. Genetic differences between the stocks suggest that each is optimally adjusted to undergo the life cycle under their respective natural climates.

CHAPTER EIGHT

PUPAL COLD SHOCK AND GENETIC ASSIMILATION

Introduction

Despite suggestions that Waddington's (1953) experiment be repeated using Lepidoptera (Nijhout, 1984), and that assimilation-like phenomena have been reported in a number of species (Harrison, 1928a; Vuillaume & Berkaloff, 1974), genetic assimilation as such remains to be explored in the group. This, despite the fact that the animals are easy to rear in captivity and are extensively farmed, even to the extent that the establishment of breeding institutes has been suggested to conserve rare species (Morton, 1983). The gap in our knowledge may be due to difficulties attendent with strictly *laboratory* regimes (Shapiro, 1976).

The present chapter examines the possibility that the prolonged pupal development of P. aegeria under cold shock could form a basis for genetic assimilation. In view of the relational nature of the LGD (Chapter three), the possibility of correlated assimilation of other life cycle features is also examined. In most studies, genetic assimilation has been evaluated through evident changes in life cycle timing (Lloyd & White, 1974) or complexity (Matsuda, 1982), and, in the laboratory, involved consistent lineages (Waddington, 1953; Ho *et al.*, 1983c). However, minor changes might pass undetected, whilst natural breeding structures may be considerably more complex; and it has been suggested that an extraneous individual be introduced to captive programmes to prevent excessive inbreeding (Morton, 1983). Moreover, should the fecundity of laboratory phenocopies be poor (Shapiro, 1976), it would seem wise to have a means of evaluating assimilation in terms of phenotypic response *vis-a-vis* lineage past treatment rather than the number of treatment generations; and such an index is developed.

A number of rearing methods were employed to ascertain an optimum breeding regime; and this was conducted concurrently with assimilation. This produced a large number of interacting factors, and the dissection of their causal relationships is explained. The

efficacy of the protocol is also evaluated here.

In addition to STOCK 01, a number of other stocks were entered into the protocol. Assimilation is firstly evaluated for the protocol overall to ascertain the main effects; then individual samples are given closer examination; and the relative merits of these two approaches are compared.

Likely modes of inheritance are presented. The possibility that genetic assimilation of cold shock effects might occur naturally in the species is considered. The extent to which the protocol reflects such conditions is also assessed. The chapter concludes by evaluating the role of genetic assimilation in the species evolution.

8.1. Experimental stocks

Six further stocks were established from STOCK 01 (Table 8.I). STOCK 01 survived to the F_4 oval stage, but hybridisation of the F_2 adults with STOCK 02 yielded STOCK 03 thus enabling continuation of the lineage for two more generations. These two stocks were also examined for population differences and the effects of hybridisation within subspecies tircis. STOCK 04 was established to compare the life cycle of subspecies aegeria. This stock was hybridised with STOCK 03 to yield STOCK 05 in an attempt to continue the lineage and to examine the effects of hybridising the two subspecies.

STOCKS 06 and 07 were established to repeat the assimilation procedure with tircis from locations different from STOCKS 01 and 02.

8.2. Rearing techniques

Three kinds of rearing environment were used: indoor room; incubator; and greenhouse. The rearing environment of captured animals is classified as wild. Dactylis glomerata provided cut or growing foodplant in all cases.

Indoor rearing of STOCK 01 F_1 has been discussed individually by Winokur (1988). The light : dark regime in all other cases comprised 16h photophase commencing at 06.00h, although rearing regimes differed somewhat in temperature. With wild samples the mean January (vernal) or July (aestival) temperature experienced by the respective subspecies (Dennis, 1977) in generations 1.i-ii and 2.i-ii was entered into analyses of temperature influences on the life cycle.

Members of each family were reared under identical regimes except for comparisons between different temperatures. Rearing regimes used with the respective families are listed in Appendix III.

Table 8.I

Stocks used in the investigation of genetic assimilation of life cycle characters in Pararge aegeria

STOCK 01 : Derivative of two pairings (=Parentals$_{01}$) from Glen Eyre Hall, Southampton (U.K.) (50° 53' N, 01° 25' W) established 28.iv.1985 - 4.v.1985.

STOCK 02 : Derivative of 283 larvae (=Parentals$_{02}$) from Bardney Forest, South Lincolnshire (U.K.) (53° 06' N, 00° 16' W) established 22.x.1985.

STOCK 03 : Derivative of a hybrid pairing (=Parentals$_{03}$) (STOCK 02 P 20°C 'E' x STOCK 01 F_2 14.7°C 'C') established 18.xii.1985.

STOCK 04 : Derivative of twelve adult (=Parentals$_{04}$) P. a. aegeria (Linnaeus, 1758) from Banyuls-sur-Mer (S.W. France) established 18.iv.1986 - 28.iv.1986.

STOCK 05 : Derivative of a hybrid pairing (=Parentals$_{05}$) (STOCK 03 F_1 16.70C 'C' x STOCK 04 P) established 29.iv.1986.

STOCK 06 : Derivative of 450 larvae (=Parentals$_{06}$) from Doncaster (U.K.) (23° 27' N, 01° 07' W) established 29.x.1986.

STOCK 07 : Derivative of 3 pupae (=Parentals$_{07}$) and 131 ova (=F1$_{07}$) from Doncaster (U.K.) established 26.v.1987.

(i) Tubs

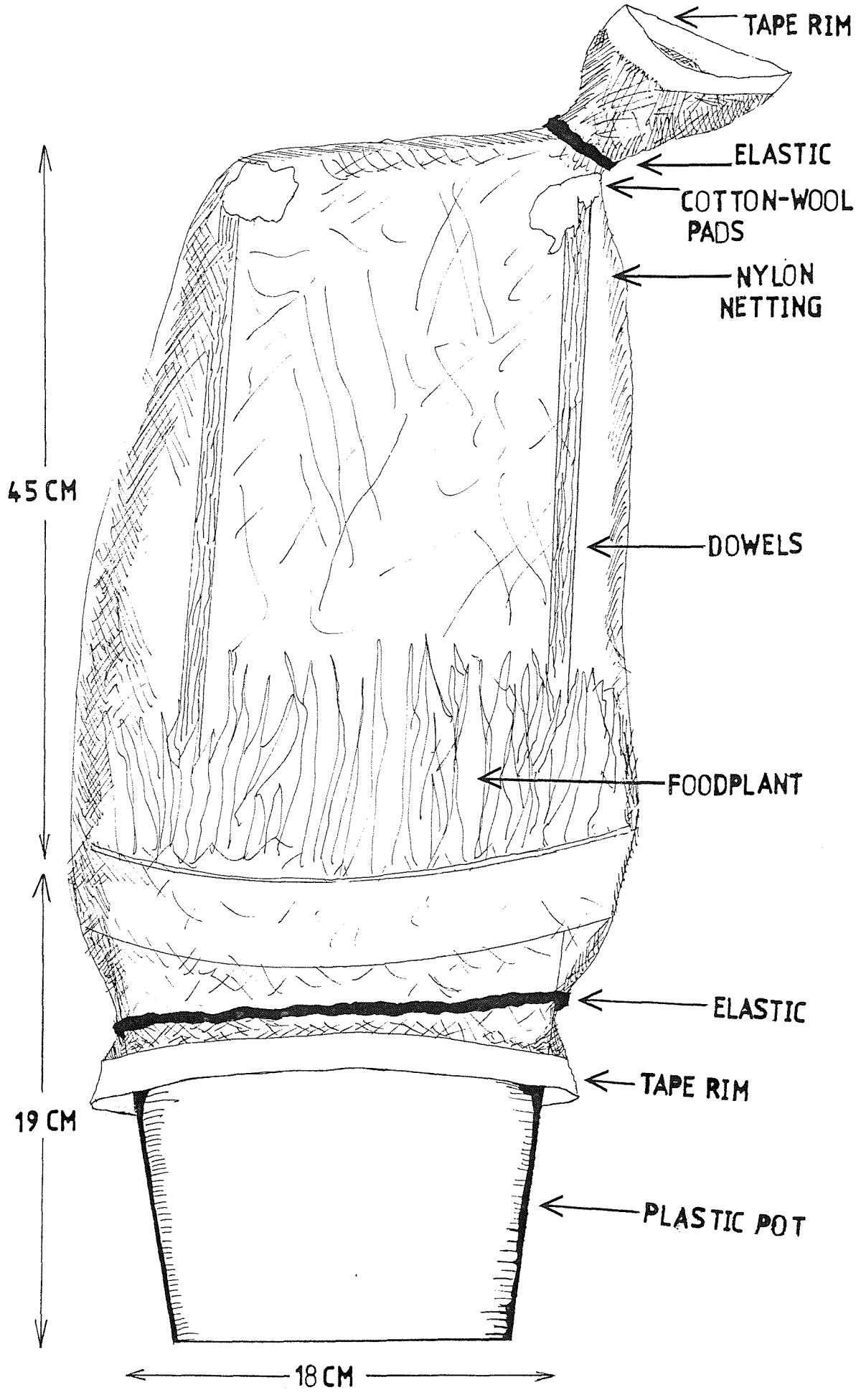
Rearing tubs supplemented clear view breeding cages to accommodate the large number of pairings. Tubs consisted of 19cm x 18cm diameter plastic pots sown with D. glomerata (Plate 6) into which four 45cm dowels were inserted as cover supports. 75cm x 45cm rectangles of parasite-proof black nylon netting (R.E. Stockley) were folded (short end on) into cylinders and the joins sealed on both surfaces with masking tape. The cylinder rims were similarly reinforced. Covers were lowered over the foodplant and secured top and base with elastic bands (Fig. 8.1). Covers were easy to clean and reassemble and the butterflies could be conveniently introduced at the bases (they tended to fly upwards). Tubs were used for indoor rearing with STOCKS 02 and 03 and in greenhouse regimes (Appendix III).

(ii) Indoor Room

Adults were maintained as described in section 6.1. Further indoor rearing was used solely for pairing and oviposition by STOCK 01 F_1 and to maintain STOCKS 02 and 03 *from 22.xii.1985 - 6.i.1986 inclusive when greenhouse and incubator rearing were unavailable.*

During this period 16h photophase was provided as fluorescent light. A domestic gas appliance maintained temperature at (mean $\pm$ SD) 14 $\pm$ 5.6°C. The minimum temperature was 9°C (during scotophase) but did not interfere with oviposition on the foodplant.

PLATE 6 (opposite). Greenhouse cultivation of Dactylis glomerata


TAPE RIM
ELASTIC
COTTON-WOOL PADS
NYLON NETTING
45 CM
DOWELS
FOODPLANT
ELASTIC
TAPE RIM
19 CM
PLASTIC POT
18 CM

(iii) Greenhouse

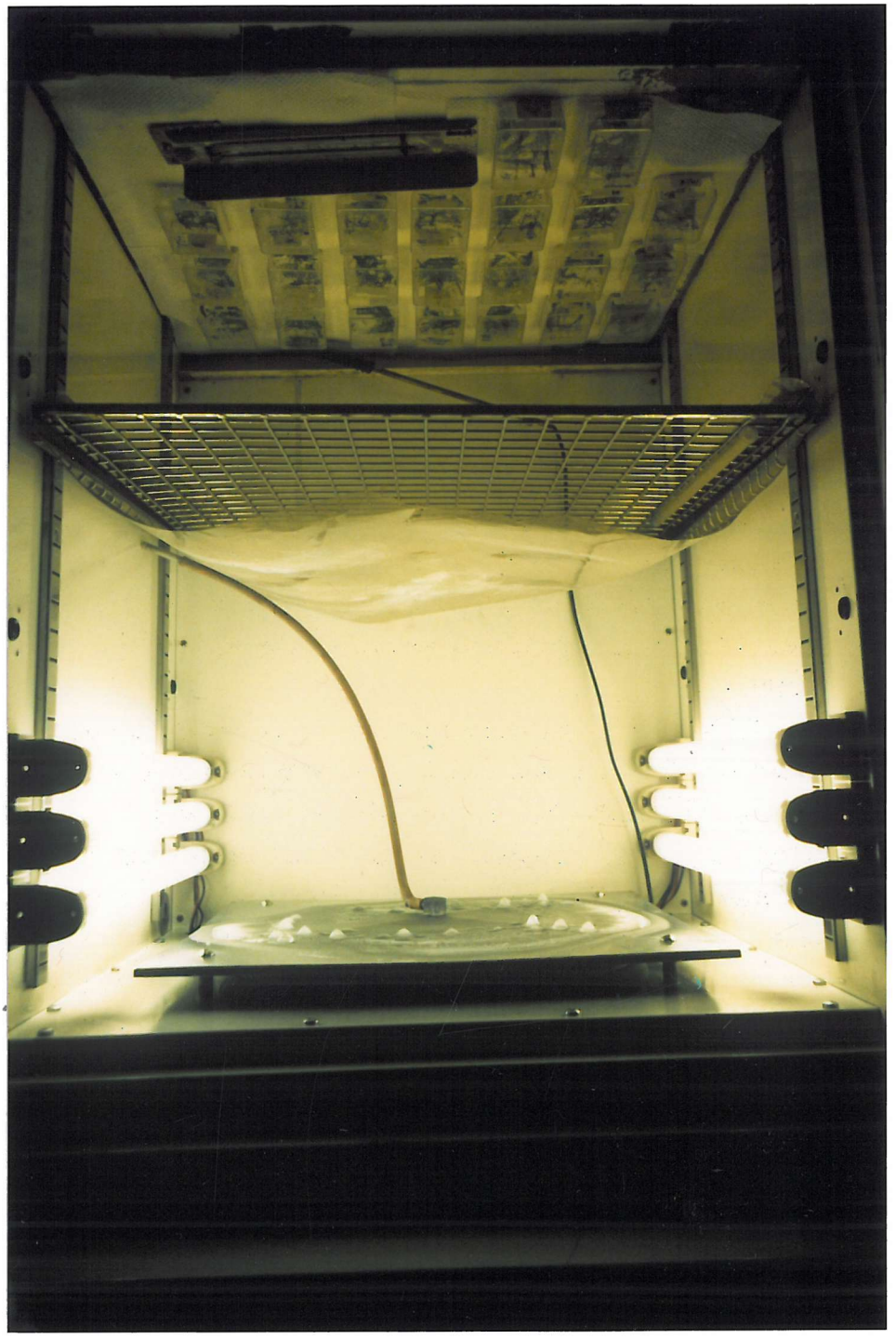
STOCK 01 F_2 were paired and left to oviposit; and the resultant F_3 plus STOCK 02 P reared to the pupal stage, in a greenhouse at 17 + 2.1°C. Larvae were maintained at a maximum density of 40 per tub. Pupae were always transferred to incubators but a few passed undetected amongst the grass and were classed with treatment 'N'.

On germination of the foodplant, feeding pads were positioned and the foodplant then covered with netting to exclude fungus flies (Mycetophilidae: Sciarinae) which can damage the roots and spread infection. Water was provided from the absorbent worktop which was wetted twice daily. Sucrose was applied to the pads through the netting by syringe and alternated daily with water to prevent it becoming too concentrated. There was occasional growth of mildew when larvae were transferred to fresh tubs.

A more serious pest, however, were Pharoah ants, Monomorium pharoensis, which on being attracted by the sucrose, penetrated the netting and interfered with the adults. The rearing of STOCK 01 F_4 and the pairing of STOCK 03 P and STOCK 04 P were therefore conducted in a separate greenhouse at 20 + 2.1°C. Foodplant was watered under the base of netting and the tubs sprayed twice daily to provide the adults with moisture.

Remaining families were reared in incubators (although STOCKS 02 06 and 07 had been tub reared in an unheated greenhouse prior to their establishment at Southampton; M.C. White, pers. comm.).

FIGURE 8.1 (opposite). Rearing tub used to culture Pararge aegeria



(iv) Incubator

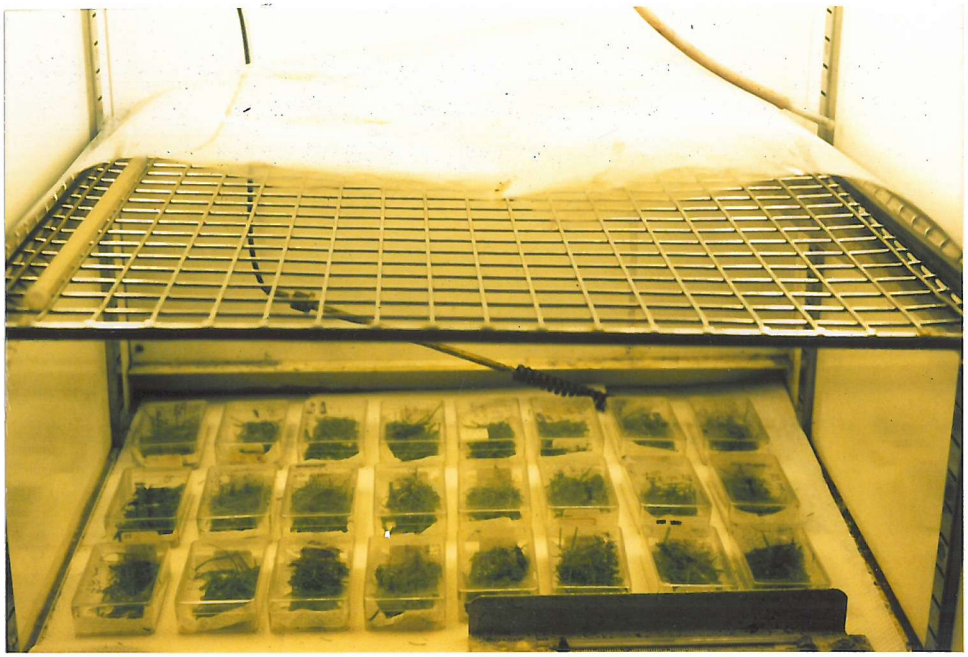
Rearing was conducted in a LEEC artificial environment cabinet at approximately 18°C ('high' temperature range) under a 16h photophase comprising fluorescent light of spectral composition similar to daylight. This is understood to be the optimal light/temperature regime for rearing P. aegeria (E. Lees, pers. comm.). A further incubator was maintained at approximately 15°C ('low' temperature range) and samples from a number of STOCK 01 F_2 and STOCK 03 F_2 families were so reared to enable comparison of the two temperatures; precise rearing temperatures are given in Appendix III

Tub-reared ova were transferred with the cut leaf to plastic boxes as described in section 6.1. Boxes were placed in a single layer on the incubator shelf and covered with a raised dome of clear perspex to prevent water falling on them from the cooling plate. Temperature minima and maxima and spot readings were recorded daily. Fluctuations during the light/dark switchover produced condensation so the incubator bases were lined with absorbent paper and dried daily (Plate 7 & 8a).

Larvae were otherwise reared as described by Winokur (1988). Prepupal boxes were placed on edge to facilitate pupation. Prepupae from tubs were similarly secured by attaching their silk pads to the box sides with adhesive tape. Humidity was maintained as described in section 6.1.

3" x 2" x 2" pairing chambers were constructed from the bases of two plastic bases secured end on with adhesive tape. The joins were prised open and adults (one pair per chamber) introduced body first with wings held closed. The joint was resecured and three 1cm strips of absorbent paper charged with 10% sucrose were suspended 1cm through the gap (Plate 8b). Wicks were recharged three times per day and replaced on alternate days, when the adults were put in fresh chambers and the boxes cleaned. Cut foodplant provided a substratum for oviposition and facilitated the transfer of ova. Adults were sacrificed and stored as per section 6.1.

PLATE 7 (opposite). Incubator culture of Pararge aegeria.




13-4-57

8.3. Experimental Techniques

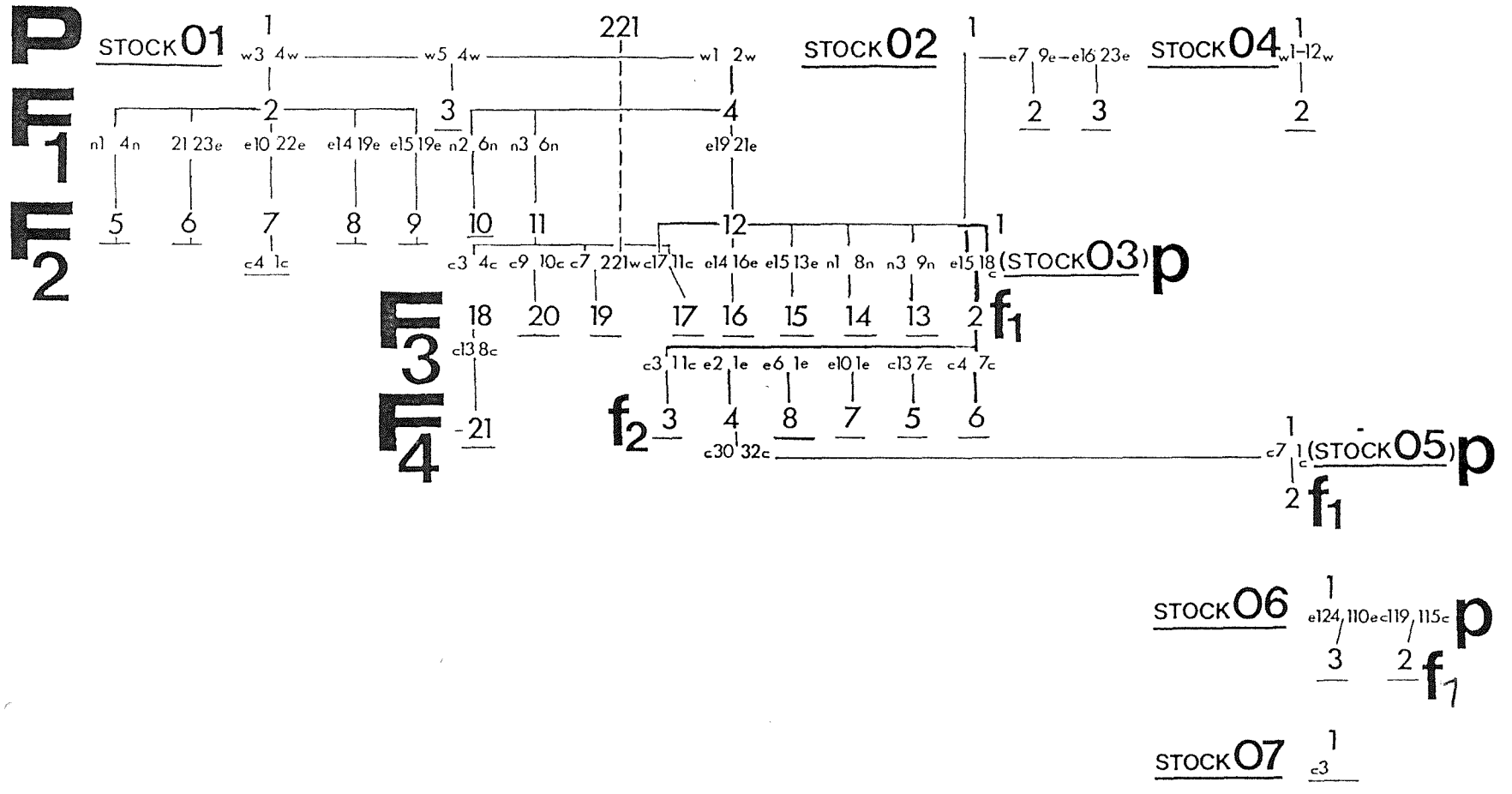
Except for the few tub-reared pupae above, the assimilation protocol used only foil control ('C') and cold shock ('E') treatments.

STOCKS 01 to 05 were cold shocked as described in section 4.2.1. Small differences in the freezing temperatures precluded repetition of *precise* cold shock conditions although STOCKS 01 and 02 always ranged between -1.0°C and -2.0°C, STOCK 03 between -3.0°C and -4.0°C.

STOCKS 06 and 07 were cold shocked in a LEEC artificial environment cabinet at -2.0° under total darkness. Temperature was very constant.

In STOCK 01 F_2 cold shock was used only in conjunction with 'high' rearing temperatures. In STOCK 03 F_2, however, cold shock was used in conjunction with both temperature ranges. The precise cold shock temperatures applied are listed in Appendix III.

PLATE 8 (opposite). Breeding Pararge aegeria in a pairing chamber (A) incubator chamber (B) pairing tub with adults.


P
F1
F2
F3
F4
STOCK 01
STOCK 02
STOCK 04
(STOCK 03) p
f1
f2
(STOCK 05) p
f1
STOCK 06
p
f1
STOCK 07

8.4. Genetic assimilation

It was planned to repeat Waddington's (1953) experiment except with HIGH and control lines only (cf. section 3.7). However, the number of visibly distinct phenocopies was very low and the urgency of the breeding programme necessitated the setting up of pairings before the effect of cold shock on pupal development rate could be confirmed. Thus, pairs were not always mated assortatively with respect to *visible* pattern or to evident increase in prepharate or pupal duration.

Two types of lineage were therefore set up, according to criteria of *treatment*. All families within each lineage were divided into both foil and cold shock samples. The control line used only foil treated (control) animals for pairing, while the experimental line used only cold shocked animals for pairing. Such lineages will be termed true to treatment (TT) lineages.

Half-sib pairings were originally planned to minimise inbreeding (Falconer, 1981) but the limited number of animals available within each lineage necessitated mostly full-sib pairings. Moreover, a number of mixed-treatment pairings (cold x control) proved necessary to maintain the pedigrees. The pairings and crosses involved are presented in Fig. 8.2.

An index was therefore developed that would provide a measure of the extent to which any lineage had previously been subjected to a defined environmental perturbation on the basis of *treatment* (as opposed to *response*) and also allow for deviations from TT-lineages.

FIGURE 8.2. (opposite). Family pedigrees of the assimilation protocol. Preceeding zeros are omitted from family and specimen numbers for brevity. Females are shown to the left within each pairing. Treatments are indicated following the specimen number: w = wild-caught; n = untreated; c = foil treated; e = cold shock. Generation number is shown along the vertical axis. A horizontal bar truncating the genealogy indicates extinction of the lineage. For further explanation see text.

8.4. The genetic assimilation coefficient

A reaction of the *kind* induced by an environmental stress will be termed the stress response (SR). When considering its expression by virtue of environmental induction (SR_E) the presence of stress is given the value 1 and its absence the value 0.

When considering the production of the stress response as a result of heredity (SR_H) the value of SR is the propensity of an individual to express SR by virtue of its having received (assimilated) a propensity to express SR from the parents. This propensity (SR_H) is the Assimilation Coefficient (A_i).

The lineage at the first generation of treatment has had no previous exposure to the stress so

$$SR_H = A_i = 0 \quad \ldots\ldots\ldots\ldots \quad (1)$$

The propensity to express SR, or P(SR) now depends only on the presence or absence of the stress

$$\begin{aligned} P(SR) &= SR_H + SR_E \quad \ldots\ldots\ldots \quad (2) \\ &= A_i + SR_E \\ &= 0 + SR_E \\ &= + SR_E \quad \ldots\ldots\ldots \quad (3) \end{aligned}$$

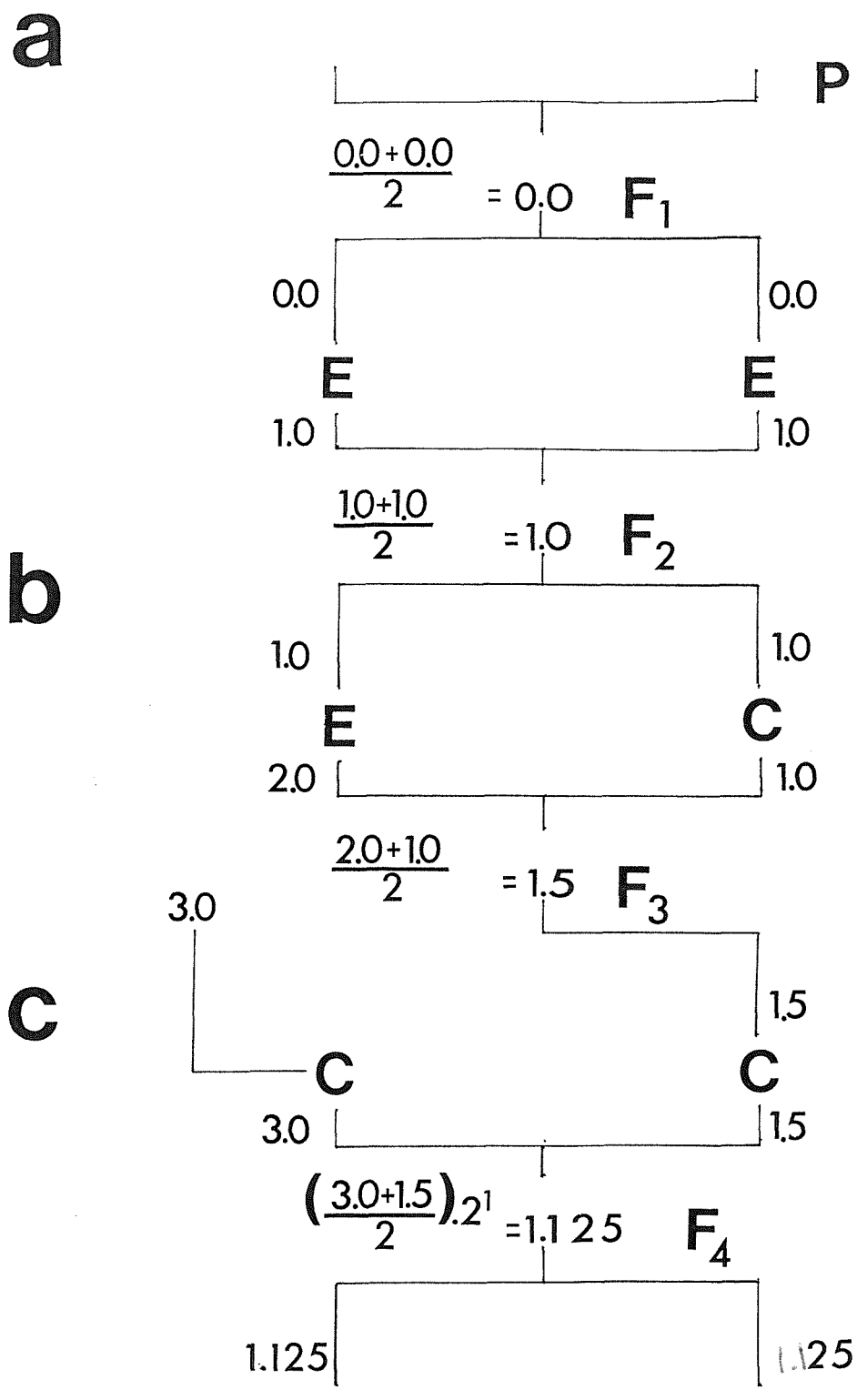
hence current environmental stress contributes additively to P(SR).

The probability of any individual inheriting SR from its parents is the probability that its parents will pass on SR, which is simply the mean of the parents' P(SR):

$$SR_H = (P(SR)_{female} + P(SR)_{male})/2 \quad \ldots\ldots \quad (4)$$

A_i prior to stress exposure is given by expression (4); with the first treatment generation a special case in which expression (4) equates to zero. P(SR) for any individual following stress exposure (A_iP) is given by the sum of expressions (3) and (4).

A_i is computed by simply working through the lineage(s) on the basis of three tenets. The first is that environmentally induced SR's


a
P
0.0+0.0
2
= 0.0
F1
0.0
0.0
E
E
1.0
1.0
b
1.0+1.0
2
=1.0
F2
1.0
1.0
E
C
2.0
1.0
2.0+1.0
2
=1.5
F3
3.0
1.5
c
C
C
3.0
1.5
(3.0+1.5)
2
.2^1
=1.125
F4
1.125
1.125

be transmitted for at least one generation. The second is that exposure to stress in the treated parent is the overiding factor; so that as long as at least one parent is exposed to the stress assimilation will occur. The third is that *expression* of the stress response is a dauermodification in which assimilation has not been completed and so the stress response is temporary only (Ho & Saunders, 1982a).

TT-treatment crosses always increase A_i by 1.00 whilst mixed crosses increase A_i by 0.5. TT-control crosses leave A_i at its present level with the proviso of the third tenet; *viz.*, when the change to TT-control continues beyond its first generation the value of A_i is halved at each generation subsequent to that at which the cessation of stimulus ocurred, so that

$$A_i = A_i' / 2^n \quad \ldots\ldots\ldots\ldots (5)$$

where A_i' is the assimilation coefficient at cessation and n the nth *subsequent* generation. Hypothetical lineages are worked through in Figure 8.3; and the assimilation values of each family tabulated in Appendix III.

FIGURE 8.3 (opposite). Computation of the assimilation coefficient. A hypothetical genealogy is shown. The coefficient (A_1) is an index of the propensity for an individual to show a reaction of a kind induced by a defined environmental stimulus. The A_i of the offspring is the mean A_i of the parents. The base pair forms the starting point in the analysis. (A) Neither parent has been treated; their A_i and that of the F_1 is thus zero. Application of cold shock, 'E', raises the index by 1. A_i among the F_2 is thus 1. (B) Similar reasoning applies as long as at least one parent is treated. Hence A_i among the F_3 is 1.5. None of the F_3, however are treated. (C) The propensity thus diminishes at each *succesive* generation. A_i among the F_4 is thus only 1.125. The index provides a measure relative to which the expressivity of traits can be compared. For further explanation see text.

8.6. Data compilation

Sixty-two parameters were recorded (Table 5.II) and classified into four main types:

1) Rearing and Environmental Parameters (REPS)
2) Pupal Treatment
3) Constitutional parameters (COPS)
4) Interactive Parameters (IMPS)

REPS define rearing environments and temperatures. Pupal treatment (PLTT: control or cold shock) defines the second class. COPS define a priori properties of the individual or sample such as sex and inbreeding coefficient. IMPS define life cycle characters whose value may depend on environmental conditions or constitution. IMPS are further divided into:

i) Growth parameters
ii) Survival parameters

Growth parameters include linear and temporal aspects. In addition to characters recorded directly, were computed genetic loads (Freire-Maia & Freire-Maia, 1974) and effective family sizes (Falconer, 1981). The assimilation coefficient was also computed.

8.7. Analysis and computation

The preliminary analysis used sample means which, unlike in the previous analyses, were based on all individuals present for that character. Means were used in order to condense the data for preliminary analysis. This enabled the data to be entered in a form from which, given the large number of possible (but unknown) interactions, groups for *analysis* could be most readily extracted. Besides, certain variables such as survival ratios and treatment differences necessarily refer to sample parameters. The main limitation from using means is that, because they summarise individual

data, some information may be lost and so are more likely to incur TYPE II errors (overlook significant effects) (Sokal & Rohlf, 1981).

Nonparametric tests were applied in all cases as the nature of the distributions was not known. The properties of each of the four types of variable can be summarised thus:

PLTT : Independent discrete
REPS : Independent discrete (environment) or quantitative
COMPS : Independent discrete (STK FAM SEX) or quantitative
IMPS : Dependent quantitative;

and statistical analysis explored two main types of relationship among variables namely:

1) Association
2) Discrimination

Samples were distinguished by one or more of four parameters during analysis:

Family
Rearing environment
Pupal treatment
sex

but often not all of these parameters distinguished particular variables (Appendix I). For example, the cases for each sex in family 01002 would both have the same entry for family size. Data were analysed using the SPSS^{x} statistical package (SPSS Inc., 1988), which requires, that *all* variables be entered for each case (it will not run with excessive missing value entries), and hence several cases contained redundant data.

Codes were therefore developed that instructed SPSS^{x} to select for analysis only those cases for which the values of respective variables refer to separate samples. The particular sample classes for each variable and the respective SPSS^{x} commands in Appendix IV.

Associations among quantitative and meristic characters were examined with Pearson rank correlation coefficients and 2-tailed tests

applied as no prediction was made as to their direction. Associations between life cycle characters with A_i, A_iP and temperatures were examined using Kendall's coefficient of concordance (the nonparametric counterpart of regression, Sokal & Rohlf, 1981).

Differences between means were tested with the Mann-Whitney U-test (two samples) or Kruskall-Wallis test (more than two samples).

Certain variables (PLTT $REPS_{env}$) describe qualitative parameters not amenable to quantitative analysis. Generation assumes numerical values which refer to temporal ordering only but cannot itself be quantified (although it may relate directly to characters such as inbreeding coefficient which can); while the numerical values assigned to stock and family serve only as identifiers. Relationships between such variables were explored using Canonical Discriminant Functions (CDFs). CDFs define the axis(es) that best demarcate the separation between groups. $REPS_{env}$ and PLTT were converted to numeric codes for such analyses. The CDF values cited give each group mean position perpendicular to the relevant axis(es). These are then compared with those expected from a uniform distribution and tested for significance using Chi^2 (SPSS^x Inc., 1988).

Significant ($P < 0.05$) interactions were entered onto one of two matrices. Matrix I (Table 8.III) comprises interactions among *independent* variables. Matrix II (Table 8.IV) comprises interactions between *dependent and independent* variables. When a dependent variable interacted with more than one independent variable (Matrix II) the relevant independent variables were themselves examined for interaction (Matrix I). If significant then exact probabilities were calculated for the original Matrix II interactions. The interaction with the lowest exact probability identifies the main effect(s). The data summarised in these two matrices are presented in Appendix V. More detailed analyses of samples and stocks were then performed. The rationale behind these is explained in the respective sections.

8.8. Inbreeding depression

Inbreeding depression represents the loss of fitness (as survival) due to increasing homozygosity at loci affecting viability or fecundity, which can result in greater mortalities in later generations. Genetic load is measured in lethal equivalents per zygote, defined as a group of mutant genes of such number that if dispersed in different individuals they would cause on average one death eg. two mutants each with a 50% probability of causing death (Dobzhansky, 1963). Large inbreeding depressions can result from even a few loci which are very lethal as homozygotes (Oliver, 1981) but whose effect is masked in heterozygotes (segregational load); so that natural populations may have large loads (Dobzhansky, 1963).

Load was therefore estimated for the P. aegeria stocks using Freire-Maia & Freire-Maia's (1964) equation:

$$B = \frac{\log (S_2/S_1)}{\frac{4 \log [1 - (F_2-F_1)]}{4}}$$

where B = genetic load, S_1 = survival of the less inbred sample, S_2 = survival of the more inbred sample and F_1 and F_2 their respective inbreeding coefficients.

The variance of B (S_B) can also be estimated:

$$S_B^2 = \frac{B^2 \left(\frac{M_2 S_2}{N_2} + \frac{M_1 S_1}{N_1}\right)}{(M_2-M_1)^2}$$

where S = survival, N = sample size and M = a measure of some (unspecified, Freire-Maia & Freire-Maia, 1964) damage of the more inbred (2) and less inbred (1) groups respectively (*ibid.*). Survival was entered as oval hatchability (No. hatching / No. laid) (Oliver, 1981) but samples of hatchability = 0.000 were excluded as this usually results from inseminary failure (*ibid.*).

Table 8.II

Parameters recorded during the analysis of genetic assimilation in *Pararge aegeria*

Character	Description
STK	Stock
FAM	Family
SEX	Sex
GEN	Filial generation
FIN	Inbreeding coefficient F_i
A_i	Assimilation index
A_iP	Post-treatment A_i
PLTT	Pupal treatment
OVRR	Egg rearing environment
TLRR	Larval environment
PDRR	Pupal environment
ILRR	Adult environment
OVTT	Egg rearing temperature
TLTT	Larval temperature
PPHT	Prepharate pupa temperature
PDTT	Pharate pupa temperature
ILTT	Adult temperature
OVDUR	Egg duration
I1DUR	1st larval instar duration
I2DUR	2nd instar duration
I3DUR	3rd instar duration
I4DUR	4th instar duration
I5DUR	5th instar duration
I4I5	Proportion of 5-instar larvae
TLDUR	Entire larva duration
PPDUR	Prepupa duration
E1LEN	Larval length at 1st ecdysis
E2LEN	Length at 2nd ecdysis
E3LEN	Length at 3rd ecdysis
E4LEN	Length at 4th ecdysis

Table 8.II (cont.)

Character	Description
PLEN	Pupa length
PPHDUR	Prepharate pupa duration
PHDUR	Pharate pupa duration
PDUR	Entire pupa duration
PNBT	Proportion of blotched pupae
LFCY	Life cycle duration to eclosion
ILONG	Adult longevity
NOVA	Size of starting egg batch
HTCH	Hatchability
I1RS	Relative survival of 1st instar
I2RS	2nd instar survival
I3RS	3rd instar survival
I4RS	4th instar survival
I5RS	5th instar survival
TLRS	Larval survival
PPRS	Prepupal survival
PPHRS	Prepharate survival
PHRS	Pharate survival
PRS	Overall pupal survival
LFCRS	Survival to eclosion
FNE	Effective family size
FNS	Effective sample size
PF	Proportion of females in family
PFS	Proportion of females in sample
PECOP	Eclosion-pairing interval
PAIRS	Number of pairings
OVPR	Number of pairs yielding ova
FECUND	Proportion of pairs yielding ova
PROV	Pairing-oviposition interval
DOVIP	Number of days over which oviposited
MOV	Mean ova per ovipositing pair
NOHTC	Number of pairs yielding fertile eggs
FERT	Proportion of egg batches hatching
MHTCH	Mean hatchability of hatching batches
MHTCH	Mean hatchability over all batches

TABLE 8.III (MATRIX I)

Relationships among
constitutive parameters, pupal
treatment, rearing environments and temperatures

	GEN	FIN	AI	AIP	PLTT	OVRR	TLRR	PDRR	ILRR	OVTT	TLTT	PPHC	PPHE
FIN	****												
AI	*	*											
AIP	***												
PLTT		*	*										
OVRR	*	*	*	***	****								
TLRR				***	****								
PDRR				***	****	****	****						
OVTT	*					****	**	**					
TLTT						**	***	**		***			
PPHC			*			**	**			***	***		
PPHE			*						*	***	***		
PDTT						***	***	*		***	***	***	***
ILTT									*				

Asterisks indicate a significant relationship between the respective variables: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $0.0001<P<0.001$; **** $P<0.0001$. Empty cells are left blank. PPHC is control prepharate temperature and equivalent to the pharate temperature of both control and cold shocked pupae. PPHE is cold shock prepharate temperature. For further explanation see text.

TABLE 8.IV (MATRIX II)

Relationships between life cycle characters and constitutive parameters, pupal treatment, rearing environments and temperatures

	STK	SEX	GEN	FIN	AI	AIP	PLTT	OVRR	TLRR	PDRR	ILRR	OVTT	TLTT	PPHTC	PPHTE	PDTT	ILTT	
OVDUR			*-	**-	****-							****-						OVDUR
I1DUR					***							*-	*-					I1DUR
I2DUR					***							*-	*-					I2DUR
I3DUR					***-								*-					I3DUR
I4DUR					***							**-	**-					I4DUR
I5DUR					**													I5DUR
I4I5												***-	***-					I4I5
TLDUR					****-							****-	****-					TLDUR
PPDUR					****-							****-	****-					PPDUR
E1LEN					***-							***-	***-					E1LEN
E2LEN					**-							**-	**-					E2LEN
E3LEN					***-							***	***					E3LEN
E4LEN					**-													E4LEN
PLEN		**	*-		****-							****-	****-					PLEN
PPHDUR	*				****+	****+	**	**	**			**-	****-	**-	****-	****-		PPHDUR
PHDUR	***		***+	***+	****+			**				****-	****-	****-	****-	****-		PHDUR
PDUR					****+	****+	**	**	**	**			*-	**-	****-	**-		PDUR
PNBT							**						*-	**-	****-	**-		PNBT
LFCY					****-	***+												LFCY
ILONG					****+	****+	**	*	*	*	*		*	***	***	**-	**-	ILONG
NOVA			**-		****	****						***-	***-	***-		***-	***	NOVA
HTCH												****						HTCH
I1RS			*-	***-								***	***					I1RS
I2RS			***-	***+								***-	***-					I2RS
I3RS												***-	***-					I3RS
I4RS												***	***-					I4RS
I5RS				*+								***	***					I5RS

Only significant interactions are shown: * 0.01<P<0.05; ** 0.001<P<0.01; *** 0.001<P<0.01; **** 0.0001<P. The direction of correlation and regression is indicated as positive '+' or negative '-'. Treatment-specific effects are denoted by subscripts: C = foil control, E = cold shock. PPHTC and PPHTE refer to prepharate temperature of foil and cold treated samples respectively.

TABLE 8.IV (continued i)

	STK	SEX	GEN	FIN	AI	AIP	PLTT	OVER	TLRR	PDRR	ILRR	OVTT	TLTT	PPBTC	PPBTE	PDTT	ILTT	
TLRS				*+					**			****	****					TLRS
PPRS	*											****	****					PPRS
PPHRS				***-E					*	*	*							PPHRS
PHRS								*	*	*	*							PHRS
PRS				**-E														PRS
LFCRS									*	*	*							LFCRS
FNE							*	*										FNE
FNS			*-	*-	*-				**	**		****-	****+	****+	***+	****-		FNS
PF			**-					**										PF
PFS			**-	*-		****-						****+	****+	****-	***+	****-		PFS
PECOP	*				***-	**-						****-	****+	***-	***+	****+	****+	PECOP
PAIRS					*-	***-						****-	****+	****-		****+	****+	PAIRS
OVPR				*-								****-	****+	***-	**-	****-	****+	OVPR
FECUND					***	****-						****+	****-	***-	**-	****-	****-	FECUND
PROV					***							***+	***-	*-	*+	***-	***-	PROV
DOVIP					**	**						*+	*-		*+	*+	*-	DOVIP
MOV					****	****						*-	**-	**-	**-	*-C		MOV
NOHTC					**	***						****-	****-	**-	*-	****-	****-	NOHTC
FERT				*		****					**	****-	****+	**-	*+	****	****-	FERT
MHTCH			*-			****-						**-	***-	*-	**-	***-	***	MHTCH
MHTCA						****-						****-	****-	**-	**+	****-	****-	MHTCA

8.9. Assimilation

(i) *Developmental parameters*

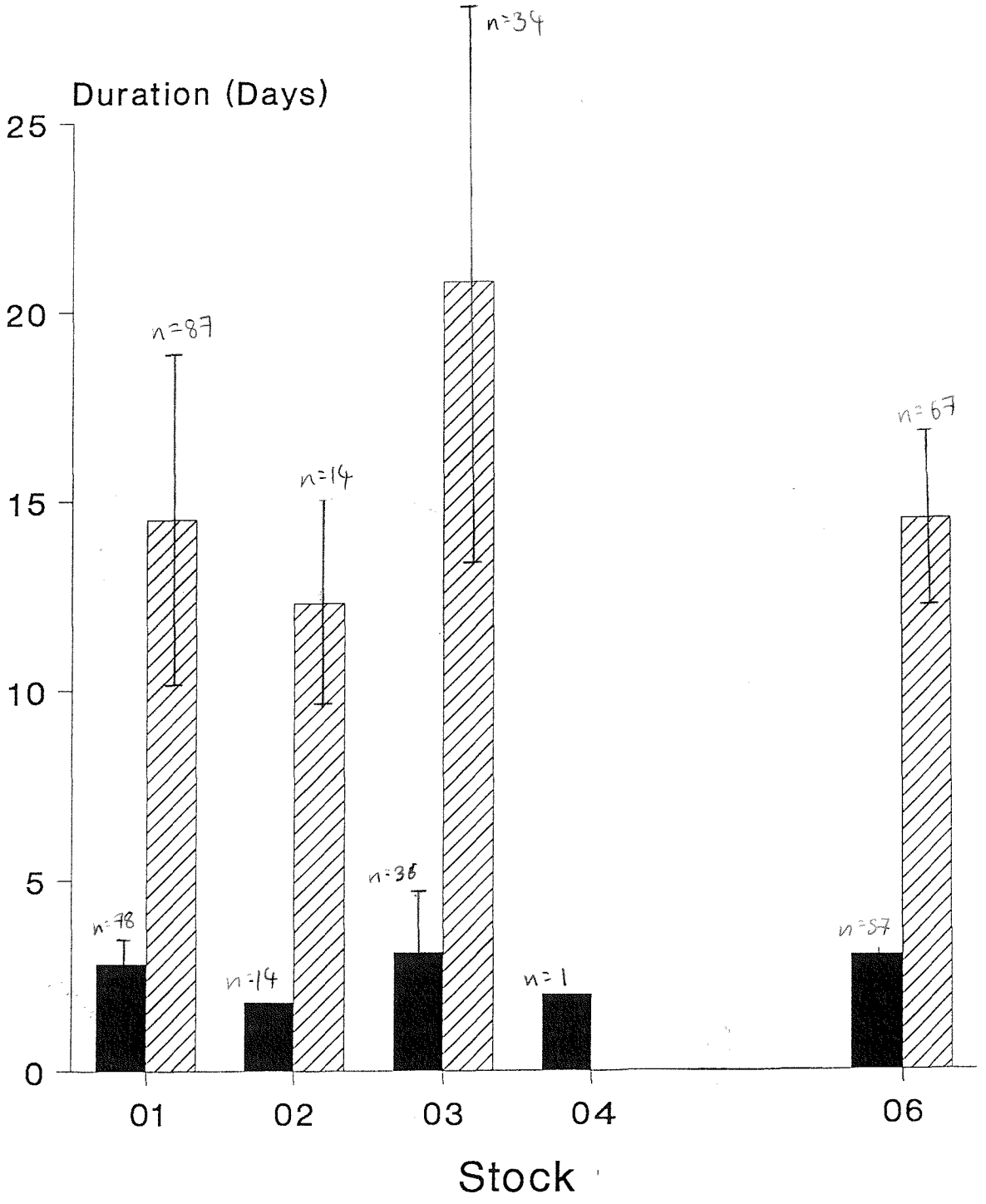
Results are listed in Appendix V and summarised in Tables 8.III & 8.IV. Larval length at 4th ecdysis and 5th instar duration increased with A_i. Length after each 1st to 3rd ecdysis, and the durations of the first four instars, entire larva, and prepupa were concordant with A_i; the assimilation is not an artifact of their concordance with egg or larval rearing temperatures as these themselves were not correlated with A_i (oval: $R_{(44)} = 0.1245$ns; larval: $R_{(31)} = 0.0344$ns). The same reasoning applies to other parameters affected by oval and larval temperature in addition to A_i.

Egg duration and pupa length decreased with larger A_i and also with generation; egg duration also decreased with inbreeding and pupa length differed between the sexes (mean $\pm$ SD: males = 11.4 $\pm$ 0.64mm; females = 12.0 $\pm$ 0.59mm). However, the exact probabilities with generation and inbreeding were 10 - 10^2x larger than with A_i, so A_i had a genuine (and the main) contribution; nor did A_i differ between the sexes (mean $\pm$ SD: males = 0.489 $\pm$ 0.5911; females = 0.488 $\pm$ 0.0.5964; Mann-Whitney U-test, Z = -0.1235ns).

In all the above cases, the main egg and larval (including prepupa) durations and pupal length decreased with A_i. *Individual* ecdysis lengths, however, *increased* with A_i except that at 3rd ecdsysis. The latter is in accord with the change in growth dynamics around the 3rd instar and the relationship between lengths at 3rd ecdysis and pupation (6.3).

That the durations of 4th and 5th instars increased whilst lengths at 4th ecdysis and pupation decreased, is explained by 4th and 5th instar larvae contracting before pupation (4th instar is usually the ultimate; 5th instar always is). That egg duration decreased but 1st instar duration increased is explained by these being intra-oval and extra-oval 'stages' of 1st instar growth. But eggs of shorter duration have a faster pre-hatch and hence slower post-hatch linear growth. This could explain the negative relationship between the duration and

Prepharate and pharate pupal durations of each stock overall



length following the 1st (and also somewhat the 2nd) instar - until the change in growth dynamics at the 3rd, whose duration and following lengths both decreased (expected as the longer the instar, the more growth achieved).

All pupal durations increased with A_i. Those of the prepharate and entire pupa, but not pharate stage, also increased with A_iP. This is explained by the lack of direct effect of pupal treatment on pharate duration; with the changes in A_i *value* (but *not* effect) following treatment masking the relationship between thc effect and value of *pre-treatment* A_i. The samples assigned to each treatment did differ *slightly* in A_i (mean $\pm$ SD; class'N': 0.000 $\pm$ 0.0000; class 'C': 0.500 $\pm$ 0.5684; class 'E': 0.672 $\pm$ 0.6737) but the exact probability was much greater than for the influence of A_i effect on duration. The assimilated increase in pharate duration is therefore not an artifact of pupal treatment.

Prepharate and pharate durations differed among thc stocks, but there was no trend with the order in which stocks entered the protocol (Fig. 8.4a-b). Pharate duration increased with generation. This most likely reflects the effect of inbreeding; which too was highly concordant with generation. It is possible, however, that the effect of A_i on pharate duration might be an artifact of inbreeding ($P<0.0001$). The durations of the pupal stages at each generation

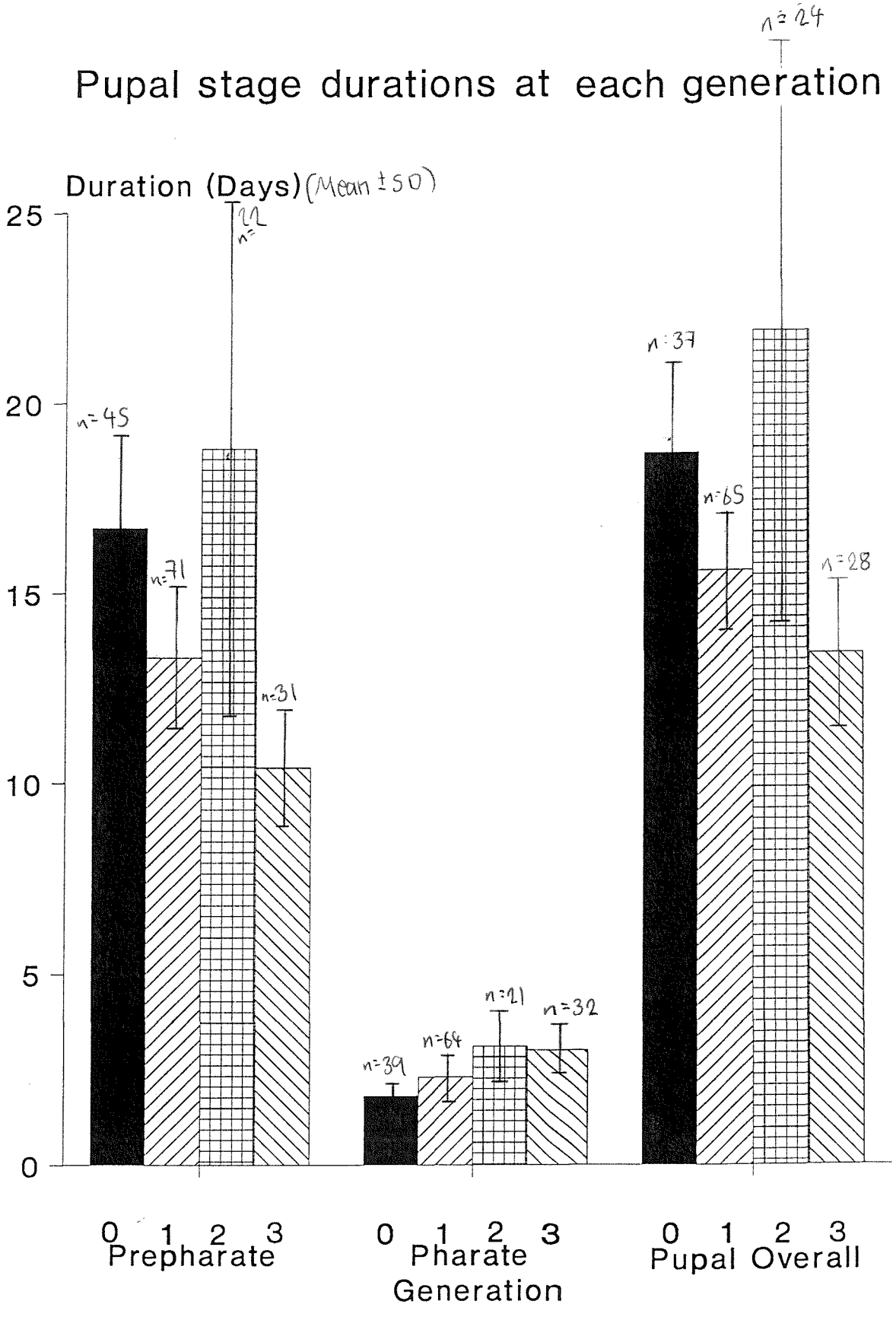
---

Generation	F_i (mean $\pm$ SD)
0	0.046 $\pm$ 0.1011
1	0.071 $\pm$ 0.2673
2	0.267 $\pm$ 0.0440
3	0.292 + 0.1514
4	0.500 $\pm$ N.A (n=1)

TABLE 8.V. The level of inbreeding (F_i) at each generation

FIGURE 8.4 (opposite). Prepharate and pharate durations of each stock overall. Solid shading gives mean pharate durations; line shading gives mean prepharate durations. For further explanation see text.

Figure 8.5


Pupal stage durations at each generation
Duration (Days) (Mean ± SD)
25
20
15
10
5
0
n=45
n=71
n=31
n=39
n=64
n=21
n=32
n=37
n=65
n=28
n=24
0 1 2 3
Prepharate
0 1 2 3
Pharate
0 1 2 3
Pupal Overall
Generation

are shown in Fig. 8.5 (opposite).

Prepharate and entire pupal duration differed among the pupal treatments but, as with pharate duration, the effect of A_i is unlikely to be an artifact of these treatments. Moreover, the concordance of pupal and prepharate duration with A_iP ($P<0.0001$) was considerably more significant than the difference between treatments ($P=0.0021$). Thus A_i has a contributory effect.

An analysis of covariance (ANCOVA) was therefore carried out to examine the relative contributions of A_i and pupal treatment to the pupal effects of A_iP. The test was run using classes 'C' and 'E' only; and then also including class 'N' (STOCK 01 and tub-reared pupae). The effects of A_i are clearly much greater than those of pupal treatment. However, the effects of A_i or treatment *alone* fell short of significance ($P<0.05$), except for pupal duration over all treatment classes considered. These strongly suggests that it is *sensitivity* to cold shock that is assimilated.

		Ai			Treatments		
	Treat	F-value	Df	P-exact	F-value	Df	P-exact
PPHDUR :	N C E	3.899	1,34	0.057	0.108	2,33	ns
PPHDUR :	C E	3.779	1,32	0.061	0.032	1,32	ns
PHDUR :	N C E	3.895	1,37	0.056	0.080	2,36	ns
PHDUR :	C E	3.813	1,33	0.060	0.024	1,33	ns
PDUR :	N C E	4.244	1,34	0.050	0.100	2,33	ns
PDUR :	C E	4.112	1,32	0.051	0.021	1,32	ns

TABLE 8.VI. Results of ANCOVA on the relative contributions to pupal stage durations of A_i and pupal treatment (treat): N = no foil control; C = foil control; E = cold shock. Exact probabilities (P-exact) of non-significant differences are omitted.

The (just) significant effect of A_i alone on the pupal stage might represent the combined assimilation of increased prepharate and pharate durations. The increase with A_i in pharate duration (Table 8.IV) may represent the assimilation of a cold effect that manifests later in pupal development ie. some determining event. This would be in *addition* to its immediate effect on prepharate development.

Correlations among the pupal stages were therefore examined within each foil and coldshock treatments using samples of $A_i < 1.000$, $A_i \geq 1.000$, and $A_i > 1.000$ (Table 8.VII). They were similarly examined for each sex, although only foil controls were used as pharate and entire pupal duration differed inherently between treatments (Chapter seven).

The concordances between the pupal stage durations and A_i were examined for each sex, but there were no differences between the sexes nor among the stages ($P \leq 0.001$ in all cases). Pupal stage durations were then regressed on A_i for each treatment 'C' and 'E' ie. for pupae that would *then* be assigned to either class 'C' or 'E'; where if A_i increases sensitivity to cold the concordance would be expected to be greater in class 'E'. No difference, however, was found; but $P < 0.0001$ in all cases so any such difference might have been masked by its appearing only at *still* greater significance levels.

The effects of A_i on pupal durations are probably not an artifact of their difference among rearing environments (Fig. 8.6a). A_i differed among egg rearing environments only (mean A_i + SD: room = 0.000 ± 0.0000; greenhouse = $0.482 + 0.7103$; incubator = $0.634 + 0.5923$); and although it increased from room through greenhouse to incubator as did the pupal durations, it more likely reflects the way in which samples of differing A_i were *then* assigned to these environments. Nor was it an artifact of the temperature differences between them (Fig. 8.6b). Firstly, A_i was not concordant with either oval or larval temperature; and secondly, the rank decreases in temperature among environments were not concordant with the rank increases in pupal durations (durations decreased with temperature).

TABLE 8.VII. Correlations among the pupal stage durations

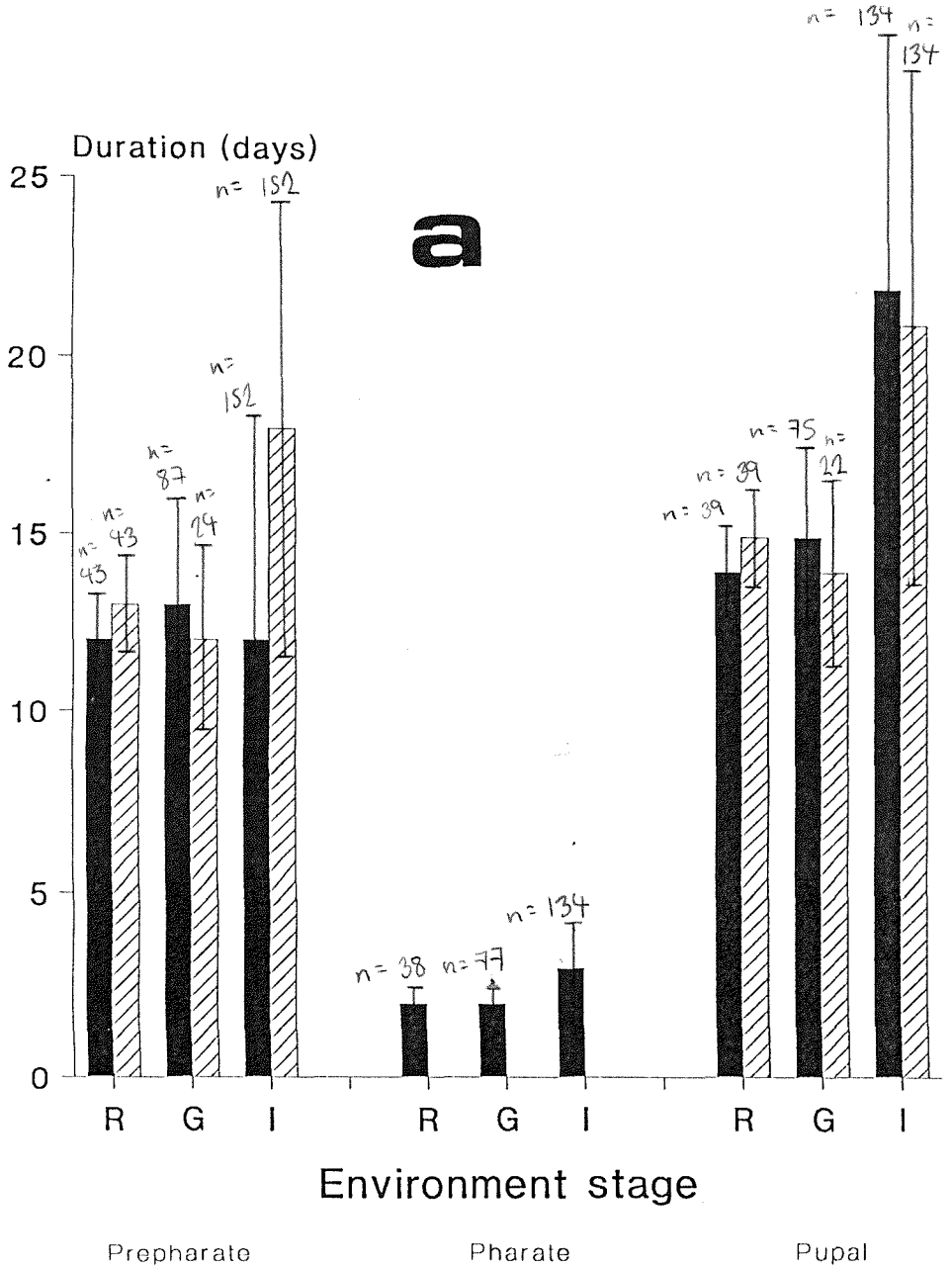
FOIL ONLY: MALES FEMALES (TRUE-TO-TREATMENT LINEAGES) Males and females from the same lineages

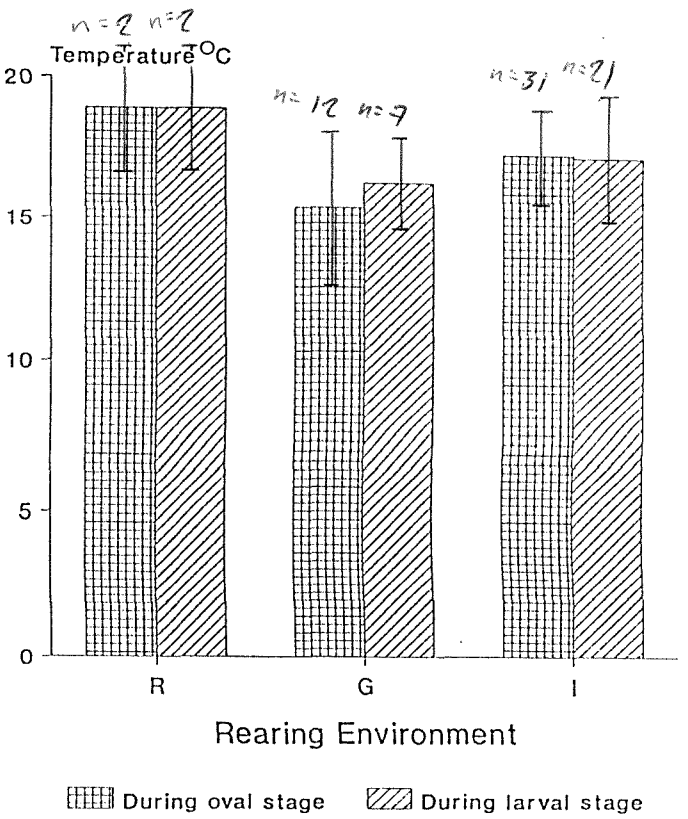
	n	R-value	Sig	P-exact	n	R-value	Sig	P-exact
Ai<1.000								
PPH PH	12	0.3403	ns	0.140	10	0.2083	ns	0.282
PPH P	12	0.9701	***	0.000	10	0.9634	***	0.000
PH P	12	0.4430	ns	0.750	10	0.3535	ns	0.150
Ai≥1.000								
PPH PH	9	0.5865	*	0.048	7	0.4818	ns	0.137
PPH P	9	0.9958	***	0.000	7	0.9910	***	0.000
PH P	9	0.6135	*	0.039	7	0.5225	ns	0.114
Ai>1.000								
PPH PH	3	0.5000	ns	0.333	3	0.5000	ns	0.333
PPH P	3	1.0000	***	0.000	3	1.0000	***	0.000
PH P	3	0.5000	ns	0.333	3	0.5000	ns	0.333

FOIL COLD SHOCK (TRUE-TO-TREATMENT LINEAGES)

	n	R-value	Sig	P-exact	n	R-value	Sig	P-exact
Ai<1.000								
PPH PH	22	0.2833	ns	0.101	16	0.3091	ns-	0.122
PPH P	22	0.9707	***	0.000	16	0.9741	***	0.000
PH P	22	0.4045	*	0.031	16	0.2209	ns	0.205
Ai≥1.000								
PPH PH	16	0.5426	*	0.015	8	0.6281	*	0.048
PPH P	16	0.9940	***	0.000	8	0.9493	***	0.000
PH P	16	0.5753	**	0.010	8	0.7950	**	0.009
Ai>1.000								
PPH PH	6	0.5000	ns	0.156	4	1.0000	***-	0.000
PPH P	6	1.0000	***	0.000	4	1.0000	***	0.000
PH P	6	0.5000	ns	0.156	4	1.0000	***-	0.000

for further explanation see p232


a
Duration (days)
25
20
15
10
5
0
n= 43
n= 43
n= 87
n= 24
n= 152
n= 152
n= 38
n= 77
n= 134
n= 39
n= 39
n= 75
n= 22
n= 134
n= 134
R
G
I
R
G
I
R
G
I
Environment stage
Prepharate
Pharate
Pupal


b
n=7 n=7
Temperature °C
n=12 n=9
n=31 n=21
20
15
10
5
0
R
G
I
Rearing Environment
During oval stage
During larval stage

The considerations for the effects of A_i as artifacts of differences between control and cold shock temperature ranges are essentially those of pupal treatment; and hence the effects of Ai are not such artifacts. Besides, the concordance of A_i with temperature *within* each control and cold range was much less significant ($0.01<P<0.05$) than the concordance between A_i or these two temperatures with pupal durations.

All pupal durations increased with cooler temperature within both ranges. That the concordance of prepharate duration with temperature was less within the control than cold range, suggests that within the

Time	Stage	>> temperature	<< duration
Env			
Oval	PPHDUR	R – G – I	R – I – G
Oval	PHDUR	R = G – I	R – I – G
Oval	PDUR	R – G – I	R – I – G
Larval	PPHDUR	G – R – I	R – I – G
Larval	PDUR	G – R – I	R – I – G

TABLE 8.VIII. Egg and larval environments ranked by increasing temperature and decreasing pupal durations.. Left to right: rank increases in temperatures and rank decreases in pupal stage durations among the rearing environments (Env): R = room; G = greenhouse; I = incubator. Note that the rank increases in duration were similar for oval and larval environments.

FIGURE 8.6 (opposite). Differences in mean ±SD pupal durations and temperature among egg and larval rearing environments: (a) solid shading shows the respective pupal stage durations of animals reared as eggs under each environment, line shading the durations associated with rearing as larvae under reach environment; (b) The mean temperatures ±SD experienced by eggs and by larvae reared under each environment: R = indoors, G = greenhouse, I = incubator. For further explanation see text.

control range, prepharate development can 'buffer' against the prolonging effect of cooler temperature. In other words, it appears that cold shock temperatures exceed the limit of prepharate canalisation. Yet pharate duration showed a stronger concordance with prepharate control range temperature than did the prepharate stage.

That life cycle duration decreased with A_i but increased with A_iP is most likely due to the magnitude of the decrease with A_i being much less than that of the increase with A_iP. Indeed, the direction of the relationship was ascertained using spearman rank correlation which in itself was, in fact, non-significant ($P>0.05$). The prolongation with A_iP most likely reflects the additive component of pupal treatment (whose own effect increased with A_i).

Adult longevity also increased with assimilation. This was not an artifact of prepharate temperature (control or cold range) because longevity decreased at cooler temperatures. Indeed when adult longevity was compared for each treatment class, it was greatest in the foil controls. It did however increase with cooler pharate temperature, and it may reflect a slowing of metabolic rate that continues in the adult. Adult longevity is known to be limited by their activity (Goddard, 1962).

The following scheme is proposed. Cooler temperature slows pupal development rate; and this has two components. The first is an immediate slowing of development rate which can be buffered within the control range. Cold shock, however, exceeds the limit of this canalisation, a canalisation which further breaks down with assimilation. Hence the increased sensitivity to cold, and the assimilated prolongation of prepharate development even in the absence of cold shock. The second is a slowing of development rate in the offspring but *not* the immediate generation. That it can prolong the duration of even distal stages (even adult longevity) suggests that development rate is determined early on.

(ii) *Survival*

The most significant finding is that none of the survival parameters, including hatchability, were affected by A_i or A_iP. Nor were any of them affected by pupal treatment.

Sample size, however, did decrease with A_i. In accord with this, sample sizes were smaller with cooler prepharate temperatures within both control and cold shock ranges. This suggests that cold shock exerts a lethal effect that becomes manifest as assimilation ensues; and which might be exacerbated by inbreeding (below). However, sample size *increased* with cooler pharate temperature. This suggests that cold shock interacts with some determining event that improves survival later in the immediate generation; concurring that the lethal effect of A_i is *early* in pupal development.

It is possible that the prolonged pupal durations with A_i reflect an assimilated decrease in metabolic rate during the pupa. This might actually help the *pupae* survive the cold shock (Masaki, 1980); and could explain why sample size did not decrease with A_iP. Effective sample size is given by

$$\frac{4N_m\ N_f}{N_m\ +\ N_f}$$

where N_m is the number of males in the sample and N_f is the number of females (Falconer, 1981); and hence deviations from an equal sex ratio can be important.

The proportion of females in the samples decreased markedly with A_iP but *not* A_i or pupal treatment alone. This suggests that there is an assimilated increase in sensitivity to cold shock which exacerbates its potentially lethal effect (note that in the first generation (chapter four) there was no difference among treatments in pupal survival); and that females are more sensitive than males. Indeed, the sex ratio was closer to its normal 1:1 ratio (cf. Davies, 1978) as shock range temperature got warmer. However, the sex ratio became more equal as *control* range temperature got *cooler*. This suggests an optimum temperature for survival between the lower range of control temperatures and the upper range of shock ones. The proportion of females too improved with cooler pharate temperature.

The proportion of females also decreased with generation and

inbreeding. Since inbreeding in aegeria has more to do with genic balance of the individual than population structure as a whole (Oliver, 1981), it might destabilise development and so increase sensitivity to the effects of cold shock.

(iii) *Fecundity*

The interval between eclosion and copulation differed among the stocks, but there was no trend with the order in which they entered the protocol (Table 8.IX).

Stock	Interval (mean±SD)
01	2.1 ± 1.98d
02	1.2 ± 0.21d
03	0.8 ± 0.96d
05	0.0 ± 0.00d
06	8.2 ± 0.40d

TABLE 8.IX. The interval (mean ± SD) between eclosion and copulation in each stock overall.

The eclosion-copulation interval decreased with Ai and, to a lesser extent, with AiP. The adults might be reproductively more mature at eclosion. In Polygonia c-aureum, summer daylength and temperature in the larva cause medial neurosecretory cells in the pars intercerebralis to activate the corpora allata; and it has been suggested that these might control sex pheromone production. Indeed summer P. c-aureum mate within three days but vernal ones not for three weeks (Shapiro, 1976). Such an effect might occur in P. aegeria - indeed the males emit a chocolate like scent (Ford, 1957), and be amenable to assimilation.

Mating was sooner with cooler control but warmer shock range temperatures, suggesting an intermediate temperature optimal for pheromone production. That warmer pharate temperature resulted in

later copulation may be due to it increasing metabolic rate; this continuing in the adult so they remain active rather than settle to mate.

Egg yield increased with A_i and A_iP suggesting that cold exerts a direct and assimilated effect that improves egg production. Egg yield increased with cooling within both control and shock range temperatures. In P. c-aureum the corpora allata also promote ovarian follicle maturation throughout adult life. But if the CA are not activated within 28h post-pupation (as under vernal conditions) then ovarian diapause ensues (*ibid.*). The promotion of ovarian maturation is most likely mediated by ecdysone, as this controls most metamorphic development (Nijhout & Wheeler, 1982). That cold shock and assimilation, however, *increased* egg production, could be explained by the post-shock ecdysone surge (chapter six). It is speculated that should the prolongation of the life cycle under cold winters become assimilated (possibly leading to univoltinism), the greater egg yield would enable the species to maintain its numbers (in much the same way as Swedish univoltine populations undergoing larval aestivation result in larger adults that lay more eggs, Wiklund *et al.*, 1983).

Cooler pupal temperatures, in particular the *pharate*, were associated with better hatchability amongst hatching batches. This suggests that in addition to its effect on egg *production*, it improves egg *maturation*; which is facilitated by cooler temperature *subsequent* to shock application.

Hatchability of hatching batches did, however, decrease with greater A_iP values although *not* with Ai or cold shock *alone*. It would appear that just as cold has a potentially lethal immediate effect but a beneficial later (offspring) one on the adult, so too does it have a potentially lethal immediate effect and a beneficial later one on the egg. Indeed, hatchability over *all* batches actually *decreased* with colder shock temperature, ie., the number of batches where none of the eggs hatched increased with colder shock temperature. And just as with the adult, both effects on the egg are assimilated. To summarise, cold shock improves egg *production* via the ecdysone surge; then given that they have been produced, cold can exert an immediate lethal effect on the eggs; but if they escape this lethal effect then cold improves their following maturation. It is possible that this change in maturation remains throughout their (the F_1) subsequent development,

thus accounting for the large number of assimilated changes in stage durations and dimensions. Indeed, their trends with A_i imply *faster* growth. While cold shock might diminish the adults' ability to copulate, that, total hatch failures apart, hatchability was greater following cold shock would argue against it interfering with fertilisation *per se*.

That the number of pairings became fewer as A_i and A_iP increased reflects the smaller sample sizes and greater disparity in the sex ratio, so with fewer potential pairings available; although males were sometimes mated more than once (Fig. 8.2).

The longer oviposition period with increasing A_i and A_iP most likely reflects prolonged adult longevity, since oviposition duration increased with longevity ($R_{(9)}$ =0.4604; Kendall-W =0.6400, $Chi^2_{(1)}$ =6.400, $0.01<P<0.05$). The number of eggs laid increased with oviposition duration ($R_{(9)}$ =0.6707; Kendall-W =0.5444, $Chi^2_{(1)}$ =5.444, $0.01<P<0.05$) but *not* longevity ($R_{(13)}$ =-0.0475; Kendall-W =0.0816, $Chi^2_{(1)}$ =1.143, P-exact =0.2851ns). This too suggests that egg yield is established before oviposition, with prolonged life span giving time for more of them to be laid. The assimilated increase in oviposition duration probably results from the slower life cycle and hence greater longevity. This would explain why oviposition duration was shorter at warmer adult temperature: they are more active so die sooner. Indeed, it has been suggested that adult longevity in the species be meaured in terms of potential activity time, rather than 'chronological' lifespan (Goddard, 1962); and is commensurate with the concept of talandic time-scales (section 6.11). That oviposition duration correlated most strongly, and positively, with increasing shock range temperatures suggests that slowing is best effected by an optimum prepharate cold temperature.

That the number of hatching batches increased with A_i and A_iP results from the assimilated slowing of metabolism and the assimilated benefit of cold shock to later egg maturation. Indeed the number of hatching batches increased with cooling post-shock temperature. That fewer batches hatched as adult temperature increased, might be due to warm temperature impeding post-eclosion egg development rendering them less amenable to fertilisation, as total hatch failure is most commonly caused by inseminary failure (Oliver, 1981).

(iv) *Selection*

To examine whether the assimilated changes in development rates and linear dimensions might have been artifacts of differential mortality at each stage of the protocol, selective bias was examined using the Chi-squared test of Manly *et al.* (1972):

$$(P_1 - P_2)^2 / (Var_{P1} + Var_{P2})$$

where P_1 = mean of sample no.1, P_2 = mean of sample no.2, Var_{P1} = variance of sample no.1, and Var_{P2} = variance of sample no.2.

The test compared sample* means based on *all* animals completing the respective stages with those based only on data from animals eclosing after each of foil and cold treatments; which has two advantages over comparison with animals simply surviving to the *next* developmental stage. Firstly, it takes into account *all* factors that might contribute to bias, including non-random assignment of particular individuals to the various pupal treatments. Secondly, as it does examine such (albeit undefined) factors simultaneously, it is more likely to detect the combined effect of factors which individually might pass as non-significant or undetected. Selection could not be assessed for pharate or entire pupal durations since their measurement requires that the pupae survive to eclosion. The data and results of the Chi-squared test are presented in Appendix VI; and the results are summarised in Table 8.X.

Selection in cold shock samples is given particular attention as it could have caused directional changes in the nth+1 generations being mistaken for A_i effect (cold shock increases A_i; control treatments do not).

*according to rearing temperature within each family

	OVDUR		I1DUR		I2DUR		I3DUR		I4DUR		I5DUR		PPDUR		E1LEN		E2LEN		E3LEN		E4LEN		PLEN		PPHDUR	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
01002*	›	›	›	‹	›	›	›	›	›	‹	‹	‹	‹	›	›	‹	›	‹	‹	‹	=		‹	‹	=	=
01004*	›	›	‹	‹	›		›	›	›	›			‹	›	›	‹	‹	=	‹	‹			‹	‹	›	=
01007B	‹		‹		‹		‹		‹				›				‹	=	‹				‹		=	
01007C	›		›		**‹**		=		›		=		‹		›							›	=		=	
01010B	›		=												›		‹		‹				‹	‹	=	=
01011B	›		›		‹		›		‹				‹		›		‹		‹				‹	‹	=	=
01011C	‹		=		›		›		›		=		=		›								‹		=	
01012B	›		›	‹	›	‹	›	›	‹	›			‹		**›**	**›**	›	‹	‹				‹	‹	=	=
01012C	=		›		‹		‹		=		=		=				=		‹				=		=	
01014																							=		=	
01018																							‹	‹	=	=
02001																							‹	‹	=	=
03002	‹	‹	›	‹	=	›	›	›	›	‹			=	›	›	=	‹	›	‹	›			›	‹	=	=
03003K																							‹	›	=	=
03003L																							›	‹	‹	‹
03004K																							‹	›	=	‹
03004L																							›	‹	‹	‹
03007K																							‹		=	
06001																							‹	‹	›	›
06003	›	›	›	›	›	›	›	›	›	‹			›	‹	‹	‹	‹	›	‹	›	‹		‹	›	=	=
OVERALL	›	›	›	‹	›	›	›	›	›	‹	‹	‹	‹	›	‹	‹	‹	›	‹	›	‹		‹	›	=	=
C and E	›		›		›		›		›		‹		‹		›		‹		‹		=		‹		=	

TABLE 8.X. The direction of selection at each stage of the life cycle within each sample and over the protocol as a whole, for each treatment calss and both classes together: C = foil controls, E = cold shock; *families 002 and 004 comprise the no foil group. Postscripts following sample listing denote rearing temperature as coded in Appendix III. Direction of selection: › towards smaller value; ‹ towards greater value; = no bias; bold type indicates significant selection (Appendix VI).

The only significant selection among cold shock samples in the direction of assimilation was towards shorter length at 1st ecdysis in family 01012. The respective sample was incubator reared at 19.3°C, one of the warmest rearing temperatures, but temperature is unlikely to have been responsible since warmth *favoured* 1st instar survival (including hatchability). Nor in this instance is it likely to have resulted from poor survival of *newly hatched* (and hence shorter) larvae (Winokur, 1988), as selection favoured shorter lengths. The direction of selection for this character over *all* cold shock samples, however, favoured *longer* length, opposite in direction to assimilation (when there may have been a greater mortality of newly hatched larvae). Indeed, among cold shock samples overall, *all* length parameters showed a selective bias that was *opposite* in direction to assimilation (1st and 2nd ecdyses and pupal length), or else *neutral* (3rd and 4th ecdyses, although the latter showed no trend with assimilation). Of course, given the large number of sample comparisons, it is quite possible that the above significant result arose simply by chance.

Over the cold shock samples as a whole, all durations except that of the 2nd instar showed a selective bias in the same direction as assimilation, but *none* of the *individual* samples were significant.

8.10. Inbreeding

Egg duration decreased with greater inbreeding. The apparent decrease in egg duration over the generations may have resulted from samples over each successive generation having been reared at warmer temperatures, since egg duration became shorter with warmth. Egg duration did, however, become more *variable* over the generations (Table 8.XI).

Generation	Oval duration	n	V*	s_{v*}	Temperature
P	9.1 ± 0.07d	2	0.8%	0.40%	8.6 + 9.95°C
F_1	10.5 ± 1.16d	6	10.8%	3.12%	17.1 + 1.99°C
F_2	9.3 ± 1.63d	11	17.3%	3.69%	17.3 + 1.99°C

TABLE 8.XI. Oval duration and rearing temperature (mean + SD) at each of the first three generations (F_3 and F_4 data unavailable). The coefficient of variation (V*) of oval duration is also indicated with its standard error (s_{v*}) and sample size (n).

Pupal length appeared to decrease over the generations, but was uninfluenced by inbreeding and so probably reflects the greater A_i. It is not an artifact of decreasing temperature over the generations which would be expected to have increased pupal length (Table 8.IV).

Pharate duration showed a highly concordant increase with inbreeding and the trend over the generations reflects this (Table 8.XII). It is possible that inbreeding might facilitate assimilation. Indeed, pharate duration became less *variable* over the generations, which, with the prolonging effect of assimilation, could effect the canalisation of prolonged pharate duration. It is suggested that since genetic load in the species relates to genic balance of the individual (Oliver, 1981), inbreeding may render development even more susceptible to the destabilising influence of cold shock, and so more likely to evoke some *alternative* pathway; whose *own* canalisation might be facilitated by any further, and concomittant, inbreeding. This might be an example of what Rechenberg (cited in Wagner, 1981) called

Generation	Pharate duration	n	V*	s_{V*}
P	1.8 + 1.08d	5	63.0%	19.92%
F_1	2.3 + 0.52d	6	23.6%	6.81%
F_2	3.1 ± 0.89d	7	29.7%	7.94%
F_3	3.0 ± 0.58d	4	20.5%	7.25%

TABLE 8.XII. Pharate pupal duration (mean ± SD) at each of the first four generations (F_4 data unavailable). The coefficient of variation (V*) is also indicated with its standard error (s_{V*}) and sample size (n).

a 'genetic gear' - which relates the phenotypic response of functionally related characters to genetic changes.

Initial family size decreased with generation. This probably resulted from the combined effects of inbreeding *and* assimilation, since initial family size showed no trend with inbreeding (although the latter increased with generation) while it increased with assimilation (but which showed no trend with generation). Initial family size did, however, become less *variable* over the generations, which would suggest at least *some* contribution of inbreeding (Table 8.XIII). It must be remembered that initial family size reflects the number of eggs *produced* and so the inbreeding coefficient of the *previous* generation.

The survival of first instar larvae (extra-oval first instar) decreased with inbreeding; and it also appeared to decrease over the generations. However, it showed no concordance with hatchability (intra-oval first instar survival) (Kendall-W = 0.1111, $Chi^2_{(1)}$ = 1.6667, P-exact = 0.1967ns), suggesting that inbreeding renders larvae more susceptible to some aspect of the early hatch environment (see below) rather than to (if any) *inherently* lethal loci. Second instar survival, however, *increased* with inbreeding, suggesting that inbreeding indeed renders 1st instar larvae more susceptible to environmental (rather than ontogenetic) selection - albeit unbiased with respect to size or duration; after which relative survival *increases* with inbreeding. The latter phenomenon, in contrast to

	Initial family size			
Generation	mean ± SD	n	V*	sV*
P	210 ± 155	6	76.9%	22.20%
F_1	44 ± 33	14	80.4%	15.19%
F_2	38 ± 19	13	50.1%	9.83%
F_3	37 ± 9	4	25.8%	9.12%
F_4	18 ± 0	1	0.0%	0.00%

TABLE 8.XIII. The number of eggs (mean ± SD) starting the families at each generation. Its coefficient of variation (V*) is also shown with the standard error (s_{V*}) and sample size (n).

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might be regarded as inbreeding facilitation, which, together with the genetic gear and selection could result in very rapid evolution (cf. Wagner, 1981).

First instar larvae may be particularly susceptible to changes in regime, since rearing environments were varied during the protocol to ascertain the optimum regime. Morton (1981), for example, reports an initially high mortality among 1st instar aegeria larvae in the first generation of rearing on artificial diet. (The decreased survival of 2nd instar instar larvae with *generation*, however, most likely reflects the increasing temperature as 2nd instar survival is better at a cooler temperature).

Prepharate survival decreased with inbreeding in *cold shocked* samples *only*. This suggests that inbreeding exacerbates the immediate lethal effect of cold shock, concurring with the hypothesis that inbreeding increases sensitivity to environmental stimuli. Moreover, pharate survival was unaffected by inbreeding, even with cold shock, supporting the contention that cold has an immediate lethal but a delayed prolonging effect. The decrease in overall pupal survival is most likely due to the decreased prepharate survival.

Sample size and the proportion of females therein decreased with greater inbreeding, suggesting that females are more sensitive than

males to the lethal effect of cold. Table 8.XIV shows sample and family proportions of females at each succesive generation (which provides an indication of inbreeding effect: see Table 8.V). Although the latter showed no *significant* relation to inbreeding, the trend was very similar to that for samples, indicating that samples were representative of the families. The decrease in female proportion can be considered an increase in female mortality; where variability *increased* with inbreeding. Thus it appears that there is a tendency to de-canalise the *lethal* effect of cold, and this has important implications for the full assimilation of *beneficial* cold shock effects (such as greater egg production and hatchability). The decrease in female proportion leads to an asymmetry in the breeding structure. Since males, but usually not females, are polygamous (Wiklund & Persson, 1983), a shortage of females is likely to be more serious than a shortage of males. The decreasing proportion of females with inbreeding and the decline in initial family size over the generations is the most likely cause of lineage extinction (Fig. 8.2). Yet it is conceivable that with a large population, however, the lineages most canalised against this effect of inbreeding and assimilation might remain, and so be amenable to further assimilation (and canalisation) of their *beneficial* effects. Yet even with small populations (such as in the current protocol), the least affected samples from within the range of *variability* of sex-ratio disparities could be amenable to such beneficial assimilation.

That the number of egg-laying pairs decreased with inbreeding suggests that it leads to a decline in egg *production*. The number of batches hatching per egg-laying pair, however, *increased* with inbreeding. As with assimilation, it would appear that having 'escaped' the decline in egg production, and then having also 'escaped' the immediate lethal effect, inbreeding results in *improved* egg maturation.

The above three fecundity parameters all responded similarly to increased inbreeding as to increasing assimilation. It is therefore suggested that inbreeding and assimilation may act synergistically; components of genetic load are certainly known to. For instance, in Drosophila virilis it was found that at high levels of inbreeding the genetic load was greater than expected from simple additivity of the load components (Kosuda, 1972). It is conceivable, therefore, that

Generation	P.F.	(P.F. -.50)	n	V*	s_{v*}
Family					
P	0.39 ± 0.284	-.11	7	75.4	20.15%
F_1	0.59 ± 0.284	+.09	5	50.5	15.97%
F_2	0.23 ± 0.190	-.37	12	84.3	17.21%
F_3	0.06 ± 0.058	-.44	2	100.0	50.00%
Sample					
P	0.41 ± 0.316	-.01	15	60.4	14.50%
F_1	0.53 ± 0.339	+.03	11	78.8	16.80%
F_2	0.23 ± 0.224	-.27	19	98.7	27.49%
F_3	0.06 ± 0.056	-.44	3	100.0	40.82%

TABLE 8.XIV. Family and sample proportions of females (P.F.) (mean±) at each generation (F_4 adult data unavailable) and their deviations from 0.50 (significant figures only are shown). The variabilities (V*) of the female proportions are reported with their standard error (s_{v*}) and the number of families or samples representing each generation (n).

having escaped their immediate lethal effects, synergism between inbreeding and assimilation could amplify their *beneficial* effects.

Genetic load was estimated for stocks 01 and 03 (estimates were not made for stocks 02, 04 or 06 for which hatchability of their parental generations was unavailable; nor stocks 05 or 07 as hatchability = 0.000). Estimates were based on the mean hatchabilities of the stock 01 F_1 (n=2) and F_2 (n=4); and in stock 03 on F_1 family 002 and the mean of its F_2 families (n=3). All estimates of the variances of the genetic loads substituted $M_1 = 1$ into the Freire-Maia & Freire-Maia (1964) equation.

Genetic load in stock 01 was estimated as 0.156 ± 0.0155 LE per zygote; that of hybrid stock 03 as 0.709 ± 0.5504 LE per zygote. Both these values are lower than that estimated by Oliver (1981) for a population from North Hampshire (1.406 LE per zygote). Assuming the stock 04 parentals (subspecies aegeria) to have had 100% hatchability (F_1 hatchability = 0.900), then load in the stock would have been not

more than 0.421 LE per zygote; and within a similar range to that of the ssp. tircis stocks above.

The hatchability of stock 03 family 002 (= 0.857) was higher than that of parent family stock 01 012 (= 0.755); though it may simply represent a value intermediate between those of stock 01 and (hatchability unknown) stock 02. On the other hand, the mean hatchability (over hatching batches) of the stock 03 F_2 (= 0.714) was lower than that of the stock 01 F_2 (= 0.832); but this is not due to stock 03 having been reared at lower temperatures than stock 01 since hatchability improved with cooling (Table 8.IV). It is therefore not possible to ascertain the nature or degree (if any) of heterosis.

8.11. Temperature relationships

(i) *Development rate*

The durations of the egg, 1st and 2nd larval instars decreased with warmer egg temperature, but 3rd instar duration was *not* affected by egg temperature (Table 8.IV). Development to completion of the 2nd instar may belong to one dynamic , 3rd instar development to another (indeed the first two instar durations appear to have been affected more by oval than larval temperature while the exact probabilities with post-2nd instar stages were similar for both temperature). 4th instar and prepupa duration also decreased with warmth.

Prepharate duration decreased with warmer *larval* temperature in particular (even more significantly than with warmer prepharate control temperature), but entire pupal duration (which includes the pharate stage) decreased more significantly with warmer prepharate than larval temperature (Appendix V.iii). These findings suggest that each stage is most infuenced by temperature during the preceding stage. (Pharate duration decreased with warmer larval, prepharate and pharate temperatures).

Length at 1st and 2nd ecdysis and pupal length were shorter at warmer temperatures (here the exact probabilities with the first two larval stages *were* similar for egg and larval temperature). Thus the shortening durations of the 1st, 2nd and 4th instars are associated

with shorter lengths at their completion (pupal length is essentially that post-4th instar since 5-instar larvae decreased in frequency with warmth and were often absent, Table 8.XXII). But length at 3rd ecdysis *increased* with warmer larval temperature, even though 3rd instar duration decreased.

It appears that as temperature increases, linear growth in the 1st and 2nd instar proceeds along the same linear dynamic only *faster*, so that the first two ecdyses occur sooner. Their shorter *lengths*, however, might be explained by some parameter that relates ecdysis to growth *rate*. This parameter appears to change in the 3rd instar, whose duration is associated with a *longer* length at its completion. The latter requires that linear growth become steeper with increasing temperature, in effect pulling the prepupational deviation towards t_o.

(ii) *The linear growth dynamic*

The prevention of ingestion as the integument approaches its elastic limit imposes metabolic stress so increasing the talandic temperature (θ_T) (section 6.7). At this point it is important to distinguish between linear growth rate,

$$dL/dt$$

where L is some metrical parameter such as length or weight, and development rate,

$$d\theta_T/dt.$$

Now integumental stress becomes greater with increasing length (L) so that:

$$\theta_T = kL \quad \ldots\ldots\ (1)$$

where k is a constant. Thus, because linear growth rate (dL/dt) increases with temperature, it is expected that the rate of increase

in talandic temperature too will increase with absolute temperature (θ_A):

$$d\theta_T/dt = k'\theta_A \quad \ldots\ldots\ (2)$$

This could explain why development rate increases with absolute temperature. Development involves the system continually attempting to minimise θ_T, this cooling rate increasing with the talandic temperature differential ($d\theta_T/dt$), so that, from (2), as absolute temperature increases, so does the rate of increase in structural complexity. This could account for the shorter prepupal duration at warmer temperature.

That maximum larval length is constrained by the elastic limit of integument implies that elasticity (e) decreases with length, *ie.*

$$e = k''(1/L)$$

$$= k''/L$$

(with k'' a constant), a decrease that may be disproportionate so that

$$e = k''/L^x$$

$$= k''(L^{-x}) \quad \ldots\ldots\ (3)$$

until it reaches zero at maximum length (L.max), when

$$e_{L.max} = 0.$$

Thus, as L.max is approached, an increasing proportion of energy that would otherwise be input to linear growth becomes stored as integumental contractile energy, which might explain the hysteresis (chapter three). This might also explain the shorter pupal length at warmer temperatures, the larvae having undergone more of their linear growth (and so nearer L.max) at the ultimate ecdysis.

Since pre-pupational linear growth becomes steeper with increasing temperature, and since $d\theta_T/dt$ tends to zero as L.max is approached, the

rate at which (2) itself changes, ie.

$$d(2)/dt$$

$$= d(d\theta_T/dt)/dt$$

$$= d^2\theta_T/dt^2$$

also increases with temperature. Thus

$$d^2\theta_T/dt^2 = k'''\theta_A \ldots\ldots\ldots (4)$$

A corollary of this is that as temperature increases, development can be expected to become more unstable and hence susceptible to perturbations such as cold shocks. Indeed, in Aglais urticae (L.) (Nymphalidae) (Winokur, unpublished data) pupae reared at >30°C produced more extreme phenocopies when given cold shocks identical to those applied to pupae reared at 15-25°C. Furthermore, their prepupal duration was shorter and mortality among control pupae greater at the warmer rearing temperature. Expression (4) suggests that still higher temperatures may sufficiently destabilise the system as to be in effect a shock itself. Indeed, phenocopies appeared in A. urticae reared without pupal cold shock at nearly 45°C (L. Winokur, unpublished data).

The precise dynamics of equations (3) and (4) will depend, of course, on the values of k, k', and x in equations (1) and (2) and L.max. That larvae of P. aegeria (and A. urticae) reared at higher temperatures pupated at shorter lengths would appear to refute the postulate that pupation ensues only when linear dimension reaches a *fixed* L.max *viz.* L.max as ascertained at the cooler temperature. Rather L.max may itself depend on linear growth rate, decreasing with warmer temperature. If elasticity too is a decreasing function of the rate of *change* in length, ie.

$$e = k''''.(dL/dt)^{-y}$$

where y is some power, then as temperature, and hence growth rate, increase, e will equate to zero at increasingly shorter lengths.

That pupation in Manduca sexta, however, depended on a 'pre-coded' threshold of absolute larval size (Nijhout, 1975), led Nijhout (*loc. cit.*) to propose an allometric size-monitoring system involving stretch-receptors whose rate of neural firing increased with integumental stretching. It is now suggested that in P. aegeria (and possibly other species), integumental growth involves the breakage and rejoining of molecular cross-links of given inherent flexibility, such breakage-rejoining occuring at a constant rate. Thus, as linear growth *rate* increases, breakage-rejoining alone becomes less able to accommodate increasing length, so that at warmer temperature the strain placed on the elastic component increases much more quickly with absolute length and, hence, reaches its limit (L.max) at a shorter length. Indeed, in M. sexta the growth *ratio* (as measured by head capsule size) was constant between each ecdysis (*ibid.*).

The change from larval to pupal moult at L.max could result as follows. Assuming a constant growth *ratio* as measured by larval mass, then the *rate* at which absolute mass increases will itself increase with mass, *ie.*

$$dm/dt = Km$$

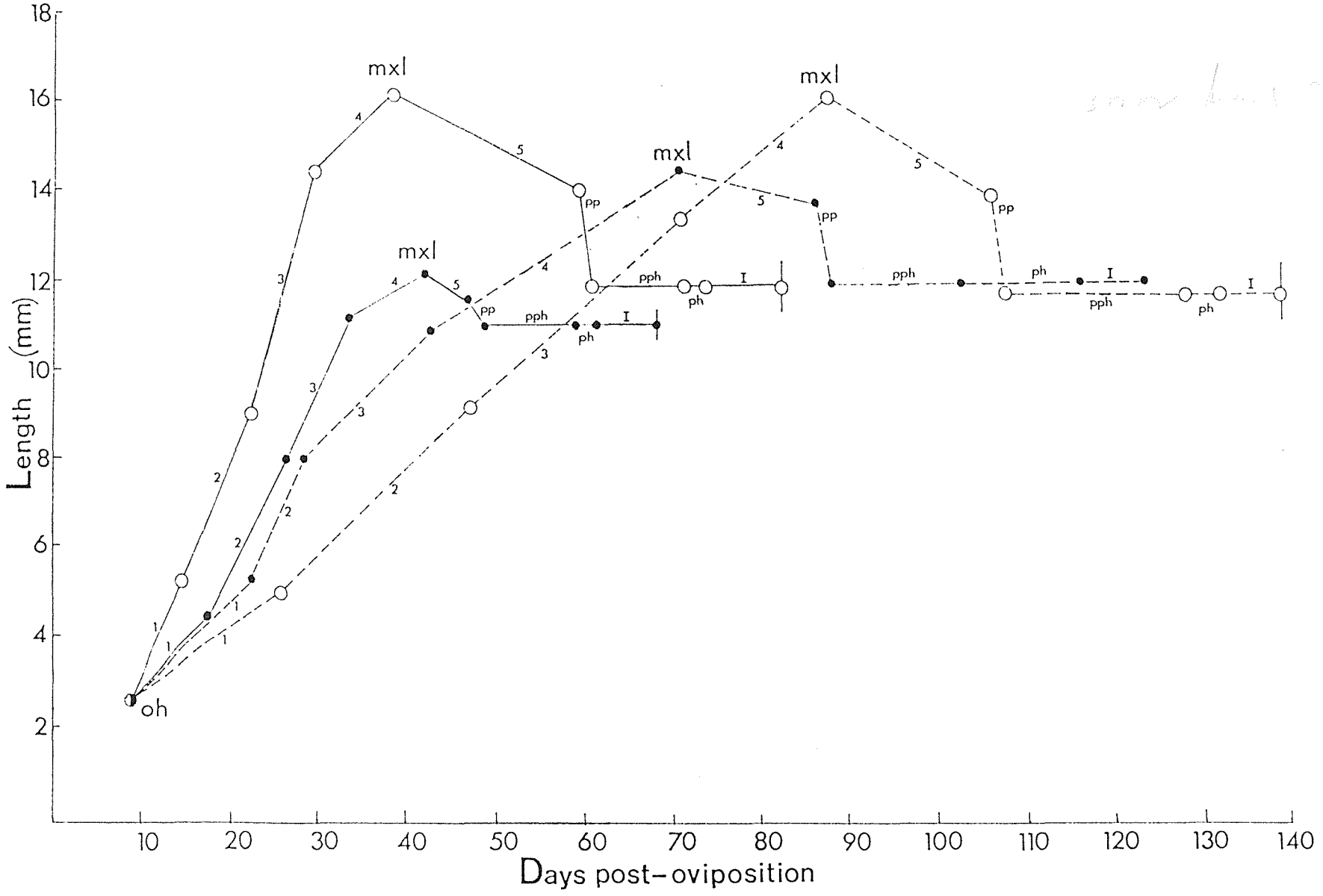
(where K is a constant). Now, linear growth concerns mainly integumental area and, since

$$\text{mass} = K'\text{area}^{1.5} \text{ (approx.)}$$

(with K' a constant), an increasing proportion of linear growth is enabled by integumental elasticity of the integument. It is possible that such strain might not appear *until* bulk has reached a certain level, which could account for *minimum* sizes for pupation as in M. sexta (*ibid.*).

The linear growth dynamic (LGD) was plotted for STOCK 01 families 007, 011 and 012 for samples reared at 19.3°C and 14.8°C respectively (STOCK 03 families 003, 004 and 007 are excluded here as only pupal data is available). Families 007 and 012 are grouped as A_i = 1.000 in both cases. With family 011 A_i = 0.000. The linear growth dynamics are shown in Figure 8.7.

With A_i = 0.000 (Fig. 8.7a) it was found that the post-hatch


Length (mm)
Days post-oviposition
oh
mxl
mxl
mxl
mxl
pp
pph
ph
I

larval dynamic was indeed steeper at 19.3°C than at 14.8°C, but that once the larvae had reached 14.2mm the forms of the dynamics to the pupal moult were similar in both cases - including L.max, prepupal duration and pupal length. This suggests that although the higher temperature induces faster linear growth it is not sufficient to exceed the limit of integumental elasticity. This might be expected given that 19.3°C is within the temperature range experienced by the species in Britain (Lees & Tilley, 1980). This temperature did however pull the prepupational peak towards t_o, the time taken for the larvae to reach L.max and then pupate (30.5d and 52.5d respectively) being about half that at 14.8°C (79.5d and 99.5d). Similar effects were found with samples of A_i = 1.000 (Fig. 8.7b), the time taken for the larvae to reach L.max and then pupate at 19.3°C (33.5d and 40.5d respectively) again being about half that at 14.8°C (62.5d and 79.5d). Figure 8.7 indicates some interaction between the influences of temperature and assimilation coefficient on the LGD (below).

FIGURE 8.7 (opposite). The influence of assimilation and temperature on the linear growth dynamic. The linear growth dynamics of STOCK 01 F_2 samples of A_i = 0.000 (open circles) and A_i = 1.000 (closed circles) each reared at 14.8°C (dotted line) and 19.3°C (continuous line) are shown. Life cycle stages are indicated thus: oh = oval hatching; pp = prepupa; pph = prepharate pupa; ph = pharate pupa; I = imago, vertical bars indicated adult longevity. Larval instars are numbered 1-5 respectively, mxl = maximum measured length. For further explanation see text.

(iii) *survival*

The survival of all stages *except* the 2nd and 3rd instar *increased* with temperature. This suggests that the insects are more intolerant of cooler rearing temperatures. However, the coolest rearing temperature was 13.9°C 2.87°C (Appendix III.ii), yet the species can undergo the entire life cycle at 11°C with *no* decrease in survival (Wiklund & Persson, 1983). Moreover, the species is actually commoner in wet seasons when daytime temperature is cooler (but when the cloud cover affords warmer nights) (Lees, 1962). Thus, the above results probably reflect some secondary effect of cooler temperature, most likely that the slower development rates prolong the risk of exposure to potential infection.

8.12. Further effects of cold shock and assimilation

(i) *Pupal blotching*

Of 64 cold shocked pupae of STOCK 06 parentals, 8 were found to have developed dark brown to black abdominal blotches. These were variable in size and random in position but not present in any of the 45 control pupae. The frequencies of blotched pupae in each treatment class were compared with their expected frequencies ($f\hat{}$) using the G-test (Sokal & Rohlf, 1981) and the following results obtained: controls: $f\hat{} = 3.303$; cold shock: $f\hat{} = 4.697$; $G_{(1)} = 8.520$, $P \leq 0.01$; $G_{adj(1)} = 8.019$, $P \leq 0.01$; $G_{Yates(1)} = 5.132$, $0.01 < P \leq 0.05$. The result was significant even with the highly conservative Yates correction. Corrections reduce the likelihood of TYPE-I error, *ibid.*.

All blotched pupae eclosed so blotching is not associated with mortality. The cold shock sample eclosed to yield 27 males and 19 females of which four of each sex derived from blotched pupae; blotching frequency did *not* differ between the sexes ($G_{(1)} = 0.246$ns).

Low temperatures are known to promote melanin sythesis in other insects (Needham, 1974). In the fly Corethra, cold induces the production of a darkening hormone which it has been suggested may be

neotenin from the corpora allata, since neotenin analogues cause excess pigmentation in locust embryos. Now neotenin counteracts ecdysone which *inhibits* melanin synthesis (*ibid.*). Thus direct cold-inactivation of ecdysone receptors (chapter seven) could also explain the pupal blotching.

(ii) *Assimilation and the linear growth dynamic*

That increasing levels of assimilation prolonged the 1st and 2nd instars but shortened the egg, larval (including 3rd instar) and prepupal durations, would suggest that assimilation too results in a steeper LGD subsequent to the 3rd ecdysis. Thus assimilation might increase susceptibility to cold shock in a similar manner to increasing temperature. The actual growth dynamics obtained with each A_i = 0.000 and A_i = 1.000 were therefore compared (Figure 8.7a-b).

At 14.8°C it was found that linear growth up to the 3rd ecdysis was steeper with A_i = 1.000 than A_i = 0.000 but that 2nd and 3rd ecdysis and L.max occured at shorter lengths. The prepupational deviation was pulled towards t_o when L.max and pupation occured 17 days and 20 days sooner respectively with A_i = 1.000. But pupal duration (28 days) was 3.5 days *longer* with A_i = 1.000. Hence there appears to be some correction (albeit slight) for the decreased larval duration at A_i = 1.000, in accord and with the notion that stage durations are regulated relative to one another (section 6.14).

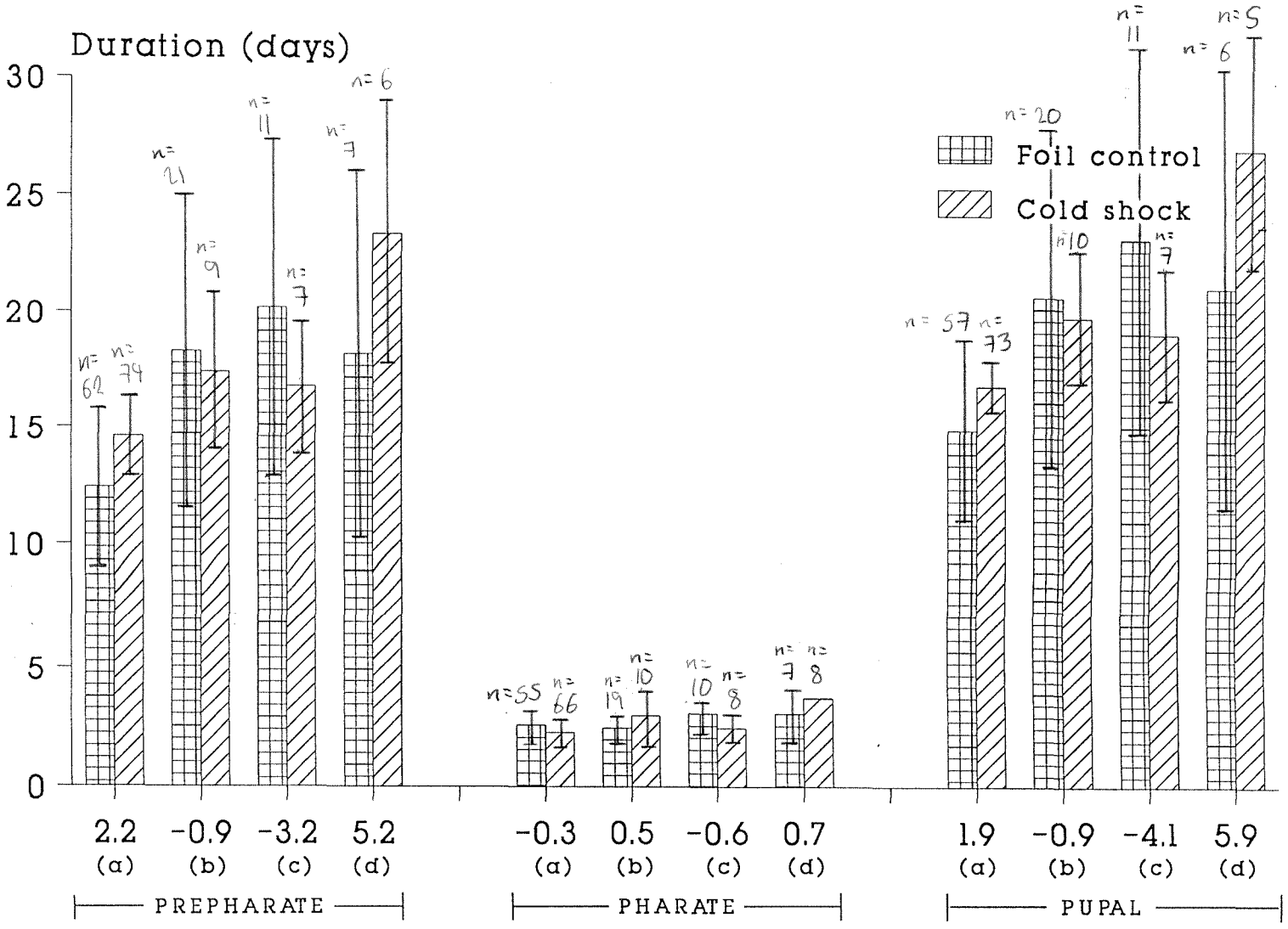
At 14.8°C linear growth between the 3rd ecdysis and L.max was *less* steep with A_i = 1.000 (the shorter L.max perhaps due simply to the 3rd ecdysis having occurred at a shorter length). But *pupal* length was now *greater* (albeit marginally so), supporting the contention that linear growth *rate* to L.max is a factor in determining pupal length. The stage during which larval length corresponded to that of the pupa was the 3rd instar with A_i = 0.000 but the 4th instar when A_i = 1.000. This suggests that correspondence occurs at a given *relative* position along the LGD (about two-thirds from hatching towards L.max) irrespective of the *particular* instar.

(iii) *Assimilation and temperature*

The effect of A_i = 1.000 at 19.3°C in comparison to A_i = 1.000 at 14.8°C (Fig. 8.7b) was that L.max and pupation occured both earlier and at shorter lengths. Its effect in comparison to A_i = 0.000 at 19.3°C (Fig. 8.7a-b) was similar except that L.max was shortened but not attained sooner. It is suggested that there is a limit to which L.max may be shifted towards t_o whether by temperature or assimilation. Thus, the higher temperature having already pulled L.max to this limit, increasing A_i to 1.000 has no further such effect. This also implies that once L.max has reached its early limit, the larval dynamic can not be steepened either. Indeed the larval dynamic was, if anything, *less* steep with A_i = 1.000 than A_i = 0.000.

Figure 8.8 shows that increasing A_i from 0.000 to 1.000 produced a much more drastic decrease in L.max (-3.8mm) than in pupal length (-0.7mm). The shallower dynamic with A_i = 1.000 may mean less contractile energy being stored in the integument for the post-L.max contraction (section 6.7). The decrease in L.max with assimilation (at 14.8°) and then warming (Fig. 8.7a-b) may be due to changes in the efficiency of the integumental *break-rejoin* process so that strain becomes *imposed* on the elastic component at progressively shorter lengths, while elasticity *per se* is unaltered so that the post-L.max dynamic (pupation) is not steepened. If anything, it became shallower with progressive assimilation.

Increasing assimilation appears to shorten the duration of the prepupational deviation (as measured by the interval between corresponding larval and pupal lengths) with a concomitant shift towards t_o, but that once the early limit of L.max is reached the prepupational deviation is truncated from the t_o side. With linear growth also becoming less steep, the net effect of increasing assimilation (and then temperature) is that the prepupational deviation is scaled down.


Duration (days)
30
25
20
15
10
5
0
Foil control
Cold shock
2.2
(a)
-0.9
(b)
-3.2
(c)
5.2
(d)
PREPHARATE
-0.3
(a)
0.5
(b)
-0.6
(c)
0.7
(d)
PHARATE
1.9
(a)
-0.9
(b)
-4.1
(c)
5.9
(d)
PUPAL

(iv) *Assimilation and pupal cold shock*

The durations of the prepharate, pharate and entire pupal stages with each treatment were first examined within each of four ranges of A_i: $A_i = 0.000$; $0.000 < A_i < 1.000$; $A_i = 1.000$; $A_i > 1.000$ (Figure 8.8). Treatment differences were compared with the Kruskal-Wallis test (Sokal & Rohlf, 1981). The only significant differences were with $A_i = 0.000$ in prepharate ($Chi_{(1)} = 6.1078$, $0.01<P<0.05$) and pupal duration ($Chi_{(1)} = 8.6159$, $0.001<P<0.01$) which were longer under cold shock. Treatment aside, there was a general increase with A_i in the durations of all the stages; and that the *difference* between treatments was greater with $A_i > 1.000$ than $A_i = 0.000$ supports the notion that cold *sensitivity* also increases with A_i.

FIGURE 8.8 (opposite). Differences between treatments in pupal stage durations at four levels of assimilation. The prepharate, pharate and entire pupal durations (mean ± SD) under foil and cold shock treatment are shown. Horizontal numbering refers to the *difference* between treatments in the respective stage duration; a negative value indicates that duration was longer in the *control* sample. The differences are shown for each (a) $A_i = 0.000$; (b) $0.000 < A_i \leq 1.000$; (c) $A_i = 1.000$; (d) $A_i > 1.000$. For further explanation see text.

8.13. Individual stocks and samples

(i) *Overview*

The durations of the prepharate, pharate, and entire pupal stages obtained with each sample are shown in Figures 8.9 to 8.11. These figures also serve as an aid to interpretation in the following sections. Sample parameters are listed in Appendix VII. In all cases treatments were compared pairwise with the t-test (2-tailed), modified as necessary to compare individuals with a sample (Sokal & Rohlf, 1981).

Samples exhibiting significant differences in any of the pupal stages are shown in Table 8.XV. It can be seen that not all samples differed significantly between treatments and that some underwent longer durations under control treatment. All *significant* differences however were towards longer duration under cold shock, and most involved the prepharate and entire pupal durations. With the exception of STOCK 03 004 reared at 17.6°C, samples with longer pupal durations under cold showed no significant differences in pharate duration. This suggests that even in assimilated lineages the prolonged pupal duration under cold is attributable to its effect on the prepharate stage, which might be expected given that pharate duration increased with assimilation alone but not when considered in conjunction with

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FIGURES 8.9 to 8.11 (overleaf). The prepharate, pharate and entire pupal durations of each family under the respective rearing temperature and treatments. Points show the means; vertical bars give the range, cases with zero range denoted by a horizontal bar. Lettering below the bars denotes pupal treatment: N = untreated, C = foil control, E = cold shock. Stocks (large numerals) and families (small numerals) are listed left to right in the sequence in which they entered the assimilation protocol: The pedigrees are shown to aid interpretation. Rearing temperatures are shown only where necessary to distinguish samples reared under different temperatures within a family: B = 19.3°C; C = 14.8°C; K = 17.6°C; L = 13.9°C. NA = data unavailable on account of mortality. For further explanation see text.

Figure 8.9

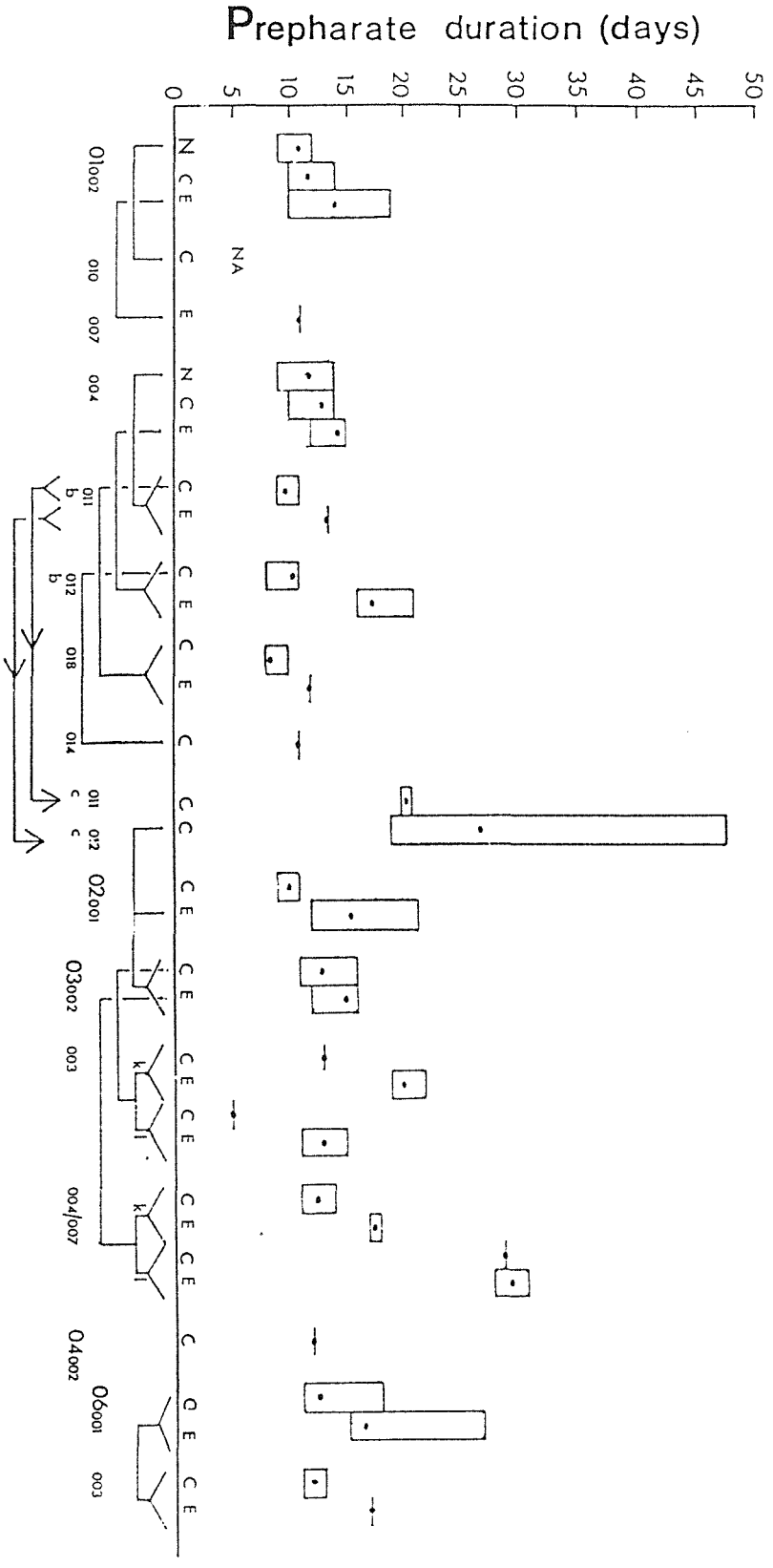

Prepharate duration (days)
0
5
10
15
20
25
30
35
40
45
50
NA
O1002
010
007
004
011
b
012
b
018
014
011
c
012
c
O2001
O3002
003
k
l
004/007
k
l
O4002
O6001
003

Figure 8.10

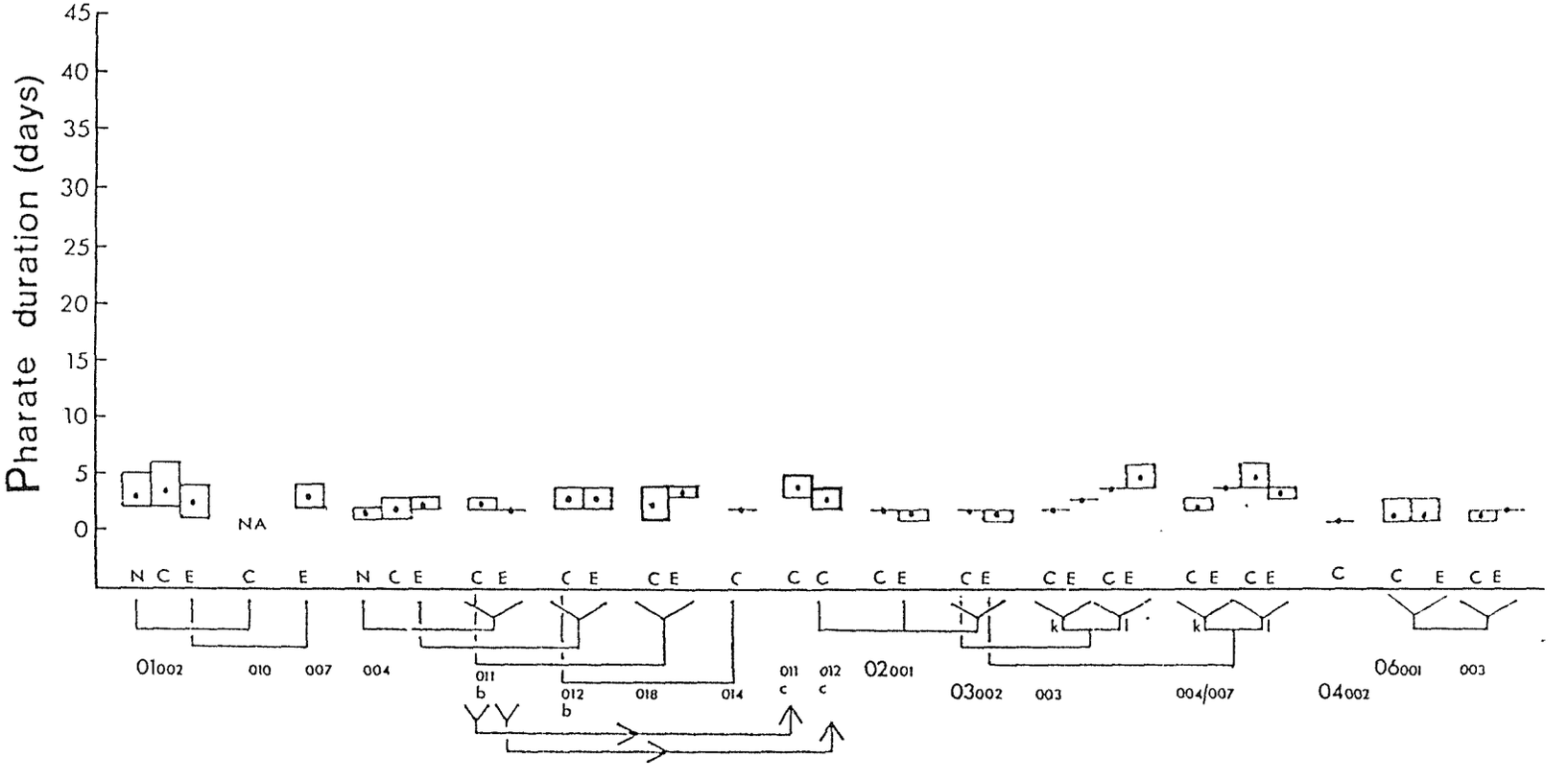
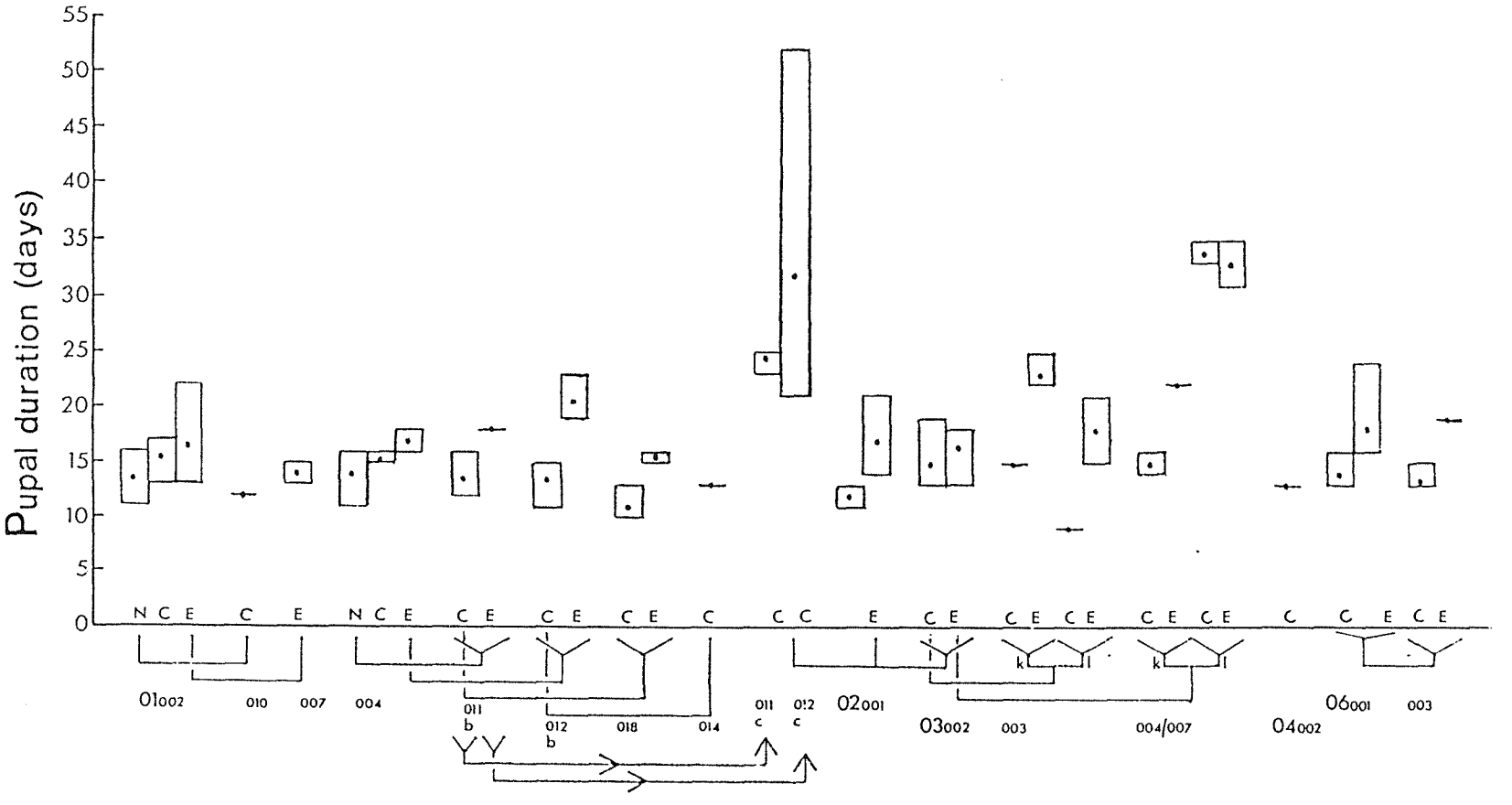

Pharate duration (days)
45
40
35
30
25
20
15
10
5
0
NA
N C E C E
N C E C E C E C E C C C E C E C E C E C E C E C E C E
01002
010
007
004
011 b
012 b
018
014
011 c
012 c
02001
03002
003
004/007
04002
06001
003

Figure 8.11


Pupal duration (days)
55
50
45
40
35
30
25
20
15
10
5
0
N C E C E N C E C E C E C E C C C E C E C E C E C E C C C E C E
01002
010
007
004
011
b
012
b
018
014
011
c
012
c
02001
03002
003
004/007
04002
06001
003

pupal treatment (Table 8.IV). This is consistent with the hypothesis that assimilation increases both immediate sensitivity to cold (manifest as prolonged prepharate duration) and pharate duration (independently of cold in the immediate generation). The prolonged pharate duration under cold in the STOCK 03 F_2 suggests that the immediate effect of cold shock may become manifest at the pharate stage only at high A_i values since, for reasons outlined below, these F_2 may in fact represent the fourth generation of treatment and so be of greater A_i than originally estimated.

Sample	PREPHARATE	PHARATE	PUPAL
01002	E ns	C ns	E ns
01004	E ***	E ***	E ***
01011	E *	C ns	E ns
01012	E **	=	E ***
01018	E **	E ns	E ***
02001	E ***	C ns	E ***
03002	E ns	C ns	E ns
03003K	E **	E ***	E ns
03003L	E ns	E ns	E ns
03004K	E **	E ***	E **
03004L	E ns	C ns	C ns
06001	E ***	=	E ***
06003	E **	E ns	E **

TABLE 8.XV. Summary of differences bewteen treatments in pupal stage durations among the samples. Two-tailed significance: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$. The treatment yielding the longer duration is shown: C = foil controls; E = cold shock; '=' denotes no difference. The respective t-values are given in Appendix VII.i. For further explanation see text. Sample sizes are given in Appendix VII.

(ii) *STOCK 01*

F_1 family 004 gave rise to F_2 families 011 (from no-foil controls) and 012 (from cold shocks). The pupal durations in 004 were compared with those in 011 and 012 (Table 8.XVI).

The pupal stage durations in 011 were no longer under either treatment than under the corresponding treatment in 004 (if anything, they were shorter). But the pupal stage durations in 012 were significantly longer under cold than in 004 under cold. This applied particularly to the prepharate and entire pupal durations whose ranges did not even overlap (prepharate duration: 004 = 13-16 days, 012 = 16-21 days; pupal duration: 004 = 16-18 days, 012 = 19-23 days). This implies an assimilated increase in cold sensitivity after only *one* generation of treatment. That the control durations in 012 too were shorter than in 004 controls strengthens the argument for assimilation (also ruling out selection for longer durations *per se*). And this not withstanding the fact that 012 (and 011) controls were foil treated (which prolongs development) while the 004 controls were not (section 7.7).

That pharate duration under cold shock was longer in 012 than 004 (Table 8.XVI) yet unaffected by immediate cold treatment in 012 (Table 8.XV) supports the contention that a propensity for prolonged development *per se*, not just increased sensitivity to cold, is inherited from the previous generation. And the similar pharate durations under both treatments suggests that its prolongation results *entirely* from this propensity. Hence the effects of even the first generation of treatment are assimilated, and this assimilation is manifest significantly after only one generation.

There was no such assimilation, however, between F_1 family 002 and either F_2 families 010 (from no-foil controls) or 011 (from cold shocks). This implicates a genetic difference or, more accurately, a difference in the inherited particulars between families. Hence there is variation within populations in the capacity of their lineages to undergo assimilation. There were no significant differences in pupal stage durations between families 011 and 012 *per se* under corresponding treatments.

	PREPHARATE		PHARATE		PUPAL	
control						
004 x 011	**	004	ns	004	*	004
004 x 012	**	004	*	012	*	004
011 x 012	ns	=	ns	=	ns	=
cold shock						
004 x 011	ns	=	ns	=	ns	=
004 x 012	**	012	*	012	***	012
011 x 012	ns	=	ns	=	ns	=

TABLE 8.XVI. Differences in pupal stage durations between STOCK 01 F_1 family 004 and its derivative F_2 families of A_i = 0.000 (family 011) and 1.000 (family 012) under corresponding treatments. Two-tailed Significance levels: * $0.01<P<0.05$; ** $0.001<P<0.01$. Note that in family 004 untreated pupae served as the controls while in families 011 and 012 foil treated pupae served as the controls. The respective t-values are listed in Appendix VIII.ii. For further explanation see text.

Controls from F_2 families 011 and 012 gave rise to F_3 families 018 (A_i= 0.000) and 014 (A_i= 0.500) respectively. The durations of each pupal stage in the F_3 were compared with those in the F_1 and F_2 under corresponding treatments (Table 8.XVII).

When controls were compared between families of A_i = 0.000 there were no significant *increases* in the F_3 relative to the earlier generations. A similar trend was found among the respective cold shocked samples (the only significant difference here being longer pharate duration in the F_3 than F_1). Nor were there any significant differences between controls in F_3 family 014 (A_i = 0.500) and in earlier generations of A_i = 0.000 (cold shock data are not available for family 014).

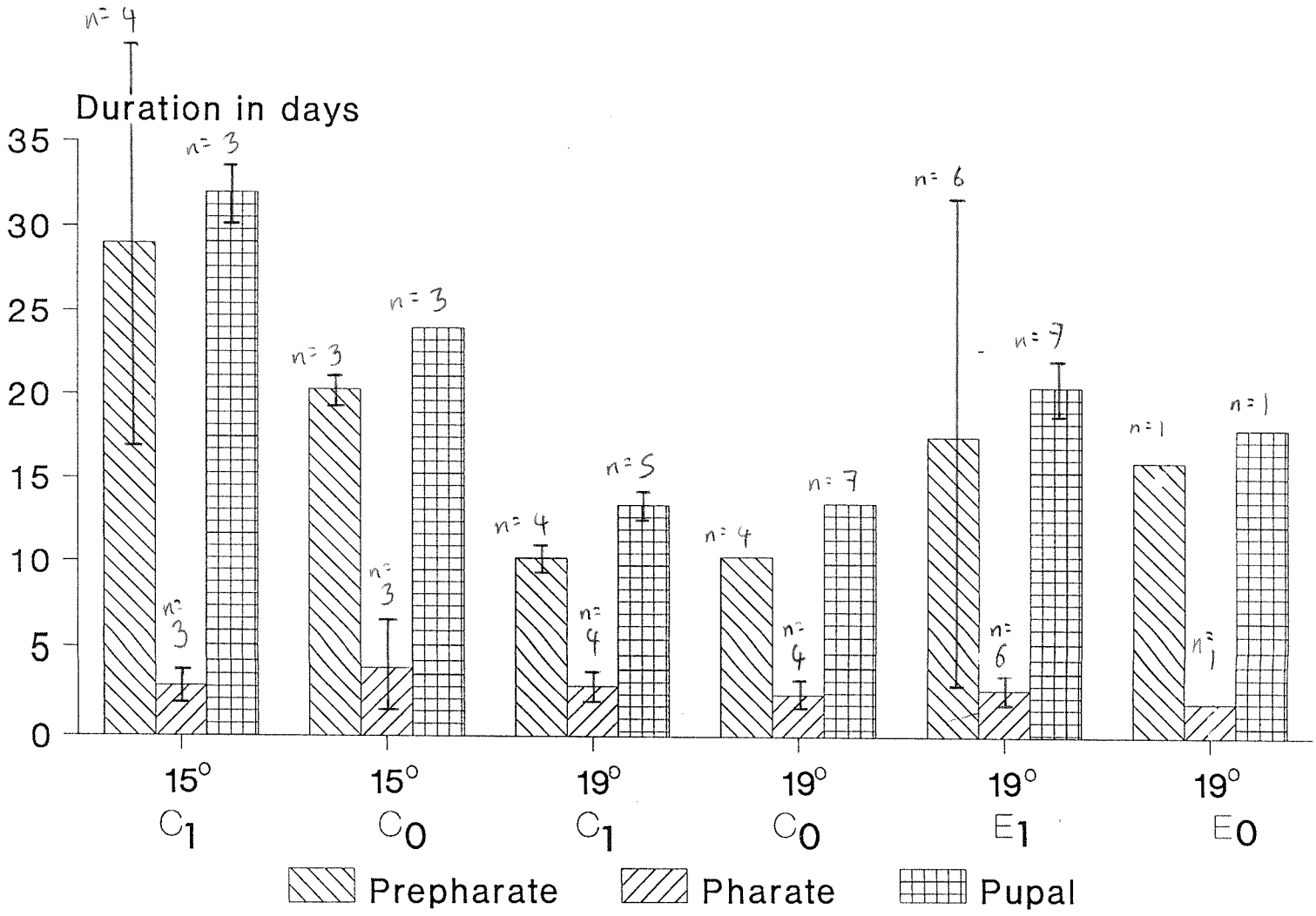
However, when controls were compared between the two F_3 families (018 and 014), it was found that prepharate duration was significantly

longer in 014 than in 018, even though both of these were derived from *control* samples, control samples that did not themselves differ in duration at that. This suggests that a propensity for prolonged prepharate duration *per se* (not just cold sensitivity) is transmitted from the cold shocked F_1 to the F_3 even *without* further cold shock in the intervening F_2 (012 controls).

		PREPHARATE	PHARATE	PUPAL
(a)				
Control:	F_3 018 x 004 F_1	*** 004	ns 018	*** 004
Control:	F_3 018 x 011 F_2	* 011	ns =	** 011
Cold:	F_3 018 x 004 F_1	** 004	** 018	* 004
Cold:	F_3 018 x 011 F_2	*** 011	ns 018	ns 011
(b)				
Control:	F_3 018 x 014 F_3	** 014	ns 018	ns 004
(c)				
Control:	F_3 018 x 012 F_2	* 012	ns 012	* 012
Cold:	F_3 018 x 012 F_2	* 012	ns 012	* 012

TABLE 8.XVII. Differences between STOCK 01 F_3 (families 014 and 018) and earlier generations in their pupal stage durations under corresponding treatments. (a) with A_i = 0.000 (DOWN-line); (b) between A_i values 0.000 and 1.000 within the F_3; (c) between F_3 of A_i = 0.000 and F_2 of A_i = 1.000. The sample of greater duration is indicated with the two-tailed significance level: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$. None of the comparisons between F_3 family 014 with F_2 families 011 and 012 or with F_1 family 004 were signifcant. The respective t-values are listed in Appendix VI.iii. For further explanation see text.

The trend under either treatment in the *unassimilated* line (004-011-018), however, was towards *decreasing* pupal stage durations. This might have resulted from the increasing level of inbreeding (with


Duration in days
35
30
25
20
15
10
5
0
n= 4
n= 3
n= 3
n= 3
n= 3
n= 4
n= 5
n= 4
n= 7
n= 6
n= 7
n= 1
n= 1
15°
C1
15°
C0
19°
C1
19°
C0
19°
E1
19°
E0
Prepharate
Pharate
Pupal

a possible concomitant drift). Such an inbreeding effect might accelerate or even bring about the extinction of a dauermodification following the cessation of treatment. Thus the extent to which the propensity for prolonged prepharate duration *per se* is actually assimilated might be stronger than suggested by its (already significant) level of *manifestation*.

The greater prepharate and pupal durations in F_2 family 012 (A_i = 1.000) than in F_3 family 018 (A_i = 0.000) most likely reflects simply its greater assimilation coefficient.

(iii) *Pupal summer diapause*

Inspection of Figures 8.10 and 8.12 shows that STOCK 01 F_2 underwent longer prepharate and pupal durations at 14.8°C than at 19.3°C, and suggests that the prolonging effect of cooler rearing temperature is greater with A_i = 1.000 (family 012) than A_i = 0.000 (family 011).

The durations of each pupal stage were thus compared between rearing temperatures at each A_i value, between control and cold shock treatment at each A_i value, and between A_i values at each rearing temperature (Figure 8.12). Similar comparisons were also made for larval duration and the entire life cycle (oviposition to eclosion) (Fig. 8.13). The results of the comparisons are summarised in Table 8.XVIII.

With animals reared at 14.8°C only control (foil) pupal treatment was applied. Cooler rearing temperature generally prolonged pupal duration, and the degree of prolongation (with respect to controls reared at 19.3°C) was greater than that resulting from cold shock alone.

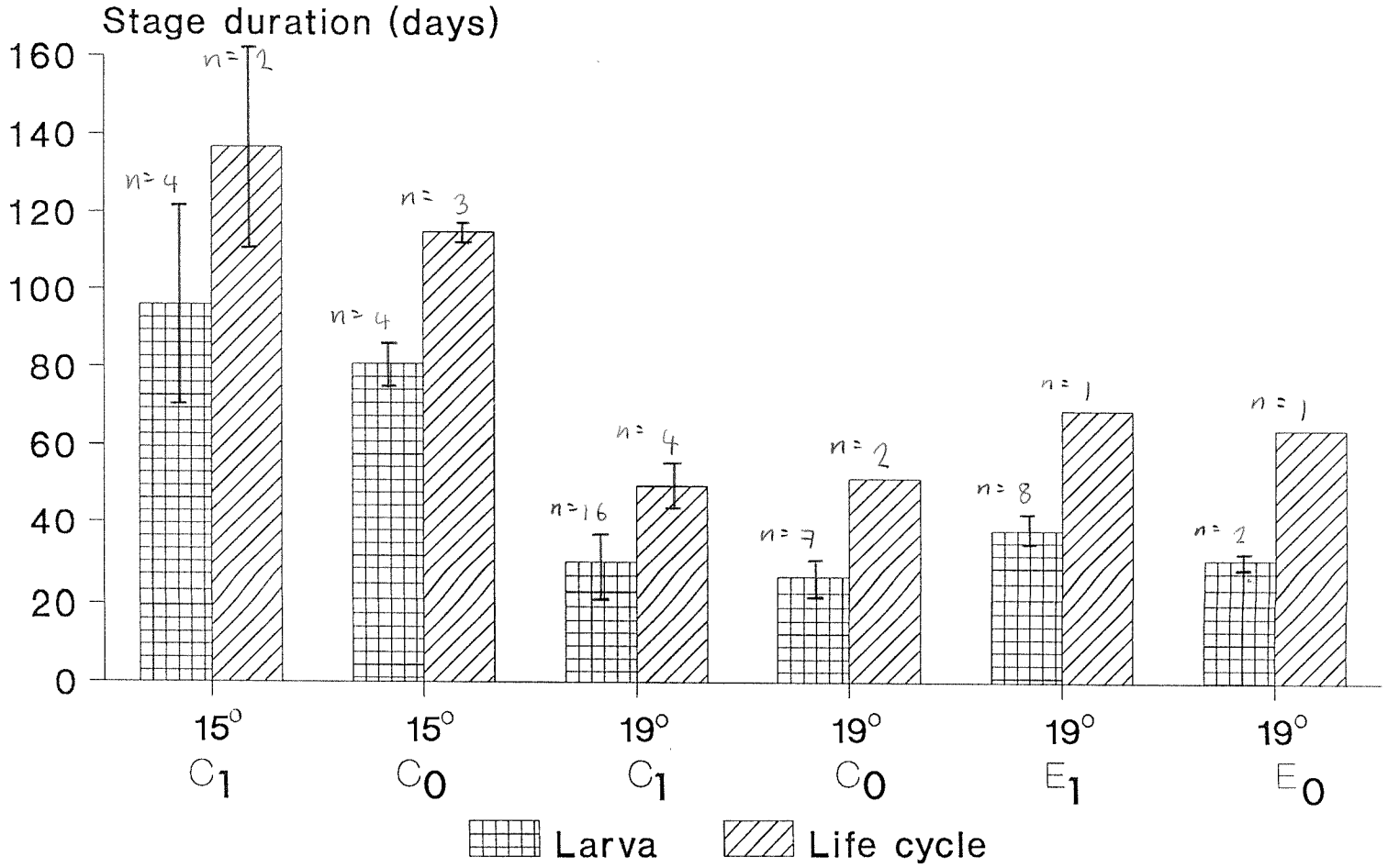
FIGURE 8.12 (opposite). Pupal stage durations (mean ±SD) of STOCK 01 F_2 samples of A_i = 0.000 and A_i = 1.000 reared at each 14.8°C and 19.3°C under each respective pupal treatment. Temperatures: 15° = 14.8°C, 19° = 19.3°C; pupal treatment: C = foil control, E = cold shock; subscripts denote Ai: 0 = 0.000, 1 = 1.000. For further explanation see text.

With animals reared at 14.8°C only control (foil) pupal treatment was applied. Cooler rearing temperature generally prolonged pupal duration, and the degree of prolongation (with respect to controls reared at 19.3°C) was greater than that resulting from cold shock alone. But the extent to which cooler rearing temperature prolonged the prepharate and pupal stage durations was greater when A_i = 1.000 (by 18.8d and 18.6d respectively) than when A_i = 0.000 (by 10.1d and 10.9d). Thus cold shock at the previous generation also increases the extent to which cooler *control*-range temperature prolongs prepharate duration.

The 012 sample (A_i = 1.000) reared at 14.8°C yielded three pupae, one of which spent 48 days (about 7 weeks) in the prepharate stage. (The prepharate durations of the other two were 19d and 20d respectively, similar to the unassimilated 011 sample reared at

		(PPH)	(PH)	(P)	(LVA)	(LFC)
Constant	Variable					
A_i=0 C	Temperature	***	*	***	***	***
A_i=1 C	Temperature	*	ns	*	***	***
19°C A_i=0	Treatment	*	ns	ns	ns	ns
19°C A_i=1	Treatment	**	ns	***	**	***
15°C C	Assimilation	ns	ns	ns	ns	ns
19°C C	Assimilation	ns	ns	ns	ns	ns
19°C E	Assimilation	ns	ns	ns	ns	ns

TABLE 8.XVIII The contributions of rearing temperature, pupal treatment and level of assimilation to differences in larval, pupal and life cycle durations in STOCK 01 F_2. Each variable was compared with the others held constant: rearing temperature = 15°C or 19°C; assimilation coefficient A_i=0 or A_i=0; pupal treatment (where applicable) = foil control (C) or cold shock (E). Stage durations: PPH = prepharate; PH = pharate; P = pupal; LVA = larval; LFC = life cycle. Two-tailed significance levels of the differences: * $0.01<P<0.05$. Respective t-values are listed in Appendix VIII.iv. For further explanation see text.


Stage duration (days)
160
140
120
100
80
60
40
20
0
n=4
n=12
n=4
n=3
n=16
n=4
n=7
n=2
n=8
n=1
n=1
n=1
15° C1
15° C0
19° C1
19° C0
19° E1
19° E0
Larva
Life cycle

14.8°C *ie.* 20.3d ± 0.5d). Now Lees & Tilley (1980) hold that a pupal duration longer than one month defines pupal diapause. Now P. aegeria can undergo *winter* diapause as either larva or pupa (*ibid.*) but summer diapause has so far been reported only in larvae (Wiklund *et al.*, 1983). Thus, it is suggested that the above pupa (012 male no.018) represents a case of *pupal* diapause, implicating a further developmental strategy for the species. Although even at 22°C (unspecified) short daylength in the fourth *larval* instar induces pupal diapause (Lees & Tilley, 1980), this can be ruled out here since larval culture at 16L:8D regime did not induce pupal diapause at 19.3°C even with Ai = 1.000, nor at 14.8°C when A_i = 0.000. Foil treatment can be discounted in this context because it was applied only at the *pupal* stage. And since it was applied to *all* the above pupae it could not have been responsible for any difference between them.

Adults that become fully developed under long but cold days may wait in the pupae for warmer temperature before eclosing (Goddard, 1962). However pharate duration in family 012 (A_i = 1.000) showed no difference with rearing temperature; which in family 011 (A_i = 0.000) was only just significant. (There was no family difference in pharate duration at either temperature.) The pharate duration of the diapause pupa (4d) was within the range of pharate durations exhibited by each STOCK 01 F_2 sample. Thus pupal summer diapause is undergone in the *prepharate* stage.

Larvae from Swedish univoltine populations can be prevented from aestivating by keeping them at just above their usual ambient temperature (Wiklund *et al.*, 1983) which in continental Europe *and* Britain is 12°C (Dennis, 1977). Thus any larval aestivation in 012 at 14.8°C is unlikely to be just the result of rearing temperature. Moreover, in the related species Lasiommata petropolitana F. there is

FIGURE 8.13 (opposite). Larval and life cycle durations (mean ±SD) of STOCK 01 F_2 samples of A_i = 0.000 and A_i = 1.000 reared at each 14.8°C and 19.3°C under each respective pupal treatment. Temperatures: 15° = 14.8°C, 19° = 19.3°C; pupal treatment: C = foil control, E = cold shock; subscripts denote Ai: 0 = 0.000, 1 = 1.000. For further explanation see text.

no obligatory association between larval aestivation and pupal hibernation (*winter* diapause). Thus the pupal diapause in specimen 01 012 018, even if it *were* associated with larval aestivation, need not be specifically the *winter* type. Nor is the specimen's prolonged pupal development likely to be just a direct cooling effect, since Shreeve (1985) reports 9.15°C as the lower threshold temperature for direct pupal development in males.

Specimen 01 012 018 is illustrated on Plate 9. The phenotype resembles a wild example of ab. Cockaynei captured by R.P. Milman on 5.xi.1932 (*cf.* Russwurm, 1978). In 01 012 reared at 14.8°C, the larval duration ranged from 71 - 125 days (mean = 96d ± 22.2d) and the life cycle (oviposition to eclosion) ranged from 101 - 156 days (mean = 137d ± 25.2d). This gives a mean life cycle duration of four and half months of which three are spent as the larva. In specimen 01 012 018 the life cycle was 153 days (five months) of which 92 days were spent as a larva (three months). Assuming that the above ab. Cockaynei (Russwurm, 1978) underwent a similar life cycle, it would have derived from generation 1.ii which tails off in early June (section 5.4). This would place it as a late emerger of generation 2.ii (*cf.* Winokur, 1988). It is possible that it derived from wild cold shocked adult(s), and developed during a cool summer to pupate in early September when daylength (similar to that in early April) would still be long enough to prevent pupal *winter* diapause.

Family 012 yielded a further phenocopy at 14.8° (male no.019) in which the life cycle (156 days) was again about five months. Its pupal duration (23d) was much shorter than in specimen no.018 (52d) and within the range of pupal durations at 14.8° in unassimilated family 011 (23d - 25d), but its larval duration (125d) was nearly five weeks longer than in specimen no.019(92d) and four weeks longer than the mean larval duration at 14.8°C in unassimilated family 011 (81d ± 5.4d). It is likely that this larva underwent aestivation (*cf.* Wiklund *et al.*, 1983). Specimen 01 012 019 is illustrated on Plate 9. Larval and pupal aestivation in family 012 suggests that although the spring or early summer eclosion of wild cold shocked pupae means their immediate (F_1) offspring might not in turn encounter frost, a cool summer would prevent these eclosing and ovipositing the F_2 till late autumn, so *increasing* the likelihood of the latter encountering *winter*

frost at pupation.

Figure 8.13 shows that, assimilation apart, the mean larval and life cycle duration at 14.8°C (81d and 115d) are three and two times as long respectively than at 19.3°C (26d and 51d).

The evolutionary implications of the above findings are discussed in chapter 10.

(iv) *Lincolnshire STOCK 02 and hybrid STOCK 03*

The above STOCK 01 F_2 pupal diapause male 01 012 018 was mated to STOCK 02 parental cold shocked female 02 001 015 to generate hybrid STOCK 03. (As the originators of stock 03 this pair is denoted parental 03 001.) Family 03 002 derived from this pairing thus represents the third generation of treatment (given its genealogical continuity with STOCK 01) and is an UP-line family of A_i = 0.750.

Prepharate duration under control treatment was longer in family 03 002 than under control treatment in family 02 001 ($t_{(11)}$ = 2,758, $0.01<P<0.05$) which might suggest assimilation given that family 01 012 had also in effect been cold treated (rearing at 14.8°C) when prepharate duration was again much longer than in 02 001 ($t_{(9)}$ = 3.793, $0.001<P<0.01$). But prepharate duration in 03 002 under cold shock was actually *shorter* than in 02 001 under cold shock, although not significantly so ($t_{(10)}$ = 0.447). Pharate durations under corresponding treatments were similar in families 02 001 and 03 002, and the relationships among entire pupal durations in these families mirrored those among prepharate durations. Thus no firm conclusions can be drawn concerning assimilation. Since all control pupal durations were greater in 01 012 (at 19.3°C) than in 02 001 (Figs. 8.10 to 8.12), control pupal durations in 03 002 might be *expected* to be intermediate between 01 012 and 02 001 and so longer than in 02 001.

Although prepharate and entire pupal duration within 03 002 were longer under cold shock than control treatment, the difference was not significant, even though cold shock significantly prolonged prepharate and entire pupal duration in each parent family (01 012 at 19.3°C and 02 001). Thus hybridisation appears to buffer pupal development against the effects of cold shock and so effect positive heterosis

(*cf.* Falconer, 1981). This could be expected given that hybridisation decreases the level of inbreeding (*ibid.*) and that genetic load in the species mainly concerns genic balance (Oliver, 1981). It is possible that as the level of inbreeding again increases, the effects of immediate cold shock *or* any underlying (assimilated) propensity towards prolonged development will re-appear.

(v) *Assimilation and hybrid STOCK 03*

F_1 family 002 gave rise to F_2 family 003 (from controls) and families 004 and 007 (from cold shocks). Each F_2 family was divided into two samples of which one was reared at 17.6°C and the other at 13.9°C.

Pupal stage durations were first examined *within* the F_2. Differences between the two levels of assimilation were compared for each combination of rearing temperature and treatment, and differences between the two rearing temperatures were compared for each combination of assimilation level and treatment. Data from families 004 and 007 (A_i = 1.750) were pooled for comparison with family 003 (A_i = 0.375). The results are shown in Table 8.XIX.

The results show that in general all pupal stage durations were longer with the greater A_i (=1.750) and at the cooler temperature (13.9°C). Table XVI shows that all pupal stage durations were longer under cold shock than control treatment.

The prolonging influence of the greater A_i was predominantly at the cooler temperature (Table 8.XIXa) while cooler temperature prolonged pupal durations only at the greater A_i (Table 8.XIXb). This suggests that the greater A_i enhances the prolonging effect of the cooler rearing temperature (13.9°C) , and resembles the enhanced prolonging effect of cooler rearing temperature (14.8°C) at greater A_i in STOCK 01 F_2 (section 8.13.iv).

Table 8.XV shows that in STOCK 03 F_2 cold shock exerted an *immediate* prolonging effect only at the higher rearing temperature (17.6°C). Here the pattern of response was similar for A_i = 0.375 (family 003) and A_i = 1.750 (families 004 and 007), suggesting that assimilation here did *not* increase sensitivity to cold shock. (The longer prepharate duration under cold shock in the sample of A_i =

1.000 at 13.9°C is simply an immediate effect). Since the F_1 family 002 exhibited heterosis, it is most likely that the hybridisation which gave rise to STOCK 03 resulted in a gene complex better canalised against the propensity for prolonged pupal development under cold shock, and, hence, also against any underlying increase (assimilation) in this propensity. (In fact, Figures 8.10 and 8.12

		variable	PREPHARATE		PHARATE		PUPAL	
(a)								
17.6°C	C	Ai	ns	0.4	ns	1.8	ns	0.4
17.6°C	E	Ai	ns	0.4	***	1.8	ns	0.4
13.9°C	C	Ai	[1]	1.8	ns	1.8	*	1.8
13.9°C	E	Ai	***	1.8	ns	0.4	**	1.8
(b)								
Ai=0.375	C	°C	[2]	18°	[2]	14°	[2]	18°
Ai=0.375	E	°C	*	18°	ns	14°	ns	18°
Ai=1.750	C	°C	***	14°	**	14°	***	14°
Ai=1.750	E	°C	***	14°	ns	18°	ns	14°

[1] both samples had zero variance
[2] one individual only per sample

TABLE 8.XIX. Differences between (a) levels of assimilation and (b) between rearing temperatures in the pupal stage durations of STOCK 03 F_2. The differences were examined for each combination of pupal treatment with rearing temperature and with assimilation coefficient respectively. The assimilation coefficient or temperature yielding the longer stage duration is shown with the respective 2-tailed significance level: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$. Pupal treatments: C = foil control, E = cold shock. The respective t-values are listed in Appendix VIII.v. For further explanation see text.

suggest that the *magnitude* of the difference between treatments in prepharate and pupal durations *decreases* with assimilation).

That cold shock did not significantly prolong pupal development at 13.9°C may be due to slowed ecdysone production at cooler rearing temperature meaning that its extracellular titre does not build up sufficiently during cold shock to serve as a 'pulse' on subsequent re-warming (section 7.12).

Pupal durations in each F_2 sample were then compared with those in F_1 family 002 under corresponding treatments (Table 8.XIX). The only F_2 controls to differ significantly from F_1 controls were those of A_i = 1.000 reared at 13.9°C when all pupal stages were longer in the F_2. Hence assimilation predisposes pupae to prolonged development at cooler rearing temperature after just one generation of cold shock treatment.

When F_2 cold shocked samples were compared with F_1 cold shocked pupae, the significant differences again indicated longer durations in the F_2 but were now generally distributed over both rearing temperatures and levels of assimilation. The longer prepharate and entire pupal duration under cold shock in F_2 family 003 (A_i = 0.375) at 17.6°C than under cold shock in the F_1 (A_i = 0.750) probably indicates the breakdown of canalisation against immediate cold shock effect as inbreeding ensues. This aside, only in F_2 families 004 and 007 of A_i = 1.750 did cold shock prolong the prepharate and entire pupal stages more than in the F_1, suggesting that assimilation increases sensitivity to cold shock. These *increases* in prepharate and pupal durations were greater at 13.9°C than at 17.6°C but are not artifacts of the prolonging effect of 13.9° *alone*, since there were no such differences between the F_1 and F_2 family 003 of A = 0.375 (at either temperature). Thus cold shock together with cooler rearing temperature interact with the higher level of assimilation in the F_2 to effect prepharate and entire pupal durations *much* longer (by 14.4d and 16.1d respectively) than under cold shock in the F_1. But since these durations did not differ between control and cold shock treatment *within* the F_2 sample of A_i = 1.000 reared at 13.9°C, assimilation would appear to interact much more strongly with cool rearing temperature than with pupal cold shock.

				PREPHARATE		PHARATE		PUPAL	
Controls									
F_1	F_2	A_i	Temp						
03002 x 03003		0.4	18°C	ns	F_2	ns	=	ns	F_2
03002 x 03004		1.8	18°C	ns	F_1	ns	F_2	ns	=
03002 x 03003		0.4	14°C	ns	F_1	[1]	F_2	ns	F_1
03002 x 03004		1.8	14°C	***	F_2	***	F_2	***	F_2
Cold shock									
F_1	F_2	A_i	Temp						
03002 x 03003		0.4	18°C	**	F_2	ns	F_2	***	F_2
03002 x 03004		1.8	18°C	*	F_2	***	F_2	ns	F_2
03002 x 03003		0.4	14°C	ns	F_1	***	F_2	ns	F_2
03002 x 03004		1.8	14°C	***	F_2	***	F_2	***	F_2

[1] both samples had zero variance

TABLE 8.XX. Differences between STOCK 03 F_1 and F_2 samples in pupal stage durations under corresponding treatments. Differences are examined for each combination of assimilation coefficient (A_i) and rearing temperature (Temp). The generation yielding the longer duration is shown; an '=' denotes identical durations in both generations. Two-tailed significance levels: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$.

A_i values: 0.4 = 0.375, 1.8 = 1.750; Temperatures: 18°C = 17.6°C; 14°C = 13.9°C. F_1 family 03002: A_i = 0.750; rearing temperature = 16.7°C. Samples denoted '03004' comprise families 03004 *and* 03007. For further explanation see text.

By virtue of their genealogical connection with STOCK 01, the STOCK 03 F_1 represent the third generation of treatment and its F_2 the fourth. Thus, the level of assimilation in family 03002 may have been even higher than originally estimated, with the canalising effect of heterosis also therefore even stronger than originally assumed. Thus, through its canalising effect, heterosis could facilitate the assimilation of an underlying developmental propensity whose *expressivity* within a population might therefore increase very rapidly as inbreeding ensues, or when selection is relaxed.

(vi) *Subspecies aegeria STOCK 04 and hybrid STOCK 05*

P. a. aegeria STOCK 04 originated with 9 males and 3 females (parental family 001) and yielded 30 ova (F_1 family 002) of unknown precise parentage (the females may have mated pre-capture). These were laid on location on cut grass (unidentified) in a flight cage under natural conditions (Table 8.I). The eggs and the leaves to which they were attached were brought to Southampton in a 3" x 2" x 1" clear plastic box. The live adults (family 001) were transported in paper envelopes. The eggs were maintained in an incubator at 17.9°C ± 1.19°C as described in section 8.2.iv. and hatchability was 0.900. The larvae were maintained on Dacylis glomerata as for incubator-reared subspecies tircis samples but their survival was only 10%, about half the average of other incubator-reared samples (23% ± 12.7%). All the three surviving larvae pupated, although two of these died during the prepharate stage. The stage durations undergone by the surviving female were as follows: larva = 34d; prepharate pupa = 11.5d; pharate pupa = 1.3d; and within the range of those undergone by the British subspecies tircis stocks.

Only one of the STOCK 04 001 adults (male no. 001) reached Southampton in a sufficiently healthy condition to attempt pairing. A hybrid pairing was thus set up with STOCK 03 F_2 female 03 004 007 in a greenhouse rearing tub at 20.3°C ± 2.08°C to generate subspecies-hybrid STOCK 05. (As the originators of stock 05 this pair is denoted parental 05 001.) 35 eggs (F_1 family 05 002) were laid on the grass, when they were transferred to an incubator at 17.6°C ± 1.26°C. They were otherwise maintained under identical conditions to

STOCK 04 002 but none hatched. Family 05 002 represented the F_5 (given its genealogical continuity with STOCK 01).

(vii) *Doncaster STOCKS 06 and 07*

Parental family 06 001 gave rise to F_1 families 06 002 (from controls) and 06 003 (from cold shocks). The F_1 were incubator reared, 06 002 at 18.1°C ± 2.28°C and 06 003 at 18.0°C ± 8.81°C, but none of 06 002 survived beyond the larval stage.

The durations of each pupal stage were compared between F_1 family 06 003 (A_i = 1.000) and parental family 06 001 under corresponding treatments. There were no *significant* differences between the generations in their mean durations, although all the pupal stages were longer in the F_1 under cold shock than in the parentals under cold shock (*control* prepharate and entire pupal durations were actually *shorter* in the F_1 than parentals although again not significantly so); the respective t-values are listed in Appendix VIII. However, the degree to which cold shock prolonged the *immediate* prepharate, pharate and entire pupal durations was greater in the F_1 (by 5.0d, 9.3d and 5.3d respectively) than in the parentals (by 3.9d, 0.0d and 4.0d), which would suggest that assimilation increases *sensitivity* to early pupal cold shock (that control durations were shorter in the F_1 argues against assimilation prolonging pupal development *per se*). Family 06 003 comprised but 6 pupae and it is possible that a larger sample would have yielded *significant* results.

The cold shocked parental animals that gave rise to F_1 family 06 003 eclosed from unblotched pupae (section 8.12.i.). Three 06 003 pupae were in turn cold shocked of which one was blotched. It thus appear that blotching is an inherent capacity of the stock and present in 15% - 30% of individuals. Such blotching too might be amenable to genetic assimilation. The Himalayan rabbit and Siamese cat have black tips to their extremities which can be phenocopied in other breeds and species (Needham, 1974). Here, melanin is deposited in the pelage wherever local temperature falls below a critical value, and genetic assimilation of cold-induced darkening has been suggested for its constitutive expression in the cat and rabbit above (*ibid.*).

STOCK 07 parental family 001 comprised but 3 pupae and neither F_2 family 002 nor 003 (incubator-reared at 18.0°C ± 8.81°C) survived beyond the larval stage. It is therefore not possible to draw any conclusions concerning pupal development in this stock.

8.14. Developmental differences between stocks

(i) *Pupal development*

Pupal stage durations were compared among STOCKS 01, 02, 04 and 06 using families all of A_i = 0.000 and reared at similar temperatures, thus:

Family	Rearing temperature
01 002 and 004	19.3°C ± 2.26°C
02 001	20.8°C ± 4.24°C
04 002	17.9°C ± 1.19°C
06 001	19.1°C ± 2.99°C

Stocks were compared on a pairwise basis for each corresponding pupal treatment (foil and cold shock) as available. The results are shown in Table 8.XXI.

All control durations were longer in STOCK 01 than STOCK 02. That the longer pharate duration of STOCK 01 was evident only in family 002 suggests that pharate duration is under a genetic control that differed between families 002 and 004. This probably accounts for the longer 'cold shock' pharate duration in STOCK 01 (002) than STOCK 02 since pharate duration is unaffected by cold shock. Hence there was no difference between STOCKS 01 and 02 in their cold shock durations. Since STOCK 02 is of northern origin, it may have become adjusted to undergo the life cycle at cooler temperatures than southern STOCK 01, which might explain the faster development of STOCK 02 controls at comparable temperatures. Hence its rate of concomitant ecdysone production (and so magnitude of the post-shock pulse) might too be greater than in STOCK 01 at comparable temperatures and so render it

more susceptible to cold shock. Indeed this could explain why the *otherwise* shorter prepharate (and pupal) duration of STOCK 02 was similar in both stocks under cold shock.

	STOCKS		PREPHARATE		PHARATE		PUPAL	
(002)	01 x 02	C	**	01	*	01	***	01
(004)	01 x 02	C	***	01	ns	=	***	01
(002)	01 x 02	E	ns	02	*	01	ns	02
(004)	01 x 02	E	ns	02	ns	01	ns	02
(002)	01 x 04	C	ns	01	ns	01	ns	01
(004)	01 x 04	C	ns	01	ns	01	*	01
(002)	01 x 06	C	ns	06	***	01	**	01
(004)	01 x 06	C	ns	01	ns	01	**	01
(002)	01 x 06	E	***	06	***	01	ns	06
(004)	01 x 06	E	**	06	***	01	ns	06
	02 x 04	C	ns	02	***	02	ns	04
	02 x 06	C	***	06	ns	02	***	06
	02 x 06	E	ns	06	ns	02	ns	06
	04 x 06	C	ns	06	ns	06	ns	06

TABLE 8.XXI. Pairwise differences between stocks in their pupal durations under corresponding treatments. The respective STOCK 01 family is indicated in parentheses. The relevant treatments are also given: C = foil control; E = cold shock. The stock of longer stage duration is indicated with the two-tailed significance level: 0.01 <P<0.05; ** 0.001<P<0.01; *** P<0.001. The t-values are listed in Appendix VIII.vi. For further explanation see text.

Only control pharate and pupal durations were longer in STOCK 01 than STOCK 06. Again the longer pharate duration of STOCK 01 was evident only in family 002 and, as above, probably accounts for the longer 'cold shock' pharate duration in STOCK 01 (002) than STOCK 06. The shorter control pharate and pupal durations in STOCK 06 than 01 at comparable temperatures may reflect northern STOCK 06 too having

become adjusted to develop under cooler climatic conditions. Again its greater rate of ecdysone production (and so magnitude of the post-shock pulse) than in STOCK 01 under comparable temperatures could be expected to render it more susceptible to cold shock. Since STOCK 06, however, unlike STOCK 02, did not differ from STOCK 01 in its *control* prepharate duration, any such difference in cold sensitivity *could* be expected to manifest as a significantly longer cold shock prepharate duration in STOCK 06, and indeed the results support this hypothesis.

That STOCK 06 but not STOCK 01 developed faster than usual at ~19°C might be conceptualised as STOCK 06 having developed at an *effectively* higher temperature than STOCK 01, which could account for its lesser degree of assimilation (cold shocked 06003 v 06001) than in STOCK 01 (cold shocked 01012 v 01004, Figures 8.9 and 8.11). The effectively higher temperature having already advanced L.max nearer or to its limit, the extent to which increasing assimilation can *further* steepen larval linear growth is reduced (section 8.12.iii.).

STOCK 02 (Bardney, Lincolnshire) and STOCK 06 differed only in control prepharate and pupal durations which were longer in STOCK 06. Both stocks are of northern origin and hence likely to have become adjusted to develop under similar climatic conditions. The longer durations in STOCK 06 probably reflect some genetic difference in development rate, although it could conceivably have resulted from its *slightly* cooler rearing temperature (especially were such a difference to involve a threshold *cf.* Lees, 1962; Shreeve, 1985).

All pupal stage durations were generally shorter in subspecies aegeria STOCK 04 than in any other stock (Figures 8.10 to 8.12). Control pharate duration was significantly shorter than in Bardney STOCK 02 and control pupal duration just significantly so than in Hampshire STOCK 01 (TABLE 8.XXI and Appendix VIII.vi). STOCK 04 originated from south-west France, where although the mean July temperature (12°C) is similar to that throughout the species' British range, the mean January temperature (possibly as low as -13°C, *cf.* Dennis, 1977) is much colder than that (2°C) experienced by British subspecies tircis. In subspecies aegeria it is the pupa which hibernates (Novak, 1974) and this is adaptive since P. aegeria larvae (in general) die if they freeze before pupation (Wiklund *et al.*, 1983). It is therefore likely that, winter diapause apart, pupae of

subspecies aegeria have become adapted to develop at cooler temperatures than their British counterparts, so that when reared at comparable *aestival* temperatures they develop more rapidly.

(ii) *Larval development and the fifth instar*

The frequency of 5-instar larvae and the number of each sex undergoing five instars was recorded for all STOCK 01 F_1 and F_2 families at each rearing temperature and for STOCK 03 F_1 family 002 and STOCK 06 F_2 family 003 (Table 8.XXII).

Family	5-instar frequency	M	F	A_i
01002 (18.9°C)	6/45 = 13.3%	1	2	0.000
01004 (18.9°C)	1/38 = 2.6%	-	-	0.000
01007 (14.8°C)	3/3 = 100.0%	1	?	1.000
01007 (19.3°C)	3/15 = 20.0%	-	-	1.000
01010 (19.3°C)	0/1 = 0.0%	-	-	0.000
01011 (14.8°C)	1/4 = 25.0%	0	1	0.000
01011 (19.3°C)	1/10 = 10.0%	?	?	0.000
01012 (14.8°C)	1/4 = 25.0%	1	0	1.000
01012 (19.3°C)	0/23 = 0.0%	-	-	1.000
03002 (16.7°C)	0/15 = 0.0%	-	-	0.750
06003 (18.0°C)	10/23 = 43.5%	1	4	1.000

TABLE 8.XXII. The frequency of five-instar larvae in STOCK 01 F_1 and F_2 and families 03002 and 06003. The frequency of 5-instar larvae (Fr, as 5-instar larvae/total larvae and as percent) is shown for each respective rearing temperature (in parentheses). The number of each sex (M = males, F = females) surviving to eclosion is also reported: '?' denotes that the larvae survived but their sex not ascertained. The final column gives the level of assimilation in the respective sample. For further explanation see text.

The differences between families 01002 and 01004 suggests a genetic component in 5-instar development that differs between families within STOCK 01. The high frequency of 5-instar larvae in 06003 also suggests a genetic difference between stocks. However, since A_i = 1.000 in 06003, the frequencies of 5-instar larvae at A_i = 1.000 and A_i = 0.000 were compared within STOCK 01 (where such data was available). Family 01010 was excluded from all analyses as the single datum could have produced misleading results[1].

Sample means (±SD) were compared using two-tailed t-tests as described in section 8.13.i. There was no significant ($P \leq 0.05$) difference between the two levels of assimilation (A_i = 0.000: Fr = 12.7% ± 8.08%, n=4; A_i = 1.000: Fr = 36.2% ± 37.98%, n=4; $t_{(6)}$ = 1.176ns). Rearing temperatures were then classified as <15°C or >15°C and the two temperature ranges compared. It was found that the frequency of 5-instar larvae was significantly greater at <15°C (<15°C: Fr = 50% ± 35.36%, n=3; >15°C: Fr = 9.2% ± 7.24%, n=5; $t_{(6)}$ = 2.539, $0.01<P<0.05$). Differences between the two levels of assimilation were therefore compared within each temperature range but were not significant at either <15°C (A_i = 0.000: Fr = 25.0%, n=1; A_i = 1.000: Fr = 62.5% ± 37.50%, n=2; $t_{(1)}$ = 0.707ns) or >15°C (A_i = 0.000: Fr = 8.6% ± 4.46%, n=3; A_i = 1.000: Fr = 20.0% ± 10.00%, n=2; $t_{(3)}$ = 1.670ns). Thus the high frequency (in nearly half of all larvae) of a fifth instar in STOCK 06 indeed appears to be a genetic feature of the stock.

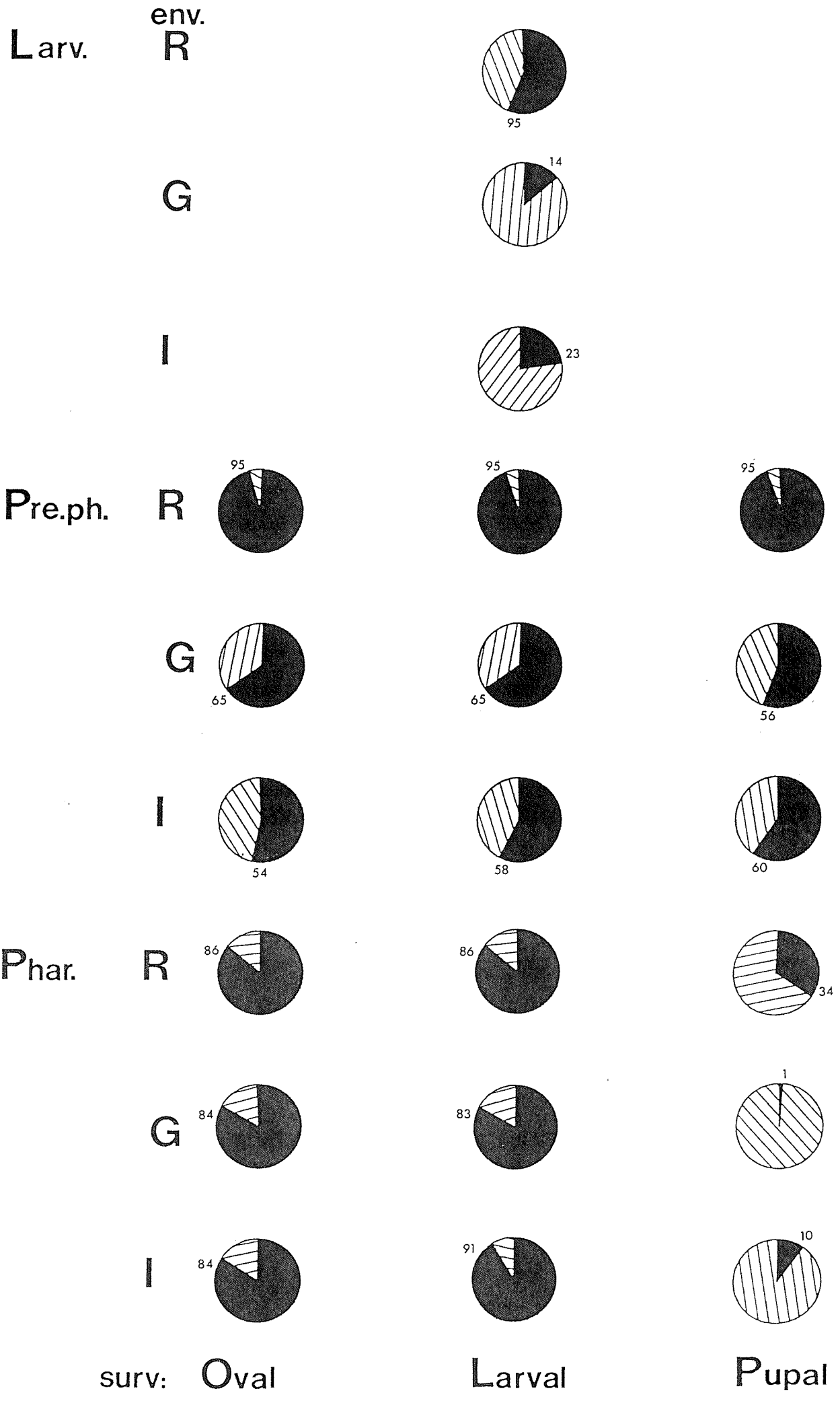
The frequencies of 5-instar larvae in parental family 01002 and its F_1 derivatives were compared with those in family 01004 and its F_1 derivatives although only F_1 samples reared at 19.3°C were included on account of the above influence of rearing temperature. The two lines were found to differ significantly (01002-line: Fr = 16.7% ± 3.34%, n=2; 01004-line: Fr = 4.2% ± 4.23%, n=3; $t_{(3)}$ = 3.160, $0.01<P<0.05$). This supports the contention that there is a genetic component in the control of 5-instar development that differs between STOCK 01 families 002 and 004.

The number of 5-instar larvae of each sex surviving to eclosion was recorded for each sample (Table 8.XXII). The results would indicate that at 19.3°C the respective female:male ratio is between 2:1 (in STOCK 01) and 4:1 (in STOCK 06). These ratios are similar to that reported for a stock from Bernwood Forest, Oxfordshire, in which

all females underwent a fifth instar at 19.8°C while only 56% of males did so (Shreeve, 1985). However, Shreeve (*loc. cit.*) reports that 5-instar *males* were less frequent at cooler temperatures, whereas the above results suggest that in STOCK 01 the sex ratio might have been more equal at the cooler temperature (14.8°C) although larger samples would be needed before a statistical analysis could be performed.

It thus appears that populations differ primarily in the *total* frequency of 5-instar larvae, the sex ratio among such larvae remaining relatively constant (at least at around 19°C). The disparate sex-ratio suggests that genetic determination of the 5th instar involves a sex-limited component. In Lepidoptera it is the female which is of XY karyotype, the male being XX (Ford, 1957). But the determination of the 5th instar cannot rest *solely* on the Y-chromosome or males would never undergo five instars. However, determination of the 5th instar interacted with temperature but more so in *males*, where it appeared (in STOCK 01) that five instars might be more frequent at cooler temperatures. Now under cool summer conditions, a fifth instar might allow for a protracted larval development and hence for the production of larger (vernal) pupae. This would be particularly adaptive in females, whose resulting adults would carry more eggs so compensating for their lack of bivoltine reproduction (Wiklund *et al.*, 1983). It is therefore suggested that male and female 5-instar larvae carry similar determinants for such development but that it is more *canalised* in females.

[1]similar results were obtained with family 01010 included


env.
Larv.
R
95
G
14
I
23
Pre.ph.
R
95
95
95
G
65
65
56
I
54
58
60
Phar.
R
86
86
34
G
84
83
1
I
84
91
10
surv:
Oval
Larval
Pupal

8.15. Rearing environments and survival

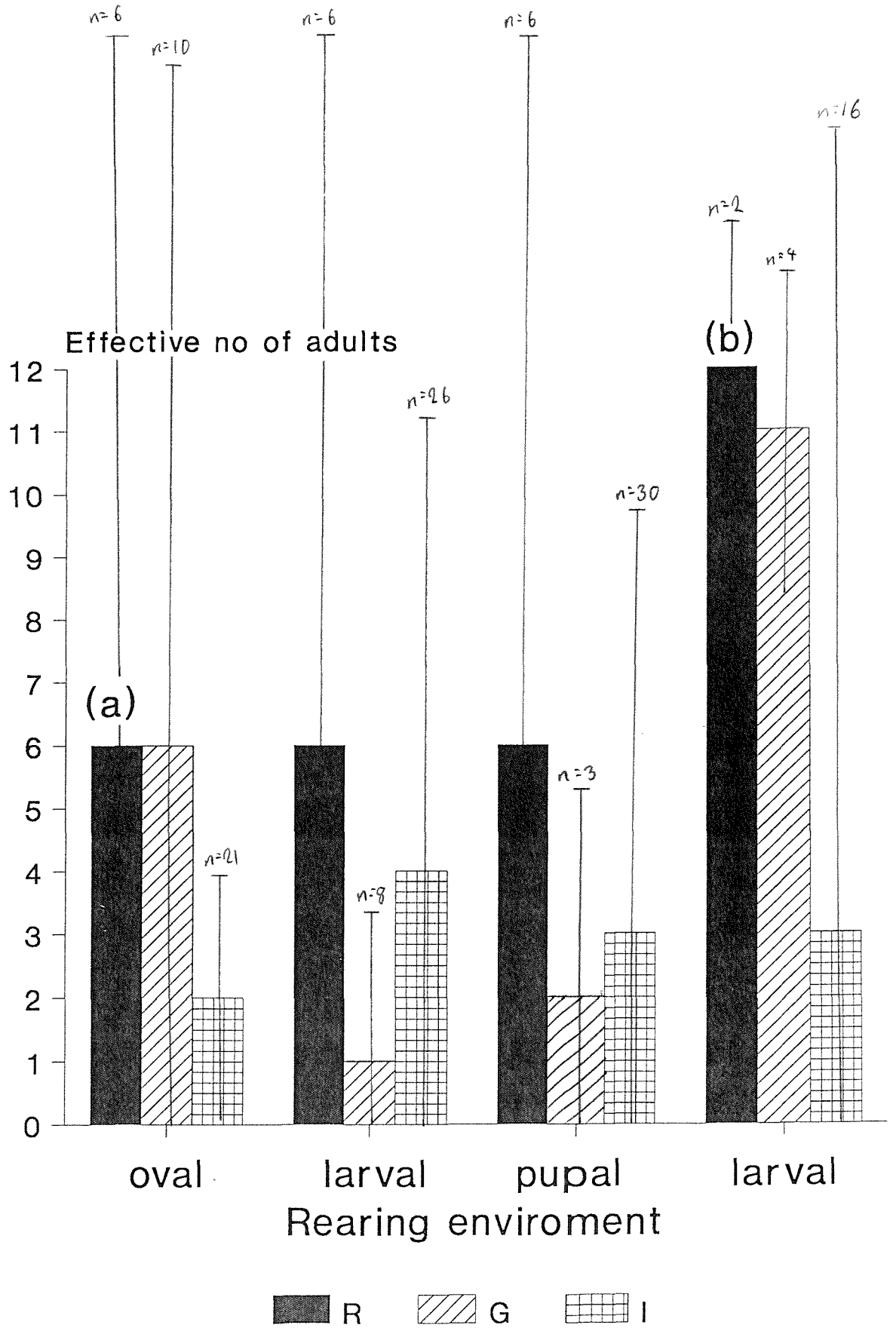
The relative survivals of the main life cycle stages under each rearing protocol are shown Figure 8.14. The environments resulting in the best and poorest survivals at each stage are summarised in Table 8.XXIII.

Hatchability was unaffected by egg rearing environment, although incubator-reared eggs resulted in smaller family and sample sizes (Figure 8.15). Incubator temperatures were similar to those indoors (Appendix III.ii) but radiation from the fluorescent tubes may have raised the boxes' internal temperatures (the thermostat monitored the ambient temperature, Plate 5). Eggs are particularly susceptible to

	BEST			WORST		
	oval	larval	pupal	oval	larval	pupal
Stage						
Larva	–	R	–	–	G	–
Prepharate	R	R	R	I	I	G
Pharate	R	I	I	G=I	G	G
Life cycle	R	R	R	G	G	G

TABLE 8.XXIII Rearing environments yielding the best and poorest relative survivals at each stage of the life cycle. Environments: R = indoors; G = greenhouse; I = incubator.

FIGURE 8.14 (opposite). Relative survival of the main life cycle stages under each rearing environment. Survival is indicated by solid shading. Rows refer to the stage (Larv = larva, Pre.ph = prepharate pupa, Phar = pharate pupa) at which rearing was conducted under the respective environment (R = room, G = greenhouse, I = incubator). Columns refer to survival of stages so reared. For example, 60% gives the mean pupal survival among samples reared as pharate pupae in an incubator; 58% gives the mean larval survival among samples subsequently reared as prepharate pupae in an incubator.


n=6
n=10
n=6
n=6
n=16
n=2
n=4
Effective no of adults
(b)
12
11
10
9
8
7
(a)
6
5
4
3
2
1
0
n=26
n=30
n=3
n=21
n=8
oval
larval
pupal
larval
Rearing enviroment
R
G
I

dessication at high temperature on account of their large surface:volume ratio (Masaki, 1980).

Greenhouse rearing of larvae and pupae resulted in *sample* sizes noticeably smaller than under other environments (Fig. 8.15a). Larval survival was particularly poor here (14% + 12.7%) and it appears to be at this stage that optimum conditions are most necessary. The greenhouse was very humid (being maintained primarily for plant propagation) and the poor ventilation encouraged mildew growth so necessitating frequent re-potting of foodplant. Poor quality food can weaken larvae (Stone & Midwinter, 1975), possibly through its influence on the gut flora (Brakefield, 1979), while the high density of larvae in the rearing tubs could have facilitated the spread of infection, the latter exacerbated by aphids and fungus flies (Winokur, 1988). This might also explain the poor prepupal survival in STOCK 02 (27%) which was also greenhouse reared, since prepupae are in effect late larvae. Otherwise, prepupal survival in each stock was >80% when there was no trend with the order in which they entered the protocol (Figure 8.16), the difference between them being only just significant.

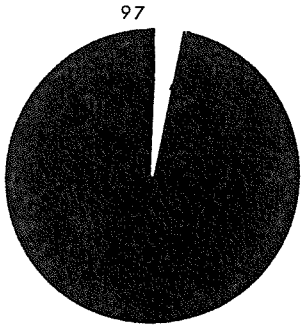
Pupal survival was also poorest in greenhouse reared samples, and this is most likely attributable to disease contracted as larvae since the incidence of mortality was higher during the prepharate than pharate stage (Fig. 8.14). Pupal mortalities were often discoloured brown, typical symptoms of bacterial disease (*cf.* Winokur, 1988).

Greenhouse-cultivated foodplant was also used for incubator rearing, where amongst both larval and pupal mortalities the most prevalent symptoms were again those typical of bacterial disease. That survival was better, however, than under greenhouse conditions

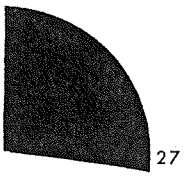
---

(means ±SD)

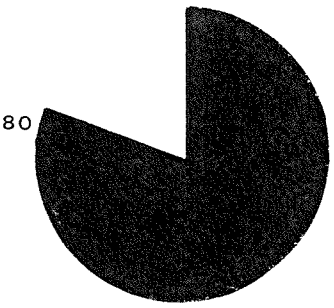
FIGURE 8.15 (oppposite). Adult sample and family sizes resulting from each rearing environment: (a) sample sizes resulting from rearing under each oval, larval and pupal environment; (b) family sizes resulting from each larval environment. The effective number of adults was computed as described by Falconer (1981, *cf.* also section 8.9) and is show rounded down to the nearest integer. Rearing environment: R = room, G = greenhouse, I = incubator. For further explanation see text.



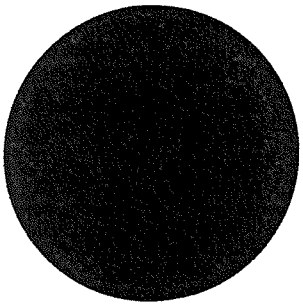
01



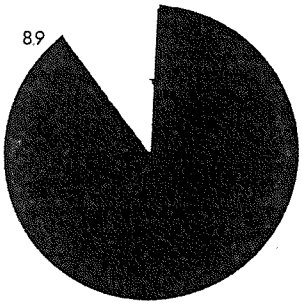
02



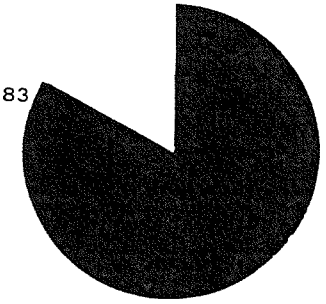
03



04



05



06

therefore most likely reflects the greater segregation of larvae. The particularly poor survival of STOCK 04 family 002 may have resulted from their also being reared at a temperature (17.9°C) lower than that usually experienced by subspecies aegeria during April and May. Certain other thermophilic species, notably the moth Daphnis nerii (L.) (Sphingidae), are particularly susceptible to otherwise non-pathogenic foodplant micro-flora when reared at sub-optimal temperatures (Friedrich, 1983). That the two pupal mortalities died during the prepharate stage would support this contention, prepharate survival (33%) here being about half the overall average (60%).

Environment	(1)	(2)	(3)
Indoor	2 ± 0.9	0.51 ± 0.456	1.02
Greenhouse	1 ± 0.6	0.94 ± 0.177	0.94
Incubator	2 ± 1.2	0.37 ± 0.346	0.74

(1) = Egg-laying pairs per family
(2) = Hatching batches per egg-laying pair
(3) = Hatching batches per family

TABLE 8.XXIV. Adult pairing environment and fecundity. Note that (2) and (3) are proportions. For example, 0.51 means that 51% of all egg batches laid by a family whose adults were paired indoors hatched, and 1.02 means that 100 families whose adults are paired indoors will yield 102 hatching batches. Adult pairing environment includes the environment under which they oviposited. For further explanation see text.

FIGURE 8.16 (opposite). Prepupal survival among the stocks. The mean survival among the families of each stock is indicated by solid shading. Numbers below the sectors refer to the stock number (*cf.* Table 8.I). For further explanation see text.

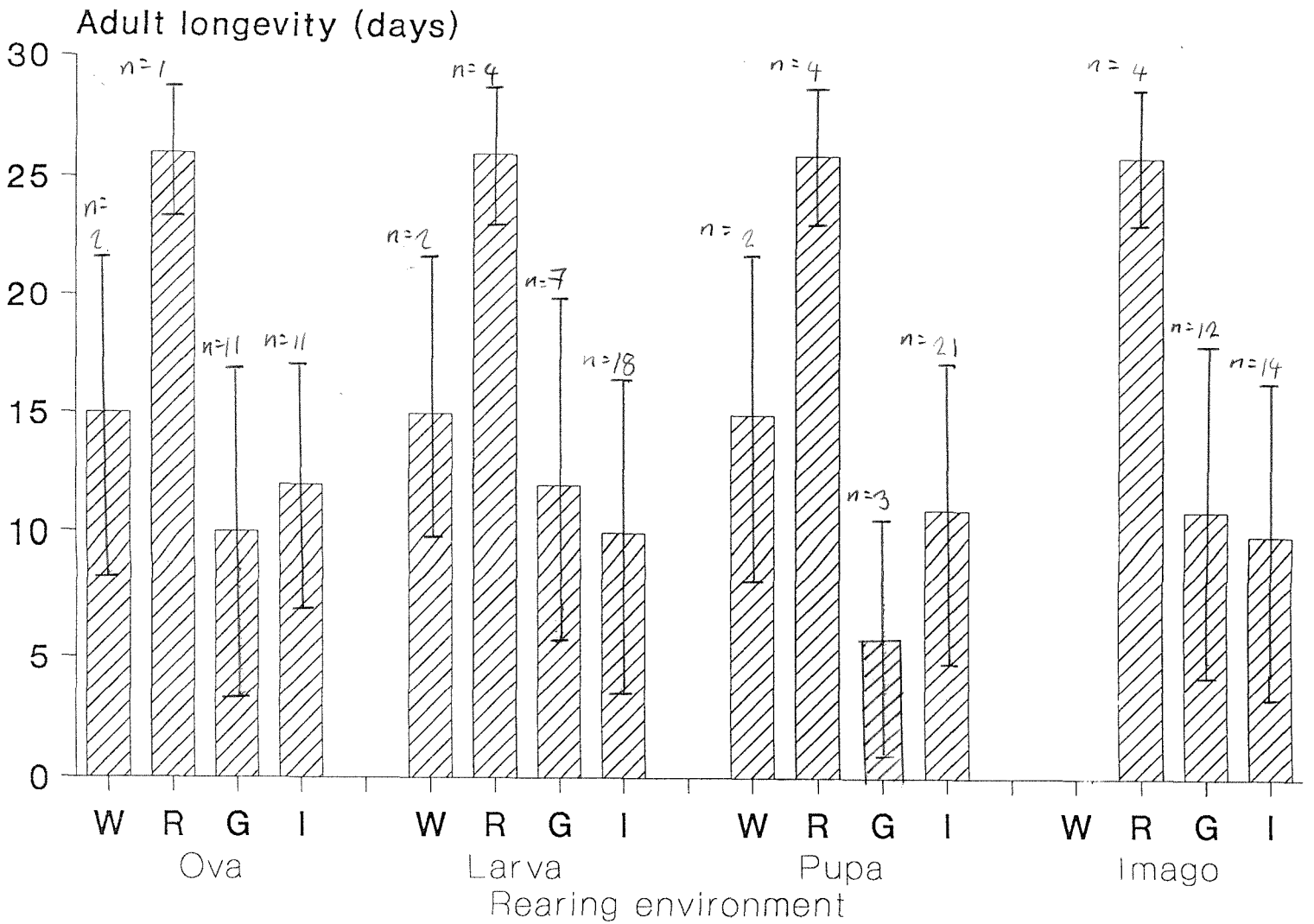
The larval foodplant for indoor rearing was cultivated outdoors and animals fed such foodplant survived best. Wild P. aegeria favours damp areas of woodland for oviposition (Wiklund & Karlsson, 1984) which may maximise hatchability and 1st-instar larval survival by preventing dessication (*cf.* Winokur, 1988), the latter susceptibility perhaps also accounting for the species' requirement for shade (*cf.* Ford, 1957). Under natural conditions, however, the freer air flow might prevent a build up of *excessive* local humidity, while the antiseptic qualities of incident ultra-violet radiation might help check disease.

Adult longevity of indoor-reared samples was about double (26d) that of samples reared under greenhouse or incubator conditions (both about 11d), and this trend was similar for each stage at which the respective environments were encountered (Figure 8.17). The shorter adult longevity of greenhouse-reared samples may have been due to predation by Pharoah ants which in the cooler greenhouse predated the STOCK 01 F_2 adults (section 8.2.ii), although the similar longevity of incubator-reared samples would implicate their poorer larval foodplant. It is unlikely that humidity or mould affected the adults directly, however, since mildew growths on living plant matter form part of their natural diet (Shreeve, 1986).

The number of egg-laying pairs per family was smallest in greenhouse reared families, but the number of batches hatching per egg-laying pair was greatest here. The product of these two variables gives the number of hatching batches per family (Table 8.XXIV).

The low proportion of batches hatching in the incubator most likely reflects the damaging (heating) effect of fluorescent light, since the eggs were oviposited in pairing boxes (section 8.2.iv) where the gaps left for feeding wicks would have rendered them more prone to dessication (Plate 8). It is also possible that sucrose dried out more quickly on the wicks than on cotton wool pads, thus becoming unduly concentrated and interfering with maternal egg development.

That only a few pairs oviposited in the greenhouse may be due to the adults not having had adequate access to sucrose solution - which stimutes oviposition (Stone & Midwinter, 1975), since the feeding pads were placed at the top of the netting to prevent the solution coming into direct contact with the foodplant and so damaging any eggs (section 8.2.iii).


Adult longevity (days)
30
25
20
15
10
5
0
n=2
n=1
n=11
n=11
n=2
n=4
n=7
n=18
n=2
n=4
n=3
n=21
n=4
n=12
n=14
W R G I
W R G I
W R G I
W R G I
Ova
Larva
Pupa
Imago
Rearing environment

The total hatch failure of hybrid STOCK 05 F_1 family 002 (section 8.13.vi) was probably due to insemenary failure as this is the most usual cause of zero hatchability (Oliver, 1981). The parent male 04 001 001 had been confined in its paper envelope for at least 24h prior to setting up the pairing during which time it may have been damaged. Damage to the legs can interfere with copulation in other species (Cribb, 1983). Although a wild male P. aegeria has been observed mating post mortem (following a suspected bird attack, Lipscomb, 1971) it would almost certainly have already been in copulo when it died. Nonetheless, several species can be successfully hand-paired following decapitation or paralysis (Friedrich, 1983), a technique which might therefore prove useful with P. aegeria. Although the total hatch failure of 05 002 may have resulted from genetic incompatability, since the parent subspecies differ in chromosome number, they have been known to yield viable hybrids (Robertson, 1959).

FIGURE 8.17 (opposite). Adult longevity associated with rearing the early stages under each regime. The mean adult longevity of animals reared as ova, larvae or pupae or maintained as adults under each rearing environment are shown. Rearing environments: W = wild, R = indoors, G = greenhouse, I = incubator. 'Wild' refers to the longevity (post-capture) of wild caught adults that would have undergone the life cycle under natural conditions. For further explanation see text.

8.16. Efficacy of the protocol

(i) *Empirical considerations*

The main considerations here concern the maintenance of lineages and the maximisation of survival.

The frequent changes of regime probably contributed to mortality (and ultimately lineage extinction). Larvae of the satyrid Maniola jurtina become adapted to local microfloras and become diseased if transplanted to foodplant from unfamiliar sites (Brakefield, 1979). Moreover non-optimal temperature and foodplant quality can aggravate these susceptibilities (Friedrich, 1983). Protocol changes would also have prevented those individuals not susceptible to a particular regime from then becoming establishing as an appropriately *adapted* lineage (*cf.* Morton, 1981).

It is suggested that foodplant be propagated in a greenhouse for rapid establishment but maintained outdoors where freer ventilation reduces the risk of (aphid-borne) disease. It is likely that a better-ventilated greenhouse would also prove suitable. For the rearing of individual batches (necessary if reliable life cycle data is to be recorded), the use of clear plastic boxes is suggested. Ova, larvae and pupae could be reared either indoors or in an incubator. Incubator-reared ova should be shielded from direct light, and at all stages care should be taken to ensure there is no excess moisture from the cooling plate. The use of an incubator enables temperature and photoperiod to be controlled, while indoor (laboratory bench) rearing allows the animals to develop under (more) natural temperatures and photoperiod. Humidification of the pupal boxes by exhaling on the lids proved satisfactory. Adults would be best paired in pairing chambers, but the females then transferred to rearing tubs or clear view breeding cages with potted foodplant for oviposition. Cotton-wool feeding pads might be sellotaped to the cage supports at various positions inside the netting, and would be best charged using a hypodermic syringe. This would prevent their becoming over-charged (and so

dripping sucrose on to the butterflies or foodplant) and enable water to be substituted (to prevent a build up of excessive concentration) with ease. During the removal of ova the adult could be transferred to a pairing chamber, then returned to the cage or tub.

Envelopes, on account of being compact and preventing undue activity of the adults, are probably the most practical means for transporting large numbers of live adults. However, it is suggested that such transportation be restricted to mated females. Several moth species are known to release fertile eggs even after stunning (Friedrich, 1983), but a female Aphantopus hyperantus (Satyridae) stunned in the field (by pinching the thorax) too oviposited its ova in a storage envelope (L. Winokur, pers. obs.). Although this species *usually* scatters its eggs, whereas P. aegeria attaches its eggs to various substrata, it is possible that since P. aegeria is not actually substratum-*specific*, it too might deposit its eggs in storage envelopes.

Neither cold treatment nor assimilation resulted in any decrease in survival (8.9.ii). The use of early pupal cold shock is thus a viable basis for the investigation of genetic assimilation in P. aegeria (and probably other species, Nijhout, 1984).

The survival of 1st instar larvae and cold shocked prepharate pupae did, however, become worse with inbreeding, as did a number of fecundity parameters (section 8.10). Yet excessive inbreeding might be prevented by the occasional introduction of individuals into a lineage from without, when Morton (1983) suggests one individual every second generation to keep inbreeding below a critical level of of 0.333. Although the introduction of a wild caught adults means that untreated animals might be introduced to assimilated lineages, the assimilation coefficient is such that it allows for mixed lineages (section 8.5). Of course, when the individual is introduced from a different stock to that from which the lineage is derived one should be aware of the possibility of heterosis (*cf.* section 8.13.v).

Initial family size decreased with increasing assimilation and inbreeding (sections 8.9.ii and 8.10 respectively). Thus assimilation might prove to be a limiting factor in the maintenance of protocols although, since the above effect appears to have involved synergism between inbreeding and assimilation, lineage extinction might be prevented by keeping inbreeding at a low level.

The foregoing investigations revealed that assimilation occurred in each of STOCK 01, 02 (insofar as when hybridised with STOCK 01 to yield STOCK 03 assimilation was evident), and 06. Assimilation was also shown to have occurred when the protocol was examined as a whole using *only* the sample means, and the results of the overall and specific analyses corroborated well. It is therefore suggested that both approaches be used in large-scale studies of genetic assimilation. While overall analysis tends to overlook specific effects, the conclusions drawn are less likely to be biased by (possibly misleading) spurious results. Conversely, an effect which proves non-significant in each of a number of similar instances (for instance the lack of definite assimilation in family 03002 and STOCK 06, sections 8.13.v and vii) might prove significant when samples are examined *en masse*. Besides, these conclusions can then provide a basis for understanding the results of specific analyses. Specific analyses on the other hand, may fail to pick out general trends (for example in the direction of selection, section 8.9.iv), but can identify effects overlooked in a more general analysis (for example pupal summer diapause, section 8.13.iv) and elaborate on those that are not.

Although the net direction of selection on each stage of the life cycle over the protocol as a whole was similar in direction to assimilation, there was no selection in any of the individual samples where assimilation too occurred. Thus selection was eliminated as an artifact in overall results, the observed assimilation thus being genuine.

The ova laid by 03004007 (hybrid STOCK 05 family 002) represent the fifth generation of continuous breeding within the protocol, although the generation number actually assigned to it (ie. F_1) was relative to the establishment of STOCK 05 (Figure 8.2). Thus, under the optimum regime above, a true to treatment lineage (section 8.5) might survive five generations before going extinct or requiring extraneous input (see above), resulting in samples of $A_i = 5.000$, more than double the maximum A_i obtained under the present rearing regime.

The use of only two pairings to establish STOCK 01 may have resulted in an undue increase in the rate of inbreeding, although STOCKS 02 and 06 were established by a considerably larger number of individuals. However, that lineages in STOCK 06 and hybrid STOCK 03

actually survived fewer generations than in STOCK 01 (Fig. 8.2), would rule out the number of pairings establishing the protocol as a limiting factor. Indeed the use of only two pairings can be quite adequate for studies involving breeding programmes (*eg.* Oliver, 1981). It is probably more important to maximise *survival*, as might be achieved by the regime proposed above.

(ii) *As a simulation of the species' natural biology*

The ways in which the present findings can aid the understanding of evolution in Pararge aegeria are presented in depth in chapter Ten so only factors related to the design of the protocol *per se* will be considered here.

It is likely that the establishment of a population from two (STOCK 01) or even just a single pair (STOCK 03) is a natural occurence in the wild. Females regularly cross open fields as singletons (Baker, 1984), single strays having been reported on several independent occasions (*eg.* Gibson, 1944; Davidson, 1956) at distances up to 60km from the nearest known populations (Barbour, 1986), and it has been suggested that its current western Scottish range originated from a single female (*ibid.*).

While it is likely that the species experiences natural pupal cold shocks in winter (section 7.13), genetic assimilation would be expected to require cold shock in contiguous generations, a less likely occurence given that the mean summer temperatures experienced by the species (in Britain and mainland Europe) are considerably warmer than the mean winter temperatures (Dennis, 1977). However, it is possible that under a cool summer, the offspring (F_1) of a winter cold shocked individual might in turn undergo larval or pupal summer diapause, which if not in effect substituting for cold shock (section 8.13.iii) could still increase the likelihood of frost exposure in the F_2. Indeed, that the phenotypes of the resultant laboratory STOCK 01 F_2 reared at 14.8° resembled those of reported natural aberrants (Russwurm, 1978) would suggest that this can (and *does*) occur in nature.

Hybridisation among populations is also a likely occurence in nature. In Europe the range of P. a. tircis and P. a. aegeria are contiguous and the distributions of the two colour forms not clearly defined (especially in central France, Higgins & Riley, 1975), the aegeria-form being constant only from the western Mediterranean as far east as Sicily, and again east of Lebanon (*ibid.*).

The life cycle durations undergone during the protocol under all three environments (indoors, greenhouse and incubator) were, on the whole, probably similar to those undergone by the species under natural *summer* conditions. However, this would suggest five (possibly six) *annual* generations in the laboratory, which *is* more than the maximum of two suggested for the species in the wild (Winokur, 1988). Nonetheless, the summer diapause undergone by specimens 01012018 and 01012019 (section 8.13.iii), resulting in a five months life cycle (oviposition to eclosion), probably does provide a more realistic reflection of the species' natural phenology, at least under conditions likely to effect natural genetic assimilation.

8.17. The assimilation coefficient

The assimilation coefficient quantifies the extent to which lineages have been repeatedly exposed (by application) to a defined environmental factor during their history. It is thus comparable to inbreeding coefficient which provides a measure of the extent to which lineages have been repeatedly exposed (by gene replication) to defined internal factors *ie.* alleles identical by descent, during their history.

It is derived upon the premise that the lineage has had no previous exposure to the factor *ie.* $A_i = 0$, which is assumed to have some perturbatory effect which is biologically cumulable (can be assimilated). It should be noted that reference to an environment as stressful (in contrast to typical) is arbitrary, and best defined in terms of the ecological context, or, at the individual level, in terms of whether it produces any observable change, and that it does not allude to the adaptiveness of its effect. The value of the coefficient is independent of any *observable response* in individuals (canalisation) or populations (assimilation) and so provides a

yardstick for assessing their occurence.

In much the same way, inbreeding coefficient assumes a base population of no inbreeding *ie.* $F_i = 0$, that genes can influence the development of traits, and that they can accumulate in a population by replication and heredity. The concept of mutant (versus wild-type) is similarly a relative one, which in individuals is best defined by whether it produces any detectable change, and that does not allude to its adaptiveness. Inbreeding coefficient too is independent of expressivity and penetrance - the genetic counterparts of canalisation and assimilation.

The coefficient is particularly useful, as it can test assimilation in the absence of observably responsive individuals and in the absence of TT-stress lineages.

Assimilation and inbreeding coefficients can be expected to have similarities as both refer to heritable aspects of the generative field. The loss of Dauermodifications on cessation of the repeated eliciting stimuli, and assimilation in the absence of selection (Ho & Saunders, 1983c), are comparable to gene loss and fixation by random drift in that they involve *internal* processes.

The assimilated changes in larval and pharate developmental parameters not directly affected by cold shock is comparable to genetic pleiotropism, while the assimilated increase in cold shock sensitivity rather than simply that of its effect (*ie.* that cold shock is required to *reveal* this underlying assimilation in individuals of $A_i > 0$) is comparable to genetic epistasis. Increased sensitivity to a stimulus is diagnosed by the response showing a greater correlation with post-treatment than pre-treatment A_i, or by an increase in the magnitude of some *difference* between control and exposed samples.

The *n*-th treatment generation is termed T_n and the parents of T_1 (first treatment generation) are referred to as P. Derivation of A_i assumes that the potentiality for expression of the stress response in any individual is intermediate to the potentialities of its parents (in a similar sense to quantitative genetic factors), since such a *potentiality* is assumed to depend on some inherited factor. It should be noted that in the case of environmentally-induced changes to the DNA (Vuillaume & Berkaloff, 1974), the assimilation coefficient can also encompass genetic inheritance, assuming that such DNA changes behave quantitatively and/or there is no dominance.

Varying degrees of maternal or paternal assimilation are comparable to sex-linked and sex-limited genetic inheritance, although the mechanics of assimilation will need to worked out in each case before the coefficient can be appropriately modelled. Similarly for assimilation in self-fertilising hermaphrodites, such as land molluscs and plants.

Just as the rate of inbreeding is dependent on breeding structure *eg.* full-sib pairings, so too will the the rate of assimilation depend on the nature of the pairings *eg.* true-to-treament lineage (section 8.5).

8.18. The occurrence of assimilation in the laboratory

(i) *Pupal development and the life cycle*

The durations of the prepharate, pharate and entire pupal stage all increased with the level of assimilation (A_i). Prepharate and entire pupal duration also increased with post-treatment A_i (A_iP), although pharate duration did not. The latter is attributed to the lack of any direct effect of cold shock on pharate duration throughout the protocol. An analysis of covariance (ANCOVA) showed that assimilation contributed the more to the effect of assimilation and cold shock in combination and that its relative contribution was similar for each pupal stage. The ANCOVA also showed that neither pre-treatment Ai nor cold shock alone significantly affected these stage durations, suggesting that it was *sensitivity* to cold shock which increased with the level of assimilation. That the difference in each pupal stage duration between foil and cold shock treatments was greater at higher levels of A_i (Figure 8.8) would support this view. The above trends were similar in both sexes. It appeared that cooler temperatures *in general* can slow prepharate development, but that this effect could be overcome provided temperatures remained within the control range. Cold shock temperatures exceed the limit of this canalisation, canalisation which further breaks down as sensitivity to cold shock increases.

Neither pupal cold shock nor increasing A_i or A_iP resulted in any decrease in survival as such. Cooler cold shock (and control) temperatures did however result in smaller sample sizes suggesting that cold shock exerts an immediate lethal effect. The proportion of females in samples became smaller as A_iP increased but showed no trend with A_i or under cold shock alone, implicating an assimilated increase in sensitivity to the potentially lethal effect of cold shock (*potentially* lethal because there was no survival difference between the treatments in the first generation) that affects females more than males. That the sex ratio became more equal with warmer cold shock temperature but cooler control range temperature, suggests an optimum temperature for pupal survival between -1.1°C and 13.9°C

(Appendix III.ii).

It was found that several life cycle parameters not directly affected by pupal cold shock showed trends with A_i. It appeared that parental (F_n) cold shock influenced the *overall* development of their offspring, and a direct effect on the developing F_{n+1} eggs was proposed. The influence of assimilation on fecundity was therefore examined. The number of eggs laid increased with both A_i and A_iP, suggesting that egg *production* increases with assimilation. The slowed F_{n+1} pupal development might allow more time for concomitant egg production, since the number of eggs laid increased with cooling control range temperature which too prolonged prepharate duration. The increase with A_iP might be due to an increasingly strong post-shock ecdysone pulse as sensitivity to cold increases, since egg production was also greater the colder the *shock* temperature, and ecdysone is known to promote ovarian follicle maturation in other species. Hatchability decreased with A_iP but not with either A_i or under cold shock alone, suggesting that cold shock can exert a lethal effect on the eggs produced. The hatchability of fertile batches, however, *increased* with pre-treatment A_i, suggesting that assimilation improves the *maturation* of the surviving eggs. The slowed development of the F_{n+1} might allow the concomitant egg maturation to further continue to eclosion and hence pairing and oviposition. Indeed, the hatchability of fertile F_{n+1} batches improved with cooling F_n control range temperature, especially during the pharate stage. The number of eggs laid also increased with A_i and A_iP, and is attributed to the prolonged adult longevity at greater A_i and A_iP allowing the females to complete their oviposition.

Oval and larval durations became shorter as A_i increased. In chapter six it was proposed that changes to stage durations will respect the overall form of the linear growth dynamic (LGD), and it would appear that the prolonged pupal stage durations necessitate faster larval linear growth. Two ways in which this might have increased sensitivity to cold shock were presented. The first was that faster linear growth involves an increase in metabolic rate and hence in concomitant ecdysone production, so that a stronger post-shock pulse ensues. This hypothesis was based on the findings that rearing larvae at 19.3°C (versus 14.8°C) had a similar steepening effect, and that in another species, Aglais urticae (Nymphalidae), animals reared

at higher temperatures yielded more extreme cold shock phenocopies. Indeed, increased ecdysone production might explain why *control* prepharate and entire pupal durations actually became *shorter* as A_i increased (Figures 8.9 and 8.11). Following the post-shock pulse, however, the rate of ecdysone production might be *lower* at the higher A_i. The second was that the higher metabolic rate would mean greater metabolic stress being imposed when the limited elasticity of larval integument precluded ingestion. It was suggested that the greater contractile energy stored therein might account for the shorter pupal lengths at high A_i. However, the latter could also be explained by modelling integumental elasticity as a (decreasing) function of linear growth rate. Here, integumental growth involves the breakage and reformation at a constant rate of molecular cross links of given inherent elasticity. As larval growth rate increases, it increasingly exceeds the rate of molecular break-reform, so that stress is imposed on cuticular elasticity increasingly early in each instar.

The steepening larval dynamic with increasing A_i effectively pulled the prepupational deviation towards t_0. However, there appeared to be a limit beyond which steepening larval growth was no longer possible, this limit perhaps being imposed by the mechanical properties of integument.

In STOCK 01 larval growth rate was slower at 14.8°C than at 19.3°C, but now increasing A_i was associated with a *decrease* in larval growth rate. It is therefore postulated that the assimilated decrease in pupal development rate is compensated by an increase in larval development rate only above some threshold temperature between 14.8°C and 19.3°C. Below this temperature, the already slower development is further slowed by assimilation. It is possible, therefore, that the option to diapause or not is related to development rate. This might explain the complex interactions between temperature, photoperiod, and assimilation in the control of pupal diapause. Temperature influences development rate directly, while temperature and photoperiod can also influence it indirectly through their influence on hormones. For example, the assimilated larva of 01012018 might have undergone pupal diapause despite the long daylength, because the assimilation and cooler rearing temperature counteracted the ecdysone-promoting influence of long daylength sufficiently to maintain developmental stability below the threshold for direct development.

In STOCK 03 it was found that cold shock had a lesser prolonging effect at the cooler of two rearing temperatures *ie.* 13.9°C versus 17.6°C. This was explained by the reduced rate of ecdysone production at the cooler temperature resulting in a weaker post-shock pulse.

Correlations among the prepharate, pharate and entire pupal durations were examined for control and cold shock samples at each of three levels of assimilation, *ie.* A_i <1.000, A_i ≥1.000 and A_i >1.000. In all cases, prepharate and entire pupal duration were strongly correlated. In controls pharate duration generally showed a weak correlation with entire pupal duration, but only when samples of A_i =1.000 were included (*ie.* the A_i ≥1.000 class) did prepharate and pharate durations correlate at all. This might now be explained by cold shock in the F_n prolonging overall pupal duration in the F_{n+1} but the pupational (and so prepharate) dynamic changing much more does the pharate dynamic as further assimilation ensues. In cold shock samples, prepharate and pharate durations again became increasingly correlated as A_i increased, except that at A_i >1.000 they were now *negatively* correlated, pharate duration also correlating negatively with entire pupal duration. This suggested that not only did overall pupal duration increase with assimilation, but that post-shock pupal development (in terms of duration) becomes increasingly canalised against the prolonging effect of shock *per se*.

With the exception of pupal (and larval) diapause above, there was no significant change in life cycle duration with increasing A_i, the longer life cycle in cold shocked samples simply reflecting the prolonging effect of cold *per se*. However, the interval between adult eclosion and copulation decreased with assimilation, this most likely due to assimilated adults being reproductively more mature at eclosion. Earlier pheromone production by the males was implicated, and that the eclosion-copulation interval was shorter at warmer cold shock but cooler control range temperatures impicating an optimum for pheromone production between -1.1°C and 13.9°C (Appendix III.ii).

(ii) *Inbreeding and canalisation*

The survival of first instar larvae became poorer as the level of inbreeding increased, but appears to have involved an increase in their susceptibility to adverse conditions rather than any inherently lethal loci, since the survival of second instar larvae actually *increased* with inbreeding. Hence under the improved regime (section 9.16.i) the decrease in first instar survival might be overcome.

Higher levels of inbreeding were associated with poorer prepharate survival among *cold shocked* samples and increasingly disparate family and sample sex ratios (towards a paucity of females). This mirrored the trend with increasing assimilation, suggesting that the two might operate synergistically. Higher levels of inbreeding, however, were also associated with *more variable* sex ratios, suggesting that there may be a concomitant decanalisation of the potentially lethal effect of pupal cold shock (but not of the assimilated changes in life cycle stage durations and improved egg production and maturation).

Pharate pupal duration increased with inbreeding and became less variable. It was suggested, therefore, that ensuing inbreeding might help canalise its assimilated increase in duration, and thus exemplify Reidl's (in Wagner, 1981) 'genetic gear'.

The extinction of lineages most likely resulted from the increasingly disparate sex ratios and smaller initial family sizes associated with greater inbreeding. However, since initial family sizes increased with assimilation, it is possible that concomitant assimilation might help counteract the latter inbreeding effect. Moreover, the proportion of egg batches hatching increased with inbreeding, so that pairing individuals become less likely to be members of the same family. In this way, inbreeding may itself limit the progressive decreases in survival or fecundity otherwise associated with it (and with the synergistic action of assimilation), and so in effect canalise development at the *population* level; and since natural populations may be established by singletons or pairs (Barbour, 1986), this could have important evolutionary implications. These are examined in chapter ten.

The extent to which cold shock prolonged prepharate (and hence entire pupal) duration was much less in hybrid STOCK 03 (family 002) than in either parent STOCK 01 (family 012) or STOCK 02 (family 001), suggesting that heterosis serves to canalise against immediate cold shock effect, which might be expected given that genetic load in the species is concerned primarily with genic balance (Oliver, 1981). That egg hatchability was also greater in 03002 than in parent family 01012 too pointed to possible heterosis, although no definite conclusion could be drawn on account of the absence of hatch data for family 02001. It was suggested that the canalising capacity of the hybrid gene complex might carry over into the F_2 (and possibly further generations), so allowing an underlying assimilation or build up of genetic variability to ensue. The evolutionary implications of this too are discussed in chapter ten.

Genetic load was estimated for STOCKS 01 and 03 and found to be 0.156 LE per zygote and 0.709 LE per zygote respectively, both lower than that estimated by Oliver (1981) for a population from north Hampshire. The higher load in hybrid stock 03, however, would appear to argue against heterosis with respect to egg hatchability. Genetic load was also examined in subspecies acgeria STOCK 04, and estimated to be not more than 0.421 LE per zygote, and thus within a similar order of magnitude to load in the above subspecies tircis stocks. Subspecies aegeria (at least samples from the same poplation as STOCK 04) might therefore be expected to show similar trends to subspecies tircis with respect to immediate and assimilated cold shock effects on development rate, survival and fecundity.

(iii) *Differences within and between populations*

Nonetheless, populations do appear to differ in their precise reactions to cold shock. P. a. tircis STOCK 06 from Doncaster, U.K., developed dark brown to black pupal blotches following cold shock where it appeared to be an inherent capacity of 15% to 30% of individuals. It was proposed that such blotching also results from the post-shock ecdysone pulse, since the hormone is known to promote melanogenesis in other insects, and that it too might be amenable to genetic assimilation.

Comparisons were made among stocks using samples of A_i = 0.000 and reared at similar temperatures. The results showed there to be genetic components in the control of normal pupal development, although the particular differences (prepharate, pharate or entire pupal duration) depended on the stocks under comparison. Lincolnshire STOCKS 02 differed from Southampton STOCK 01 in the durations of all three pupal stages, while Doncaster STOCK 06 differed from it only in pharate and entire pupal duration. Within STOCK 01, families 002 and 004 and their respective derivatives were found to differ genetically in pharate duration, implicating genetic differences within populations.

It was found that stocks from more northerly locations developed faster when reared at similar temperatures (*circa* 19°C). This was explained as the more northerly populations having become adapted to develop normally under cooler conditions, so that when reared at a mean temperature of *circa* 19°C (higher than the mean July temperature over the species' range in Britain, *cf.* Dennis, 1977), those from the northerly populations would *in effect* have experienced a higher temperature. It was predicted that their greater ecdysone turnover would render them more sensitive to cold shocks (on account of a stronger post-shock pulse), and the results bore this out. The shortest stage durations at *circa* 19°C, however, were those of STOCK 04 (although data was available only for one individual), and this was explained in terms of south European P. a. aegeria having become adapted to mean *winter* temperatures (-13°C) lower than *any* normally experienced by British subspecies tircis (*cf.* Dennis, 1977).

The incidence of larvae undergoing five instars was examined in the STOCK 01 F_1 and F_2, STOCK 03 F_1 family 002, and STOCK 06 F_1 family 003. Within STOCK 01 the incidence of five instars differed significantly between lineages derived from the two F_1 families 002 (frequency = 17%) and 004 (frequency = 4%), implicating a genetic component in the control of five instar development that can differ *within* a population. In northern STOCK 06 the incidence of 5-instar larvae was 44%, also implicating a genetic difference *between* populations. The high incidence here was probably *not* related to it being of A_i = 1.000, since there was no difference between samples of A_i = 0.000 and A_i = 1.000 within STOCK 01. Within STOCK 01, 5-instar larvae were more frequent in samples reared at <15°C (50%) than at >15°C (9%).

The sex ratio among 5-instar larvae surviving to the adult was examined. At *circa* 19°C the female:male ratio was between 2:1 (STOCK 01) and 4:1 (STOCK 06), whereas at *circa* 15°C the data, though scanty, suggested that the sex ratio was more equal. It was postulated that 5-instar development depends on a similar genetic determinant(s) in both sexes. At lower temperatures (here 14.8°C) it is equally expressed in both sexes but higher temperatures (here *circa* 19°C) promote faster (4-instar) development in males. It appeared that females were better canalised against the hastening influence of the higher temperature, this canalisation perhaps involving cytoplasmic components and inheritance on account of female Lepidoptera being of XY karyotype (male Lepidoptera are XX) given that sex-linked traits mostly involve X-linked inheritance (Ford, 1957; Robinson, 1971). Indeed, cytoplasmic inheritance has been implicated in the inheritance of the black mimetic form of Papilio glaucus (Burns, 1966). It was proposed that such canalisation might be adaptive in ensuring 5-instar female larvae, even when the *immediate* climate favours faster growth, in situations where later climatic conditions may be unpredictable. In the latter case, constitutive 5-instar growth ensures that should the late summer generation 2.ii be poorly represented, the subsequent generation 1.i females will be larger and so able to reinforce numbers by laying more eggs (Wiklund *et al.*, 1983). Indeed, STOCK 06, from a northern population and one thus more likely to suffer unfavourable climatic conditions, had the highest incidence of 5-instar larvae and the greatest bias towards its expression in females.

It is clear that genetic assimilation of the effects of early pupal cold shock on pupal development (and other aspects of the life cycle) can occur in Pararge aegeria tircis. It also seems possible that it could occur naturally in the species, and that an assimilated sample yielded phenotypes resembling a known natural aberration suggests that such genetic assimilation might well occur in nature.

The effects of cold shock on the wing morphology are examined in the next chapter, where a number of other species are also examined. The relationship between the level of assimilation and the incidence of cold shock phenocopies is considered in chapter ten, when the implications for the species of life cycle and wing phenotypic cold shock effects and their genetic assimilation are drawn together.

CHAPTER NINE

Summary

This chapter describes the P. aegeria wing pattern in terms of the Nymphalid Groundplan. Microscopy and photography are used to measure the effects of cold shock and darkness on 128 size and colour characters. Untreated animals are also examined, as are sex and family differences. The study sample comprises the two F_1 families from Chapters Six and Seven.

Fore- and hind-wing venation are brought into homology and the nomenclature updated. Size and colour correlations between the wing surfaces suggest an early dorsal-ventral separation. Concordances among pattern elements and wing spaces show each wing to comprise four sectors. They also suggest that pattern is established from the margin and largely dependent upon venation. There was no significant directional asymmetry or antisymmetry. Variability was measured to assess genetic variation and developmental stability, the latter also estimated by fluctuating (random) asymmetry.

The hindwing is less canalised than the forewing, and males were less canalised than females especially under dark and cold treatment. Males were more susceptible to the ecdysone pulse; while females were more sensitive to ecdysone present *during* treatment. Only two males and one female showed obvious pattern disturbances under cold shock: the female resembled a wild aberrant. There was no sex difference in hindwing spot number but this did differ between families. The more orange colour and greater canalisation of hindwing spot size in males implicated interaction between the X-chromosome and the rest of the genome.

Cold shock effects were also examined in other species and support previous findings. The various imaginal discs also appear to be coordinated. A model is presented whereby phenotype reflects the pattern of wing-cell division and growth, accounting for its morphallactic *and* epimorphic features; wing cells differ in maturity, and a substrate perfuses all wing cells together but transformed within each until it matures. It is suggested that eyespot foci mark the intersect of two nodal values of reaction-diffusion.

CHAPTER NINE

WING DEVELOPMENT AND COLD SHOCK

Introduction

This chapter firstly examines wing development in P. aegeria under normal conditions. There is considerable variation in the wild-type pattern (Cribb, 1983), and photographic (Robertson, 1980a), digital (Shreeve, 1985) and visual techniques (Packer, 1984) have been used to analyse its seasonal variation. Yet, although the complex selective interactions influencing spot-phenotype frequencies are well understood (Packer, 1984), the development of the pattern itself and the influences of hereditary and environmental factors have yet to be elucidated.

Heat shock can induce pattern modifications in the species, which also show considerable variation (Robertson, 1959). Since it is perturbation *per se*, rather than its precise nature that is important (Ho *et al.*, 1986), cold shock too might induce such modifications. Several wild aberrations have been reported (Russwurm, 1979), which, since it is likely that the pupae suffer occasional frost exposures, may represent naturally occuring phenocopies. Therefore the effects of cold shock on wing development are also examined.

Nijhout (1984) classified cold shock phenocopies of *Cynthia cardui* (L.) into six visually discrete classes. Several methods of wing pattern analysis have been used with various species in other contexts (Ford, 1940; Jarvinen & Vepsalainen, 1979; Shapiro, 1984; Brakefield & Liebert, 1985), yet particular levels of analysis differ in their respective merits (White *et al.*, 1988). This chapter employs visual and photographic methods of analysis and wing shape is also considered.

9.1. Pattern

Wing pattern is shown on Plates 1 and 2. The pale markings range from white to tan. There are also darker grey and brown areas which may engulf or obcure the pale markings. The forewing has a single eyespot in s5. The hindwing has up to five (dorsal surface) or six (ventral surface) eyespots. Both wings have a fringe pattern. The species exhibits Oudemans' phenomenon of the Argynnis type (where the ventral hindwing and forewing apex are similar to each other but distinct from the dorsal surface) (Schwanwitsch, 1935) which here involves a complex pattern of ochres and greys.

9.2. Venation

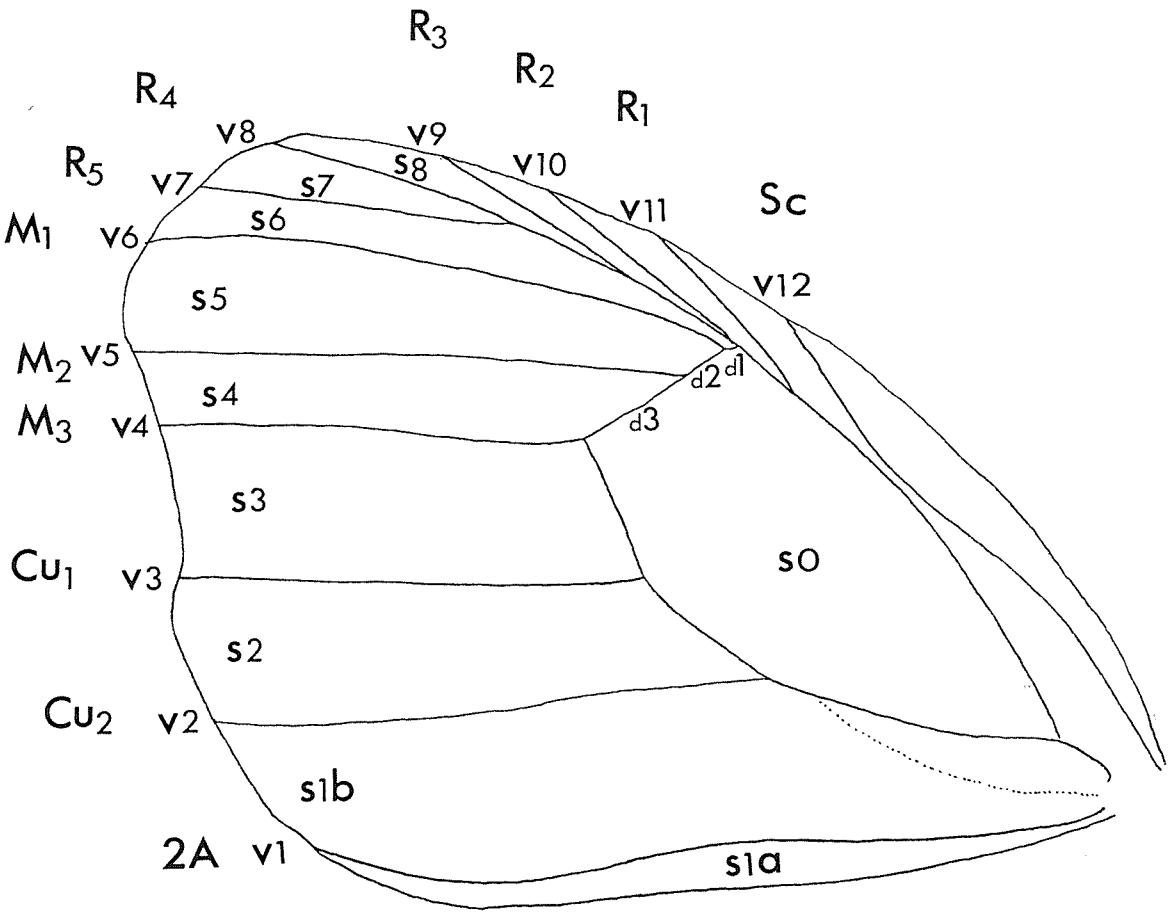
Wing venation is shown in Fig. 9.1. It is suggested that the discal cell (Higgins and Riley, 1975) be designated s0, which is closed (Ford, 1957) on the forewing. Hindwing s0 lacks the discoidal veins and is thus open (*ibid.*) being contiguous with s4 and designated s0/4 here. The 12th and median forewing veins are thickened at their bases. The hindwing precostal vein appears to be vestigial in aegeria (*cf.* Higgins, 1975).

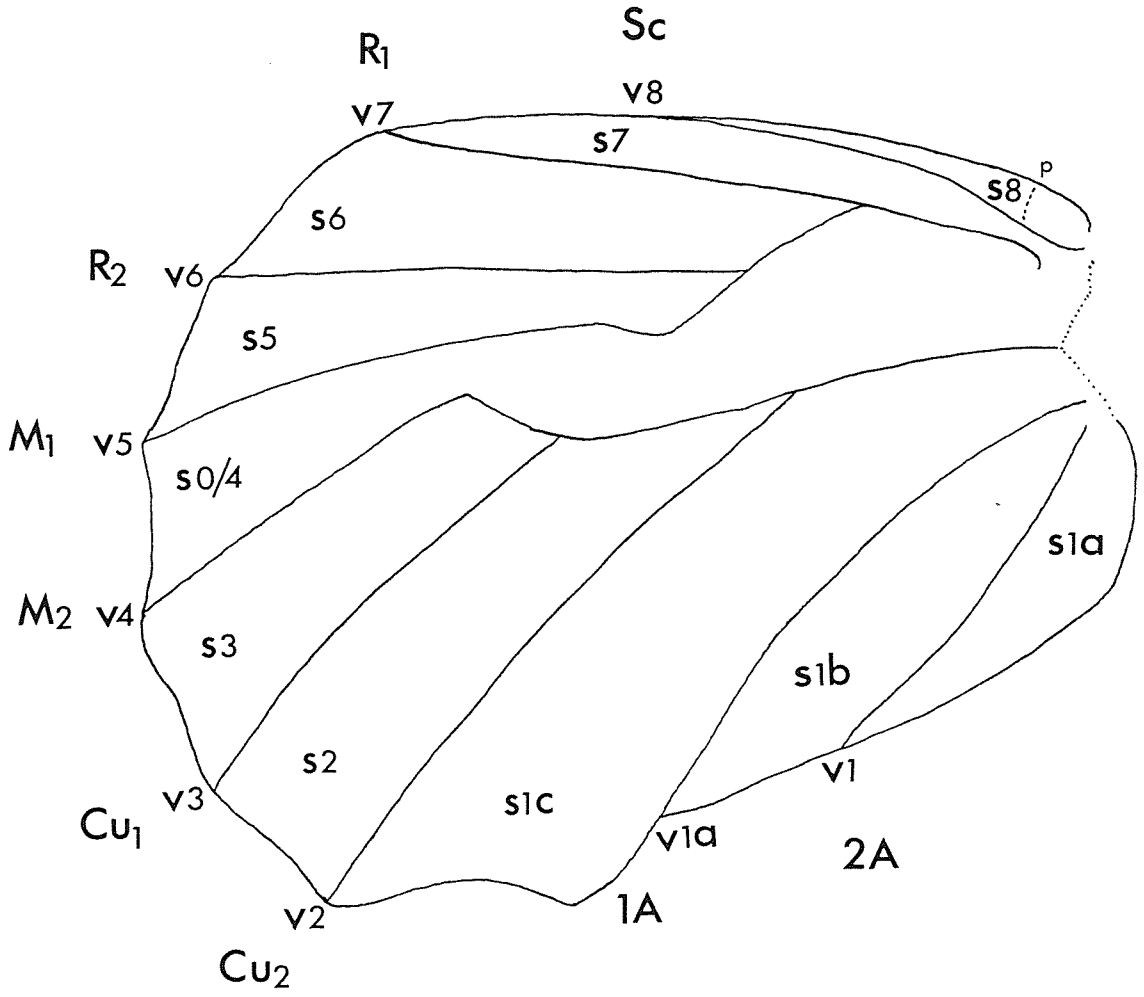
9.3. Shape

Gestalt shape differs between the forewings and hindwings and also exhibits sexual dimorphism (Plates 1 & 2). Shape is established by development of the bordering wing lacunae during the late final instar larva (Nijhout, 1985c).

9.4. Pigmentation

Aegeria lacks flavonoids (Ford, 1957), ommochromes and pterins (Nijhout, 1985c). The white of the eyespot pupils is probably structural (Nijhout, 1981) as their microscopic appearance changes with the angle of incident light. Browns and black are produced by


R3
R2
R4
R1
v8
v9
R5
s8
v10
v7
s7
Sc
v11
M1
v6
s6
v12
s5
M2
v5
d2
d1
s4
M3
v4
d3
s3
s0
Cu1
v3
s2
Cu2
v2
s1b
2A
v1
s1a


Sc
R1
v8
v7
s7
p
s8
s6
R2
v6
s5
M1
v5
s0/4
s1a
M2
v4
s3
s1b
s2
v1
v3
s1c
Cu1
v1a
2A
v2
1A
Cu2

eumelanins, yellows and reds by phaeo- and erythromelanins respectively (Nijhout, 1985c). Each scale contains one pigment only (Nijhout, 1981). Colour can also arise from different pigment concentrations (Nijhout, 1980b) or from mingling of differently coloured scales (Brakefield & Liebert, 1985).

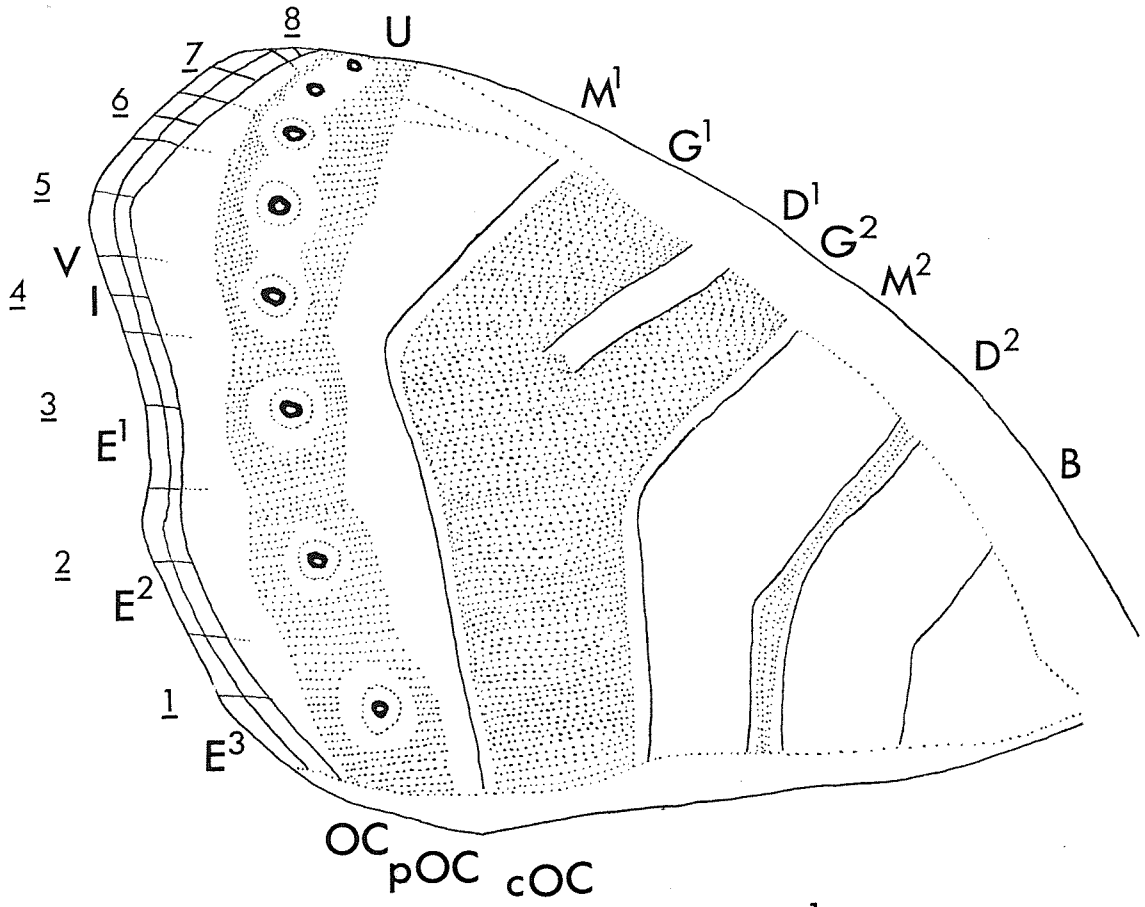
Four kinds of scale were identified: field; fringe; hair; and androconial types.

9.5. The "Nymphalid Groundplan"

Schwanwitsch (1935) used his Nymphalid Groundplan (1924) (Fig. 9.2) to interpret the P. aegeria wing morphology (Fig. 9.3); which derives from extensive nigrism (broadening and darkening) of the principal pattern elements. Schwanwitsch's terminology provides a useful system of *nomenclature* which is adopted here, being more comprehensive than that of Suffert (*cf.* Schwanwitsch, 1929).

Current understanding of pattern physiology, in particular the developmental autonomy of wing spaces (Nijhout, 1985b) and recognition of the parafocal elements (Nijhout, 1986), calls for revision of certain groundplan features.

Figure 9.1 (opposite). Wing venation of Pararge aegeria. The numeration of veins (v), discoidal veins (d) and spaces (s) is indicated. The Comstock-Needham nomenclature (upper case lettering) is also given: Sc = subcostal; R_{1-5} = Radials; M_{1-3} = Medials; Cu_{1-2} = Cubitals; 1A and 2A = Anals. The putative hindwing precostal vein (p) is shown. (After Higgins & Hargreaves, 1983; Nijhout, 1985). s0 and s0/4 are new. Designation of wing margins: Costa = wingbase - v8; Termen = v8 - v1 (v1b on hindwing); Dorsum = wingbase - v1 (v1b on hindwing). For further explanation see text.


8
U
7
M1
6
G1
5
D1
V
G2
I
M2
4
D2
3
E1
B
2
E2
1
E3
OC
pOC
cOC

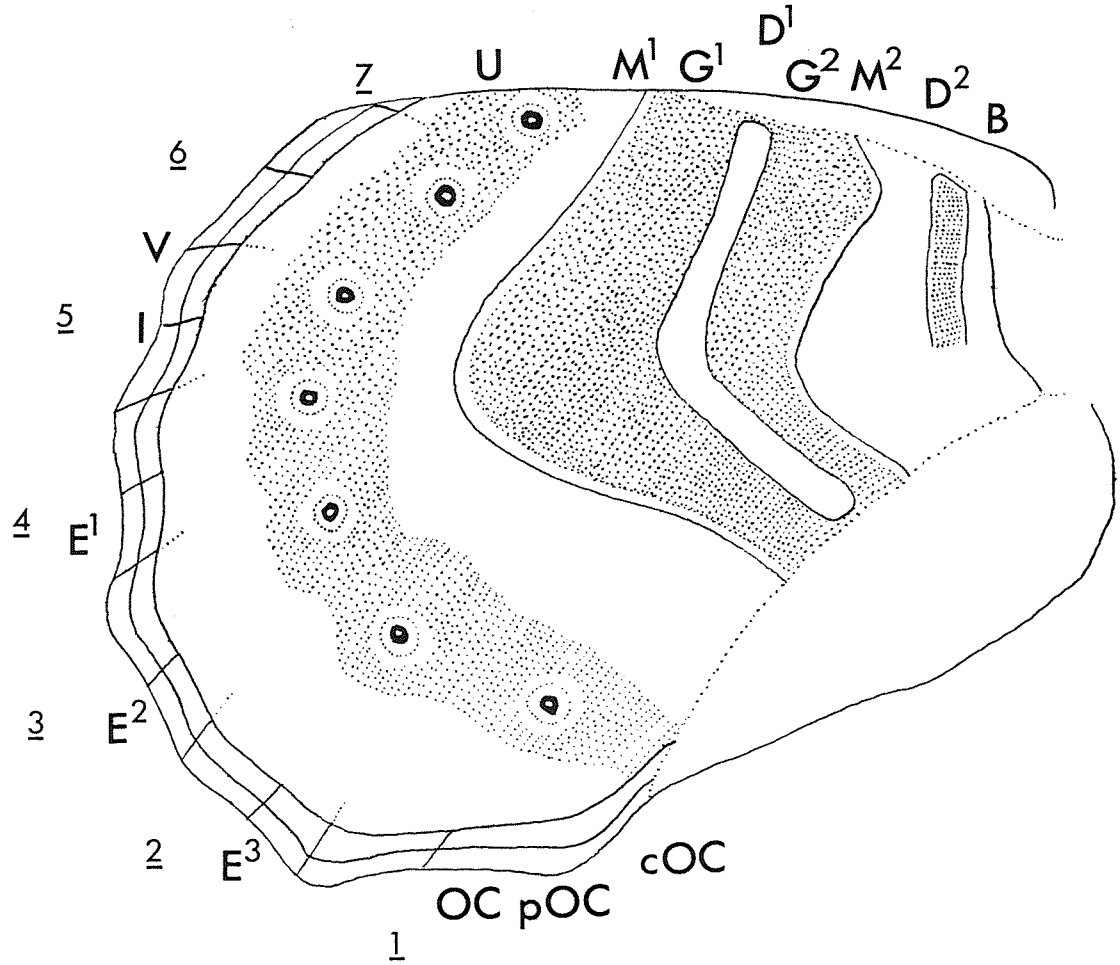

D1
7
U
M1
G1
G2
M2
D2
B
6
V
5
I
4
E1
3
E2
2
E3
cOC
OC
pOC
1

Figure 9.2 (opposite)

The Nymphalid Groundplan

Elements

B = basalis
C = circulus
D^1 = 1st discalis (discal spot)
D^2 = 2nd discalis (basal symmtery system)
E^1 = 1st externa (1st submarginal band)
E^2 = 2nd externa (1st submarginal band)
E^3 = 3rd externa (1st submarginal band)
G^1 = 1st granulata
G^2 = 2nd granulata
M^1 = 1st media
D^2 = 2nd media (CSS = central symmetry system)
OC = circuli (border ocelli)
V = venosa
I = intervenosa

Ocellar subdivision

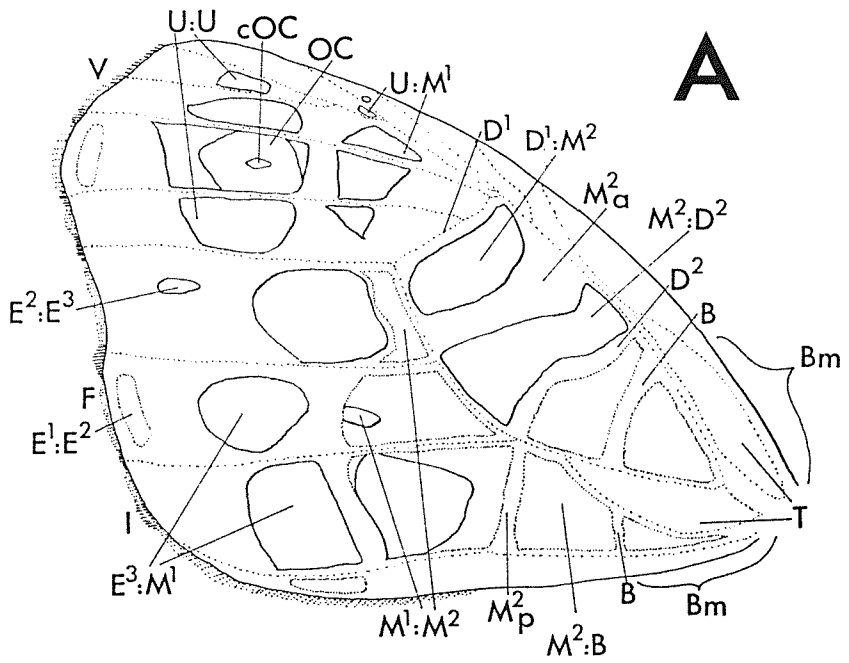
cOC = pupil
pOC = dividing ring
OC = main ring

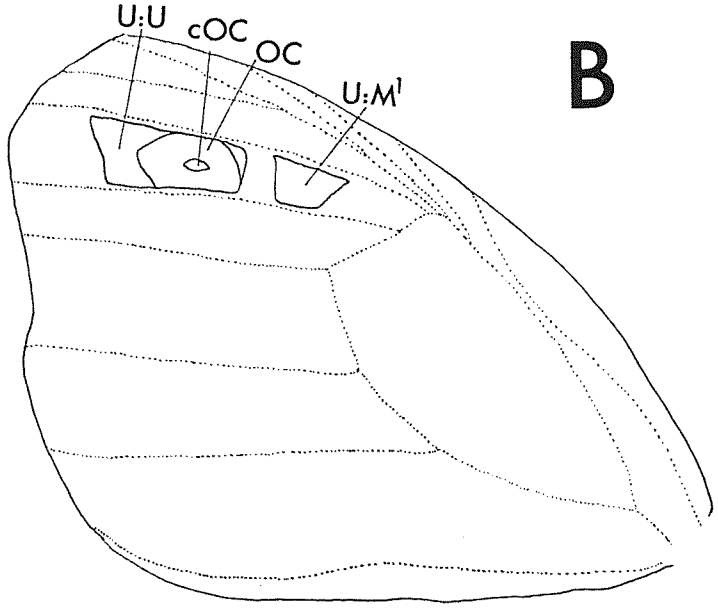
(Modified after Schwanwitsch, 1935, 1948). Marginal ciphers denote numeration of bordering spaces; underlining that the space includes an OC element. The prototype corresponds to the formula:

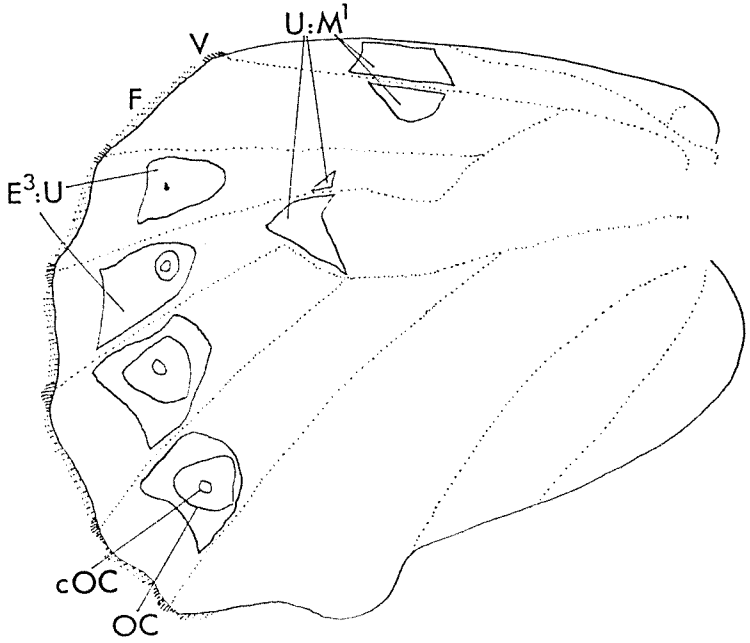
E^1 E^2 E^3 (1 2 3 4 5 6 7 8) U M^1 M^2 G^1 G^2 D^1 D^2 B : V I

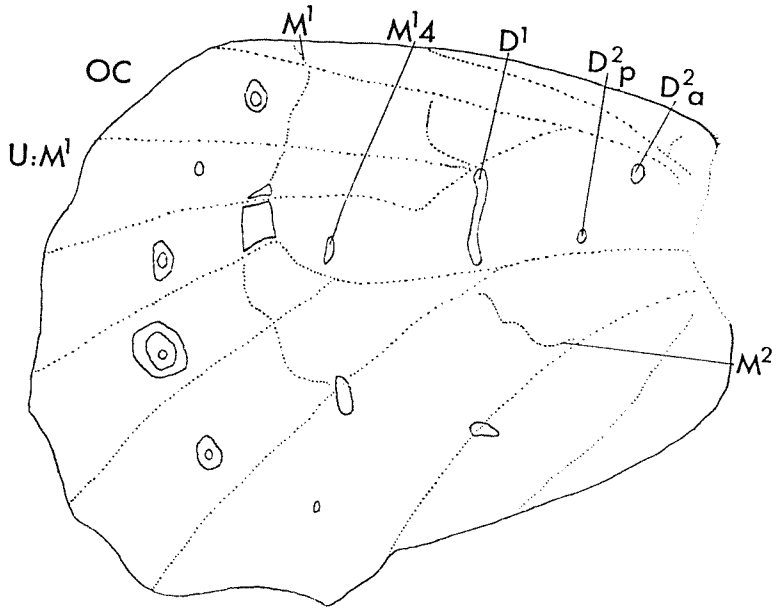
E^1 E^2 E^3 (1 2 3 4 5 6 7 8) U M^1 M^2 G^1 G^2 D^1 D^2 B : V I

Forewing above; hindwing below. Elements right of the colon are features of the anterio-posterior axis. For further explanation see text.


A
U:U
cOC
OC
V
U:M1
D1
D1:M2
M2a
M2:D2
D2
B
Bm
E2:E3
F
E1:E2
I
E3:M1
T
M1:M2
M2p
M2:B
B
Bm


B
U:U
cOC
OC
U:M1


V
U:M1
F
E3:U
cOC
OC


OC
M1
M1a
D1
D2p
D2a
U:M1
M2

9.6. Samples and treatment

This investigation used the adults of families 002 and 004 as described in chapter Six ; to which pupal cold shock and control (foil and no-foil) treatments had been applied as described in chapter Seven.

9.7. Measurement and scoring

Specimens were left to dry post-mortem at room temperature in their storage envelopes, to render them friable. The wings were then freed from the bodies by crumbling the latter away with entomological forceps. 64 wing features were selected (Fig. 9.4) and scored for one or more of (1) linear dimension; (2) frequency within a sample; (3) number present (count) on any individual; (4) gross coloration; and (5) complexity of colour (uniform or mingled scales). 128 characters

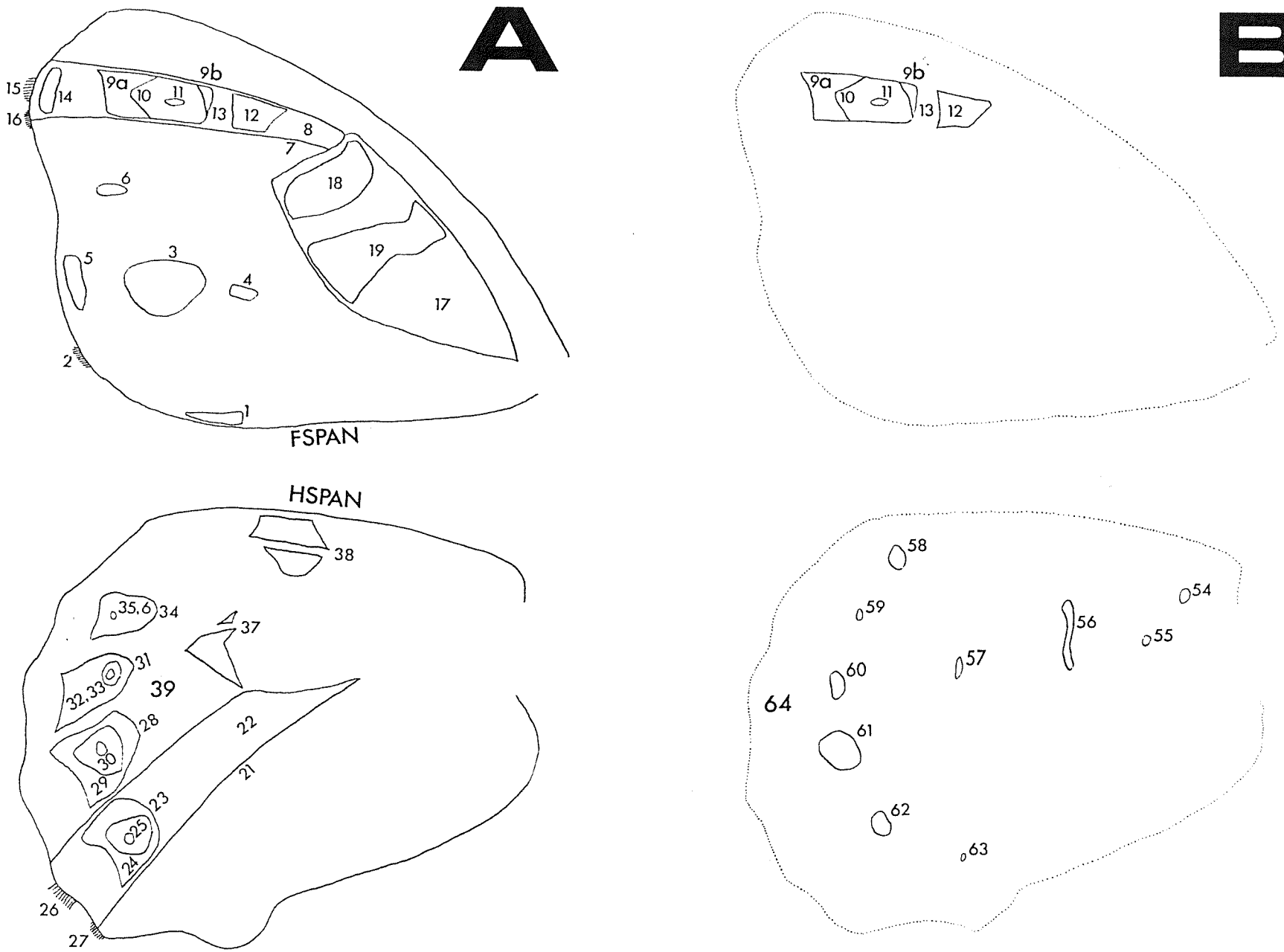
Figure 9.3 (opposite). The Pararge aegeria ground plan. A) dorsal surface; B) ventral surface. The pale markings (cf. plates 1 & 2) represent interspaces (ground colour) between the pattern elements.

Distal:Basal bordering elements define the interspaces. Fusion of elements is denoted **Distal+Basal**. Superscripts identify particular elements within similarly named series. Subscripits indicate anterior$_a$ or posterior$_p$ sectors of elements so divided within a wing surface. Wing space designation follows element listing. **Bm** denotes basal melanisation. (After Schwanwitsch, 1935, 1948).

Supplementary distinctions: forewing **FW** or hindwing **HW** are shown first; subscripts denoting dorsal$_D$ or ventral$_V$ surfaces. Subscripts following *element* (or interspace) listings denote basad$_b$ or distad$_d$ sectors. Venosae are numbered according to their anterior bordering space.

F = fringe

T = basal thickening


A
B
9a
9b
10
11
12
13
14
15
16
8
7
6
18
19
17
5
3
4
2
1
FSPAN
HSPAN
38
35,6
34
37
31
32,33
39
28
30
29
22
21
23
25
24
26
27
58
59
54
56
55
60
57
64
61
62
63

were examined on either the left or right side for all respective characters (the 1° side), the other (2°) side being scored only for characters to be used in the measurement of asymmetry.

Each wing was secured between two microscope slides at 90° to each other to hold it flat and to facilitate manoeuvre. Linear dimensions were measured using a travelling microscope with vernier scale. A 60 Watt tungsten lamp provided standard illumination; and white paper was placed beneath the microscope stage to aid visibility.

Wingspans differed between the sexes (see below); and since all linear characters proved concordant with wingspan (Kendall's-W: $P<0.001$), they were scaled to the span of the wing on which they were measured. Presence or absence was detected by inspection, but small or obscure features were confirmed by microscopic examination. This also applied to meristic characters.

A colour map was compiled for scoring continuous colour variation in terms of discrete values (Fig. 9.5).Colours were also classified as uniform (TYPE-1) or mingled (TYPE-2) scale-types. When TYPE-2 comprised more than two colours the predominant two were recorded.

The manual assembly and analysis of realistically large data sets is extremely time-consuming (White *et al.*, 1988), so the present analysis focussed exclusively on the F_1. Yet larger analyses may reveal isolated effects which when collated form a set or series, and other descriptor systems may detect hitherto unresolved global differences or morphoclines (*ibid.*).

Figure 9.4 (opposite). Wing features scored for Pararge aegeria. Numeration only is shown: (A) dorsal surface. Forewing characters FSPAN and 9A - 13, and hindwing characters HSPAN, 28 - 36 and 39, were also scored for the 2ry wing; denoted by a following letter 'A'; (B) ventral surface. Forewing characters 9A - 13 measured for the vnetral surface are denoted by a following letter 'U'. See also Appendix VII.

COLOUR	CODE
WHITE	A
PALE (BLUE-) GREY	B
PALE YELLOW-GREY	C
CREAM	D
PALE YELLOW	E
GOLDEN YELLOW (1)	F
GOLDEN YELLOW (2)	F
PALE ORANGE-YELLOW ...	S
ORANGE-YELLOW	G
ORANGE.....	H
ORANGE-BROWN	K
RED-BROWN	L
OCHRE	J
LIGHT GREY-BROWN	M
DARK GREY-BROWN	N
DARK BROWN	P
BLACK	R

9.8. Computation

The SPSS^{X} statistical package (SPSS Inc., 1988) was used for most analyses. Analyses not available on SPSS^{X} were run on a programmable calculator.

Colour codes as defined on the map were converted to two-letter codes, for example 'white' as 'AA' and 'black' as 'RR', to enable intermediate colours to be scored (for example between cream and pale yellow as 'DE'). Each letter of the two-letter code was used to position the colours on a matrix when they were assigned quasi-continuous numerical values for analysis by SPSS^{X}, TYPE-2 colours being identified by reverse-alphabetical listing (Fig. 9.6). This quasi-continuous numerical scheme constituted recoding scheme 1 or REC1TEMP.

A second recoding scheme, REC2TEMP, assigned the value 0 to TYPE-1 colours and the value 1 to TYPE-2 colours.

9.9. Statistical analysis

Each character was scored on two occasions and repeatability of scoring estimated with the Eta-squared statistic (White, 1974), where: Eta^2 = between groups sum of squares/total sum of squares. The chi-squared test was used for REC2TEMP characters.

It is essential to distinguish treatment effects from those due to samples comprising predominantly one sex or family (which may

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Figure 9.5 (opposite). The colour map. 17 colours were sequenced with priority (1) white, yellow-red and brown/black -component hues; (2) increasing saturation; and (3) decreasing purity (increasing 'greyness'). Codes deviate from strict alphabetical sequence for historical reasons. Code F comprises two poorly-resolved categories. Colour was scored when the marking and colour patch appeared as a field interrupted only by the intervening wing area. Contrasting reference backgrounds aid colour interpretation. Pigment composition is given in Appendix VIII.

COLOUR CODE

	A	B	C	D	E	F	S	G	H	K	L	J	M	N	P	R
A	01 AA	02 AB														
B	02 BA	03 BB														
C			09 CC	06 CD	08 CE										26 CP	
D				04 DD	05 DE		06 DS						15 DM		19 DP	
E					07 EE	10 EF							17 EM		22 EP	
F						11 FF		13 FG					23 FM		29 FP	31 FR
S							12 SS									
G	07 GA						34 GS	14 GG	16 GH			21 GJ	27 GM		32 GP	
H									18 HH							
K										24 KK			35 KM		36 KP	
L											30 LL			37 LN	39 LP	
J	12 JA						20 JS			28 JK		33 JJ			41 JP	
M				15 MD					27 MG				38 MM		42 MP	
N								28 NG				30 NJ		40 NN	43 NP	
P			26 PC	19 PD						36 PK		41 PJ	42 PM	43 PN	44 PP	45 PR
R															45 RP	46 RR
Z										25 KZ ZK						

themselves differ). The difficulties inherent in such dissection are discussed by Lewontin (1974). Analysis of variance (ANOVA), however, *is* suitable once additivity has been achieved by transformation (Sokal & Rohlf, 1981). Heteroscedastic and non-normally distributed data were compared with the Mann-Whitney U-test (*ibid.*). No variable was subjected to more than one transformation; applied transformations are listed in Appendix IX.

Variability and asymmetry were compared between treatments, and intra-sample correlations among the wing characters were also examined. The analysis of asymmetry used only specimens where the right side was the primary.

Figure 9.6 (opposite). The recoding matrix. Map colours and intermediates are represented by two-letter codes. Numbers give the REC1TEMP recode values. AB BA BB of *structural* origin were scored as AA.

Type-2 colours are underlined. Code Z with colour depicts hair-scales.

Construction

Colour codes were entered map-sequentially along two axes of common origin. Two-letter codes assigned position within the matrix and facilitated computational formatting. Only colours present in aegeria were coded. Type-1 colours $_{(C)}$ and intermediates $_{(I)}$ were grouped by priority-1 class and assigned numerical values increasing with precedence: left-right > top-base. Type-2 colours were given values intermediate to their components (except JA below).

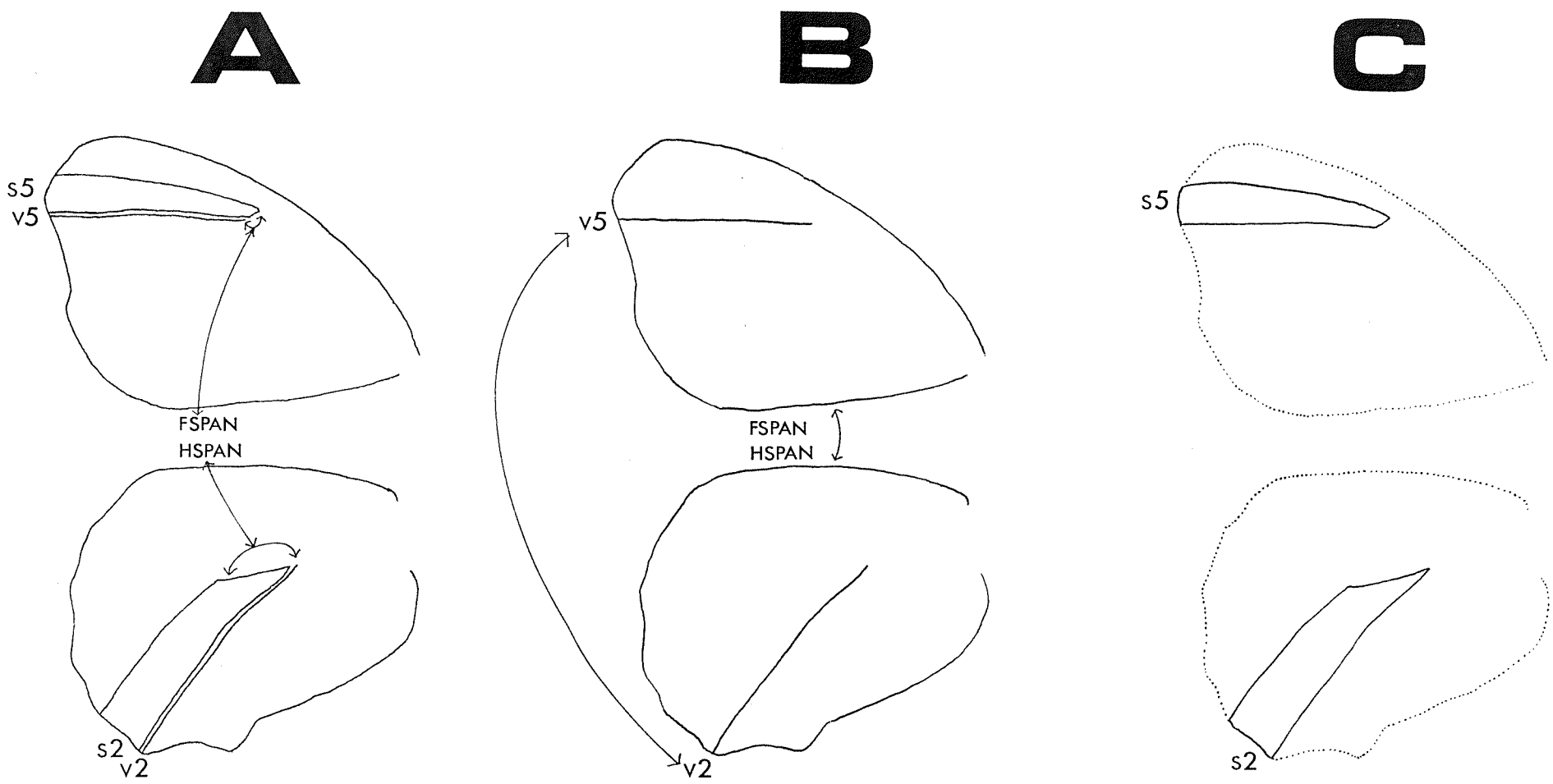
Coincident values took precedence: Type-2_I > Type-1_I > Type-1_C. Type-2 included KZ and ZK. Two-letter codes were then assigned integer values for REC1TEMP from 01-46 concordant with their real-numerical progression. GA JA NG NJ were added subsequently.

9.10. Repeatability of scoring

Six characters proved unreliable (Table 9.1) of which five are excluded from present consideration. PAT9B, however, *is* considered because it may represent an eyespot focus (cf. Nijhout, 1980a). PAT4 comprised a dusting of scales, as did PAT9B which also diffused beyond the limit of the macroscopic boundary. Individual fringe scales comprising PAT26 varied considerably in colour and at random with respect to their position. The unreliability of PAT29C, PAT29AC and PAT32AC arose through attempting to resolve TYPE-1 colour classes PP, PR and RR, and was overcome by grouping them as TYPE-1 colour PR (=RP); when PAT29C, PAT29AC and PAT32AC *were* included in the investigation.

Character	(1)	(2)	Eta^2	Df.	F-value
PAT4	12.913	113.529	0.114	1,33	4.235*
PAT9B	0.032	0.232	0.137	1,36	5.716*
PAT26C	42.344	289.081	0.146	1,35	6.007*
PAT29C	9.524	17.619	0.541	1,40	47.059****
PAT29AC	7.714	19.619	0.393	1,40	25.920****
PAT32AC	5.625	11.375	0.495	1,38	37.174****

TABLE 9.I. Repeatability of scoring. (1) Between groups sum of squares; (2) total sum of squares. F-values (one-way ANOVA) are reported with their degrees of freedom. Significance levels: * $0.01<P<0.05$; **** $P<0.0001$. All other characters produced non-significant ($P>0.05$) F-values.


A
B
C
s5
v5
FSPAN
HSPAN
s2
v2
v5
FSPAN
HSPAN
v2
s5
s2

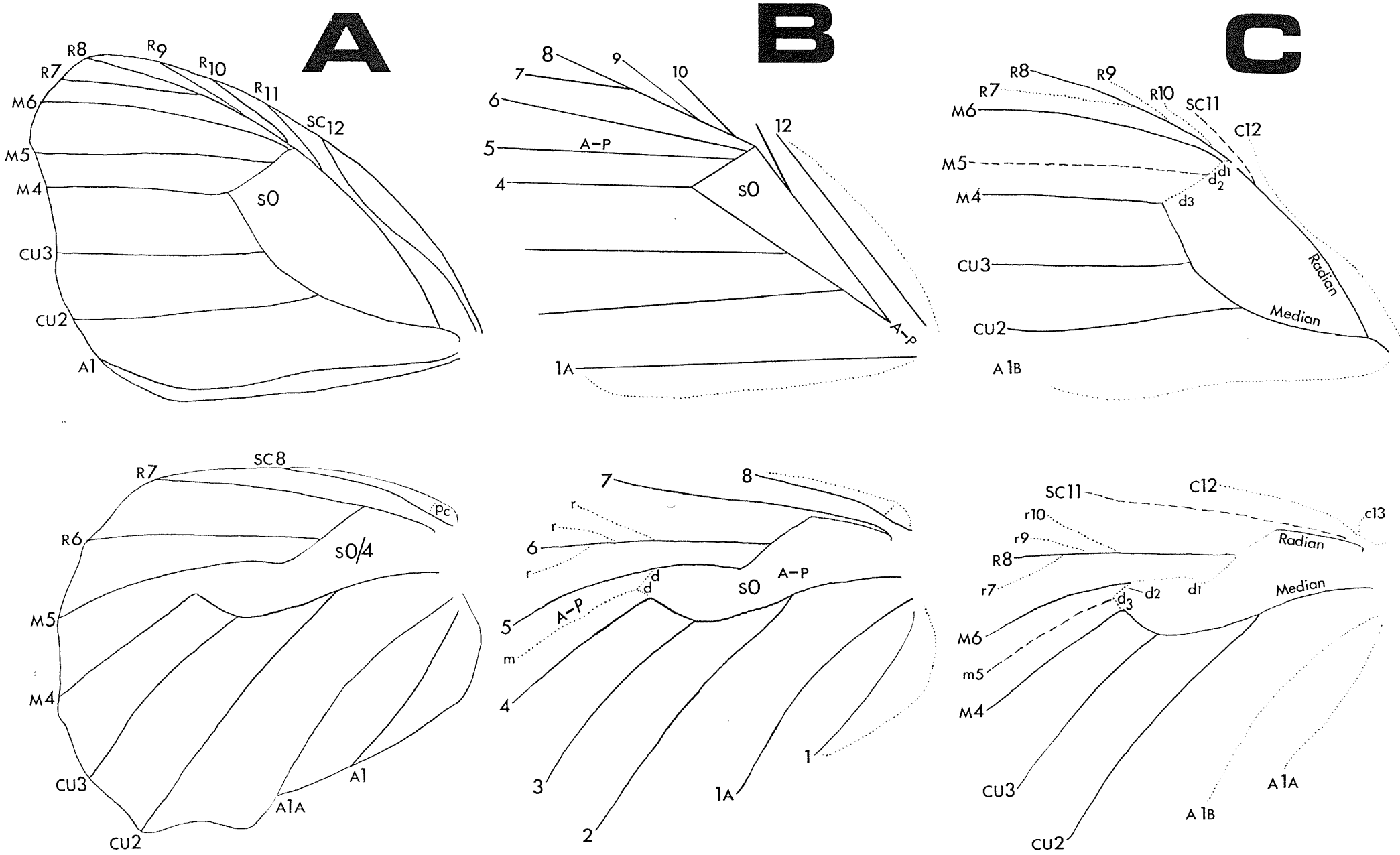
9.11. Venation

(i) *Forewing*

Forewing span, v5 and s5 length correlated with each other ($0.001<P<0.01$) and forewing span correlated with s0 length in both sexes ($W = 1.000$, $Chi^2_{(1)} = 9.000$, $0.001<P<0.01$) (Fig. 9.7a). v5, however, correlated with s0 within each sex separately ($0.01<P<0.05$) but *not* when they were considered *together*. Females had larger forewing and hindwing spans than males and, since all wing spans were highly concordant with pupal length ($W > 0.8186$, $Chi^2_{(1)} > 17.1904$, $P<0.0001$), reflects their greater pupal length. But their forewing s0 was *relatively* smaller than in males, suggesting that it is wing areas distal to it which are responsible for the larger female span; which would account for the above anomaly in s0 - v5 correlation as well as the rounder female shape.

Schematic representation reveals s0 as an origin from which the other spaces radiate (Fig. 9.8), and the extension of wing symmetry systems on to the thorax (Nijhout, 1978) would be commensurate with such radiation towards the body axis. Examination of the P. aegeria venation pattern reveals that v5 forms an axis of anterior-posterior (A-P) symmetry about s0. Sibatani (1980) found a putative compartment boundary between v5 and v6 in a number of other species, so the above axis might represent such a compartment boundary in aegeria. v7 v9 and v10 branch from v8 (*not* s0) to form a region of special complexity at the apex; while v1a and v12 originate at the margins (Fig. 9.8b).

Figure 9.7 (opposite). Correlations among wing venation features (A) within each wing and (B) between forewing and hindwing; (C) the lack of correlation between forewing and hindwing space dimensions is shown for comparison. Relevant features are shown in continuous outline and vein (v) and space (s) numbers are indicated. Dotted outlines aid orientation.


A
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R10
R11
R7
M6
SC12
M5
M4
s0
CU3
CU2
A1
B
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10
7
6
12
5
A–P
4
s0
A–P
1A
C
R8
R9
R7
R10
SC11
M6
C12
M5
d1
d2
M4
d3
Radian
CU3
Median
CU2
A 1B
R7
SC8
Pc
R6
s0/4
M5
M4
CU3
A1
A1A
CU2
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SC11
C12
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Radian
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R8
r7
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d1
Median
M6
m5
M4
CU3
A 1A
A 1B
CU2

The forewing comprises four sectors (Fig. 9.9a). s0 is the origin and comprises sector 1. v5 divides the wing into anterior sector 2 and posterior sector 3. Spaces 10-12 comprise sector 4 which is compressed along s0 giving the illusion of three wing sectors (a phenomenon apparently ubiquitous in Lepidoptera) (Fig. 9.7b). A wave of compression may pass along the costa from the base to the apex. The rounder shape of female wings may be due to its earlier extinction *ie.* before reaching the termen of sector 3 in this sex (Fig. 9.9c-d). Indeed a male of Aglaias urticae reared under similar conditions to aegeria above, had sector 3 more indented on the right than left

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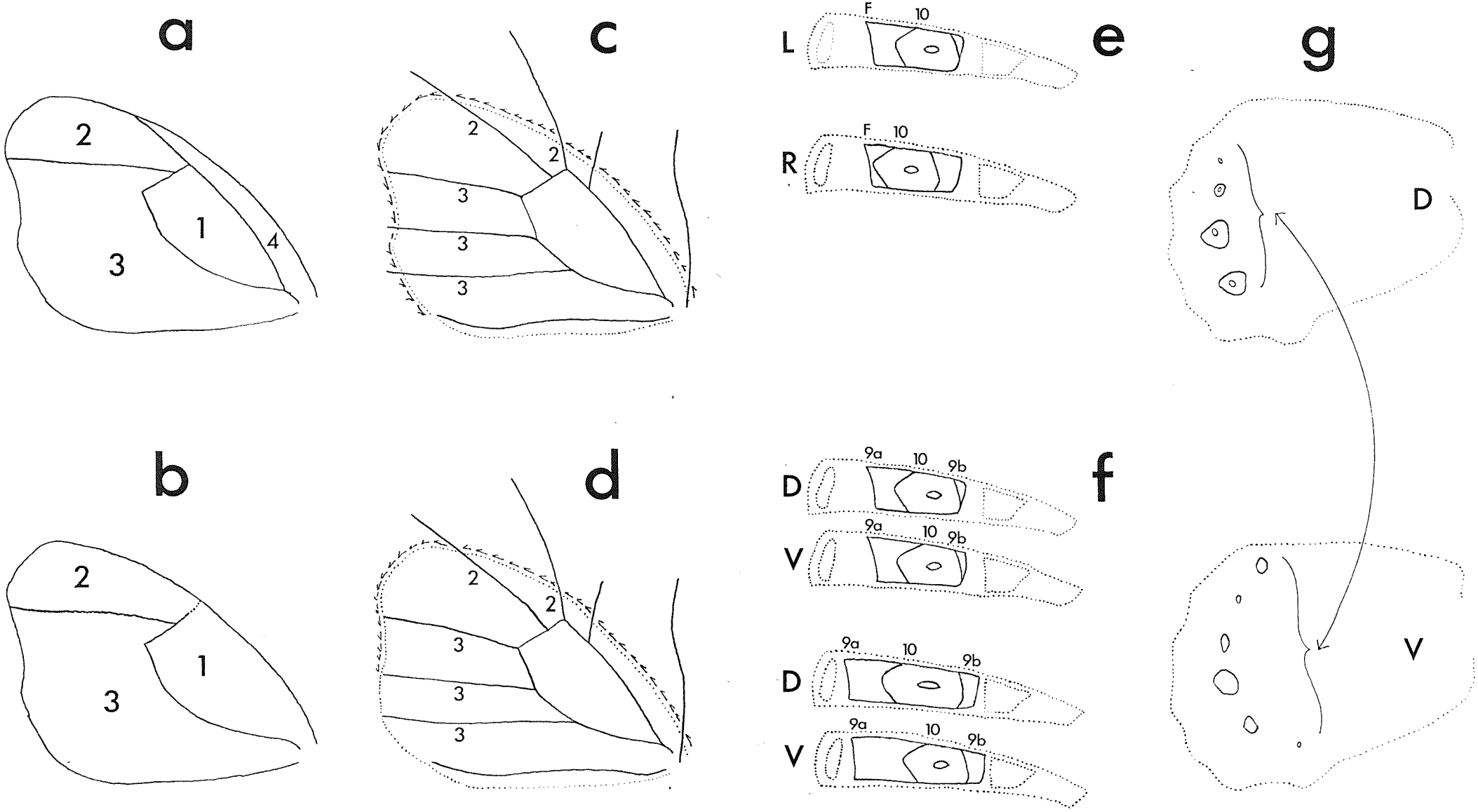
Figure 9.8 (opposite). Homology in the wing venation.

(A) Nomenclature prior to assignment of forewing-hindwing homology. Lettering corresponds to the Comstock-Needham system (Pc = hindwing precostal vein) with conventional numeration (*cf.* Fig. 9.1). s0 and s0/4 are also shown;

(B) Schematic representation of wing vein topology. The main veins are depicted in large numerals. Note the symmetry of forewing veins 1a and 12 about the anterior-posterior (A-P) axis. Small numerals highlight the branching of v7 v9 and v10 from v8. The putative hindwing radials (r) and the supplementary medial (m), with its associated discoidal veins (d) which would close s0/4 and divide the s4 component into s4 and s5, bring the two wings into accord. Note the symmetry of hindwing veins 1 and 8 about the corresponding A-P axis;

(C) Forewing-hindwing homology. The anterior vein of s0 which continues to the margin is the radian, the posterior vein being the median. Subcostal veins (SC) branch from R8 basally to the discoidals (d). Medials (M) run to the termen from the discoidals; cubitals (CU) from the median. The independent costal (C) (anterior) and anal (A) (posterior) veins are depicted. A1A and C13 are supplementary to the hindwing. Hindwing anal veins 1A and 1B are numbered 0 and 1 respectively. For further explanation see text.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9a
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9b
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9a
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9b
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9a
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9b
V
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9b
g
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forewing giving the former a distinctly elongated appearance[1].

s1a is pale grey unlike the rest of sector 3 and its position suggests homology with s12. The region comprising s7-s9 can behave as sector 2 or sector 4 (below).

(ii) *Hindwing*

Hindwing span, v2 and s2 length correlated with eachother ($0.001<P<0.01$) (Fig. 9.7a). The s0 component of s0/4 appears to be an origin from which the other spaces radiate and s0/4 forms an axis of A-P symmetry (Fig. 9.8b).

The distinct venation of forewing v7-v10 is *not*, however, repeated on the hindwing. This leaves an unbranched vein homologous to forewing v8. Were s0 to be closed, then an additional medial vein (*sensu* Comstock-Needham) could be present (Fig. 9.8b).

It is suggested that the two discoidal veins associated with the new medial vein would be homologues of forewing d2 and d3. This implies that the sector of hindwing v5 bordering s0 is equivalent to

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Figure 9.9 (opposite). Aspects of wing shape and eyespot deployment: (a-b) principle forewing regions (a) before and (b) after the fusion of regions 1 and 4; (c-d) progression of the hypothetical mechanical compression wave (dashed line) around the wing margin in (c) males and (d) females. Numbers refer to the region encompassing the respective spaces; (e) random shift between left (L) and right (R) dorsal forewing in the relative position of PAT10 (10) within FWOC (F); and (f) dorso-ventral (D-V) independence of of PAT10 from FWOC components. An increase in the size of PAT9A (9a) and PAT9B (9b) on the dorsal surface (i & iii) is associated with an increase in their size on the ventral surface (ii & iv), whilst an increase in the size of PAT10 on the dorsal surface is *not* necessarily associated with an increase in its size on the ventral surface. The space and other marking boundaries are shown in dotted outline to aid interpretation; (g) D-V concordance in hindwing spot number. For further explanation see text.

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[1]specimen available from the author

d1, making old v6 now a *radial* (Fig. 9.8c). The new medial would convert old v5 to v6 leaving the new radial v8 (putative v7 v9 and v10 being absent) homologous between the two wings. The next vein would be subcostal v11, which on the hindwing is compressed against s0 thus *appearing* as a radial, leaving the costal vein as new v12.

The region bounded by v6-v8 behaves as the rest of sector 2. s1a and s8 are pale grey unlike the rest of their respective sectors, suggesting that they are homologous about an A-P axis described by the new medial v5 and passing through s0.

(iii) *Forewing/hindwing comparisons*

The alternative numeration and nomenclature are given in Fig. 9.8c; and it is suggested that wing venation be represented by a venation formula comparable to Schwanwitsch's Nymphalid grounplan formula (Fig. 9.2) thus:

–	A	CU	CU	M^a	M^a	M^a	r	R	r	r	SC	C	–
A	A	CU	CU	M	–	M^a	–	R	–	–	SC	C	C
0	1	2	3	4	5	6	7	8	9	10	11	12	13

The discoidals are depicted as superscripts and lower case characters denote derivative veins. It can be seen that on both wings, the number of main veins (upper case lettering) is symmetrical about v5. The numeration is such that no numerical value is assigned to more than one vein; and the main veins bordering s0 are suffixed -ia*n*, to distinguish them from those outside s0, suffixed -ia*l*. The subcostal (SC) veins are functionally radials, but so named to distinguish them as branching *basally* to the discoidals in cases where s0 is closed. This resolves the disparate interpretations of (hindwing) radial and subcostal veins among the literature (eg. Ford, 1957; Warnecke, 1964; Novak, 1974; Nijhout, 1985c).

Forewing (metathorax) and hindwing (mesothorax) venation appear homologous, although the latter, since it lacks discoidal veins and the v7-v9-v10 system, is less complex. Since complexity (*sensu* the

number of kinds of component) tends to increase in evolution (Saunders & Ho, 1981), this may be due to the hindwing not yet having had time to attain a similar level of complexity to the forewing (where A0 and C13 appear to have been lost), suggesting that its *origin* may be more recent (and so still closer to the structure from which it derives ie. *structurally* more *primitive*). Indeed among Rhopaloceran families, the *least* specialised Hesperiidae have s0 *open* on both wings, while the *most* specialised Papilionidae have s0 closed on both wings (Ford, 1957).

Forewing and hindwing span correlated with each other, as did forewing v5 and hindwing v2 ($0.001<P<0.01$) (Fig. 9.7b). Forewing s5 and hindwing s2, however, did *not*, and this may be due to differences in the development of forewing and hindwing bordering lacunae which establish the geometry of their wing margins (Nijhout, 1985c).

9.12. Axial correlations

(i) *Left-right (L-R) axis*

None of the *hindwing* characters, including wingspan, showed significant L-R correlations. On the forewing, only linear dimensions, including wingspan, showed significant ($P<0.05$) L-R correlations. This suggests that the hindwings are the more susceptible to random departures from bilateral symmetry, commensurate with the contention that they are more recent (and hence less specialised), having had less time for their development to become canalised.

(ii) *Dorsal-ventral (D-V) axis*

D-V correlations were present on both the forewings and the hindwings, but only linear dimensions showed significant ($P<0.05$) D-V correlations. That L-R and D-V showed size but not colour correlations, suggests that marking size (nigrism of elements) and colour (pigmentation of interspaces) involve distinct processes.

(iii) *Anterior-posterior (A-P) axis*

A-P correlations refer to those between forewing and hindwing characters. Both linear dimensions and TYPE-1 colours showed significant ($P<0.05$) A-P correlations. It would appear that colour is determined after the establishment of the L-R and D-V axes but before that of the A-P axis.

9.13. Forewing space 5

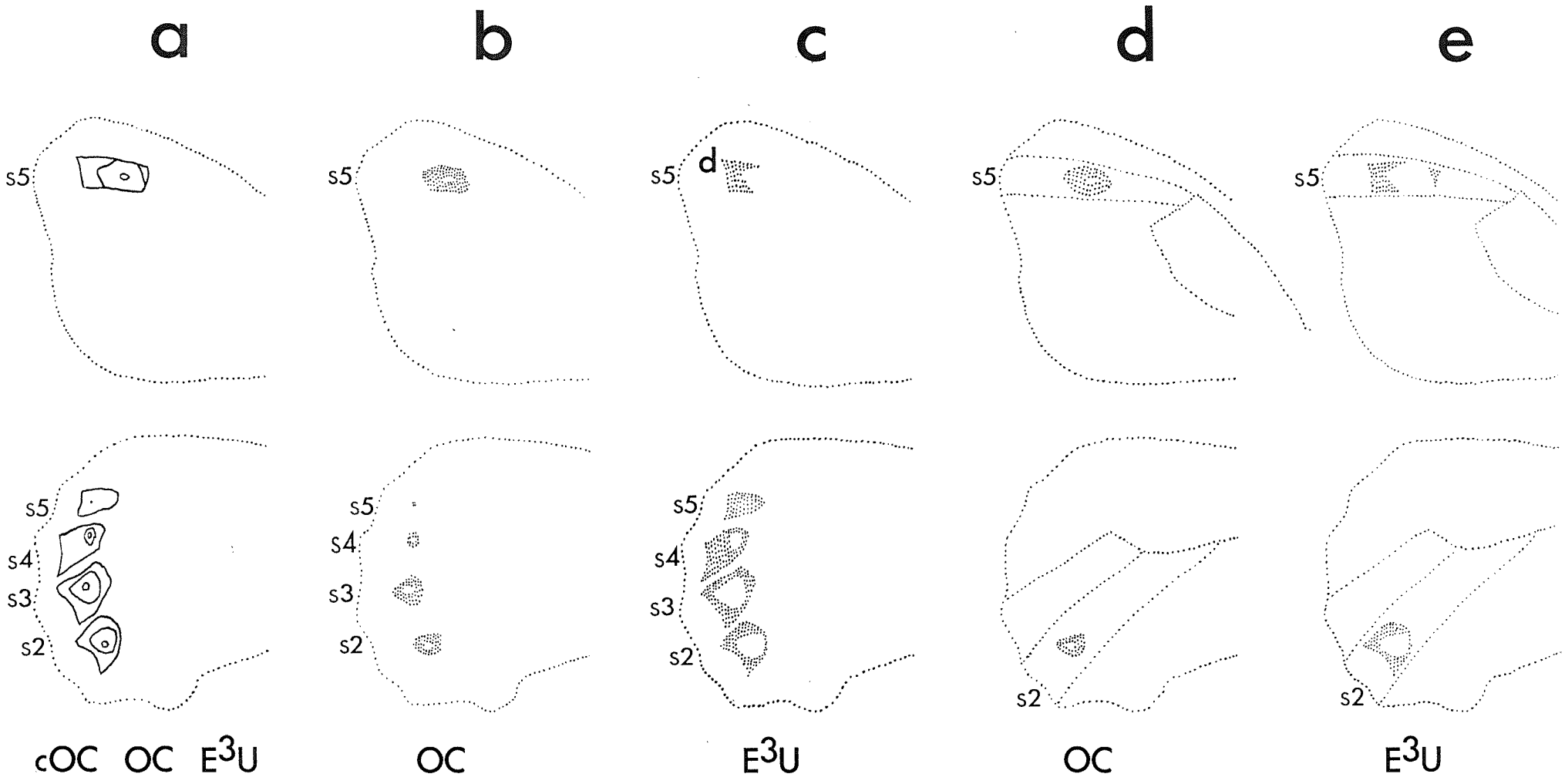
PAT12 and FWOC overall showed both D-V and L-R concordance ($0.01<P<0.05$). PAT9A and PAT9B (distal and basal components of FWOC) considered separately, however, did *not* show L-R concordance. This is most likely due to a lack of concordance in the relative *position* of PAT10 within FWOC, since PAT10 itself was concordant between left and right (Fig. 9.9e).

PAT9A, PAT9B and FWOC (FW_D U:U 5 components) showed D-V concordance, but PAT10 (OC 5) did not. This suggests that the development of OC elements may be independent of their adjacent markings (Fig. 9.9f).

9.14. Ocellar and umbral correlations

(i) *Dorsal/ventral hindwing correlations*

In males, but *not* females, the ventral and dorsal hindwing spot number were concordant ($W = 1.000$, $Chi^2_{(1)} = 6.000$, $0.01<P<0.05$) (Fig. 9.9g). It would appear that eyespot determination is independent of, or prior to, D-V compartmentalisation.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(ii) *Dorsal hindwing and forewing/hindwing*

There was strong concordance among hindwing s2 s3 s4 s5 as a group ($P<0.0001$) and between these as individual spaces in the *size* of each E^3:U, OC and cOC elements. Similar concordances were found when forewing s5 was included in such comparisons (Fig. 9.10a). It therefore appears that the unit of size determination of these elements is not the wing space (*cf.* Nijhout, 1985b) and is independent of, or prior to, A-P compartmentalisation.

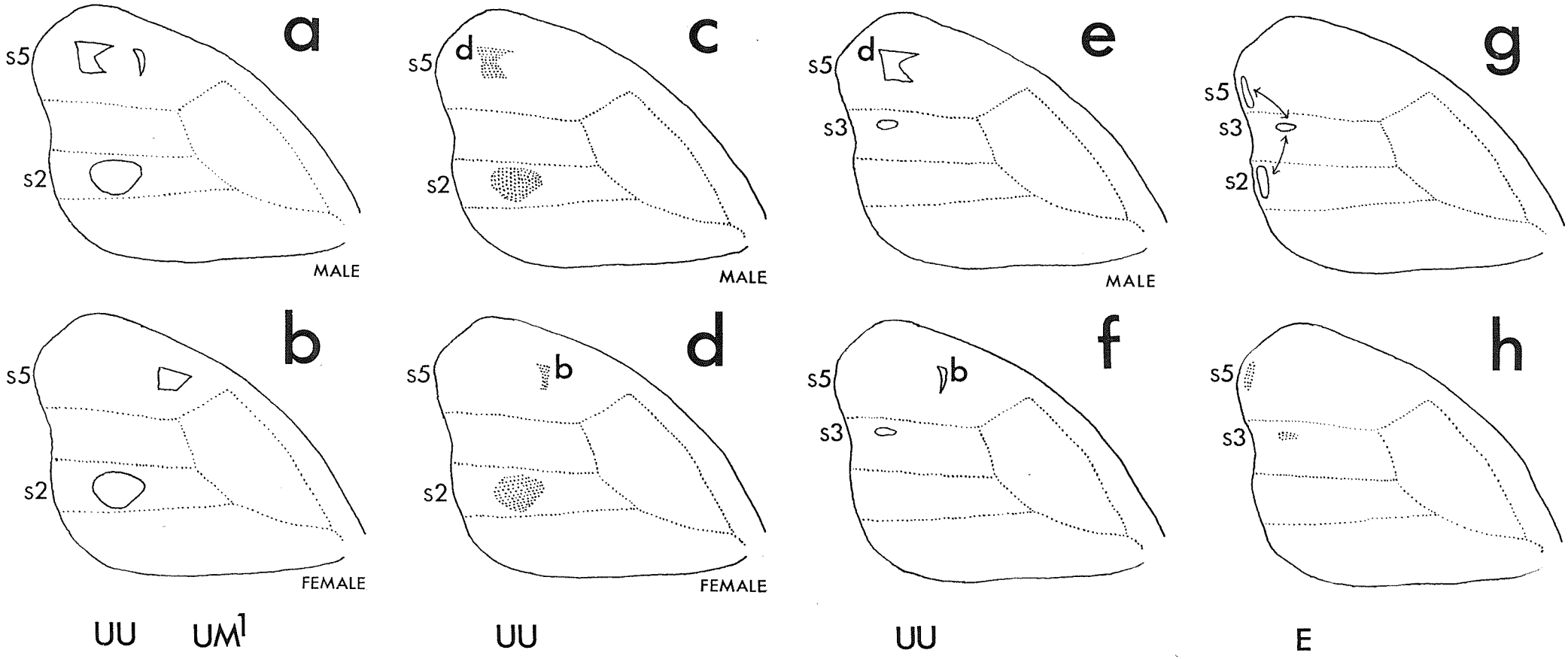
There was strong concordance among hindwing s2 s3 s4 s5 as a group ($0.001<P<0.01$) in the *colour* of OC elements. A similar concordance was found when forewing s5 was included in the group ($0.01<P<0.05$) (Fig. 9.10b). In the latter case, the colour of the hindwing E^3:U and *distal* forewing U:U (PAT9AC) elements also proved concordant ($0.01<P<0.05$) (Fig. 9.10c).

The only colour concordances between hindwing s2 s3 s4 s5 and forewing s5 as *individual* spaces were between the hindwing s2 and forewing s5 OC and U:U (E^3:U) elements (Fig. 9.10d-e). In the latter case, the concordance was greater with the *distal* forewing U:U (PAT9AC) ($W = 0.889$, $Chi^2_{(1)} = 8.000$, $0.001<P<0.01$) than basal forewing U:U (PAT9BC) ($W = 0.640$, $Chi^2_{(1)} = 6.400$, $0.01<P<0.05$). Note, that of the hindwing U:U elements, that in s2 is placed most distally within the space (Fig. 9.3). Pigment deposition in $U:U_b$ and $U:U_a$ may involve different parameters.

Figure 9.10 (opposite). Concordances of ocellar and umbral elements between forewing s5 and hindwing spaces 2-5: (a-c) as groups (shown bracketed); and (d-e) individually. Relevant spaces are numbered. Dotted wing and space outlines are shown to aid interpretation. Elements: cOC = eyespot pupil; OC = eyespot ring; E^3:U = hindwing interspace equivalent to forewing U:U (umbra).

Legend: ——— Linear dimension
▒▒▒▒ TYPE-1 colour

For further explanation see text.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9.15. Umbral and medial homologies between spaces

(i) *Dorsal forewing*

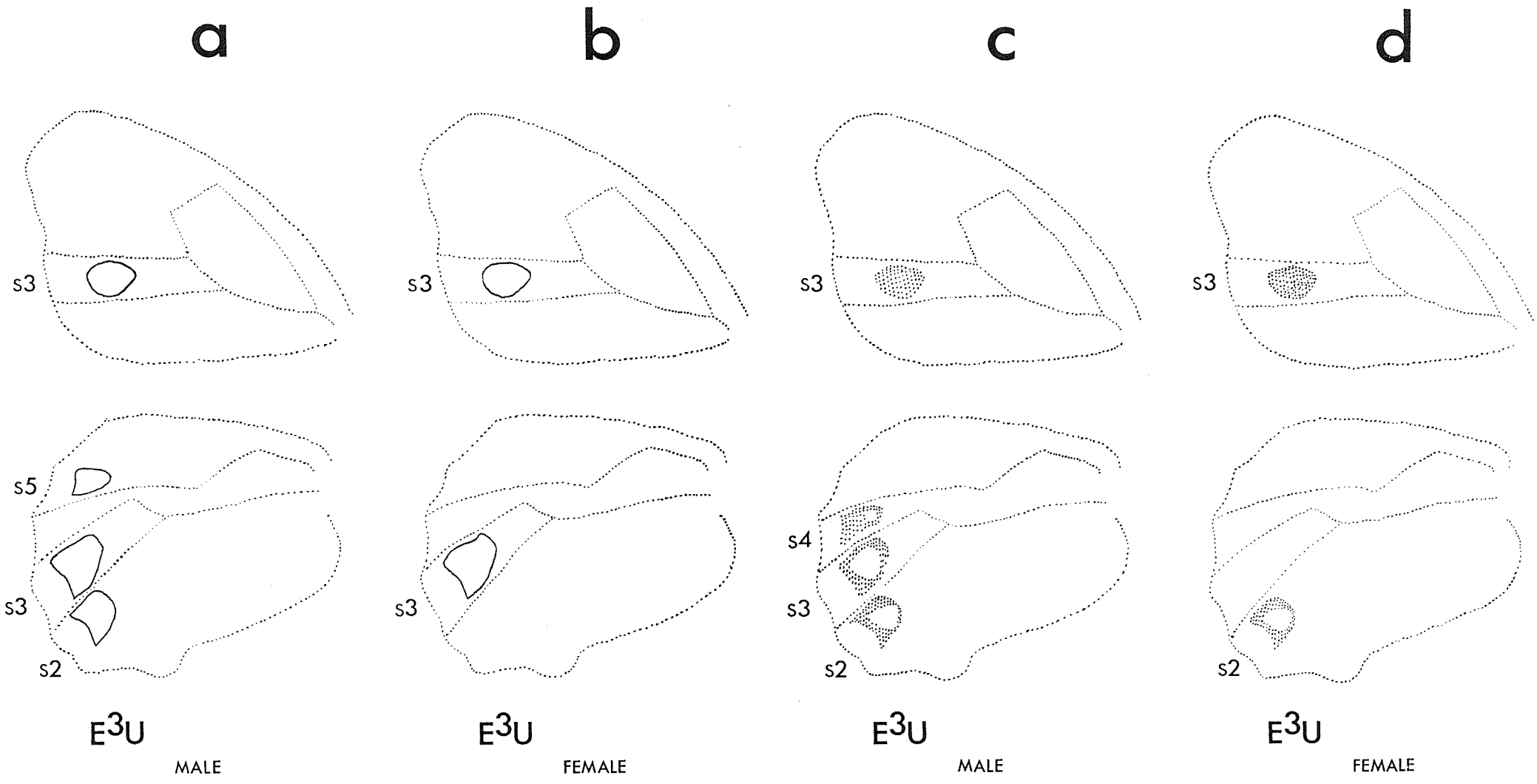
In males, the *size* of PAT3 (s2) correlated with that of FWOC (s5) including its distal (PAT9A) and basal (PAT9B) components, whereas in females, it correlated with the more basally located PAT12 (Fig. 9.11a-b). s2 lies in sector 3 which is less compressed in females (section 9.11 i); where PAT3 therefore occupies a relatively more basal position within the space. It would appear that the deployment of these interspaces within the forewing respects the wing margin; and so is determined *after* the geometry of the termen has been established.

In males, the *colour* of PAT3C correlated with that of the distal component of FWOC (PAT9AC), whereas in females, PAT3C correlated with its basal component (PAT9BC) (Fig 9.11c-d). Thus colour, too, appears to be established relative to the wing margin.

Figure 9.11 (opposite). Concordances among corresponding elements between spaces. Elements: UU = umbral; UM^1 = umbral-medial^1; E = externae. Subscripts: b = basal component; d = dostal component. Concordances: (a-d) s2/s5 and (e-f) s3/s5 for UU or UM^1; (g) s3/s5 for E (and s2/s3 when PAT6 is included under E). Relevant spaces are numbered and any sex-specificity is indicated. Dotted wing and space outlines are shown to aid interpretation.

Legend: ——— Linear dimension
▒▒▒▒▒ TYPE-1 colour

For further explanation see text.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MALE
E3U
FEMALE
E3U
MALE
E3U
FEMALE

(ii) *Dorsal forewing-hindwing*

In males, the *size* of PAT3 (FW s2) correlated with that of each of PAT23 PAT28 and PAT34 (HW s2 s3 s5), whereas in females it correlated only with that of PAT28 (HW s3) (Fig. 9.12a-b).

In males, the *colour* of PAT3C correlated with that of PAT23C PAT28C and PAT31C (HW s2 s3 s4), whereas in females it correlated only with that of PAT23C (HW s2) (Fig. 9.12c-d).

In females, the correlations with forewing s3 involved the more posterior hindwing s2 and s3 only, where, relative to the wing margin, the E^3:U elements occupy more basal positions (than in s4 and s5). This applied particularly to colour which, in both sexes, was correlated with generally *more* posterior hindwing spaces than was size.

Figure 9.12 (opposite). Concordances between the umbral elements in forewing s3 and individual hindwing spaces in each sex: (a-b) for linear and (c-d) for TYPE-1 colour characters. E^3:U = hindwing interspaces equivalent to forewing umbra. Relevant spaces are numbered. Dotted wing and space outlines are shown to aid interpretation.

Legend: ——— Linear dimension
▒▒▒▒ TYPE-1 colour

For further explanation see text.

9.16. External homologies between spaces

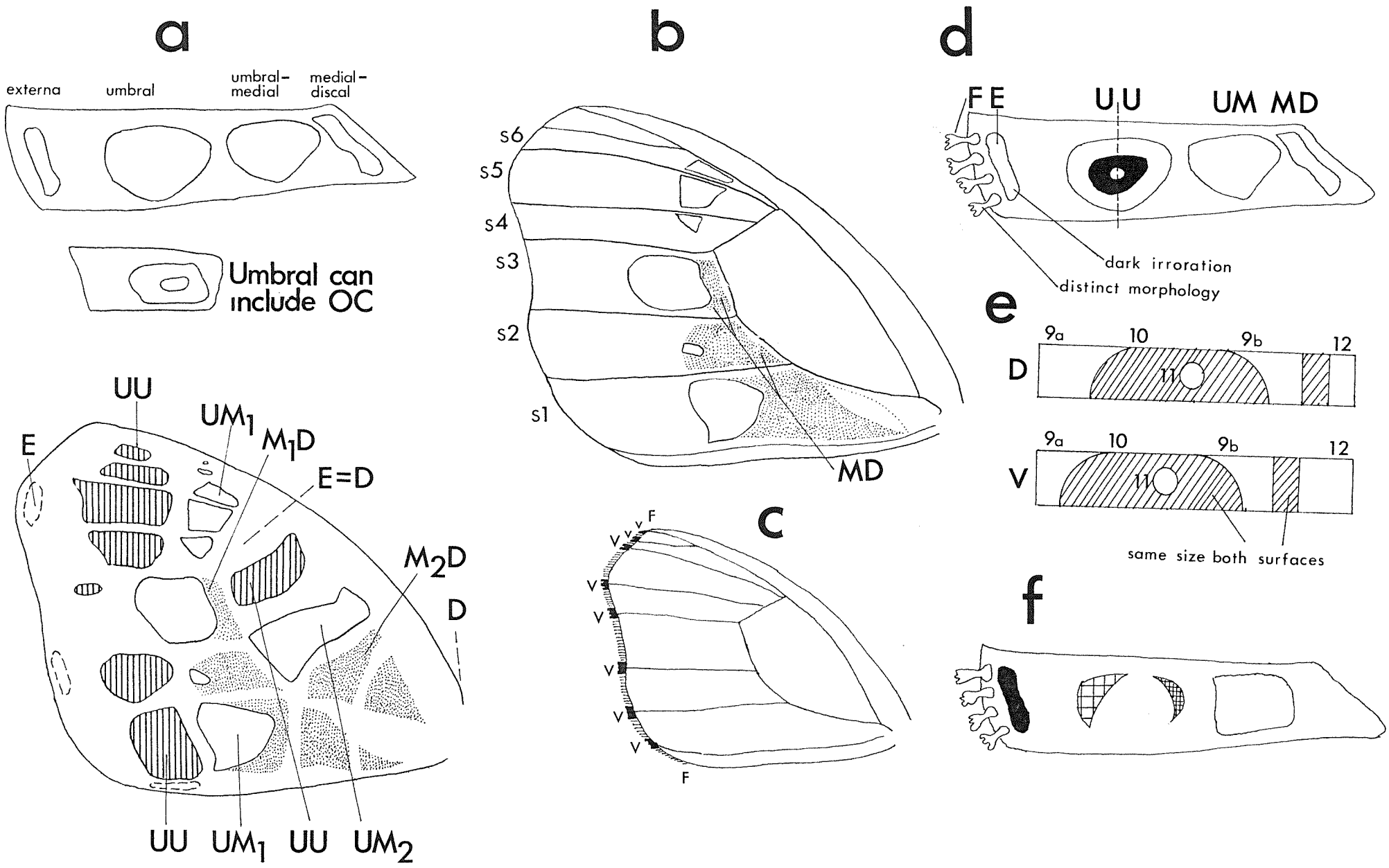
(i) Dorsal forewing

The *sizes* of PAT5 (s2) and PAT14 (s5) both correlated with that of PAT6 (s3) ($0.001<P<0.01$) (Fig. 9.11g) but *not* each other; which may reflect differences between sectors 2 and 3 in the development of the termen.

The *size* of PAT6 (s3) correlated with that of FWOC (s5) but more so with its basal (PAT9B) ($0.001<P<0.01$) than distal (PAT9A) component which was significant only in males ($0.01<P<0.05$). Since PAT6 relative to the termen is more basal in females than males, the role of the wing margin in pattern deployment is supported; and its generally greater concordance with the basal than distal U:U 5 implicates PAT6 as an umbral (U:U) rather than external (E) interspace. This would produce homology between the spaces and simplify the deployment of the groundplan (Fig. 9.13a).

Sector 1 (s0) lacks the externa and the discalis is effectively embedded in the thorax. The M:D interspaces appear to be displaced towards s0 and lost as they progress from s1 to s3; and are absent in s4 to s7 (inclusive) (Fig. 9.13b). The median and discoidal veins appear to behave as a distinct boundary; yet pattern is *not* simply scaled to the differing space sizes (*cf.* Nijhout, 1981, 1985b). Assuming that the deployment of pattern is established from the wing margin, this suggests that it does so in an epimorphic (progressive) manner until blocked at the median vein.

The *colour* of PAT6C (s3) correlated with that of PAT14C (s5) ($0.01<P<0.05$) (Fig. 9.11h), both interspaces occupying marginal positions.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U U
UM MD
dark irroration
distinct morphology
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9b
12
11
V
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10
9b
12
11
same size both surfaces
f

(ii) *Forewing and hindwing fringe patterns*

P. aegeria has a fringe pattern at the termen of both wings (Fig. 9.3) in which the positions of the dark scales correspond the wing veins. The fringe might be regarded as a border element (though absent from the costa and basal third of the dorsum); and examination of the forewing pattern suggests that the externae (including UU 1a) are similarly border elements (Fig. 9.13c).

Fringe scales are deployed from *within* the margin. If the pale intervenosae are included in the same developmental unit as the externa-umbra-media system, then the entire space (fringe included) will be symmetrical about U:U; invoking it (or the eyespot focus (cOC) when present) as the topological centre of the space (Fig. 9.13d).

Figure 9.13 (opposite). Simplification of the groundplan and size and colour relationships within the wing spaces.
(a) the four main types of interspace and the inclusion of ocellar elements within the umbra; and the entire dorsal forewing showing homology between the spaces:

Legend: ------ External : E-type interspaces
Umbral : UU-type interspaces
Umbral-Medial: UM-type interspaces
Medial-Discal: MD-type interspaces

Note the interpretation of old UU s1a as E; (b) progressive dislocation of MD towards s0 through s1 to s3 with its loss in s4 to s6; (c) the P. aegeria fringe pattern, with the fringe (F) as a border element and the venosae (V) corresponding to wing vein positions; (d) deployment of fringe scales within the space showing topological symmetry about U:U (or cOC when present); (e) dorsal-ventral (D-V) differences in the size of forewing UU 5 and UM 5 components. Numeration corresponds to the respective characters (Fig. 9.4); (f) differences in TYPE-1 colour among the interspaces of dorsal forewing s5. The heavier the shading, the greater the colour value. For further explanation see text.

9.17. Non-homologous elements concordant between spaces

This section examines correlations (among the wing spaces) between characters belonging to *different* elements of Schwanwitsch's (1924) ground plan.

(i) *Wing sectors 1 and 3*

The size of PAT3 correlated more with that of PAT19 ($0.001<P<0.01$) than PAT18 ($0.01<P<0.05$) (Fig. 9.14a-b); and the size and colour of PAT6 correlated with those of PAT19 ($0.01<P<0.05$) but *not* PAT18 (Fig. 9.14c-d). It appears, therefore, that there is greater concordance between interspaces occupying similar *relative* positions within the spaces than between those occupying different positions. The size of PAT5, however, was *equally* concordant with those of PAT18 and PAT19 ($0.001<P<0.01$) (Fig. 9.14e-f); so the disparity in concordance between corresponding and non-corresponding positions would also appear to be greater the more anterior the space within sector 3.

(ii) *Wing sectors 1 and 2*

The size of FWOC *overall* was concordant with that at both corresponding (PAT19) and *non*-corresponding positions (PAT18) in s0 (Fig. 9.14g-h). That the sizes of the *individual* distal and basal FWOC components did *not* show such concordances, most likely results from (random) shifts in the relative position of the OC element within FWOC (section 9.13). However the *colour* of the individual distal and basal FWOC components *were* concordant with that at the corresponding position in s0. This suggests that colour determination of $U:U_a$ and $U:U_b$ in P. aegeria may be independent of eyespot determination, in contrast to that in Precis coenia (Nijhout, 1980a) (Fig. 9.14g). But the colour, unlike size, of these U:U components was not significantly concordant with the non-corresponding position in s0 (Fig. 9.14h). Hence colour determination within the spaces may be established in relation to their distal border (termen) not only in sector 3, but

also (discoidal vein system) in sector 1, commensurate with simplification of the groundplan as proposed in section 9.16 (Fig. 9.13a).

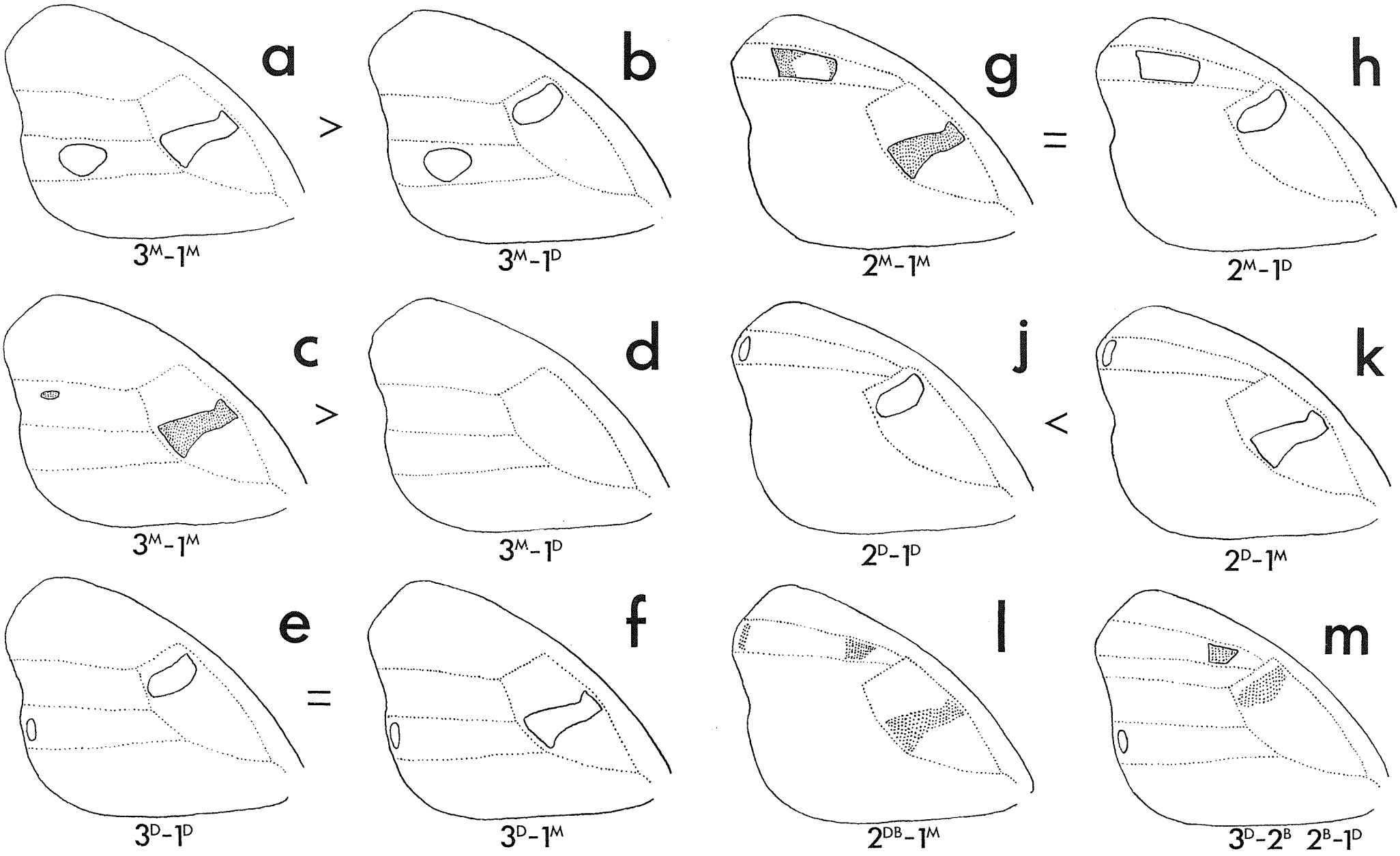
This hypothesis is complicated by the fact that the colour of interspaces at both the distal (PAT12C) and basal (PAT14C) *ends* of s5, too, were concordant with that at the *middle* of s0 (PAT19C) ($0.01<P<0.05$) (Fig. 9.14l). With the exception of colour concordance between *basal* s5 and *distal* s0 ($0.01<P<0.05$) (which may involve some parameter of symmetry about the discoidal vein system) (Fig. 9.14m), the E $U{:}U_a$ $U{:}U_b$ and $U{:}M^1$ interspaces of s5 all showed similar patterns of concordance with s0, implicating them as part of a common colour-developmental unit; whose greater concordance with middle than distal s0 suggests that its determination *is* centred on the middle of s5. This paradox is re-examined below.

The *size* of PAT14 in sector 2, in contrast to that of PAT3 in sector 3, was *less* concordant ($0.01<P<0.05$) with that of the corresponding (PAT19) than *non*-corresponding (PAT18) ($0.001<P<0.01$) position in s0 (Fig. 9.14j-k). This, anomaly, however, might be resolved by modelling *pattern* deployment in terms of serial values that progress with the establishment of the venation (below).

(iii) *Sectors 2 and 3*

The only concordance was between the *sizes* of PAT5 and PAT12 *ie.* distal s2 and basal s5. These interspaces, however, are equidistant from the intersection of the termen (border lacuna) and the hypothetical compartment boundary (v5) between sectors 2 and 3. It is possible, therefore, that they may be established by a common value in a progression wave that diverges from this intersection along v5.

There were *no* basal-basal concordances, nor forewing-hindwing concordances between non-homologous elements, for either size or colour.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l
m
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3M-1M
3M-1D
2M-1M
2M-1D
3M-1M
3M-1D
2D-1D
2D-1M
3D-1D
3D-1M
2DB-1M
3D-2B 2B-1D

9.18. Intra-space correlations

(i) *size*

There were no concordances within s0 (sector 1). FWOC PAT12 and PAT14 (forewing s5) were concordant with each other ($0.001<P<0.01$) (Fig. 9.15a-c); where the concordance between PAT12 and PAT14 (Fig. 9.15a) suggest some pattern determining process common to both ends of the space.

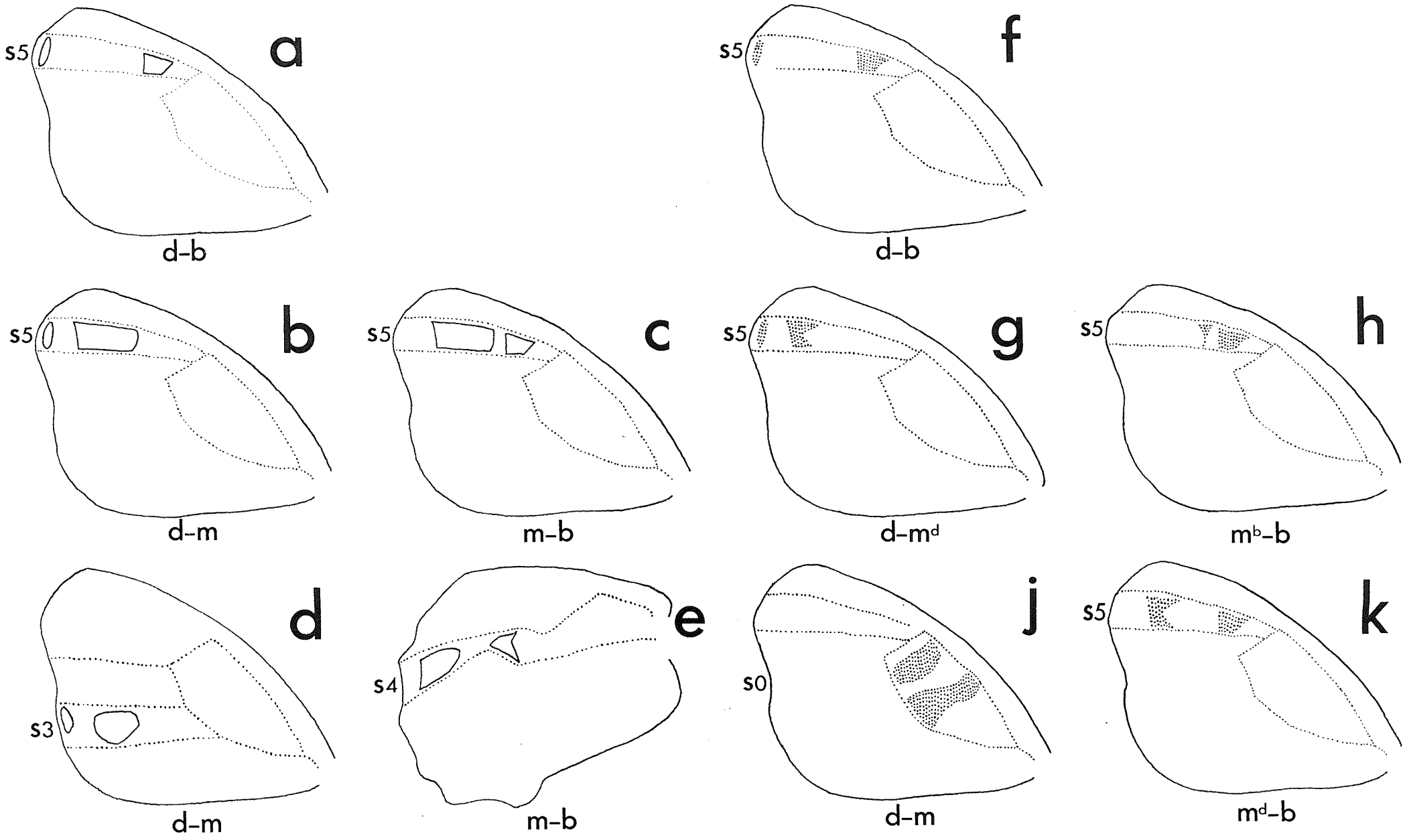
PAT3 and PAT5 (forewing s2) were concordant ($0.001<P<0.01$) (Fig. 9.15d); and appear homologous with FWOC and PAT14 respectively (*cf.* Fig. 9.15b) which, too, occupy middle and distal space positions respectively.

PAT31 and PAT37 (hindwing s0/4) were concordant ($0.01<P<0.05$) (Fig. 9.15e); and appear homologous with FWOC and PAT12 respectively (*cf.* Fig. 9.15c) which, too, occupy middle and basal space positions respectively.

Figure 9.14 (opposite). Concordances among spaces between non-homologous elements. Relationships between particular pairs of spaces are shown for corresponding (a,c,e,g and j) and non-corresponding (b,d,f,h and k) regions respectively. Which of the pair (corresponding or non-corresponding) was more (>) or less (<) concordant is so indicated; = denotes equal concordance between corresponding and non-corresponding positions. Wing markings left unillustrated indicate that there was *no* significant concordance. Numeration refers to the wing sector (Fig. 9.9b), that with the more distal feature listed first. Superscripts refer to thc distal (D), middle (M) or basal (B) regions within the sector. (l and m) show concordances not otherwise belonging to any particular type of relationship. Note that in (m) the concordances are between the two linear and between the two colour characters respectively.

Legend: ——— Linear dimension

▒▒▒▒▒TYPE-1 colour


a
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s5
d–b
b
s5
d–m
c
s5
m–b
g
s5
d–m^d
h
s5
m^b–b
d
s3
d–m
e
s4
m–b
j
s0
d–m
k
s5
m^d–b

(ii) *Colour*

In contrast to size, colours *were* concordant within s0, between its distal (PAT18C) and middle (PAT19C) positions, implying that pattern and colour determination (at least in s0) involve distinct processes.

Distal FWOC PAT12C and PAT14C were concordant with eachother (Fig. 9.15f,g & k). The concordance between PAT12C and PAT14C (Fig. 9.15a) suggests some colour determining process common to both ends of the space.

Distal s5 (PAT14C) was concordant with the distal (but not basal) component of U:U (PAT9AC) ($0.01<P<0.05$) (Fig. 9.15g). Basal U:U (PAT9BC) was concordant with the basal (but not distal) end of s5 (PAT12C) ($0.001<P<0.01$) (Fig. 9.15h). PAT9AC was concordant with PAT12C ($0.001<P<0.01$) (Fig. 9.15k).

Figure 9.15 (opposite). Concordances between elements within wing spaces: for (a-e) linear and (f-k) type-1 colour characters. All except (e) refer to the forewing and relevant spaces are numbered. Concordant regions are hyphenated, the more distal listed first: d = distal; m = middle; b = basal. Superscripts denote distal (d) or basal (b) components of the middle section when divided by an OC element. Wing and space outlines aid interpretation.

——— Linear dimension

▒▒▒▒▒ TYPE-1 colour

For further explanation see text.

9.19. Differences between elements and interspaces in forewing s5

Differences in size and colour between particular characters were examined with the t-test, as were all pairwise comparisons below. 2-tailed tests were applied as the directions of the differences were not predicted.

(i) *Dorsal-ventral differences*

There were no significant colour or size differences in females (Table 9.II). In males, PAT10 and PAT11 (OC and cOC) were of similar size on both surfaces, but were situated more distally within FWOC (U:U) on the ventral surface where PAT9A (distal U:U) was therefore smaller and PAT9B (basal U:U) larger than on the dorsal surface (Fig. 9.13e). The entire FWOC-PAT12 system was similarly sized on both surfaces, but the FWOC (U:U) component was smaller with PAT12 (U:M^1) larger on the ventral surface. It appears that the OC and M^1 elements are constant in width on both surfaces but shifted distally within the space on the ventral surface.

Character	Male		Female	
FWOC	D > V	**	D > V	ns
PAT9A	D > V	**	D > V	ns
PAT9B	V > D	*	V > D	ns
PAT10	D > v	ns	V > D	ns
PAT11	V > D	ns	V > D	ns
PAT12	V > D	**	D > V	ns

TABLE 9.II. The direction of differences between the surfaces in the size of forewing s5 elements and interspaces. Only characters scored for both dorsal (D) and ventral (V) surfaces are considered. The relationships are shown for each sex. Significance levels of the differences: * $0.01<P<0.05$; ** $0.001<P<0.01$.

These results suggest that the positions of these elements are established independently on each surface and after D/V compartmentalisation.

(ii) *Distal-basal differences*

The more marginal interspaces were darker (Fig. 9.13f) and all interspace differences (except between PAT9AC and PAT9BC *ie.* distal and basal U:U) significantly so, although more strongly in males than females and on the dorsal than on the ventral surface (Table 9.III).

It is suggested that there is a gradient of colour determination that originates from the termen and changes in value basally; it may be similar to that implicated in the deployment of pattern (section 9.16.i). Different ranges of values may activate a common pigment pathway to differing degrees. Its progressive decrease in value basad may mean progressively fewer steps in the pathway being activated, and

Characters	Overall	Male	Female
Dorsal			
14C > 9AC	*	ns	ns
14C > 9BC	*	**	ns
14C > 12C	***	**	**
9AC < 9BC	ns	ns	ns
9AC > 12C	***	***	*
9BC > 12C	***	***	*
ventral			
9AUC > 9BUC	*	ns	ns
9AUC > 12UC	ns	ns	ns
9BUC > 12UC	**	*	ns

TABLE 9.III. The direction of colour differences within forewing space 5. The significance levels of the differences are indicated thus: * $0.01<P<0.05$; ** $0.001<P<0.01$; $P<0.001$.

hence the paler basal colours. That the basal U:U, however, was darker than the distal U:U may result from some interaction between the basad progression of this gradient and another progressing radially from the eyespot focus (*cf.* Nijhout, 1981).

9.20. Ocellar and umbral size and colour differences between spaces

The relationships between hindwing s2 s3 s4 and s5 and fore wing s5 in the size and TYPE-I colours of each the dorsal E^3:U, OC and cOC components are given in Table 9.IV and illustrated in Figure 9.16.

(i) *Hindwing only*

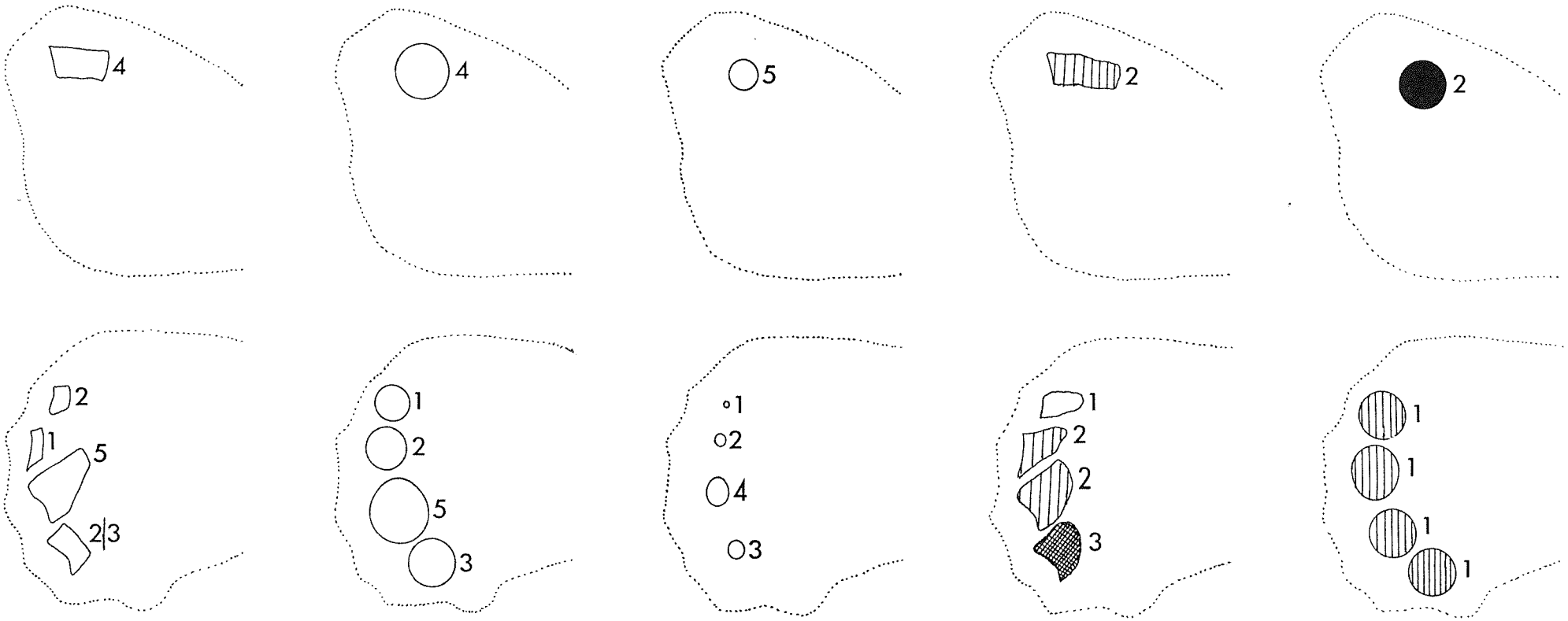
In all cases sizes were larger in posterior spaces s2 and s3 than in the anterior s4 and s5. The precise relationship between these four spaces was identical for the sizes of OC and cOC, suggesting that cOC and OC comprise a common developmental unit. The size relationship between s4 and s5 was different for E^3:U and OC, commensurate with the hypothesis that development of the OC is independent of that of the surrounding E^3:U interspaces.

All the hindwing OC and all the cOC were similar in colour. Only E^3:U differed between spaces, being darker in the more posterior ones though only significantly so between s2 and s3 ($0.01<P<0.05$).

The size and colour of E^3:U, Oc and cOC were not quantified for the ventral surface, but the ventral OC elements were visibly smaller (though more numerous) and the ventral E^3:U indistinct or absent (Plates 1 and 2).

(ii) *Hindwing and forewing s5*

The cOC element (eyespot pupil) was much larger in dorsal forewing s5 than in any of the dorsal hindwing spaces, suggesting that the forewing manifests greater eyespot focus activity (Table 9.IV). Indeed, the surrounding OC element in s5 was much darker than any of those on the dorsal hindwing albeit not significantly so (although the larger size and darker colour of the forewing s5 OC element was very


4
2
1
5
2|3
E3U
4
1
2
5
3
OC
5
1
2
4
3
cOC
2
1
2
2
3
E3U
2
1
1
1
1
OC

noticeable on the ventral surface (*cf.*Plates 1 and 2).

That the relative size and colour of the dorsal forewing s5 OC and U:U were most similar to those on the *posterior* dorsal hindwing (s2 and s3), points to an homology between the anterior forewing and posterior hindwing about an anterio-posterior (meso/metathoracic) axis of symmetry.

Size	E^3:U	s3 > Fs5 > s2 ≥ s5 > s4
	OC	s3 > Fs5 > s2 > s4 > s5
	cOC	Fs5 > s3 > s2 > s4 > s5
Colour	E^3:U	s2 > s3 = s4 = Fs5 > s5
	OC	Fs5 > s2 = s3 = s4 = s5

TABLE 9.IV. The relationships between forewing s5 (Fs5) and hindwing spaces s2-s5 in the size and colour of the umbral (E^3:U, U:U on the forewing) interspace and ocellar elements.

FIGURE 9.16 (opposite). Rank increases in size and darkening of the cOC, OC and elements and E^3:U interspaces among hindwing spaces s2 to s5 and forewing s5. Larger or darker features are also illustrated as such. Note that the ranks do not refer to absolute size or colour values (for example, the OC in forewing s5 is darker than the E^3:U in hindwing s3). For further explanation see text.

9.21. Asymmetry and developmental stability

Asymmetry was examined for forewing span and markings in dorsal s5, and for hindwing span and dorsal OC and E^3:U components in s2-s5 (Appendix IX). Three kinds of asymmetry were examined, namely directional asymmetry, antisymmetry and fluctuating asymmetry. These asymmetries were estimated for each sex and family of STOCK 01 F_1 under each of the control (untreated and foil) and cold shock pupal treatment.

(i) *Directional asymmetry*

In directional asymmetry one side always differs from the other in a particular direction (Soulé, 1967), and is estimated as the *right-left difference/mean of the two sides x 100%*. Directional assymetries are reported in Appendix XII. Only four of the 300 measurements proved significant ($P<0.01$); and their irregular distribution suggests that the directional bias more likely arises from the random direction of fluctuating asymmetry (below). Thus P. aegeria does not exhibit regular directional asymmetry.

(ii) *Antisymmetry*

In antisymmetry asymmetry is the rule but unpredictable in direction (Soulé, 1967). It is estimated as the *absolute left-right difference/ mean of the two sides x 100%*, then compared using Chi-squared with a normal distribution of the same mean and variance, when a significant result indicates antisymmetry (Van Valen, 1962). However, a significant result must be tested for skewness, which if significant at the $P<0.1$ level must be corrected and then the antisymmetry re-tested (*ibid.*). Antisymmetries are listed in Appendix XIII. None of the 300 measurements proved significant ($P<0.01$). Thus P. aegeria does not exhibit significant antisymmetry.

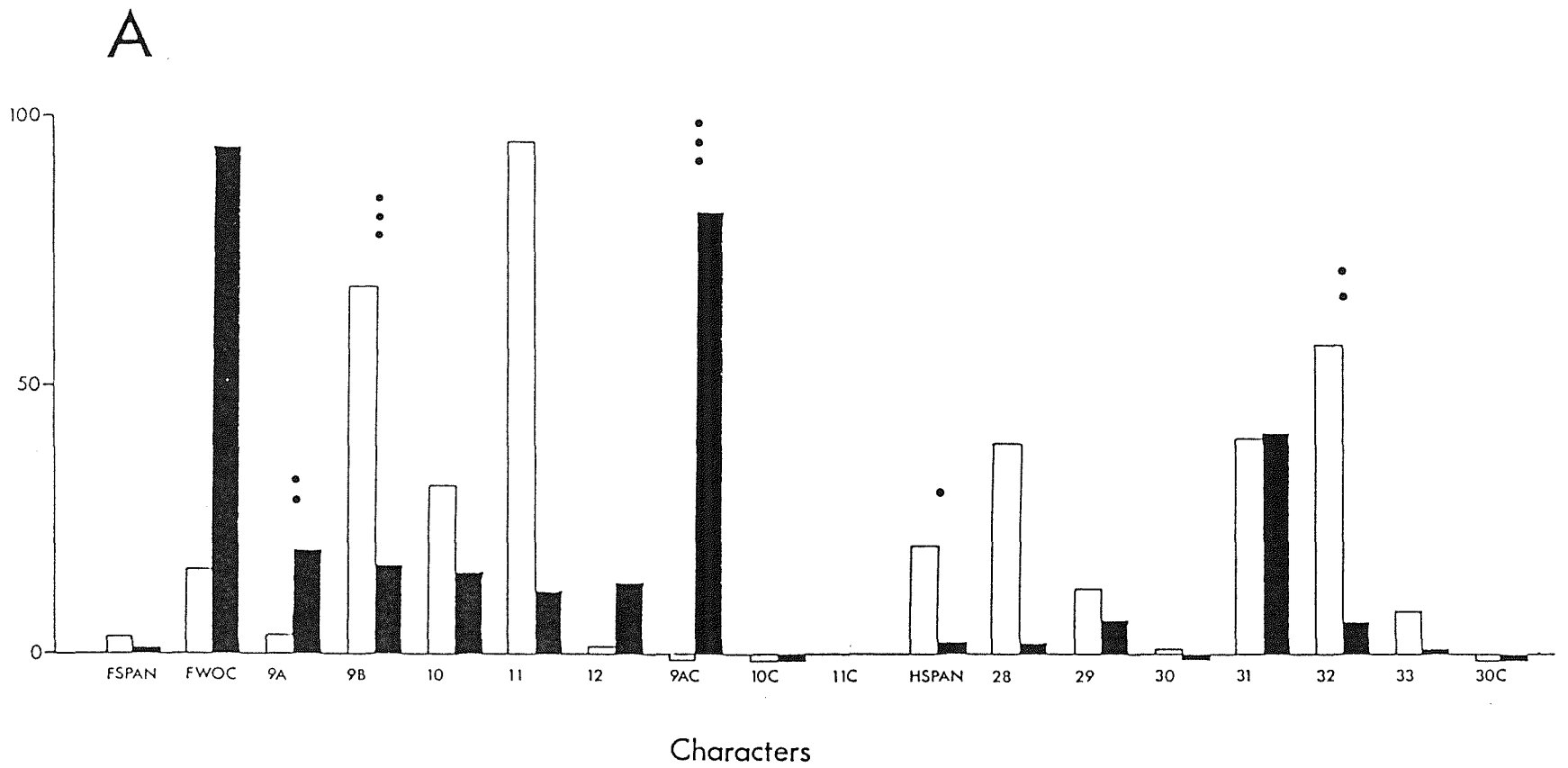
(iii) *Fluctuating asymmetry*

Fluctuating asymmetry (A_F) describes minor random departures from bilateral symmetry which can provide a measure of developmental stability (Soulé, 1967). Resistance to developmental noise (Lewontin, 1982) as measured by A_F may be related to canalisation (Van Valen, 1962). Its magnitude may be influenced by genetic components not *per se* responsible for the character in question (Reeve, 1961).

Directional asymmetry and antisymmetry are eliminated from measurement by subtracting the mean difference between the sides from the side with the larger value (Soulé, 1967), then the product-moment correlation (r) measured between them. $1-r^2 \times 100\%$ gives the level of fluctuating asymmetry. Fluctuating asymmetries were compared between the families, sexes and pupal treatments as r-values using the t-test as described by Bailey (1977). Fluctuating asymmetries are reported in Appendix XIV.i and iii, and are shown for each family under no foil treatment and for the sexes under each pupal treatment in Figure 9.17A-B and E-F.

In all cases, hindwing span exhibited greater A_F than forewing span, which is in accord with the lack of any left-right concordance among hindwing characters (section 9.12.i). In both sexes, more forewing characters were destabilised by cold (versus foil) than by foil (versus no foil) (Figs. 9.17C-F and 9.18C-F), whereas on the hindwing the number of characters significantly affected by each treatment was more equal. If this results from the hindwing being more affected by dark than is the forewing, it too implicates the hindwing as the less canalised. Here, all significant sample differences in A_F were limited to s3 and s4, the instability of s4 perhaps related to the degeneracy of the hindwing s0/4 discoidal veins (section 9.11.ii).

FIGURE 9.17 (overleaf). Levels of fluctuating asymmetry compared: between (A) STOCK 01 families 002 and 004; (B) males and females; (C) untreated and foil treated males; (D) untreated and foil treated females; (E) foil and cold treated males; and (F) foil and cold treated females. Significance levels of the differences: • $0.01<P<0.05$; •• $0.001<P<0.01$; •••$P<0.001$. For further explanation see text.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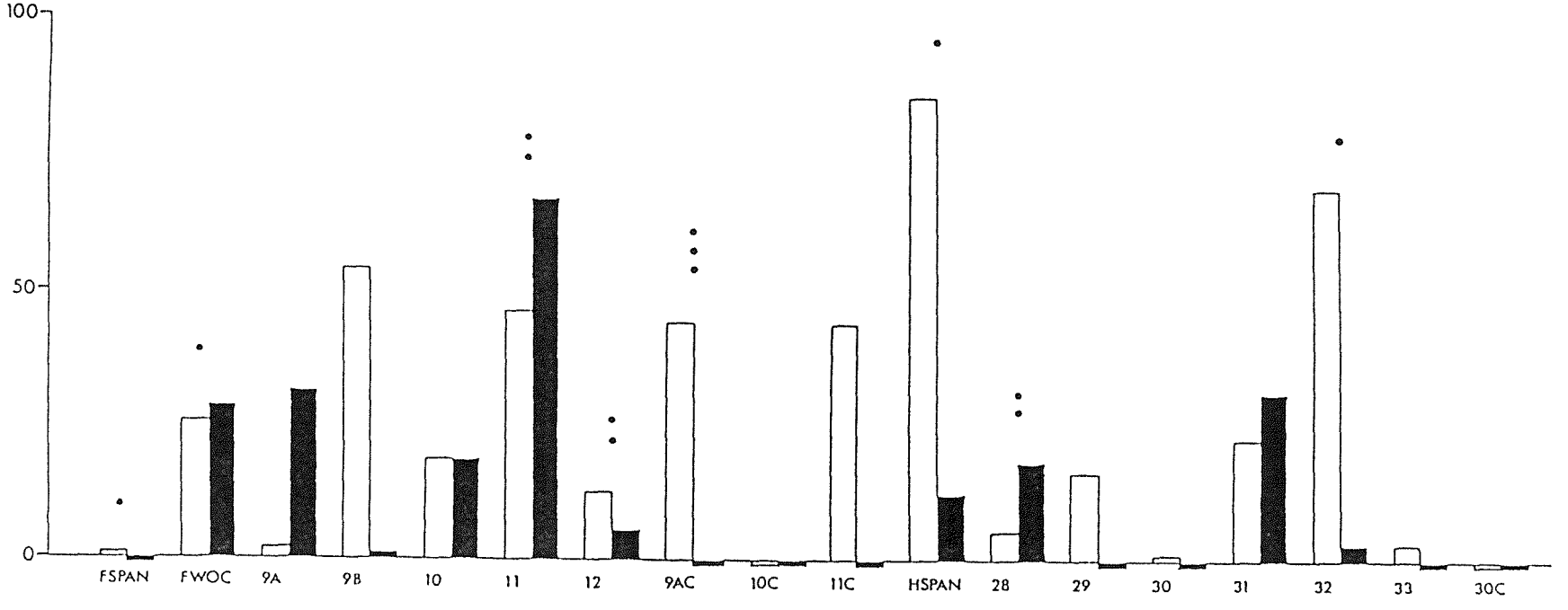
30C
Characters

B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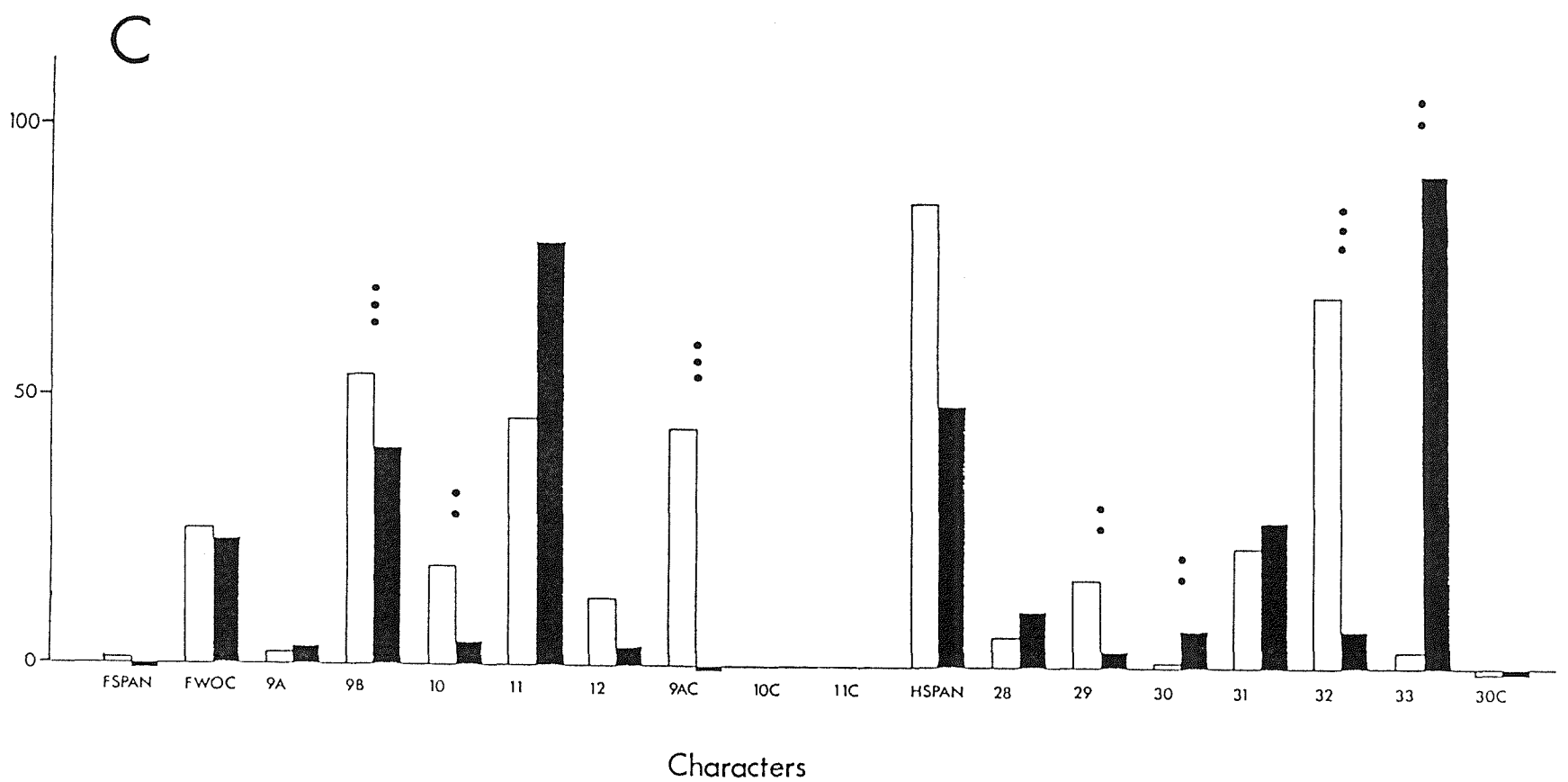
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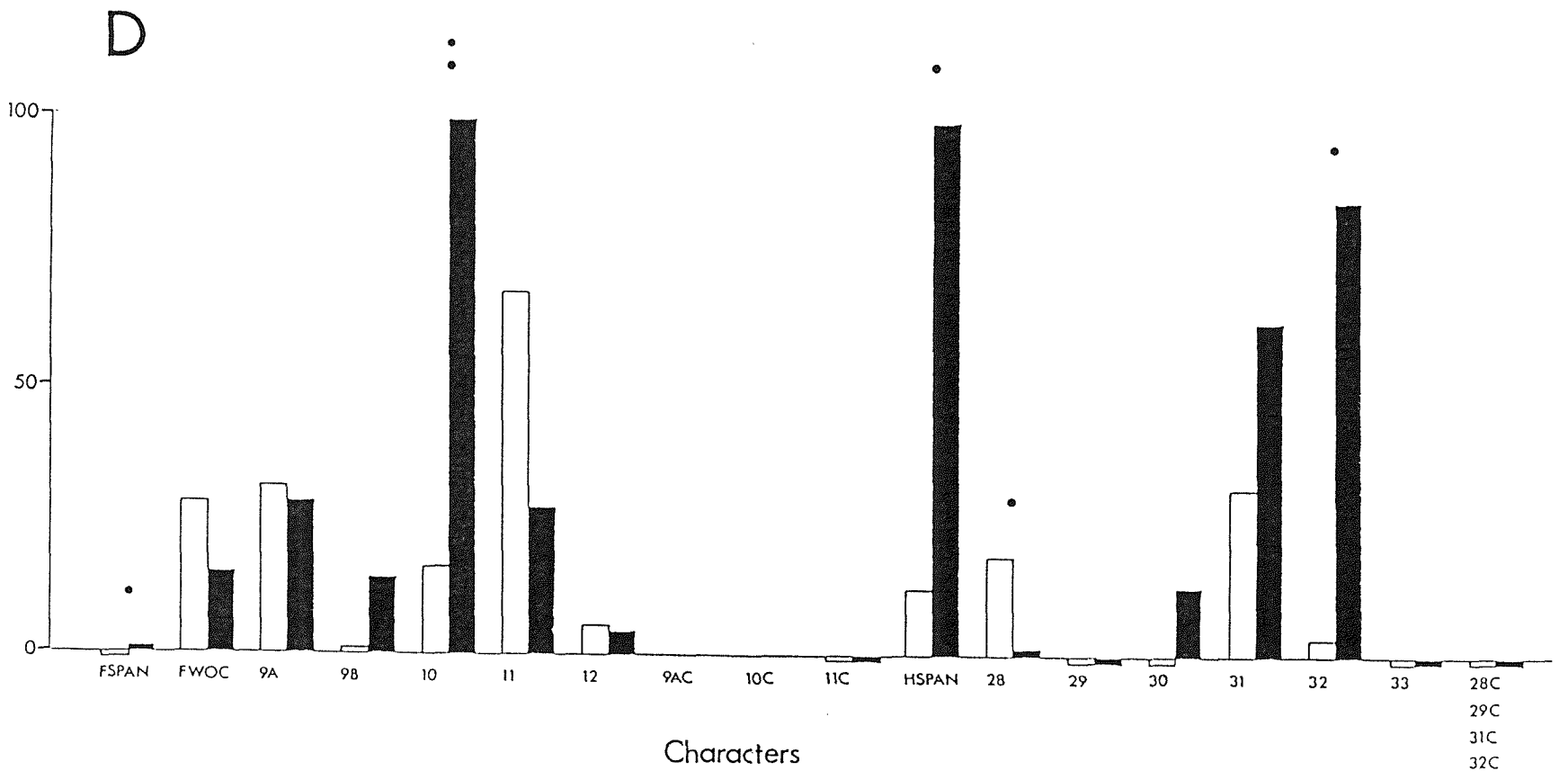
Characters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31C
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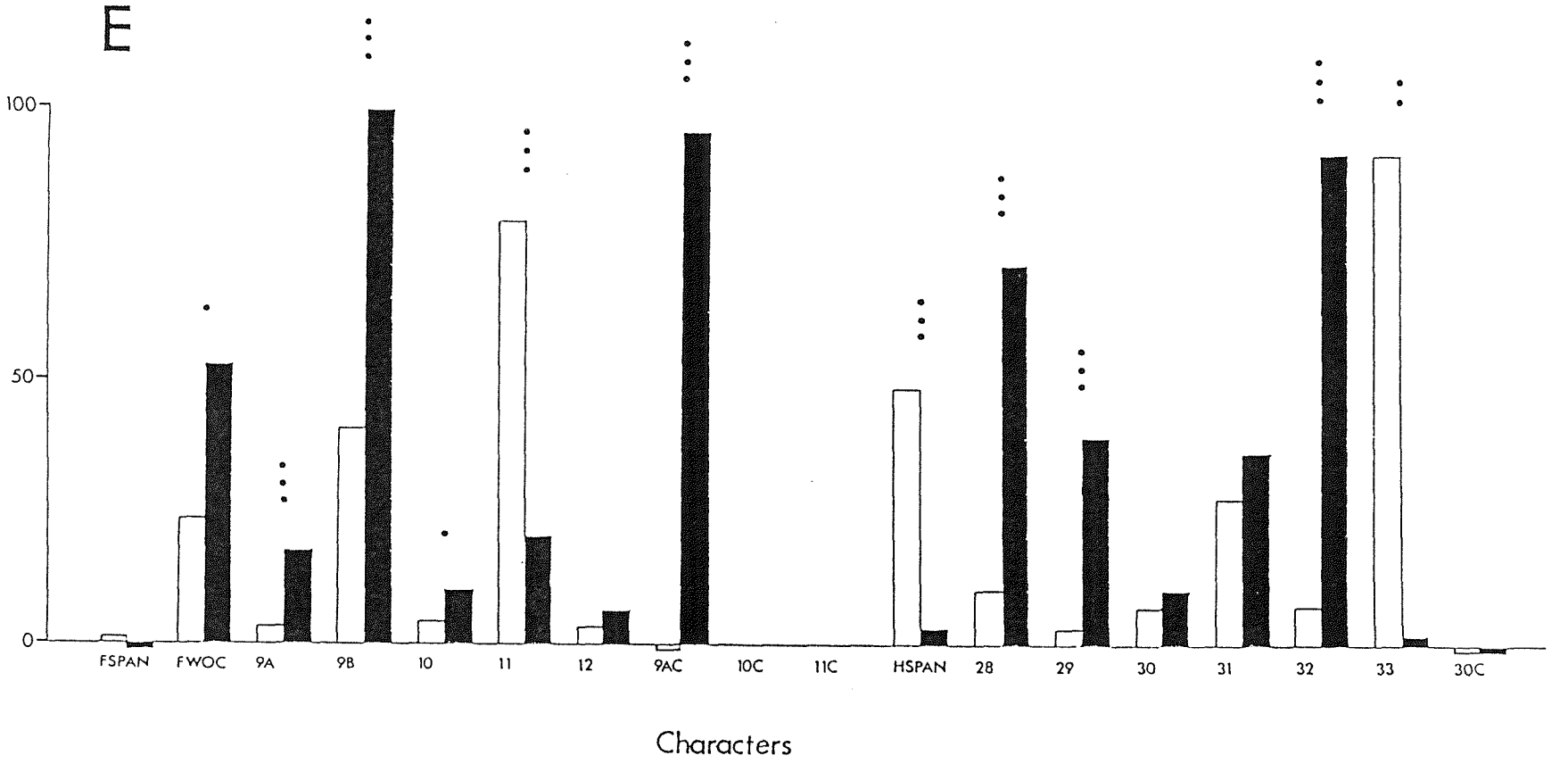
Characters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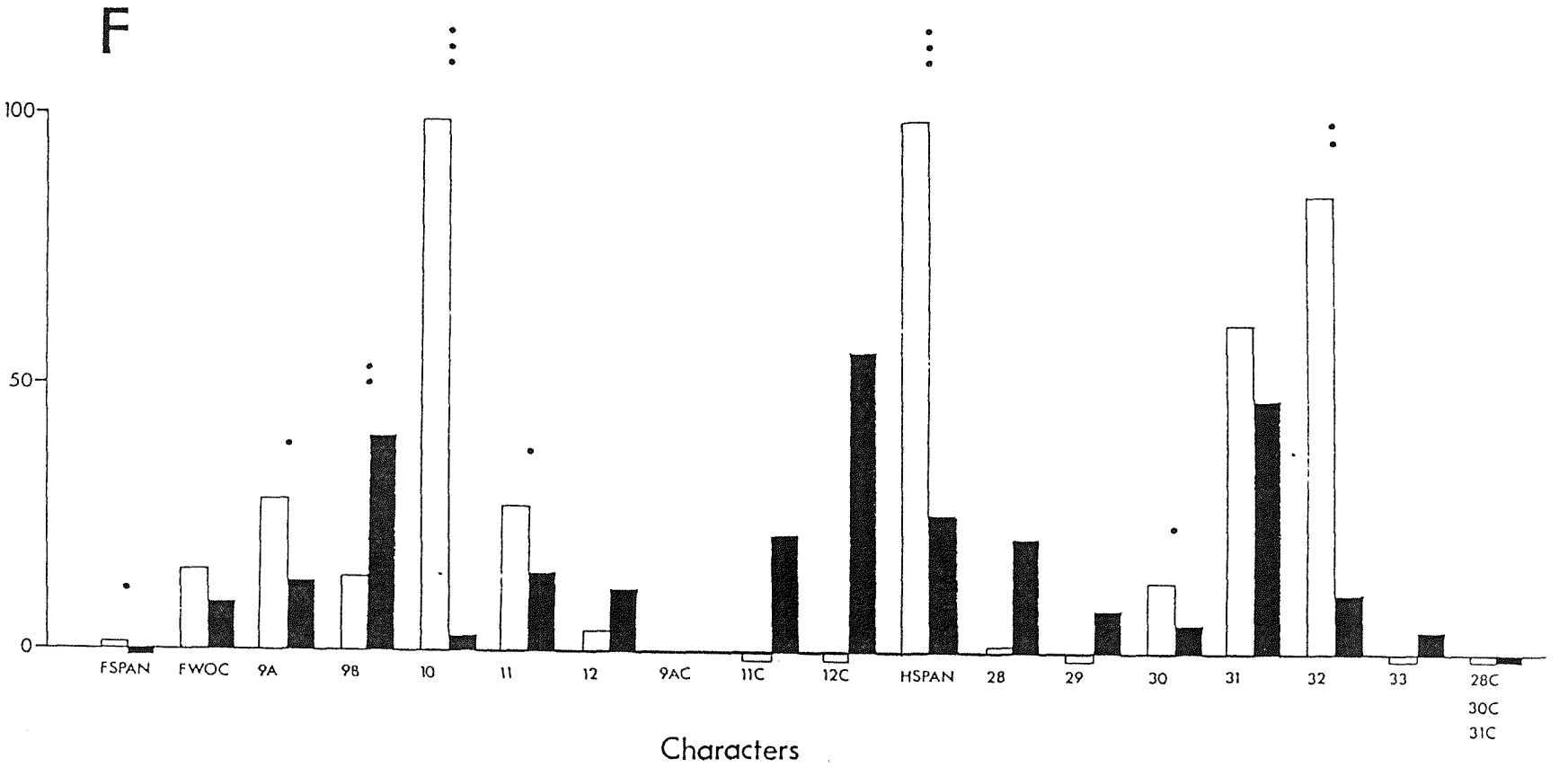
Characters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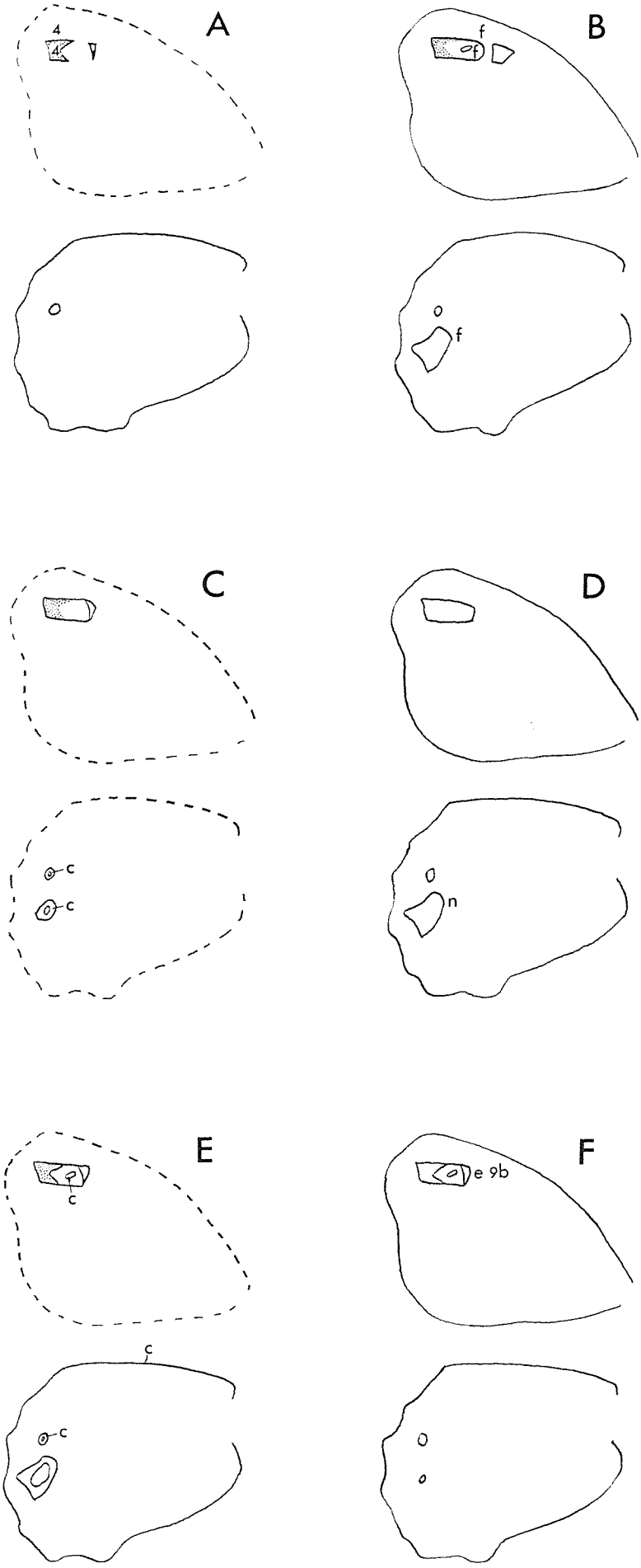
30C
31C
Characters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B
f
f
C
c
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n
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c
c
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e 9b

That cold destabilised more forewing characters than did foil might be expected given that cold shock is a more severe stimulus and less likely to be encountered in nature than dark (*viz.* nights longer than the threshold for inducing diapause).

On the hindwing, only linear characters exhibited fluctuating asymmetry (Fig. 9.17A-B and E-F), while on the forewing it was *predominantly* linear characters which did so. This suggests that linear characters (element widths) are less canalised than colour determination and/or establishment. While the forewing s5 distal and basal U:U interspace (PAT9A and PAT9B) exhibited fluctuating asymmetry, there was no bias as to which exhibited the A_F of greater magnitude. This corroborates with fluctuations in the *relative* position of the OC and cOC elements within the U:U interspace (FWOC) (section 9.13).

The only forewing TYPE-I colour characters to exhibit fluctuating asymmetry were the s5 eyespot pupil (PAT11C) and the distal U:U interspace (PAT9AC). The former may be a <u>focus</u> of active morphogen production (*cf.* Nijhout, 1978, 1981), while the colour of the latter may involve interaction of such a morphogen with the (hypothetical) progression of colour determination from the wing margin (section 9.19.ii).

FIGURE 9.18 (opposite). Characters differing in fluctuating asymmetry: between (A) the families, characters exhibiting the greater A_F in family 004 designated 4; (B) the sexes, characters exhibiting the greater A_F in females designated f; (C) untreated and foil treated males, characters exhibiting the greater A_F under foil treatment designated c; (D) untreated and foil treated females, characters exhibiting the greater A_F in untreated controls designated n; (E) foil and cold treated males, characters exhibiting the greater value under foil treatment designated c; and (F) foil and cold treated females, characters exhibiting the greater A_F under cold shock designated e. *Dotted* wing outlines are shown only to aid interpretation.

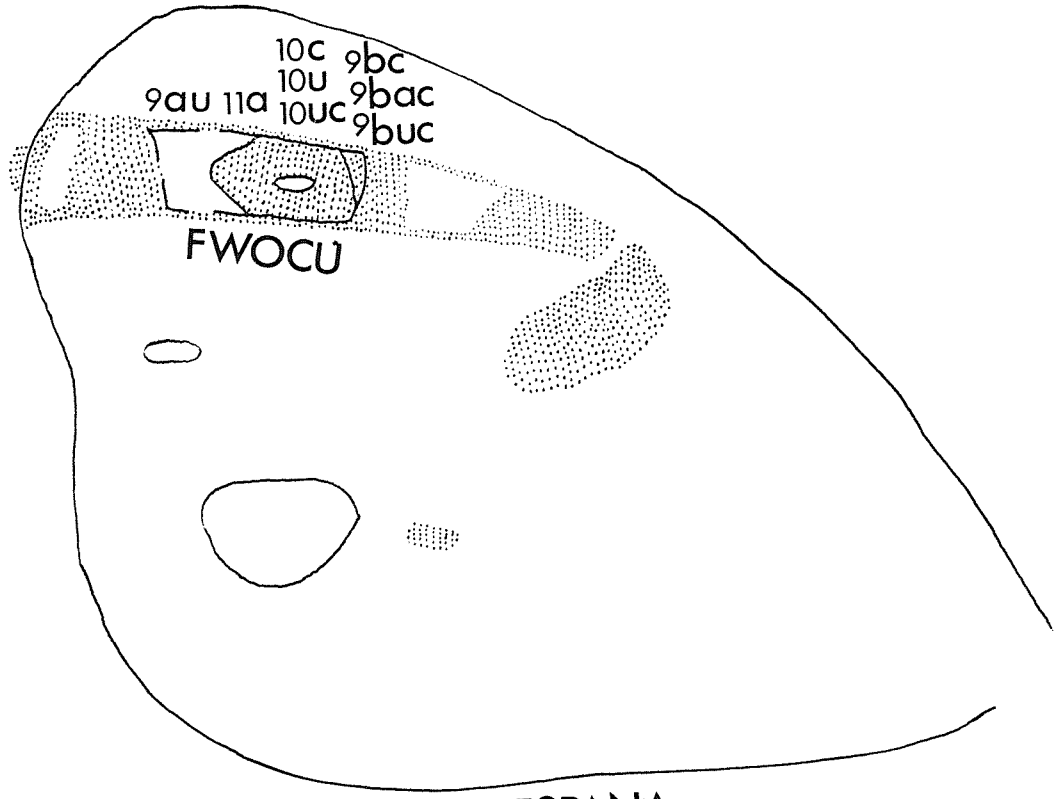
Where the level of fluctuating asymmetry appeared to be influenced by aspects of genetic constitution (as suggested by significant family or sex differences, Fig. 9.17A-B), more characters differed in A_F between the sexes than between the families (Fig. 9.18A-B). Sex, family and sample comparisons are examined more closely below.

9.22. Variabilty and developmental stability

Variability profiles provide inferences about the amount of developmental (and ultimately evolutionary) control of variability (Sokal & Braumann, 1980), characters of large selective value being expected to be more stable with lower coefficients of variation (Mather, 1953), where the coefficient of variation, V, is given as *standard deviation/mean x 100%* (Sokal & Rohlf, 1981). It is essential that the data be normally distributed, so V was based on transformed data, then corrected for bias (Sokal & Braumann, 1980) which could have resulted in misleading inferences (Lande, 1977) to yield V* (Sokal & Braumann, 1980).

Variability profiles were compiled for each sex under each pupal treatment (no foil, foil and cold shock) and tested for similarity with Kendall's coefficient of concordance (Sokal & Braumann, 1980). Individual V* values were compared using a t-test (*cf.* Sokal & Braumann, 1980), but when variances of V*, *ie.* s_{V*}, were doubted for equality or based on data not normally distributed, the degrees of freedom were U-adjusted (*cf.*Parker, 1979).

Characters manifesting significant differences in variability between any of four pairwise comparisons (between the sexes, families 01002 and 01004, no foil and foil treatments, and foil and cold shock treatments) comprise the variable character set (VCS) (Figure 9.19). The total number of characters examined for variability was very large (*cf.* Appendix IX), but, since V* will reflect variation in their determinants and in their developmental stability to perturbation (and, of course, any interaction between the two), the overall trends can be assumed from characters which differ in variability between known genetic (sex and family) and environmental (pupal treatment) states.



FSPAN FSPANA

HSPAN HSPANA

Variability profiles are illustrated in Figure 9.20. Forewing and hindwing spans were generally of similar variability, except in cold shocked females where both forewing spans (characters 1 and 15) were much more variable than hindwing spans (characters 18 and 29). The overall profiles under each treatment were more similar in males (Fig. 9.20A-C) than in females (Fig. 9.20D-F). This would suggest that female variability is more affected by pupal treatment than that of males.

Figure 9.19 shows that variable characters, with the exception of TYPE-I colour in forewing D^1:M^2 (PAT18C), are limited to forewing areas distal to s0 and to hindwing areas distal to the putative discoidal veins in s0/4. None of the hindwing E^3:U interspaces were included in the set, and hindwing variable characters were restricted to ocellar (OC and cOC) elements.

The variability of forewing s5 OC element (PAT10) and distal U:U (PAT9A) differed between treatments only on the ventral surface, suggesting that the size of this OC element is regulated independently on the two surfaces, and hence subsequent to D/V compartmentalisation (*cf.* section 9.12.ii).

Differences between samples in the colour variability of forewing s5 OC (PAT10C: characters 6 and 13) and basal U:U (PAT9BC: characters 7 and 14) were distributed over both surfaces (Fig. 9.19). This general association between forewing s5 OC and U:U_b too would suggest some common process or an interaction in their colour establishment (*cf.* section 9.19.ii)

The profiles of dorsal hindwing size characters (nos. 18-24) tended to be similar in all cases (Fig. 9.20).

In males, all dorsal hindwing size characters (nos. 18-24) were more variable in the untreated than foil treated sample

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FIGURE 9.19 (opposite). The variable character set (VCS). Characters manifesting significant differences in variability between any of four pairwise comparisons (males/females, families 002/004, untreated/foil treated, foil treated/cold shocked) comprise the VCS. Characters are numbered only where necessary to denote ventral surface (U) or 2ry wing (A).

(Fig. 9.20A-B), while in females all forewing size characters except span (nos. 2-17) were more variable in the untreated than foil treated sample (Fig. 9.20D-E). Generally, the maximum variabilities were larger in untreated than foil treated classes, when the magnitudes of their *differences* in variability were larger when it was the untreated class that was the more variable. The summer photoperiod experienced by untreated pupae may have resulted in greater ecdsyone production in this class. Ecdysone is known to effect changes in pigmentation (Needham, 1974).

Maximum variabilities were generally greater under cold shock (Fig. 9.20C & F) than under either control treatment, when the magnitudes of the *differences* in variability between cold and foil treated classes were larger when it was the cold shock class that was the more variable. In males, characters most variable under cold shock were clustered on the hindwing (characters 18-31), whereas in females they were clustered on the forewing (characters 1-17). Specific characters differing in variability between the sexes and treatments are discussed below, but the profiles were otherwise similar for the two sexes under each respective treatment (Fig. 9.20A & D, B & E, C & F). The greater variabilities under cold shock than in either control class might be attributed to the destabilising influence of the post-shock ecdyone pulse (*cf.*section 7.12).

That (in males at least) hindwing variabilities differed more between treatments than forewing ones would again point to the hindwing being the more poorly canalised of the two (*cf.* section 9.12.i).

FIGURE 9.20 (overleaf). Variability profiles for the variable character set (VCS). (A) untreated males; (B) foil-treated males; (C) cold-treated males; (D) untreated females; (E) foil-treated females; (F) cold-treated females. Coefficients of variation (%) are drawn connected (after Sokal & Braumann, 1980) to aid interpretation. Characters are grouped by wing surface and as linear or TYPE-I colour.

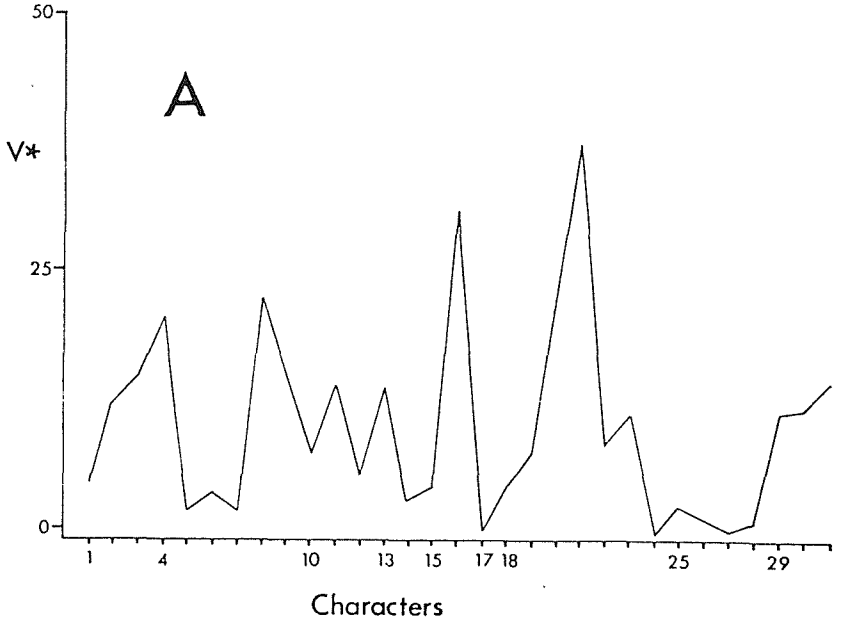
Character groupings:

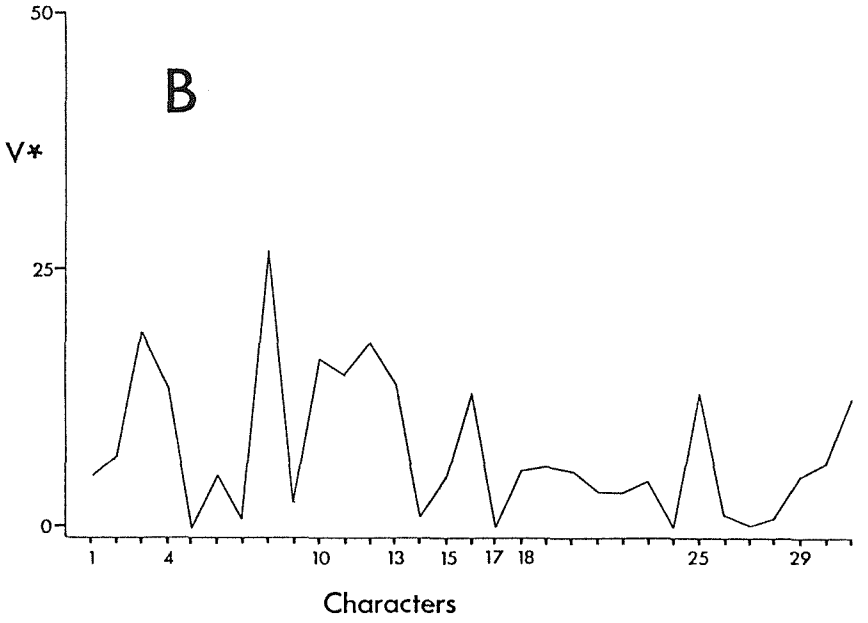
1 - 3	1ry forewing dorsal	- linear
4 - 9	1ry forewing dorsal	- colour
10 - 12	1ry forewing ventral	- linear
13 - 14	1ry forewing ventral	- colour
15 - 16	2ry forewing dorsal	- linear
17	2ry forewing dorsal	- colour
18 - 24	1ry hindwing dorsal	- linear
25 - 28	1ry hindwing dorsal	- colour
29 - 31	2ry hindwing dorsal	- linear

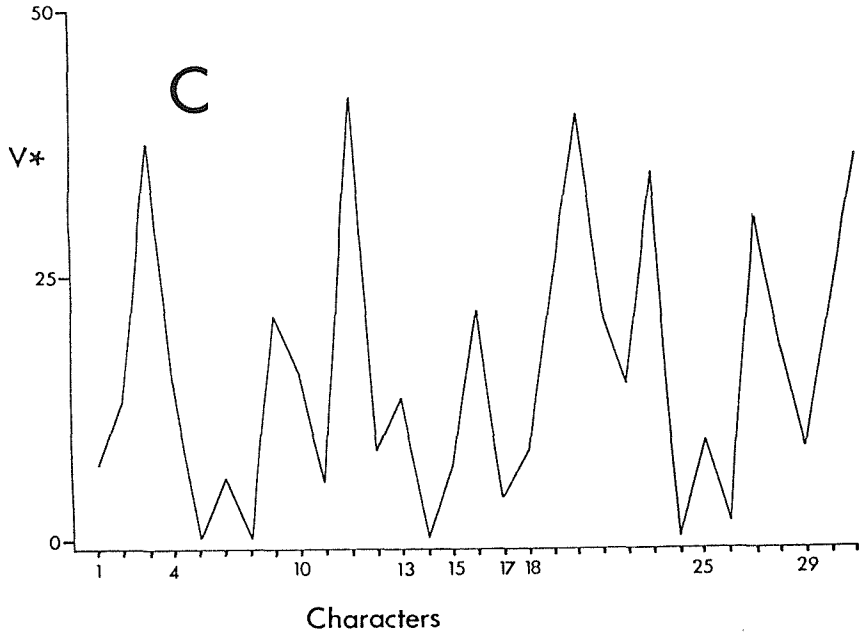
Characters:

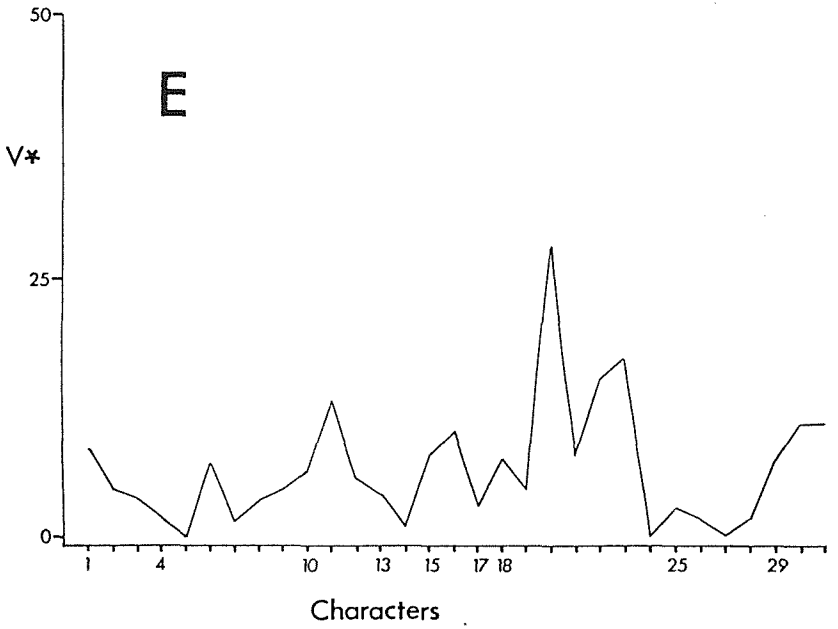
1 = FSPAN	11 = PAT9AU	21 = PAT26
2 = PAT3	12 = PAT10U	22 = PAT29
3 = PAT6	13 = PAT9BUC	23 = PAT32
4 = PAT4C	14 = PAT10UC	24 = PAT36
5 = SPC8C	15 = FSPANA	25 = SPC22C
6 = PAT9BC	16 = PAT11A	26 = PAT24C
7 = PAT10C	17 = PAT9BAC	27 = PAT27C
8 = PAT15C	18 = HSPAN	28 = PAT32C
9 = PAT18C	19 = PAT24	29 = HSPANA
10 = FWOCU	20 = PAT25	30 = PAT29A
		31 = PAT32A

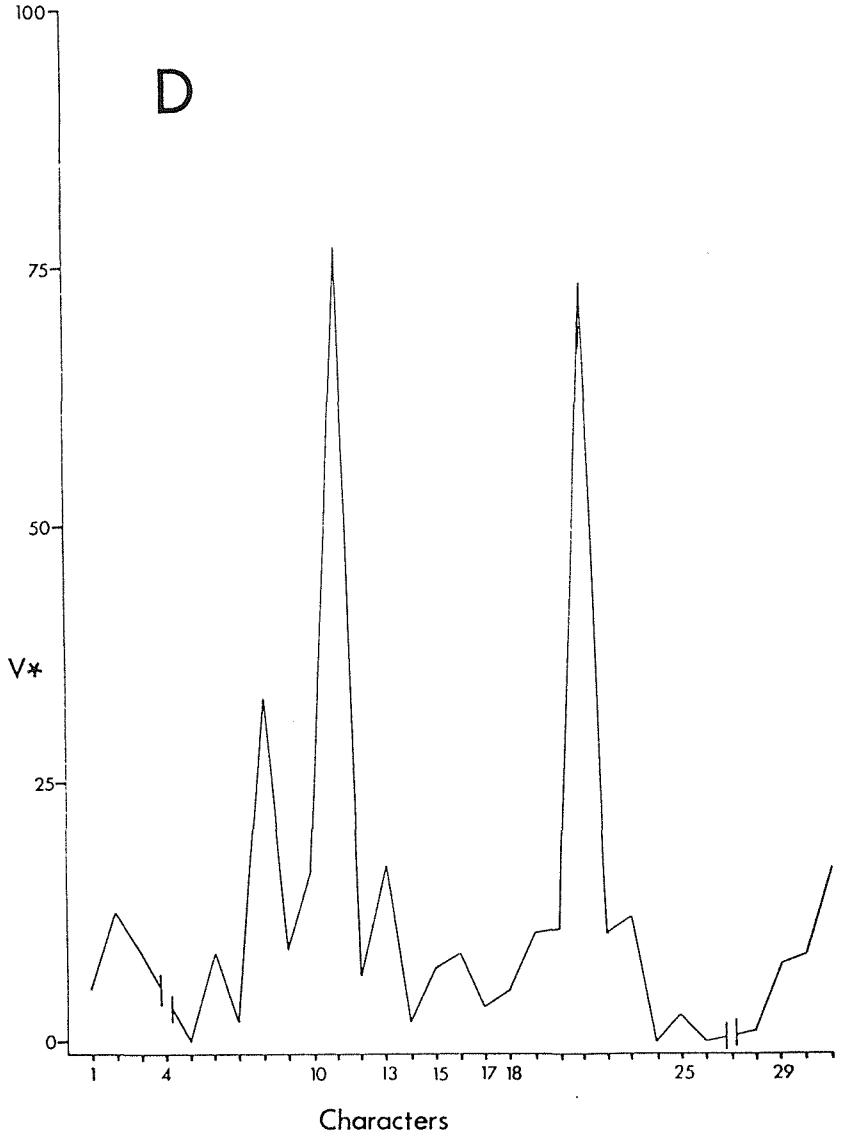
For further explanation see text.

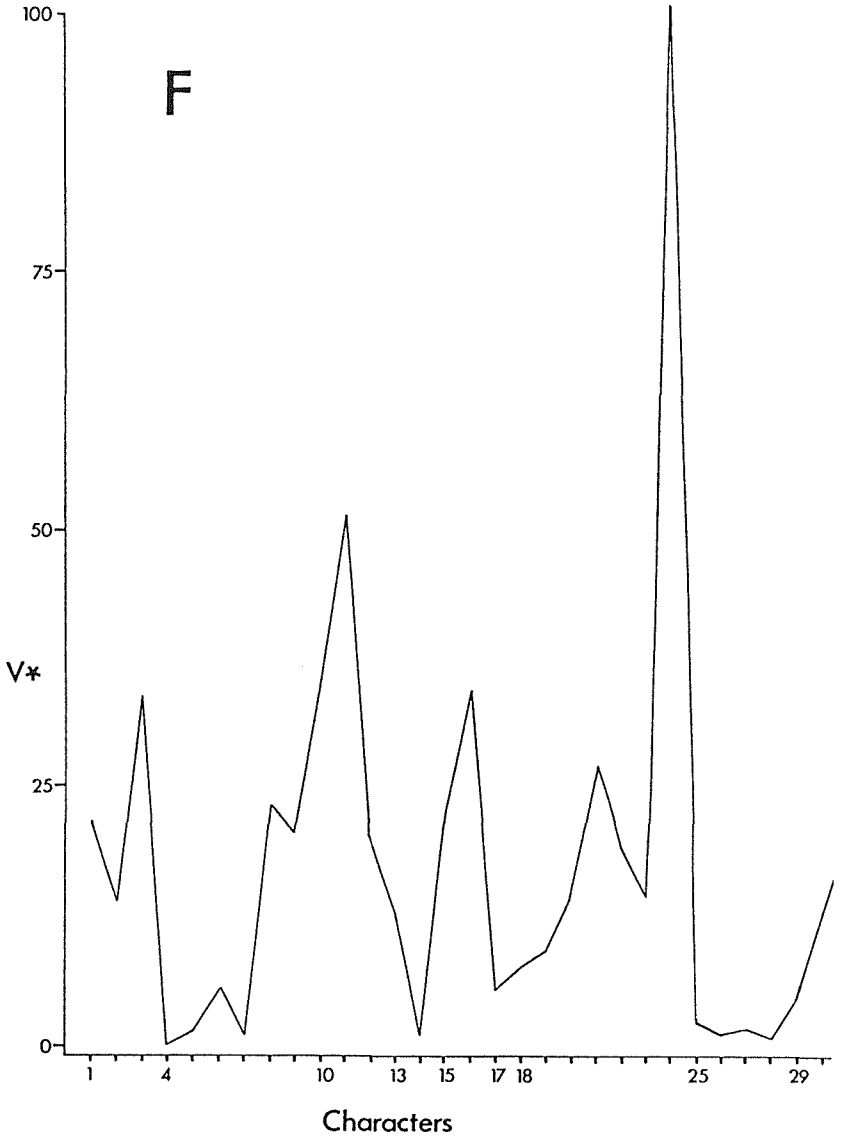

A
V*
50
25
0
1
4
10
13
15
17
18
25
29
Characters


B
V*
50
25
0
1
4
10
13
15
17
18
25
29
Characters


C
V*
50
25
0
1
4
10
13
15
17
18
25
29
Characters


E
V*
50
25
0
1
4
10
13
15
17
18
25
29
Characters


D
100
75
50
25
0
V*
1
4
10
13
15
17
18
25
29
Characters


F
100
75
50
25
0
V*
1
4
10
13
15
17
18
25
29
Characters

9.23. Sex differences

(i) *Linear and colour characters*

Characters differing in size or colour value are listed in Table 9.V and illustrated in Figure 9.21A. Size differences were present on both wings, and all sizes were larger in females except for the forewing venosa at v5 (PAT16), s0 (SPC17) and the dorsal hindwing E^3:U in s5 (PAT34). In both sexes, all size characters were concordant with pupal length (Kendall's W = 0.8186, $Chi^2_{[1]}$ = 17.1904, P<0.0001).

The *relatively* smaller s0 in females (all linear dimensions were scaled to the wingspan) supports the contention that it is wing ares *distal* to it that are responsible for the larger female forewing span (*cf.* section 9.11.i).

The venosa comprise fringe scales, and it is suggested that these are of similar size in both sexes, since the Fringe element comprises only wing *scales* (*cf.*section 9.16.ii), when it is the wing *membranes* (not the scales deployed therein) which expand on eclosion (Ford, 1957; Nijhout, 1980b). The greater wing expansion in females would thus result in the fringe length being *relatively* smaller. Indeed, in unexpanded wings the fringe appears disproportionately long.

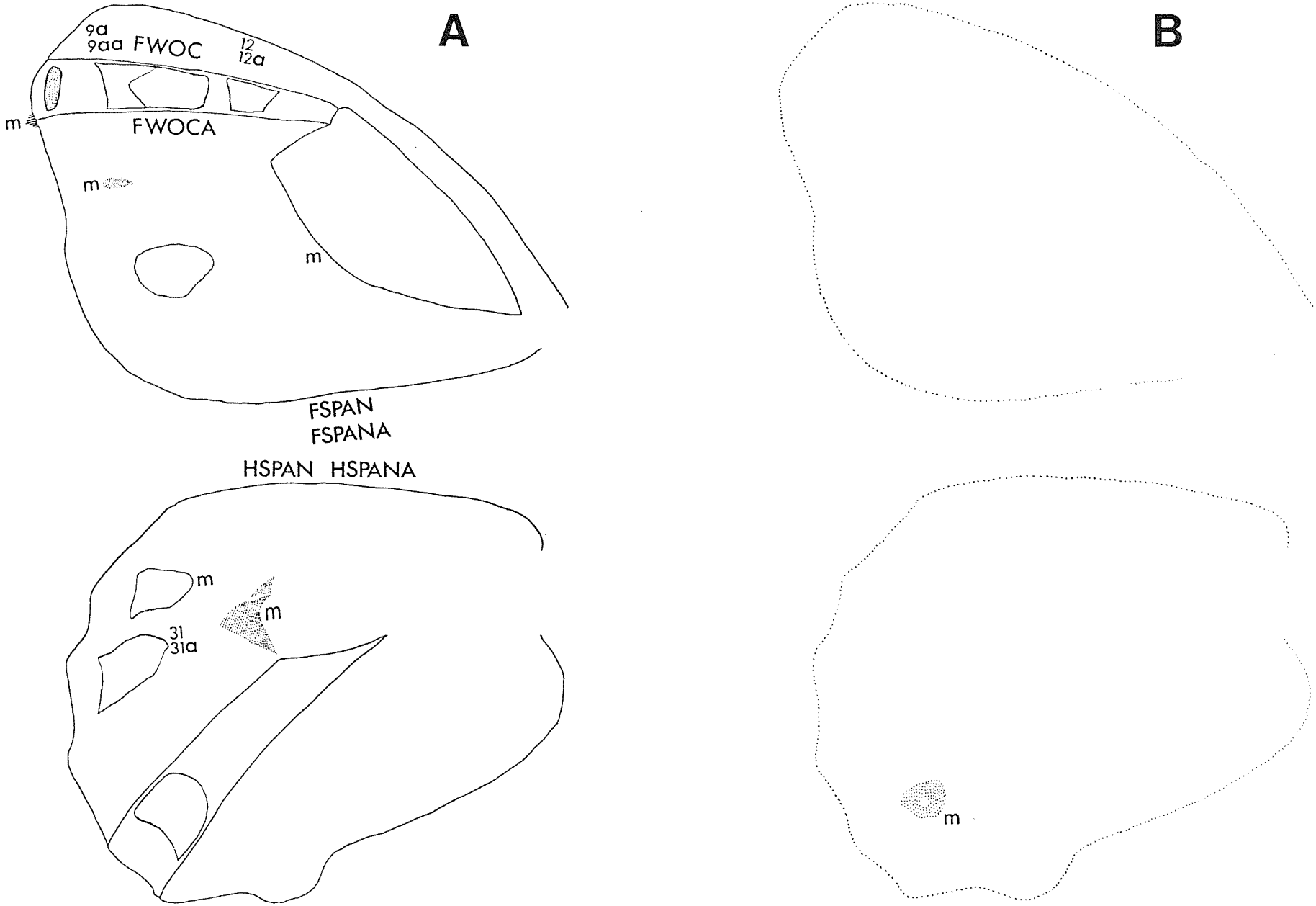
There was no difference between the sexes in the number of eyespot elements (cOC or OC) or in their individual size. This applied also to that within forewing s5 (PAT10 and PAT11 respectively) although its surrounding U:U interspace *was* larger in females. This further suggests that eyespot rings are independent of their surrounding elements. That it was the *distal* U:U which was responsible for the overall difference, suggests that wing expansion is greater distal to the eyespot focus. This might be expected given that the force of the haemolymph through the wing veins would be expected to be dissipated at the margin.

A similar phenomenon in the hindwing might explain why the E^3:U in s5 but not in the other spaces is relatively smaller in females, if the effective 'compression wave' (*cf.* section 9.11.i) round the anterior hindwing spaces means that the others are stretched towards the margin to a greater degree.

Character	Length		Colour$_1$		Colour$_2$	
	M	F	M	F	M	F
^{1}FSPAN	-	+				
^{1}PAT3	-	+				
^{1}PAT6			+	-		
^{1}VEN7	-	+				
^{1}SPC8	-	+				
^{1}FWOC	-	+				
^{1}PAT9A	-	+				
^{1}PAT12	-	+				
^{1}PAT14	-	+	-	+		
^{1}PAT16	+	-				
^{1}SPC17	+	-				
^{2}FSPANA	-	+				
^{2}FWOCA	-	+				
^{2}PAT9AA	-	+				
^{2}PAT12A	-	+				
^{3}HSPAN	-	+				
^{3}VEN21	-	+				
^{3}SPC22	-	+				
^{3}PAT23	-	+				
^{3}PAT31	-	+				
^{3}PAT34	+	-				
^{3}PAT37					+	-
^{4}HSPANA	-	+				
^{4}PAT31A	-	+				

TABLE 9.V. Sex differences in size and colour. Significant ($P<0.01$) differences are shown. '+' denotes larger size or darker colour; '-' denotes smaller size or lighter colour. Sex: M = male, F = female. ^{1}1ry FW_D; ^{2}2ry FW_D; ^{3}1ry HW_D; ^{4}2ry HW_D. For further explanation see text.

Sex differences in TYPE-I colour were limited to the dorsal forewing. E^1:E^2 (PAT14C, in sector 3) was darker (red-brown) in females than in males (light grey-brown), while E^2:E^3 (PAT6C, in sector 3) was less red (*ie.* orange) in females than in males (red-brown). Since PAT6C is relatively more basal in females than in males, its paler colour in females may reflect a general tendency for colour to become paler as it progresses basally within the wing spaces (*cf.* section 9.19.ii). Indeed it was suggested that PAT6 be designated an umbral (U:U) rather


A
B
9a
9aa
FWOC
12
12a
m
FWOCA
m
m
FSPAN
FSPANA
HSPAN
HSPANA
m
m
31
31a
m

than an external (E) interspace (section 9.16.i).

The only significant TYPE-2 colour difference was in hindwing s0/4 U:M^1 interspace, where TYPE-2 colour was more prevalent in males (44%) than females (5%) and comprised light grey-brown irroration of the cream interspace field.

Both TYPE-I colour differences were restricted to the forewing distal to s0. This would concur with the hypothesis that colour determination originates at the wing margin and depends on the basad distance from it, as it is primarily the forewing distal to s0 that is responsible for the sex difference in span. Indeed it is worth noting that the colour of D^1:M^2 in distal s0 (PAT18C) did not differ between the sexes.

(ii) *Fluctuating asymmetry*

Characters differing between the sexes in fluctuating asymmetry were present on both wings and, except for PAT9AC (FW_D U:U_d 5) which exhibited fluctuating asymmetry in males only, were restricted to size characters (Fig. 9.18B). Wingspan had a greater A_F in males than females, and the size of the hindwing eyespot rings and foci in s3 and s4 were also less canalised than in males. Males were also less canalised to the effects of foil (versus untreated) and cold (versus foil) treatment (Fig. 9.17C-F).

FIGURE 9.21 (opposite). Wing characters differing between the sexes in (A) size or colour and (B) variability. Characters taking the larger value in males are denoted m. Numeration is shown only where necessary to denote 2ry wing (A or a).

Legend: _____ Linear dimension

TYPE-1 colour

C_2 TYPE-2 colour

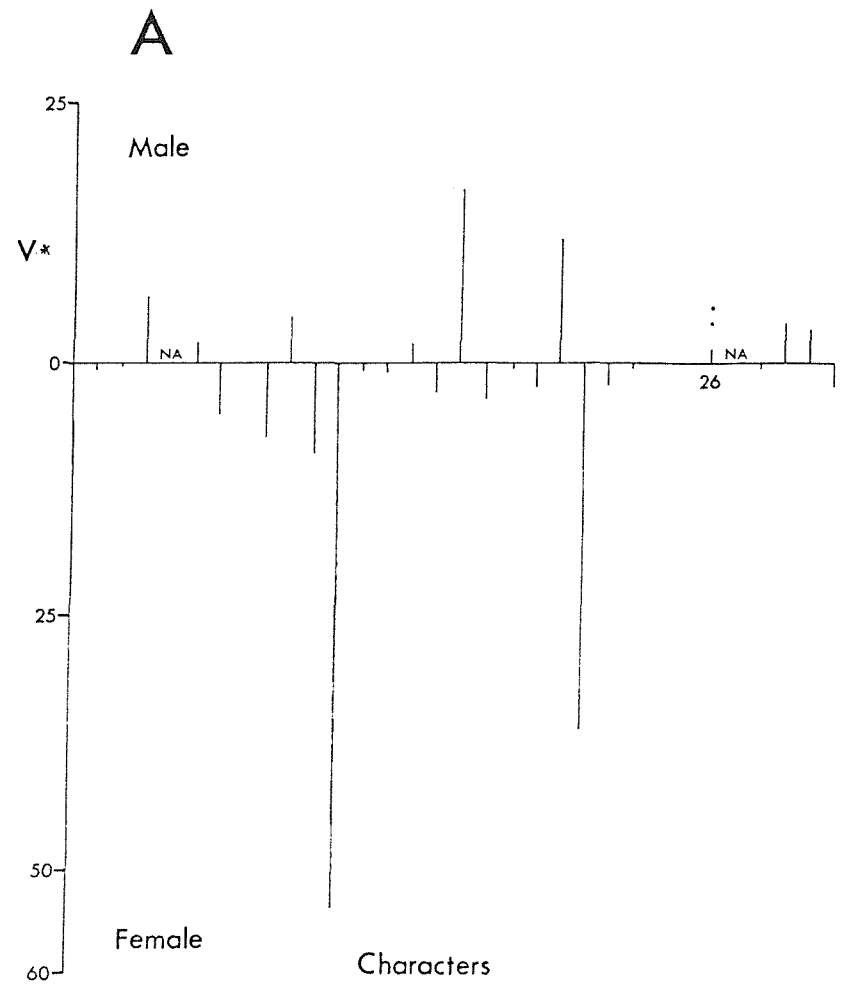
Dotted wing outlines are shown only to aid orientation. For further explanation see text.

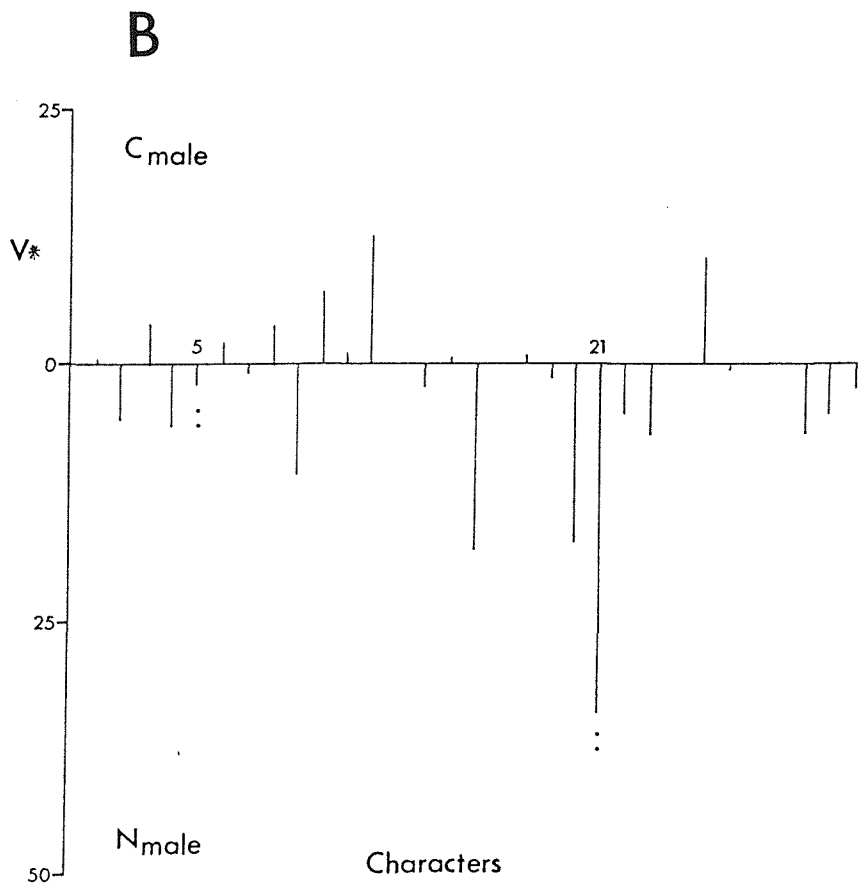
Some characters, although not showing any difference in A_F between the sexes *per se*, did show sex-specific sensitivities to particular treatments (below). The sensitivity of PAT9AC to foil and cold treatment too was limited to males (Fig. 9.17B-C & E).

(iii) *Variability*

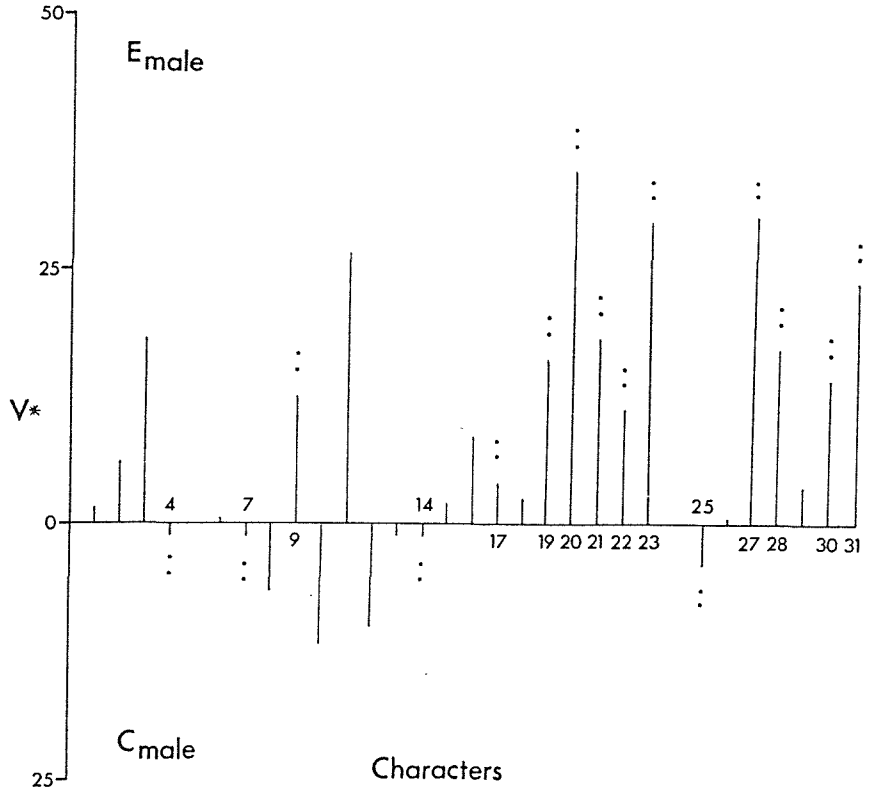
Only the TYPE-I colour of the hindwing s2 OC element (PAT24C) differed significantly in variability between the sexes (Figs. 9.21B and 9.22A, character 26). This character was variable only in males ($V^* = 1.3\%$, $s_{V^*} = 0.38\%$, n=6; *cf.* Fig. 9.20A and D), consistent with eyespot development being generally less canalised in males.

FIGURE 9.22 (overleaf). Absolute differences in the coefficients of variation of characters of the variable character set. Between: (A) the sexes; (B) untreated and foil treated males; (C) foil and cold treated males; (D) untreated and foil treated females; and (E) foil and cold treated females. Bars above or below the abscissa indicate that the respective treatment class took the greater value: N = untreated, C = foil treatment, E = cold shock. Only characters showing significant differences are numbered, numeration as for Figure 9.20. NA: V* not available owing to insufficient sample size. Significance levels: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$. For further explanation see text.

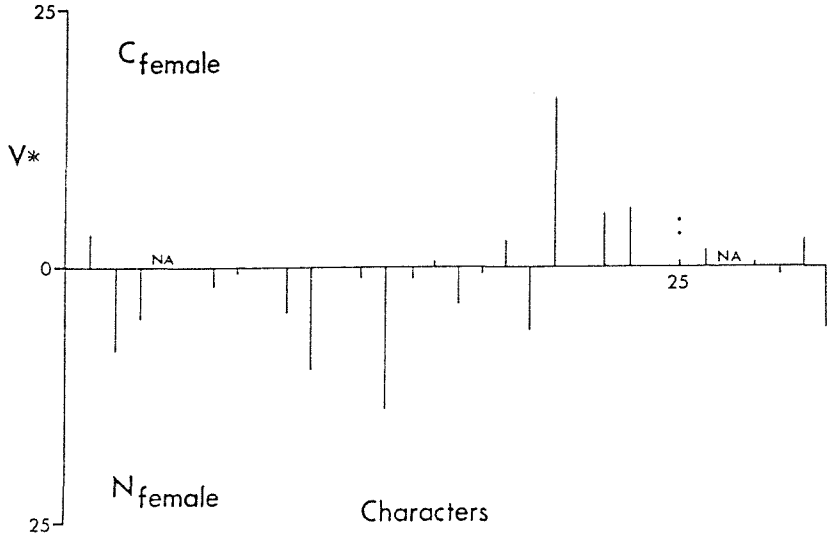

A
25
Male
V*
0
NA
NA
26
25
50
Female
60
Characters

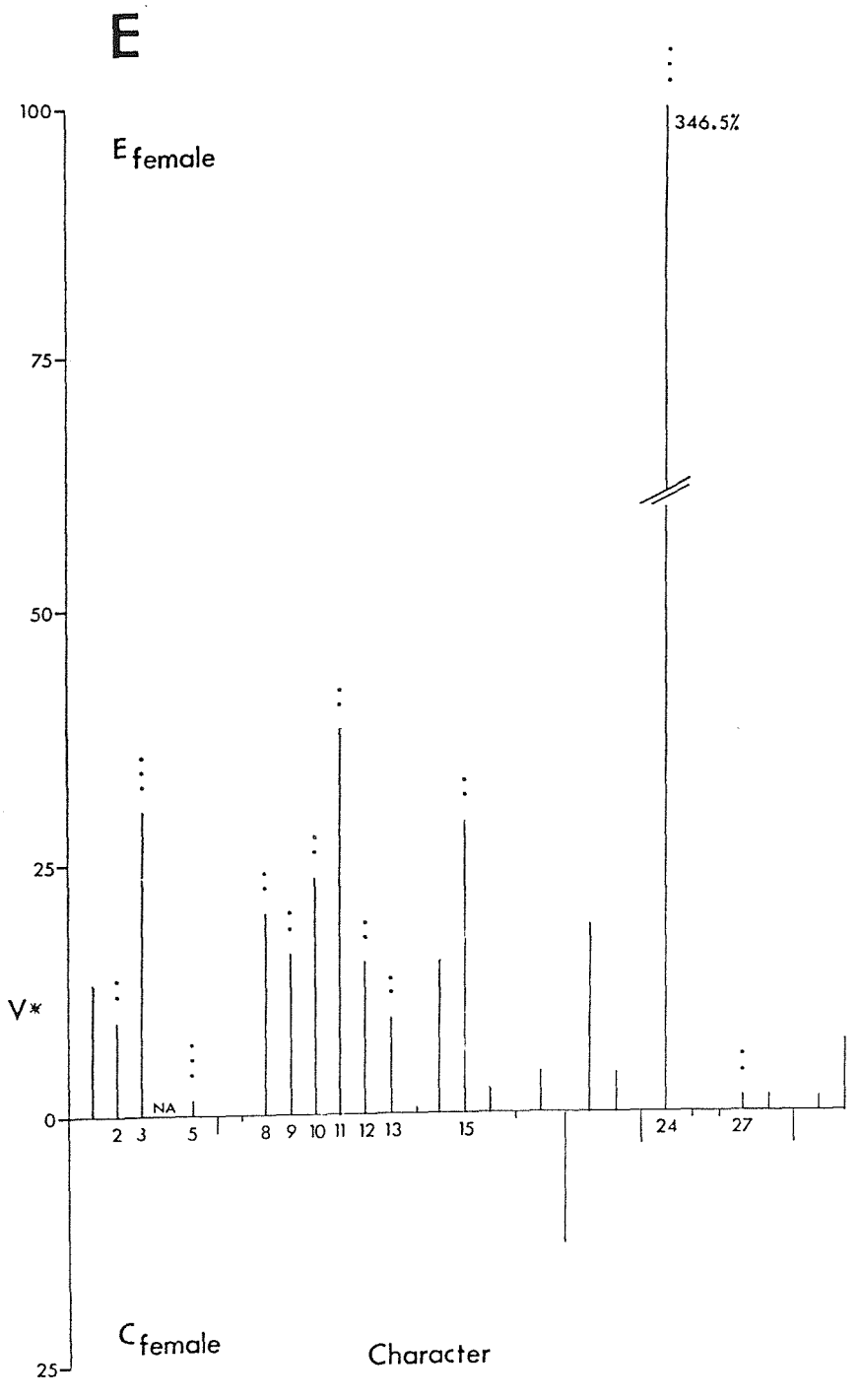

B
25
Cmale
V*
0
5
21
25
Nmale
50
Characters

C


50
25
0
25
V*
Emale
Cmale
Characters
4
7
9
14
17
19 20 21 22 23
25
27 28
30 31

D


25
0
25
V*
Cfemale
Nfemale
Characters
NA
NA
25


E
100
75
50
25
0
25
V*
E female
C female
346.5%
NA
2 3 5 8 9 10 11 12 13 15 24 27
Character

9.24. Family differences

(i) *Linear and colour characters*

Characters differing in size or colour between STOCK 01 families 002 and 004 are listed in Table 9.VI and illustrated in Figure 9.23A. Size differences were confined to the hindwing and dimensions were larger in family 002, with the exception of the Fringe intervenosa at s2 (PAT26) and the OC element in s5 (PAT35). There were no size differences on the forewing.

TYPE-1 colour differences were predominant on the forewing, all such colours being darker in family 004 except for the ground colour of hindwing s2 (PAT22C) which was darker in family 002. The darker colour of the forewing s0 M^1:D^2 interspace (PAT18C) in family 004 was due to a greater red-brown component.

TYPE-2 colour differences were confined to forewing interspaces intervening the E^2 and distal U:U elements, and this applied to both surfaces. TYPE-2 colour was predominant in s2 and s5 when it comprised dark irroration of the cream/pale yellow background (giving the marking a more TYPE-1 orange colour), and this irroration was limited to family 004. Thus, TYPE-1 and TYPE-2 colours tended to be darker in family 004. Irroration of the forewing s3 E^1:E^2 interspace, however, was light grey brown and present in both families, albeit more prevalent in family 002.

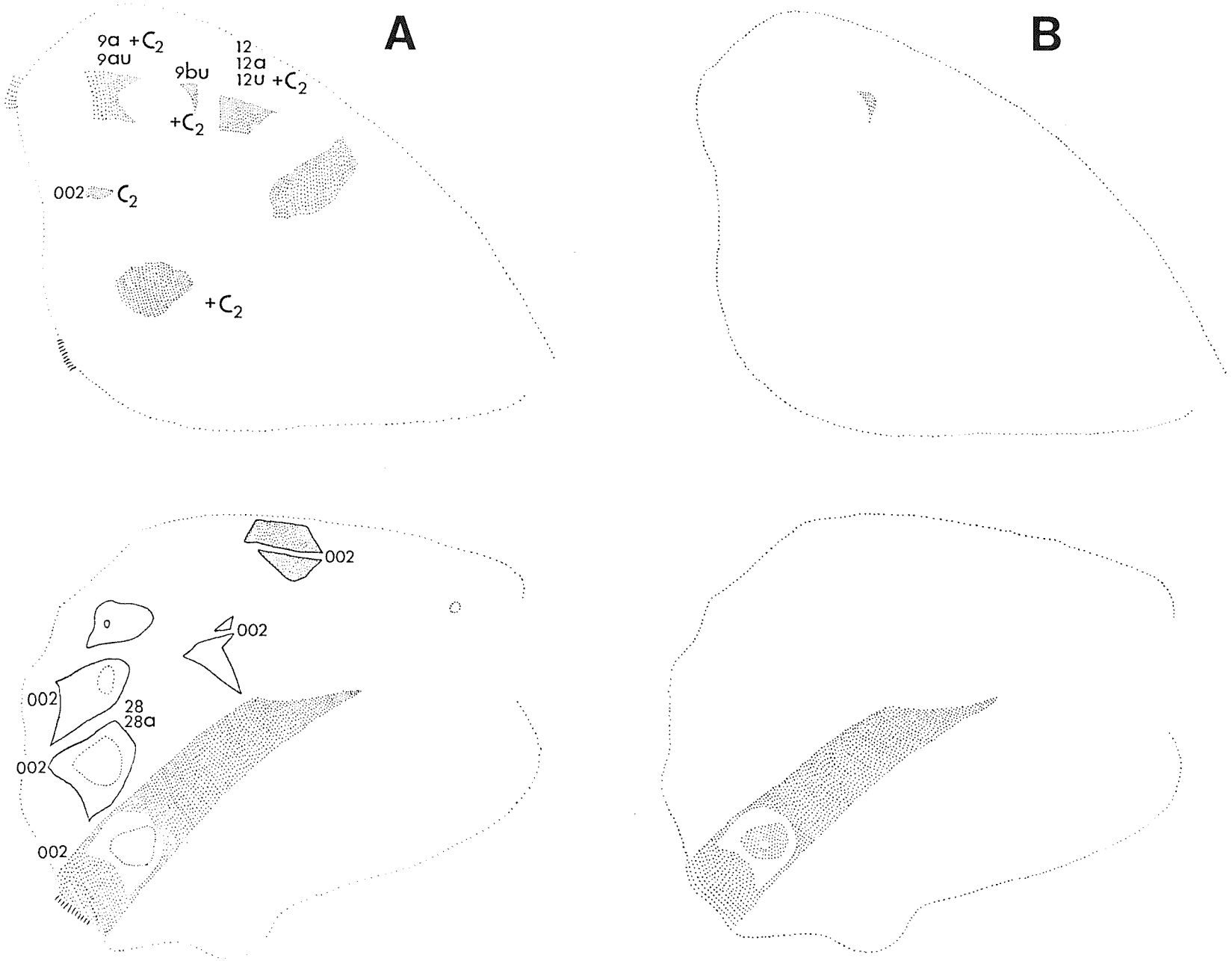
Family 004 had more frequent 4-spot (versus 3-spot) dorsal hindwing phenotypes than family 002. Thus eyespot number is under genetic control. The OC element in hindwing s5 (PAT35) was the only *individual* eyespot to differ between the families, suggesting that *it* is responsible for the difference in spot number. Its more frequent absence in family 002 may represent the limit of size diminution of its already smaller size in this family.

Thus, family 004 has smaller interspaces and darker colours. Its more frequent ocellar element in s5 would give the E^3:U region of this space a darker overall appearance. These results implicate a genetic difference between them that coordinates these three aspects of darkening, giving family 004 a more 'summer-form' phenotype (*cf.* Packer, 1984). Since both families were generation 2 part i and reared under identical conditions, this would support Packer's (1984)

Character	Length		Meristic		TYPE-1		TYPE-2	
	002	004	002	004	002	004	002	004
[1]PAT2			-	+				
[1]PAT3					-	+	0	+
[1]PAT6							+	-
[1]PAT9A					-	+		
[1]PAT12					-	+	0	+
[1]PAT15					-	+		
[1]PAT18					-	+		
[2]PAT9AU							0	+
[2]PAT9BU					-	+	0	+
[2]PAT12U					-	+		
[3]PAT12A					-	+		
[4]SPC22					+	-		
[4]PAT26	-	+						
[4]PAT28	+	-						
[4]PAT31	+	-						
[4]PAT35	-	+						
[4]PAT37	+	-						
[4]PAT38	+	-			-	+		
[4]PAT39			-	+				
[5]PAT54			-	+				
[6]PAT28A	+	-						

Table 9.VI. Family differences in size and colour. Significant ($P < 0.01$) differences are shown. Meristic includes characters scored for presence or absence. TYPE-1 = TYPE-1 colour, TYPE-2 = TYPE-2 colour. '+' denotes larger value or darker colour; '-' denotes smaller value or lighter colour; '0' denotes absence (versus presence). Families: 002 or 004. [1]1ry FW_D; [2]1ry FW_V; [3]2ry FW_D; [1]1ry HW_D; [1]1ry HW_V; [1]2ry HW_D; For further explanation see text.

contention that 3-spot ('spring form') and 4-spot ('summer form') dorsal phenotypes are determined genetically.


A
B
9a +C2
9au
9bu
12
12a
12u +C2
+C2
002
C2
+C2
002
002
002
28
28a
002
002

(ii) *Fluctuating asymmetry*

Significant differences in fluctuating asymmetry (Figs. 9.17A and 9.18A) show that forewing s5 basal U:U (PAT9B), hindwing span and s4 OC (PAT32) were more canalised in family 004. The size and colour of forewing s5 distal U:U were more canalised in family 002. It would appear that their fluctuating asymmetry is regulated by genetic components that differ between the families. Forewing s5 and hindwing s4 each describe an A-P axis within the wings that may be be homologous (section 9.11); and in several species the forewing s5-s6 boundary approximately delineates separate wing compartments (*cf.* Sibatani, 1980).

The distal U:U of forewing s5 was the only TYPE-1 colour character to differ in F_A between the families. It would appear either that any genetic components involved in the canalisation of colour development tend not to differ between the families. That the number of family differences in size or colour *value* (Fig. 9.23A) was much larger than the number of differences in *fluctuating asymmetry* (Fig. 9.18A), suggests that genetic differences between the families in the control of pattern relate more to its determination than to the stability of its development.

FIGURE 9.23 (opposite). Wing characters differing between the families in (A) size or colour and (B) variability. Characters taking the larger value in family 002 are denoted 002. Numeration is shown only where necessary to denote 2ry wing (a) or ventral surface (u).

Legend: _____ Linear dimension

▒▒▒▒ TYPE-1 colour

▒▒▒▒ C_2 TYPE-2 colour ($+C_2$ denotes TYPE-1 and TYPE-2)

Dotted wing outlines are shown only to aid orientation. For further explanation see text.

(iii) *Variability*

Three TYPE-1 colour characters differed significantly in variability between the families (Fig. 9.23B) where they varied only in family 004; the V* values are listed in Appendix XV. It is suggested that the ground colour of hindwing s2 (SPC22C) is typically dark, its paler colour in family 002 (section 9.24.i) perhaps being simply their expressing a lighter colour from its normal range of genetic variation. Hindwing s2 OC (PAT25C) and forewing s5 basal U:U (PAT9BC). These character differences most likely reflect genetic variation in their *determination* (rather than regulation) since they did not differ significantly between the families in F_A.

9.25. Darkness effects

(i) *Linear and colour characters*

Untreated and foil treated samples were first compared overall (Table 9.VII), when the only character to differ was the TYPE-1 colour of forewing s0 D^1:M^2 (PAT18C) which was darker (ochre) under foil than no foil treatment (yellow-orange).

The two treatments were therefore compared with each combination of onset and duration of foil treatment (Table 9.VIII). Significant differences and their specificities are shown in Figure 9.24A. All such differences, with the exception of hindwing s2 eyespot pupil (below), were associated with the <12h onset of treatment. PAT18C again differed, but now also the forewing s0 M^2:D^2 interspace (PAT19C) which was smaller under foil treatment. Thus darkness effected a general darkening of dorsal forewing s0. PAT18C is close to the

	TYPE-1		Length		Meristic		TYPE-1	
	N	C	C	E	C	E	C	E
Character								
$PAT5_{004}$					-	+		
$PAT15_{MALES}$					+	-		
$PAT15_{002}$					+	-		
PAT18C	-	+					+	-
$PAT18C_{MALES}$							+	-
$PAT18C_{002}$							+	-
$PAT9AAC_{004}$							+	-

Table 9.VII. Treatment differences in size and colour. Differences specific to a particular family (002 or 004) or sex (MALES) are indicated by the respective subscript. TYPE-1 = TYPE-1 colour. Meristic includes characters scored for presence or absence. '+' denotes a larger value or darker colour; '-' denotes a smaller value or paler colour. Treatments: N = untreated, C = foil, E = cold shock. For further explanation see text.

Application	Character	N	C		C	E	
<12:48	PAT18C	-	+	*			
	PAT19	+	-	*			
	PAT27	-	+	**	+	-	*
	PAT31C	+	-	*			
	PAT32	-	+	*			
<12:96	PAT9AAC				+	-	*
	PAT23	-	+	*			
<24:48	PAT15				+	-	*
	PAT25	+	-	*	-	+	*
<24:96	PAT9A				-	+	*
	PAT12				-	+	**
	FWOCA				-	+	**
	PAT9AA				-	+	*
	PAT12A				-	+	*
	PAT30A				-	+	*

Table 9.VIII. Specificities of response. Pairwise comparisons between treatments for each combination of treatment onset (<12h or <24h) and duration (48h or 96h). '+' denotes larger value or darker colour; '-' denotes smaller value or paler colour. Treatment: N = untreated, C = foil, E = cold shock. Significance levels (Mann-Whitney U-test): * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$. For further explanation see text.

discoidal veins, and its early onset and duration specificities (<12h onset and effective during the first 48h of treatment) implicate the discoidal area in early pattern development. That PAT19, also in s0 from which the other spaces are postulated to radiate (section 9.11.i), too was influenced by the earlier onset and durations, further implicates s0 as important in early pattern development.

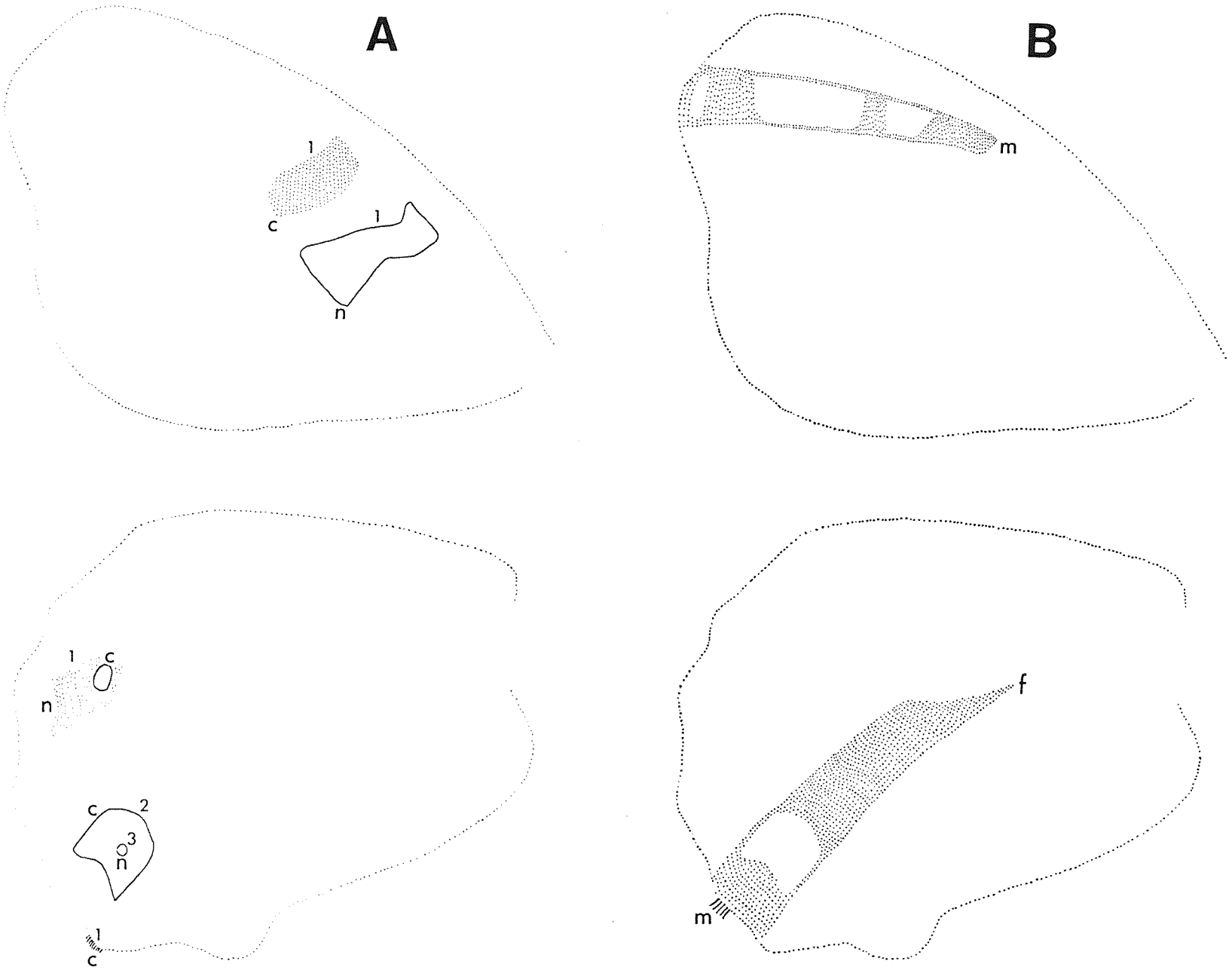
The hindwing v2 venosa (PAT27) and s4 OC (PAT32) were larger under foil treatment and the difference specific for the <12h onset and 48h duration. This implicates the wing margin and eyespot ring as being determined early in pattern development. The s2 E^3:U too was larger under foil treatment and specific for the <12h onset, but the difference now apparent only under the 96h duration. Since the size of

the E^3:U interspace is related to the width of its bordering elements (E^3 and M^1, Fig. 9.3A), then if the latter are established by some determinant that spreads radially from the eyespot focus (*cf.* Nijhout, 1981) their determination can be expected to occur later than onset of focal activity. The size of the s2 eyespot pupil (PAT25), however, was smaller under foil treatment and this difference, although specific for the <24h onset, was again effected within 48h of treatment onset. The general effect is a widening of the eyespot ring within the E^3:U interspace. The colour of the s4 E^3:U interspace was paler under foil treatment, the difference again effected by 48h of treatment starting within 12h post-pupation. This would support the contention that colour is determined early and from the wing margin (*cf.* section 9.19.ii). The overall effect of foil treatment is thus a larger and paler hindwing s4 E^3:U interspace but with a broader (dark) eyespot ring, and a generally more orange forewing s0 with broader (dark) elements.

(ii) *Fluctuating asymmetry*

Characters differing significantly in fluctuating asymmetry between the treatments are shown in Figures 9.17C-D and 9.18 C-D. All significant differences, with the exception of the forewing s5 distal U:U interspace (PAT9AC), involved linear characters.

In males (Fig. 9.18C), F_A values were generally greater in *untreated* animals, possibly reflecting the destabilising influence of a higher ecdysone level under summer daylength. Here the sizes of OC elements (eyespot *rings*) in forewing s5 and hindwing s3 and s4 were more unstable in the untreated class. The size of forewing s5 basal U:U interspace too was more unstable in the untreated class. This again suggests a possible association between the forewing s5 eyespot ring (PAT10) and basal U:U interspace (PAT9B), implying that the fluctuating size of the eyespot ring is attributable to its basal border. That TYPE-1 colour in forewing s5 distal U:U interspace (PAT9AC) too fluctuated more in untreated samples again points to a possible interaction between an epimorphic wave of colour determination and eyespot focal activity. The sizes of cOC elements (eyespot *pupils*) in hindwing s3 and s4, however, were more unstable under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foil treatment. That eyespot foci differed between the untreated and foil treated class only on the forewing, suggests that in eyespot focal activity in males is less canalised on the hindwing than forewing.

In females (Fig. 9.18D), fluctuating asymmetries were generally greater under *foil* treatment. Again the sizes of OC elements (eyespot *rings*) in forewing s5 (PAT10) and hindwing s4 (PAT32, although not in s3) differed in A_F between treatments. The size of the hindwing s3 E^3:U interspace (PAT28), however, was more unstable in the untreated class. This suggests that treatment influences the development of pattern components within the space other than just OC elements. That the E^3:U exhibited greater F_A in the untreated class while the OC exhibited greater F_A under foil treatment, again points to their respective development as involving distinct processes.

In both sexes, the hindwing characters involved were limited to s3 and s4. This would further implicate the hypothetical (A-P) intra-wing compartment boundaries as sites of developmental activity.

FIGURE 9.24 (opposite). Wing characters differing between no foil and foil treatments in (A) size and colour and (B) variability. (A) The nature and timing of treatment yielding the greater variability is indicated. Treatment: n = no foil, c = foil; Timing of application (onset,duration): 1 = <12h,48h; 2 = <12h,96h; 3 = <24h,48h. (B) The sex in which the character differed in V* between treatments is shown thus: m = males; f = females.

Legend: ——— Linear dimension

TYPE-1 colour

For further explanation see text.

(iii) *Variability*

Characters differing significantly in variability between untreated and foil treatments are shown in Figure 9.24B. In both sexes variability was greater in *untreated* animals. Again a greater rate of early pupal ecdysone production under summer daylength may destabilise development.

In males, the forewing s5 ground colour (dark elements) and the hindwing s2 intervenosa were affected; in females, the hindwing s2 ground colour (dark elements) was affected. However, in neither sex were the OC elements affected, suggesting that the regulation of OC (eyespot ring) colour is separate from that of other elements within the respective spaces. That fringe length and colour of space elements other than OC were affected suggests that the determination of such elements is related to some process at the wing margin. Since forewing s5 and hindwing s2 were the only spaces in which element colour (other than of OC) was examined, it is possible that other spaces may behave similarly.

9.26. Cold shock effects

(i) *Linear and colour characters*

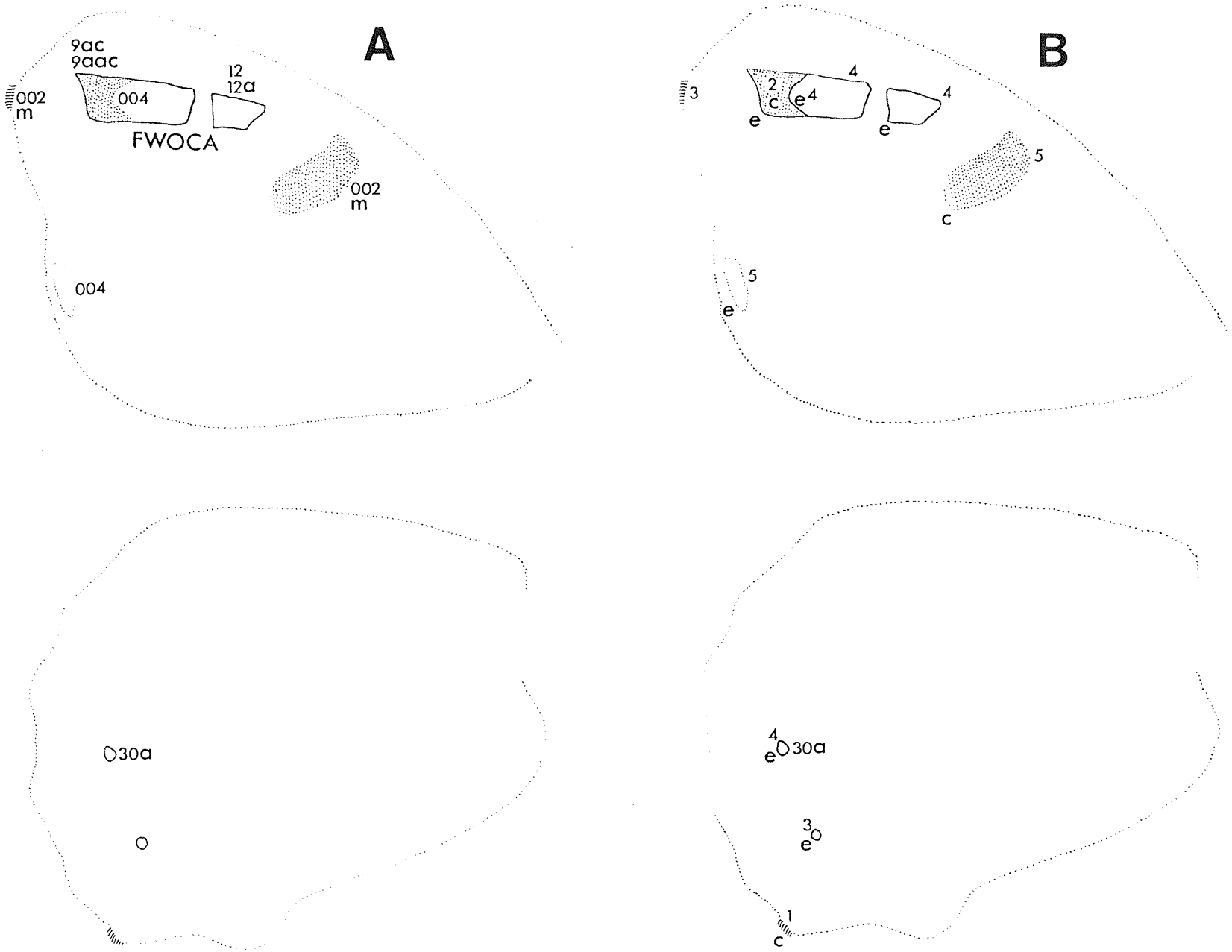
Characters differing in size and colour between foil and cold shock treatments are listed in Tables 9.VII and 9.VIII and illustrated in Figure 9.25. The colour of forewing s0 D^1:M^2 (PAT18C) was more orange under cold shock than foil treatment (when it was ochre) and hence more similar to that of untreated samples (yellow-orange). The greater orange component under cold might therefore too be connected with a higher ecdysone titre, that of the post-shock pulse. The forewing s5 intervenosa (PAT15) was larger under foil than cold treatment, but is not an artifact of any difference in wingspan as the latter did not differ between the treatments.

Sex and family specificities are shown Fig. 9.25A. Both the above effects were significant in males and in family 002, suggesting that they depend on genetic components which differ between the sexes and families. Females may be more canalised.

The presence of the forewing s3 E^1:E^2 interspace (PAT5) was more prevalent under cold (51% of individuals) than foil (8%) treatment. The colour of forewing s5 distal U:U (PAT9AC) too was more orange (yellow-orange) under cold than foil (when it was ochre) although, unlike PAT18C, it did not differ between untreated and foil treated samples. These effects were specific to family 004, suggesting that they depend on genetic components differing between the families but not the sexes.

Inspection of Tables 9.VII and 9.VII reveals untreated and cold shocked animals to be generally the more similar. This supports the hypothesis that the respective treatment differences (untreated versus foil, cold shock versus foil) are connected with higher ecdysone titres.

Treatment onset and duration specificities are shown in Fig. 9.25B. In general, characters situated more distally within the spaces differed under the earlier onset of cold treatment (Fig. 9.25A, codes 1 and 2), more basally situated characters tending to differ under the later onset (Fig. 9.25B, codes 3 and 4, code 5 indicating that the character differed under all combinations of onset and duration).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PAT18C and PAT9AC both differed under the <12h onset and showed a similar pattern of response among treatments (Tables 9.VII and 9.VIII). Both are situated distally within the respective (s0 and s5) wing spaces. PAT15, although also situated distally within s5, differed only under the 12-24h onset. While this delay might be spurious, since the hindwing s2 venosa did differ under the <12h onset, it is possible that while the *venosa* might be established first, the *inter*venosa are established through the subsequent activity of the eyespot foci (*cf.* sections 9.16.i and ii).

Treatment differences within forewing s5 were limited to the dorsal surface (other spaces were examined only on the dorsal surface so it was not ascertained whether *they* might have differed ventrally). The overall and distal (but not basal) U:U and U:M^1 interspaces were all larger under cold shock, implying that their bordering *elements* (E^3 and M^1) were *narrower* under cold shock. These differed under the later (12-24h) onset and longer (96h) duration. This again suggests that these pattern elements are determined later than the fringe and ocellar elements.

FIGURE 9.25 (opposite). Wing characters differing in size and colour between foil and cold shock treatments including specificities for (A) sex and family and (B) treatment timings. (A) Numeration is shown only where necessary to denote 2ry wing (a or A). Family or sex manifesting the difference: 002 = family 002, 004 = family 004; m = males. Unmarked characters differed overall. (B) Treatment yielding the larger value: c = foil, e = cold shock. Timing of treatment application yielding the larger value (onset,duration): 1 = <12h,48h; 2 = <12h,96h;
3 = 12-24h,48h; 4 = 12-24h,96h; 5 = all treatments.

Legend: ——— Linear dimension
····· Meristic
▒▒▒▒▒ TYPE-1 colour

Dotted wing outlines are shown only to aid orientation. For further explanation see text.

Their reduced nigrism may have resulted from a reduced ecdysone turnover *during* cold (as opposed to the sudden increase *following* shock). It is thus important to distinguish between the effects of reduced ecdysone turnover during cold and the effects of the post shock pulse. It is speculated that the more extensive nigrism of the summer-form might be due to aestival conditions or some genetic factor (*cf.* Packer, 1984) resulting in increased ecdysone production in such specimens.

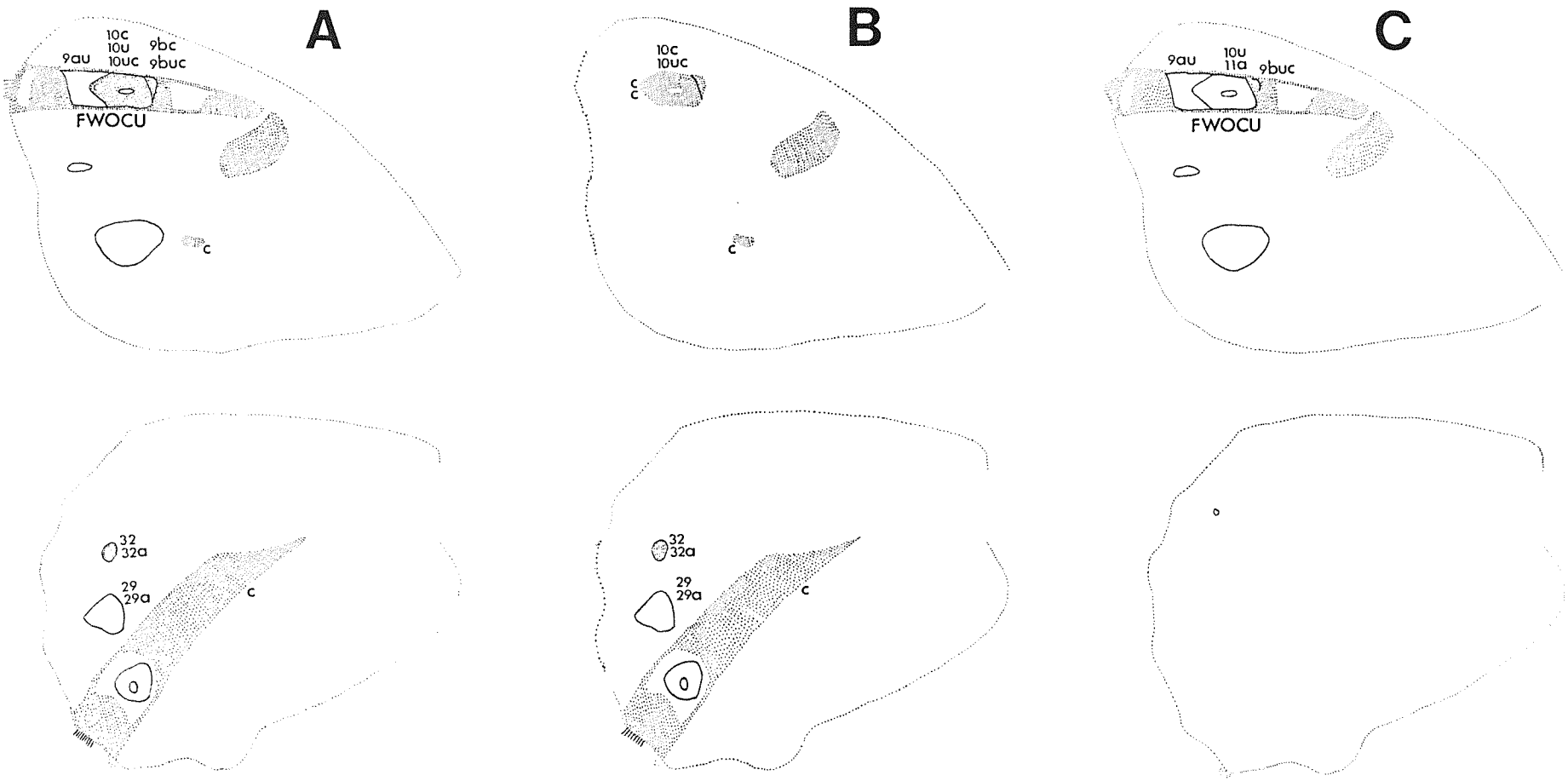
The eyespot foci in hindwing s2 and s3 (PAT25 and PAT30) were larger under cold than foil treatment. Thus, although the eyespot rings (OC) in these spaces were not affected by cold, it is conceivable that larger foci could mean the rings being narrower and hence exhibiting reduced nigrism.

(ii) *Fluctuating asymmetry*

In males, all characters differing in fluctuating asymmetry between treatments (Fig. 9.18E) exhibited greater F_A values under cold, except for hindwing span and the cOC elements of forewing s5 and hindwing s4. In this sex, the colour of the forewing s5 distal U:U (PAT9AC) also fluctuated more under cold. It was also the only colour character to differ in F_A between other sample comparisons.

In females, all characters differing in F_A between treatments exhibited greater F_A under foil, except for the forewing s5 basal U:U interspace, PAT9B (Fig. 9.18F).

That in males the F_A levels were generally greater under cold, whereas in females they were generally greater under foil, might be explained thus. Ecysone production *during* treatment is greater under foil than cold treatment and explains the greater F_A under foil in females. Ecdysone turnover immediately *after* treatment, however, is greater following cold than foil treatment, and the magnitude of the difference is greater than during treatment. Females are canalised against the post-shock pulse so their F_A levels reflect differences in ecdysone turnover *during* treatment. Males are not canalised against the post-shock pulse so their F_A levels reflect differences in ecdysone turnover *following* treatment, when the magnitude of the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FWOCU

instability induced by the post cold pulse is such that it overides the otherwise greater stability during cold.

In both sexes, hindwing span was less canalised than forewing span. That in *both* sexes hindwing span and the size of the cOC elements of forewing s5 and s4 were more unstable to *foil*, suggests that their determination is completed before the termination of treatment, possibly within 72h post-pupation (allowing for the 12-24h onset with 48h duration). That in both sexes the size of forewing s5 basal U:U (PAT9B) was more unstable to cold, suggests that its determination or, more specifically, that of its bordering elements (M^1 and OC), occurs at 96h+ post-pupation when the lability of the OC would appear to involve particularly its *basal* border (*cf.* section 9.24.ii). That the hindwing s3 E^3:U and OC (PAT28 and PAT29) were unstable only in males (and here to cold), suggests that the determination of the associated s3 elements (E^3, OC and M^1, Figs. 9.2 and 9.3) continues after the termination of treatment (72h-120h post-pupation) and is less canalised in males.

In males, forewing span showed a significant difference between foil and cold treatments in the magnitude of directional asymmetry (mean ± SD: foil = -2.04% ± 0.848%, cold shock = -0.04% ± 1.102%, $F_{(1,13)} = 13.641$, $0.001<P<0.01$), in both cases being biased towards a larger right forewing span. However, this most likely reflects a difference between their (spurious) *fluctuating* asymmetries, since directional asymmetry of forewing span was only significant *per se* in foil treated males (Appendix XII).

FIGURE 9.26 (opposite). Wing characters differing in variability between foil and cold shock treatments. (A) both sexes overall, (B) males, and (C) females. Numeration is shown only where necessary to denote ventral surface (U or u) or 2ry wing (a). Characters exhibiting the greater variability under foil treatment are designated c.

Legend: ——— Linear dimension
▒▒▒ TYPE-1 colour

Dotted wing outlines are shown only to aid orientation. For further explanation see text.

(iii) *Variability*

Characters differing in variability between foil and cold shock treatment are shown in Figure 9.26; the respective coefficients of variation of reported in Appendix XV.

In both sexes, hindwing characters differing in variability between the treatments primarily concerned size, and were much more prevalent in males than in females where only the size of hindwing s5 OC (PAT35) differed in variability. Thus hindwings are less canalised in males than in females. In males, the size of the hindwing s2, s3 and s4 OC elements and the colour of that in s4 were more variable under cold. The ground colour of hindwing s2 (non-OC elements), however, was more variable under foil. This suggests that the latter is affected during treatment (rather than at the time of the putative post-shock ecdysone pulse), when the warmer temperature under foil would be associated with a greater ecdysone production. This is consistent with still greater variability of this character in the untreated (full daylength) class (*cf.* section 9.25.iii). In males, all forewing characters differing in variability between foil and cold treatments concerned TYPE-1 colour: s0 M^1:D^2 (PAT18C) and s5 basal U:U (PAT9BC) were more variable under cold, suggesting that they are destabilised by the post-shock pulse; but s2 U:M^1(PAT4C) and s5 OC on both surfaces (PAT10C and PAT10UC) were more variable under foil, suggesting that they are destabilised by the greater ecdysone level during treatment.

In females, *all* characters differing in variability between the treatments were more variable under cold. Linear character differences were confined to elements within the Umbral region (*ie.* to the more distal wing region), which in s5 were further limited to the ventral surface; these included the size of the OC element. It is possible that differences between the surfaces in the variability of size characters might reflect an influence of cold on wing expansion, since the surfaces may already differ in expansion rate under normal conditions (section 9.32.ii). Indeed in females forewing span had greater fluctuating asymmetry under cold treatment.

That the colour of forewing s5 basal U:U was also more variable on the ventral surface, further suggests some relationship between the OC element and TYPE-1 colour immediately basad to it.

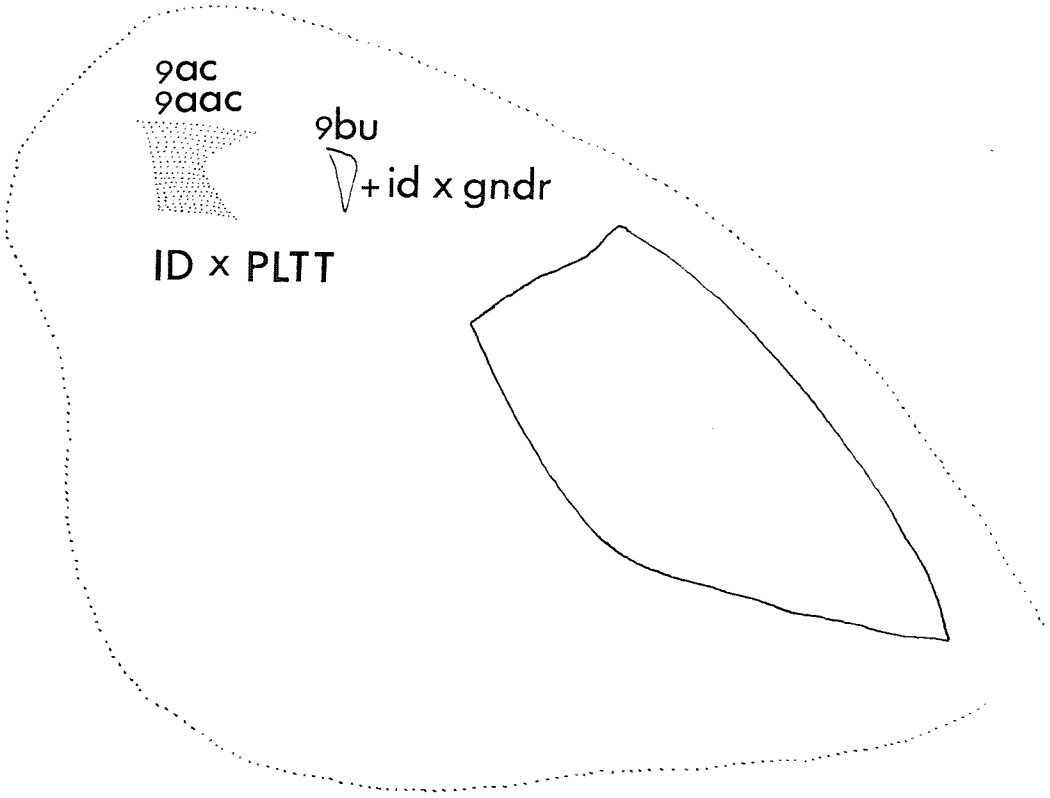
9.27. Interaction between family, sex and treatment

Linear and TYPE-1 colour character differences dependent on interaction between family, sex and treatment are listed in Table 8.IX and shown in Figure 9.27.

Characters showing interaction between family and treatment were limited to the forewing. That PAT9AC and PAT9AAC were darker under foil treatment only in family 004 was discussed in section 9.25.i. Neither the size of forewing s0 (SPC17) nor ventral forewing s5 basal U:U (PAT9BU) differed between the families or either of the treatment comparisons *per se*. However, the size of forewing s0 did differ between untreated and foil samples in family 002 (mean + SD: untreated = 10.3mm ± 0.10mm, foil-treated = 10.8mm ± 0.19mm; $F_{(1,7)} = 24.032$, $0.001<P<0.01$), implicating a hereditary component in the influence of photoperiod; while the size of forewing s5 basal U:U between the families only among cold-shocked animals (mean ± SD: 002 = 0.19mm ± 0.066mm, 004 = 0.29mm ± 0.075mm; $F_{(1,21)} = 11.431$, $0.001<P<0.01$), implicating a hereditary component whose difference between the families requires cold to render it manifest.

	Character	Sex x Family	Family x Treatment
Forewing	SPC17		$F_{(2,35)} = 5.654$**
	PAT9AC		$F_{(2,42)} = 6.524$**
	PAT9AAC		$F_{(2,41)} = 8.004$**
	PAT9BU	$F_{(1,40)} = 11.297$**	$F_{(2,39)} = 5.934$**
Hindwing	PAT37C	$F_{(1,42)} = 13.236$**	
	PAT39A	$F_{(1,40)} = 7.819$**	

TABLE 9.IX. Interaction between family, sex and pupal treatment. Significant ($P<0.01$) interactions are reported. F-values (ANOVA) are shown with their degrees of freedom in parentheses. Significance levels: ** $0.001<P<0.01$; *** $P<0.001$. For further explanation see text.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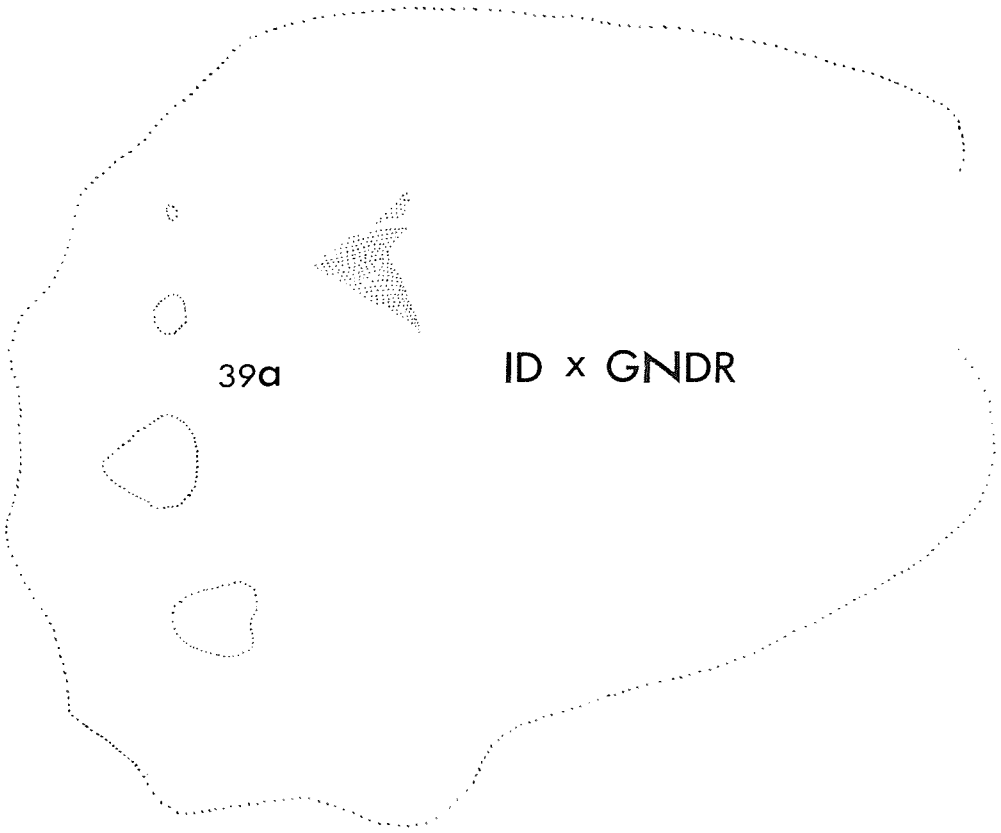

39a
ID x GNDR

Asymmetry in the total number of dorsal hindwing cOC + OC + E^3:U elements (PAT39A) and the size of ventral forewing s5 basal U:U (PAT9BU) were greater in family 004 though they did not differ between the sexes *per se*. *Within* family 004, however, PAT39A was greater in females (mean left-right difference ± SD: males = 0.0 ± 0.00, females = 0.4 ± 0.52, $F_{(1,18)} = 6.000$, $0.01<P<0.05$), suggesting that the sex-chromosomes interact with the rest of the genome (and developmental system) to canalise eyespot establishment. Since the females (XY) were less canalised than the males (XX), such an interaction would more likely involve the X-chromosomes; indeed this canalisation might depend on the quantity of some (as yet undetermined) factor. The family difference in PAT9BU proved to be confined to males (mean ± SD: 002 = 0.19mm ± 0.046mm, 004 = 0.27mm ± 0.071mm, $F_{(1,20)} = 10.450$, $0.01<P<0.05$), suggesting either that the Y-chromosome (in females) or diploidy of the X-chromosome (in males) interacted with rest of the genome to bring some family difference in one or more of these factors to manifestation.

The TYPE-2 colour of hindwing s0/4 U:M^1 interspace (PAT37C) was more prevalent in males although this did not, overall, give them a darker TYPE-1 colour, nor did either its TYPE-1 or TYPE-2 colour differ between the families *per se*.

FIGURE 9.27. Linear and TYPE-1 colour characters showing significant interaction between family, sex and pupal treatment.

Legend: ——— linear dimension
------ meristic
▒▒▒▒ TYPE-1 colour

Interacting variables: ID/id = family; GNDR/gndr = sex; PLTT = pupal treatment. Dotted wing outlines are shown only to aid orientation. For further explanation see text.

Within family 004, however, males did prove to have a darker TYPE-1 colour value than females (mean ± SD: males = 27 ± 11.0, females = 12 ± 0.0, $F_{(1,18)} = 19.800$, $P<0.001$), which proved to comprise pale orange yellow in females, and orange yellow but with light grey brown irroration in males.

The families now also differed when each sex was considered separately. Among males, TYPE-1 colour was darker in family 004 than in family 002 (mean ± SD: 002 = 6 ± 2.2, 004 = 27 + 11.0, $F_{(1,19)} = 40.272$, $P<0.0001$), which proved to comprise pale yellow grey in family 002 and golden yellow in family 004. Among females, TYPE-1 colour was again darker in family 004 than in family 002, but the difference not nearly as great as among males (mean ± SD: 002 = 6 + 3.3, 004 = 12 + 0.00, $F_{(1,20)} = 36.795$, $P<0.0001$), which again proved to comprise pale yellow grey in family 002 but now *pale orange yellow* in family 004. These results indicate that in males, the TYPE-1 colour of hindwing s0/4 U:M^1 interspace has more orange component as well as the light grey brown TYPE-2 irroration that is absent from females. They also indicate that the degree to which males have a greater orange TYPE-1 component differed between the families. Since males are diploid for the X-chromosome whereas the females are but haploid, it is possible that the production of orange pigment increases with the ploidy of the locus on the X-chromosome whose activity is regulated by the remainder of the genome. Indeed, it is worth recalling that in subspecies aegeria, where the interspaces are generally much more orange than in subspecies tircis, the haploid chromosome number is 28 rather than 27, commensurate with the above hypothesis.

9.28. Further wing phenotypic effects of cold shock

(i) *STOCK 01 F_1*

Cold shocked male no. 021 of family 004 (<12h,96h onset/duration) had orange scales within the dorsal forewing s5 OC element (Plate 10, frame 34A-35). It also lacked eyespot pupils (cOC) in hindwing s2 and s3, while the E^3:U in hindwing s4 and s5 were poorly defined with their putative OC elements apparent only as an irroration of dark brown scales (Plate 10, frame 36A). The ventral hindwing completely lacked cOC and OC elements (Plate 10, frame 1A-2). Specimens of P. aegeria with diminished hindwing eyespots (ab. parviocellata Lempke) or missing hindwing eyespot pupils (ab. postcaeca Lempke) have been recorded in nature (Russwurm, 1978). It is thus conceivable that the latter might constitute naturally occuring cold shock phenotypes.

Cold shocked female no. 016 of family 004 (<12h,48h onset/duration) had the dark brown ground colour of both dorsal forewings mottled dark grey brown (Plate 10, frames 24A-26). This phenotype was also exhibited by a wild male taken at West Wood, Hursley, Hampshire, on 9.vii.1987. The flight period of both specimens corresponded to generation 2.i (the female eclosed 1.vii.1985). In the male, the mottling was very distinct over both forewing surfaces and also present on the dorsal hindwing; there was also dark irroration of the dorsal forewing s0 M^1:D^2 interspace (PAT18C_2). The specimens are shown in Plate 11. This suggests that such cold shock phenocopies indeed occur in nature. Its greater expressivity in the male might reflect a poorer canalisation in this sex. Both cold shocked female no. 01004016 and the Hursley male (no. 08001003) were missing pupils in the dorsal hinwing s0/4 eyespots; and 01004016 had a putative eyespot within the dorsal hindwing s5 E^3:U interspace, which, though not comprising a *distinct* OC element in 08001003, did, nevertheless exhibit dark brown irroration. This suggests that the capacity to produce the phenotype under [cold shock] might be dependent on a particular genotype. Of course the phenotype might represent a *high* temperature phenocopy (*cf.* section 9.26.iii) or even a temperature-independent genetic mutant.

The latter, if homozygous, could mean the frequency of heterozygotes being considerable (*cf.* section 9.26.ii); and if temperature-dependent, then estimated frequencies of such genes very likely be under-estimates. The effects of environmental influences on development can thus indeed have important implications for population genetics (*cf.* Lewontin, 1968). The general similarity between the range of laboratory cold shock phenotypes (see Plates 10 and 11 for examples) and naturally occuring aberrations of P. acgeria (see Russwurm, 1978, for examples), suggests that both are delimited by a common developmental repertory.

In cold shocked male no. 019 of family 002 (12-24h,48h onset/duration), the forewings were rather rounded while the relative size of the hindwings was noticeably small. It thus appears that cold shock can influence wing shape, and the means in which it may do so is presented below.

(ii) *Further stocks*

A purchased stock of P. a. tircis from Doncaster, U.K. was established as 47 larvae on 14.vi.1988 and reared indoors. 41 pupated of which 25 were cold shocked. The only mortality was one control (foil treated) which was deformed at pupation; survival under cold shock was 100%. One of the cold shock males eclosed with the left hindwing apparently unexpanded which on inspection proved to be a stump. This further suggests that hindwings are less canalised than forewings. The stump resembled a haltere, and it is postulated that the hindwings are ultimately derived from a bithorax-type phenocopy process (*cf.* Sibatani, 1980). Specimens showing wing deformities fall into the general category of teratological aberrations, which too occur as natural aberrants in several species (Russwurm, 1978). However, on account of their ungainly appearance, they tend not to have been figured in popular texts (*ibid.*).

9.29. Further aspects of morphological development

(i) *Wing scale morphology*

Microscopic examination of the wing revealed three morphologically distinct classes of scale:

Field type. These are simple in gross structure and are deployed in the typical 'overlapping slate' arrangement;

Fringe type. These appear as long tassel-like projections of very variable length and range widely in colour. They are present only at the fringes and always appeared to emanate from dark brown or grey-brown field type scales. They probably represent a specialisation of the end of the scale;

Hair type. These appear as hair-like projections, usually in excess of 10x the length of field scales, and vary in colour from pale yellow through tan to light grey. They occured anywhere on thc wing surface except at the fringe and were prevalent at the base of the dorsal forewing.

In males, the basal portions of the dorsal forewing s2 and s3 M^1:M^2 interspaces were often darker and more grey than the distal portions (Plate 1). These interspaces correspond to the position of the 'sex brand' and so comprise the specialised scent scales or androconia.

Foil treated female no. 007 of family 002 (<12h,48h onset/duration) had a distinctive green mark near the s2 eyespot (OC) element on each ventral hindwing (Plate 10, frame 8A-9). Green pigment does not occur in any British Rhopalocera (Ford, 1957), although freshly emerged Satyrids often have a gold or green sheen (of structural origin) on the dorsal wing surface. Microscopic examination revealed the green ventral mark also to be structural (its precise shade varied with the angle of incident light), and it most likely represents some defect of scale gross morphology or ultra-structure.

(ii) *Wing ocellus morphology*

The forewing s5 ocellus ring (OC) did not always respect the wing space boundary as delineated by v5 and v6.

Ocellar subdivision, where the eyespot ring is divided into distinct peripheric and sub-peripheric zones (Schwanwitsch, 1948), was detected in ventral hindwing s2, s3 and s6. The phenomenon was originally described for Pararge megera with Pararge the only one of nine Satyrid genera to exhibit the phenomenon (*ibid.*), but the subsequent re-assignment of the latter species to the genus Lasiommata would place the phenomenon as occurent in two, albeit closely related, Satyrid genera.

(iii) *Pattern intercalation*

During a food change, the integument of one second-instar larva was accidentally pinched between the box and its lid. The larva showed no immediate sign of injury, but was later found to have developed two lateral projections to the dark longitudinal dorsal stripe. These projections were retained throughout the subsequent instars (Plate 5b).

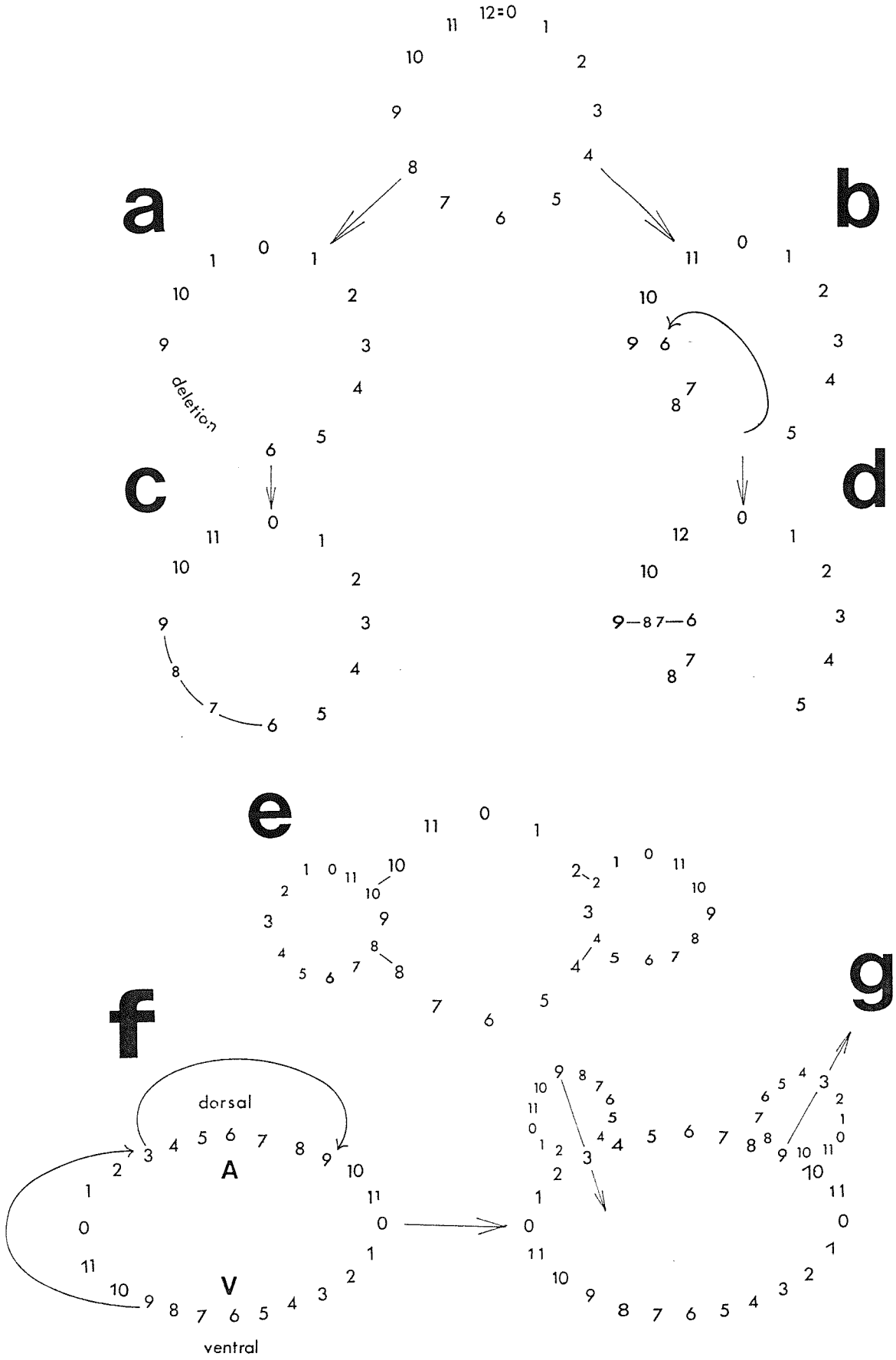
French *et al.* (1976) put forward a model based on serial circumferential values to account for the formation of supernumerary limbs following rotational limb regrafting in certain insects and in the axolotl. This clockface model is shown in Figure 9.28. If non-adjacent values are brought into juxtaposition by deleting part of the field (Fig. 9.28a) or by displacement (Fig. 9.28b), then the tissue regenerates so as to fill in the shortest possible series of intervening values (Fig. 9.28c-d), a principle is known as the shortest intercalation rule (*ibid.*). If the field is rotated through 180°, then where the adjacent values are in maximum conflict the entire circumferential series is regenerated. However, there are two such possible series, one clockwise, the other anti-clockwise, the direction of rotation always so as to prevent the new neighbouring values coming into conflict (Fig. 9.28e).

It is possible that the larval longitudinal stripes (Plate 4a-b) are established by circumferential values. It is postulated that in

the larva with the lateral projections, the integument was torn, but that the edges of the tear had become displaced before healing. This would have brought non-adjacent values into conflict, and it is suggested that lateral projections developed at two positions where the conflict was greatest. It is possible that the value series is repeated, the first corresponding to the dorsal surface, and contiguous with that corresponding to the ventral surface, although the two series do not *per se* recognise the dorsal-ventral distinctions (which more likely respect some animal-vegetal polarity, Fig. 9.28f). Thus were the displacement to have forced, say, the 9-ventral position onto the 3-dorsal position, and the 3-dorsal position onto the 9-dorsal position, then intercalation would have generated two whole series and hence the lateral extensions (Fig. 9.28g). From Fig. 9.28g it can be seen that the two new fields are similar in handedness, but in order to minimise conflict with neighbouring cells they will necessarily grow in opposite directions, so that one extension will progress anteriorly, the other posteriorly.

It is possible that the extra cells formed during intercalation could dislocate the surrounding tissues, so distorting the segment or introducing an asymmetry. It is possible that such twisting could be responsible for the 'spiral segmentation' occasionally found in nature. Aindow (1988) reports the case of Death's-Head Hawkmoth larva, Acherontia atropos L. (Sphingiidae) in which the blue and yellow stripe on the 9th segment from the head was swollen and tapered into the 10th segment instead of going into its normal 'V'. The pupa had a twist on the dorsal surface of the 5th abdominal segment, this segment remaining swollen in the emerging female where it also lacked the blue central stripe (*ibid.*). The integument may have been stretched so that the site of the usual band came to be occupied by non-stripe values. The above cases show that the hypothetical intercalation can come about through both natural and artificial (injury) causes, their effects delimited by a common developmental response. P. aegeria and A. atropos show the effect to continue through the larval and subsequent metamorphic stages respectively. Such continuity has also been reported for an abdominal asymmetry in a hybrid between two North American Phyciodes butterflies (Nymphalidae) (Oliver, 1979).

It is possible that wing pattern development might involve sequential values, since the pattern elements too are essentially


a
b
c
d
e
f
g
deletion
dorsal
A
V
ventral

longitudinal (anterio-posterior) stripes (Fig. 9.2). As in the larva, such a sequence might be repeated on each dorsal and ventral surface, since Lepidopteran wing discs comprise two cell layers (Nijhout, 1985c). Indeed, the wings are outgrowths of the dorsal body integument or tergum (Ford, 1957a), and it has been suggested that the wing root band (Basalis, Fig. 9.2) comprises a fourth symmetry system that may organise the body pattern (Nijhout, 1978). In this regard, the determination of body spiracles may even involve foci similar to those responsible for the wing border ocelli (*ibid.*). Furthermore, regulatory intercalation following local cell death has been implicated in homoeotic transformations between dorsal and ventral wing surfaces (Sibatani, 1980).

FIGURE 9.28 (opposite). The clockface model and pattern intercalation. The field is conceived as a sequence of circumferential values. If non-adjacent values are brought into juxtaposition then the shortest series of intervening values grows back or intercalates: (a) deletion of values 7 and 8 brings 6 and 9 into conflict, and (c) the shortest intervening series *ie.* 7-8 (not 5-10) is regenerated; (b) values 6 and 9 are brought into conflict through displacement, and (d) the values 7-8 are generated to eliminate the conflict, resulting in repetition of the series 6-7-8-9; (e) rotation of the field through 180° to bring values (here 3 and 9) into maximum conflict. The intervening values are regenerated so as to avoid conflict with their neighbouring values and a new complete circumferential sequence, *ie.* a supernumerary field, results. In the figure, the values 3 and 9 are juxtaposed at two positions (left and right) on the original field. The result is two supernumeraries whose handedness is similar to one another but different from that of the original field; (f) proposed aetiology of the larval lateral extensions (Plate 5b): the series 1-12 is repeated in tandem on the dorsal (D) and ventral (V) surface. The two series may reflect an early animal/vegetal (A/V) polarity. Juxtaposition of the 9-V and 3-D, and of the 3-D and 9-D, results in (g) two supernumaries of *similar handedness*, but which since they occur on *different sides*, undergo anterio-posterior growth in opposite directions. For further explanation see text.

9.30. Other species

Cold shocks similar to those used with P. aegeria were carried out in a number of other Lepidoptera. As these are discussed elsewhere (Winokur, in prep.), only the salient features will be presented here.

(i) Dendrolimus pini (L.) (Heterocera: Lasiocampidae)

A purchased stock of the Pine-tree Lappet moth, Dendrolimus pini, originating from a female taken at Avenida del Pina, Mallorca on 13.ix.1986 (Robert Woods, pers. comm.), was established as ova in Southampton on 27.ix.1987. Since the species is univoltine (Novák, 1980) these eggs would have represented the F_2 of the original female and hence been of inbreeding coefficient = 0.250 (*cf.* Falconer, 1981). The stock was reared indoors at 18.9°C ± 4.70° on Scots Pine, Pinus sylvestris. Fourteen adults eclosed and the sex ratio was precisely 1:1. Their pale colour and large size identified the stock as belonging to subspecies grisescens (*cf.*Novák, 1980). The specimens were sacrificed by freezing and the spans of each of the four wings measured while on the setting boards using a perspex rule. Wingspan was taken as the maximum distance from the base of the dorsum to the termen. The measurements were repeated and tested for reliability using Eta-squared, and all proved reliable. Pupal cold shocks were not applied but their normal wing development was investigated for comparative purposes.

Directional asymmetry and antisymmetry were estimated but, as in P. aegeria (sections 9.21.i and ii), found not to be significant. Forewing and hindwing spans were highly concordant (Kendall's W = 1.000, $Chi^2_{[1]} = 7.000$, $0.001<P<0.01$), but there was *no* left-right concordance between the forewings or hindwings (although in males it was just significant between the forewings, Kendall's W = 1.000, $Chi^2_{[1]} = 3.000$, $0.01<P<0.05$). These trends too are as in P. aegeria (section 9.12.i). The hindwing spans were also more variable than the forewing spans. Again it is possible that the hindwings are evolutionarily more recent, their development thus having had less time to become canalised, so implicating them as being the more primitive in *condition*.

Two specimens, one male and one female, eclosed with deformities at the termen. These involved up to three indentations on any wing that appeared to corresponded to wing vein positions. Other than that these deformities were also absent from the costa and dorsum, they showed no locational specificities. It is postulated that wing membrane formation is dependent on the veins (including the border lacuna), and that the transverse veins are deployed from the border lacuna. Thus, any indentation of the latter will result in the transverse veins originating at positions indented from the termen, whose contour the developing membrane will respect. The border lacuna then degenrates, leaving indented wing spaces at whose distal border the remaining veins terminate (Fig. 9.30a-c). Indeed, indented wing margins are a normal occurence in certain species, notably the British Comma butterfly, Polygonia c-album (Nymphalidae).

(ii) Lysandra coridon coridon Poda. (Rhopalocera: Lycaenidae)

A purchased stock of the Chalk Hill Blue butterfly, Lysandra coridon (Lycaenidae: Polyommatini), originating from an unidentified site in the Chiltern Hills, Buckinghamshire, U.K. (Martin White, pers.comm.), was established as 21 larvae in Southampton on 14.vi.1988. The larvae were reared indoors on cut Horse-shoe Vetch, Hippocrepis comosa L., but crushed thawed peas proved to be a convenient substitute when the vetch became depleted. 19 larvae pupated, although one was deformed and subsequently failed to eclose. Of the other pupae 14 were cold shocked and 4 were foil treated as described for P. aegeria. All of these eclosed, the relative survival in both classes thus being 100%. The controls, 3 males and 1 female, were all of normal phenotype. The cold shock sample, 7 males and 7 females, yielded 1 male and 2 females which failed to fully expand the wings, and a female with normal wing expansion but white borders to the dorsal discoidal spot on each forewing.

One of the two females exhibiting wing deformity suffered complete failure of wing expansion and the area described by the putative s0 on each hindwing was totally devoid of membrane, the latter appearing as a hole in the wing. This again implicates the wing veins in membrane formation, and that this effect was limited to s0 further points to

this space as distinct (*cf.* sections 9.11.i and ii). The secondary wing tracheae which supply air to the wings develop in the early pupa, when the primary tracheae remain as debris in the wing veins (Nijhout, 1985c). It is possible that cold shock disrupts their development so impeding subsequent wing expansion.

That in one female the colour of the dorsal forewing discoidal spot was affected by cold shock again implicates the discoidal region of s0 in early wing pattern development. In the Northern Argus, Aricia artaxerxes (Polyommatini), the wild type phenotype expresses a fully white spot. In the related Brown Argus, A. agestis agestis Denis & Schiffermüller, however, the wild type discoidal spot is black. Now within their British ranges, A. artaxerxes experiences mean January and July temperatures (0°C and 10-11°C respectively) cooler than those experienced by A. agestis (2°C and 14°C, Dennis, 1977). It is therefore conceivable that the A. artaxerxes phenotype originated as an effect of a cooler climate that subsequently became assimilated; it could even be just a pleiotropic effect of some adaptive change to the life cycle. Indeed, A. artaxerxes occasionally sports a phenotype with white rings around the dorsal forewing discoidal spots as a naturally occuring form, f. albiannulata (Dennis, 1977). This form is known *not* to be a heterozygote of known artaxerxes and black-spot phenotypes (*ibid.*). It is thus conceivable that it could represent a natural [cold shock] phenocopy (*cf.* Shapiro, 1975b). Its occurence in even 1 in 10,000 adults suggests that if a genetic mutant, the frequency of heterozygotes could be as high as 2%, even higher if sub-lethal in early development (Dennis, 1977). Of course, production of the form by the gene could depend on it interacting with a particular micro-climate.

(iii) Aglais urticae L. (Rhopalocera: Nymphalidae)

119 larvae of the Small Tortoiseshell butterfly, Aglais urticae, were collected from Twyford Down, south Hampshire, U.K. on 8.viii.1988, and reared on cut Stinging Nettle, Urtica dioica. 40 larvae were reared indoors at 15°C - 25°C and 40 in an airing cupboard with access to daylight at ≥30°C (the remaining 39 larvae were reared under darkness in a refrigerator at 0°C - 10°C but died before

pupating, though the presence of droppings showed that this was not due to feeding failure). 32 larvae from each sample pupated, of which 23 pupae in each sample were cold shocked and the remaining pupae foil treated.

Of the cold shocked pupae reared at ≥30°C, one male eclosed with somewhat 'stumpy' wings. This was not failure of wing expansion as the wings were flat (incomplete expansion results in the wing being buckled or crumpled, pers. obs.). Moreover, both antennae were also short, giving them a more clubbed appearance. However, when the specimen was compared with so-unaffected individuals, it was found that the tips of the antennae corresponded in position to the same pattern components in both cases. At ≥30°C the 'stumpy' specimen might have experienced a more severe cold shock effect than had it been reared within the lower temperature range (section 8.11.ii). Antennae and wings both derive from imaginal discs (Ford, 1957), and it is possible that cold shock affects some global parameter of disc development. Indeed in Heteroptera, cold is known to induce a short winged (brachypterous) condition in the laboratory, when changes in wing size are associated with corresponding changes in the size of the visual ocelli (Southwood, 1961).

The brachypterous A. urticae underwent normal larval-pupal metamorphosis, implicating metathely where there is excess juvenile hormone (JH) in the adult (*ibid.*). Since it is the *relative* ecdysone/JH concentration which is important in effecting metamorphosis (Nijhout & Wheeler, 1982), it is postulated that the increase in relative JH titre attendant with reduced ecdysone production during cold shock, retarded the differentiation of the imaginal discs.

A further sample of larvae from the same locality and reared without pupal cold shock under daylight at ≥40°C, yielded four adults of which three exhibited grey mottling of the normally uniform black markings over the entire dorsal surface.

(iv) Vanessa atalanta L. (Rhopalocera: Nymphalidae)

Two larvae of the Red Admiral butterfly, Vanessa atalanta were collected from Twyford Down as above and reared under darkness in a refrigerator at 0°C - 10°C. One died before pupating (though the presence of droppings showed that this was not due to feeding failure), while the other continued its development and eventually formed a prepupa, although it did not, however, pupate. The insect was inspected four weeks later, it had apparently died, but closer examination revelaed it to have developed adult structures and split. This suggests that it may have exhibited protothely where there is excess ecdysone (or perhaps more accurately an insufficiency of JH) in the larva, when the pupal moult *per se* is *not* essential for adult development, although the experiment will need to be repeated before any definite conclusions can be drawn.

(v) Diachrysia sannio L. (Heterocera: Arctiidae)

75 ova of the Clouded Buff moth, Diachrysia sannio, collected from southern West Germany during June 1988, were established in Southampton in late June 1988 and maintained as for A. urticae above. All ova hatched and the larvae were reared on cut plantain (Plantago spp.). On 31.vii.88 the 45 surviving larvae were partitioned into three batches: 10 in a refrigerator at 0° - 10°C; 27 continued indoors at between 15°C and 25°C ('room-reared'); and 10 in an airing cupboard with access to daylight at ≥30°C ('heat-reared').

Of the larvae reared indoors, 9 pupated of which 4 were cold shocked. 4 of the heat-reared larvae larvae pupated though no cold shocks were applied. The larvae in the fridge grew very slowly and died before pupating.

Of the room-reared sample, 2 cold shocked males and 4 control females eclosed. Of the heat-reared sample, 2 males and 2 females eclosed. In one of the heat-reared males (no. 4), the left hindwing was completely undeveloped while the right hindwing was represented by only a small stump, which on closer scrutiny proved to be divided into an anterior and a posterior part, the anterior one the larger. It

would appear that heat can disrupt wing development, and that wing development, on the hindwings at least, involves anterior and posterior compartments. The other heat-reared male (no. 2) was normal in shape and pattern, though the width of the dark outer hindwing band was much narrower than in either of the room-reared cold shocked males (nos. 8 and 10). The heat-reared females (nos. 1 and 6) and room-reared control females (nos. 1,2,3 and 11) were much more similar to eachother. Again it appears that males are less canalised against heat or cold shock effects, and that the hindwings are generally less canalised than the forewings.

(vi) Tyria jacobaeae L. (Heterocera: Arctiidae)

Cold shocks were also applied to pupae of the Cinnabar moth, Tyria jacobaeae, collected as 50 larvae from Southampton during July 1988. The larvae were reared indoors on Ragwort, and yielded 37 pupae of which 17 were cold shocked as for P. aegeria. The species normally undergoes pupal winter diapause, so all pupae were kept under darkness in a refrigerator at 0°C - 5°C for eight months. They were returned to the indoor room environment in late March 1989. The investigation is still in progress and it is expected to report further details on completion. At present, however, it will suffice to say that of the cold shocked adults so far to have eclosed, one female had the red hingwings tinged distinctly paler towards the anterior spaces on both surfaces, one male had an indented termen on right forewing s1 and s2, while in another male both forewings were creased near to their anterior and posterior edges.


KODAK PKR 5033
1A
2
34A
35
36A
6A
7
8A
9
24A
25
25A
26
18A
19
2 3 6
21A
22
14A
15
10A
11
15A
16
17A
18
16A
17

PLATE 9 (opposite). Wing phenotypic effects of pupal cold shock.

Frame no.	specimen	Surface	Feature
14A-15	01012018[1]	L.Fw Ventral	nigrism of sector 3
10A-11	01012018[1]	R.Fw Dorsal	suffused interspaces
15A-16	01012018[1]	R.Hw Dorsal	reduced/absent cOC
17A-18	01012018[1]	R.Hw Ventral	prominent s0/4 spotting
16A-17	01012018[1]	R.Hw Ventral	uniform ground colour
18A-19	01012019	L.Fw Dorsal	missing s5 cOC
21A-22	01012019	L.Fw Dorsal	missing s5 cOC: detail
24A-25	01004016	L.Fw Dorsal	mottled ground colour
25A-26	01004016	L.Fw Dorsal	mottled ground colour
1A-2	01004021	R.Hw Ventral	missing cOC and OC elements
34A-35	01004021	L.Fw Dorsal	yellow scales in s5 OC
36A	01004021	L.Hw Dorsal	missing cOC, reduced E^3:U
6A-7	01002008	L. & R.Hw Dorsal	asymmetry: s2 OC-E^3 fusion
8A-9	01002007[2]	R.Hw Ventral	s2 green scaling

[1]control reared at 14.8°C
[2]control reared at 19.3°C

Frame numbers correspond to those along the film perforations. Specimens were cold shocked unless otherwise indicated. For further explanation see text.

9.31. The Nymphalid Groundplan

The Nymphalid groundplan is still the only generally useful and accurate way to identify homologous pattern elements in most Lepidopteran families (Nijhout, 1986). The Lepidoptera are monophyletic, all the 100,000 or so species fitting within the Schwanwitsch-Süffert scheme (*ibid.*), and since about 50% of species have an almost perfect correspondence to it, such grounplans provide a good starting point for the study of pattern development and evolution (Nijhout & Wray, 1986). Indeed Schwanwitsch (1929) suggested that they be put to such use, yet they have been little applied since their inception (Nijhout & Wray, 1986). That Lepidopteran wings are two dimensional and develop without cell movement makes them ideal subjects for the study of pattern formation (Nijhout, 1986), and since recent insights into wing pattern have been gained largely through reference to these groundplans (Nijhout, 1978, 1981) and with only minimal revision to them (Nijhout, 1986), it is suggested that they be retained as bases for descriptive reference and comparative study.

9.32. Wing shape and venation

(i) *Normal development*

The discal spot (D^1) is almost ubiquitous in Lepidoptera and indeed is the absolute reference for identifying pattern elements by their relative position (Nijhout & Wray, 1986). Although, since it lies on the discoidal veins, it had been viewed as dependent on venation (*ibid.*), it is hereby suggested that it may be the origin from which the venation develops. We have already seen that the fore- and hindwing spaces appear to radiate from s0 and s0/4 respectively.

Wing discs start to develop around the time of larval hatching, and throughout larval growth the walls of the disc, the future wing, and epidermis remain one cell thick. The venation and shape of the adult wing are established in the final instar, when the disc grows

and folds to form a two-layered 'wing anlage'. Late in the instar, it enlarges and flattens to form a crescent-shaped lamina, when the basement membranes of the two cell layers fuse permanently *except where the veins will develop* (Nijhout, 1985c). There are two possible ways in which these arise. The first is that the membranes separate as they fill with haemolymph, to form lacunae (essentially hollow spaces), along which the primary tracheal branches then progress from the base of the anlage. The second is that the primary tracheae penetrate the wing membranes, the haemolymph then penetrating to form the lacunae as it does so. The precise mechanism of *pathfinding*, however, is not known (*ibid.*).

In Drosophila, mutant genes can shift the venation only before the wing fold is everted from the imaginal bud (Waddington, 1950), suggesting that venation is *determined* prior formation of the wing anlage. This determination may involve some wave function with nodes corresponding to the wing compartments, and whose harmonics demarcate the putative wing spaces. In Lepidoptera, basal (proximal) wing structures corespond to the periphery of the disc, the distal wing tip to the centre (Sibatani, 1980), so that the establishment of wing shape likely involves a folding of the disc along its anterio-posterior (A-P) axis followed by distortion (Figure 9.29a-c).

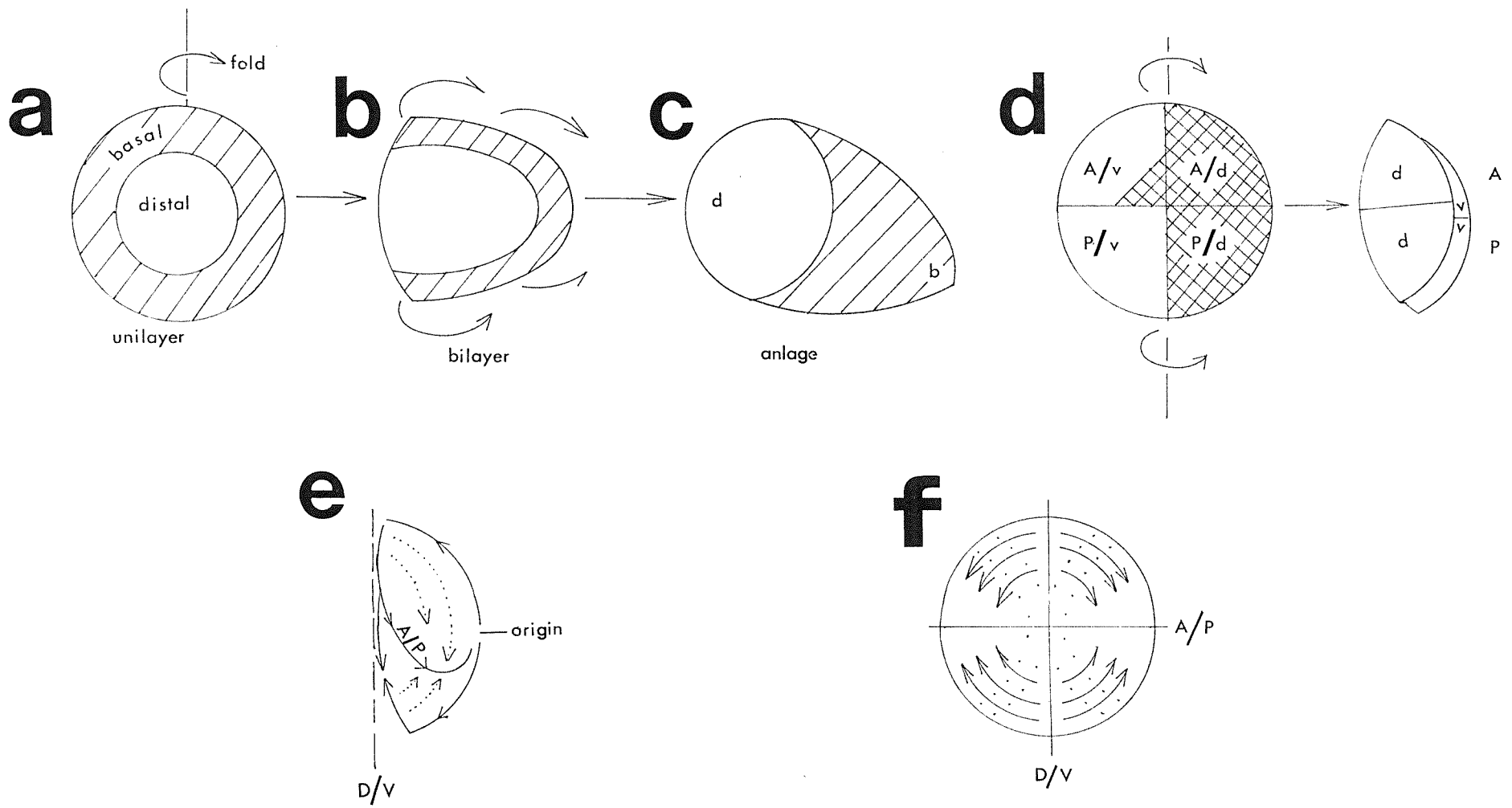
Specialised Lepidoptera have counterparts of the anterior and posterior intra-disc compartments of Drosophila (Sibatani, 1980), and Sibatani (*loc. cit.*) found from the distribution of homoeotic transformations that their boundary lies within forewing s5 and hindwing s0/4. Indeed, in the European Satyrids Hyponenphele lycaon Kuehn and Coenonympha pamphilus L. the boundaries correspond to forewing v5 and hindwing v4 (*ibid.*).

It is postulated that the establishment of the lacunae originates at the wing base and progresses towards the discal spot which acts as a sink. Firstly, there is a progression distally along the anterior edge of the posterior compartment (putative median vein) and anterior edge of the posterior compartment (putative radian vein) towards the discal spot region. There is also a concurrent progression distally around the costa and the dorsum (Figure 9.30d). Hence these progressions essentially follow the compartment boundaries, and it is suggested that they weaken in potential as they do so. The basal thickening, for example, might reflect the high potential near

the wing base. The posterior compartment may be the larger of the two, so that the anterior progression is the first to meet the A-P intersect at the termen (Fig. 9.30e). The anterior proression thus forks here, one branch continuing around the termen till it meets the posterior progression, the other returning along the posterior border of the anterior compartment to the discal spot region. The hypothetical wing sectors 2 and 3 thus correspond to the anterior and posterior wing compartments respectively. The transverse veins (not discoidals, below) might then describe parallel A-P intra-disc subdivisions (Fig. 9.30f).

The compartment (and wing space) boundaries may offer a path of least resistance to the flow of haemolymph between the membranes, which the developing tracheaa then explore; or else describe some equipotential in a (surface) field condition (*cf.* Goodwin, 1984, *re* holoblastic cleavage) that guides the primary tracheae, in so doing establishing a channel for the flow of haemolymph. In support of the determinant losing potential as it progresses is that if, as in post-eclosion wing expansion (*cf.* Ford, 1957), wing shape is established by the distad force of haemolymph through the lacunae, the less developed and so narrower veins will offer a greater resistance to such a force when a progression such as that shown in Fig. 9.30e would be commensurate with wing shape. In this way, the wing would appear to suffer the hypothetical 'compression wave' around the termen of forewing sector 3. The A/P compartment boundary anterior to vIV in Drosophila, has apprently been shifted forwards in Lepidoptera (Sibatani, 1980). In Lepidoptera, the posterior forewing (sector 3) is much deeper than in Drosophila, and it is possible that new nodes would be introduced here, when if these coincided in position with previous nodal positions they could result in an apparent overlap of vein positions. Indeed, in some Lepidoptera, medial v5 and v6 may migrate and coalesce with the radials, while the way in which the median tracheae are partitioned into anterior and posterior parts of the wing differs among various groups (*ibid.*).

The 1A lacunae and media bases then constrict to the diameter of the primary tracheae they enclose, after which the bordering lacuna disappears (Nijhout, 1985c). After pupation, new secondary tracheae branch from the main tracheal loop at the base of the wing and penetrate the lacunae. It is *these* tracheae that provide air to the


a
fold
basal
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A/v
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P/v
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origin
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A/P
D/V

adult wing during post-eclosion wing expansion. Thus the poorer developed the lacunae, the less air can penetrate to expand the wing, this perhaps further contributing to adult wing shape. Moreover the primary tracheae are torn and fragmented as the wing expands, when they remain as debris in the adult wing veins. Thus the narrower the original lacunae, the more likely will the primary tracheal remains obstruct the secondary tracheae at eclosion. The final venation corresponds to the secondary tracheae and lacunae.

FIGURE 9.29 (opposite). Compartmentalisation of the wing. Each disc starts out as a cell unilayer (a) whose centre corresponds to the prospective distal tip and its periphery to the prospective wing base (line-shaded). The disc then grows and folds to form a bilayer (b), possibly undergoing a concomitant distortion (c), to form the wing anlage, the geometry of whose basal (b) and distal (d) extremities is similar to that in the adult wing. The initial unilayer may be partitioned into four compartments (e), the anterior and posterior ventral (A/v and P/v) and the anterior and posterior dorsal (A/d and P/d) compartments corresponding to the ventral and dorsal wing surfaces subsequent to folding. Cross-hatching shows the region of unilayer corresponding to areas of similar pattern in the adult forewing showing Oudemans' phenomenon. It is possible that the determination of venation and pattern originates from two poles perpendicular to the A-P axis, poles which correspond to the dorsal and ventral adult wing base at the junction of the anterior and posterior compartments; only the prospective dorsal surface is shown. The dotted lines show the subsequent course of pathfinding by the transverse veins. Note (f) that after the first division along the basal-basal axis and the second division along the A-P axis, the third axis of subdivision (described by the transverse veins) is similar to the first, and that only *then* is there subdivision (described by the pattern elements) that effectively intersect both the basal-basal and anterio-posterior axes. The progressive perpendicular subdivisions and their preferences when a choice exists are thus reminescent of holoblastic cleavage. For further explanation see text.

(ii) *Cold shock, venation and wing shape*

Cold shock had various effects on the shape and form of the wings, although their individual natures suggest well-defined causes in each case, and so they will therefore be dealt with individually.

The absence of membrane in the putative s0 of both hindwings in one of the cold shocked L. coridon females (section 9.30.ii) suggests that the veins effect a role in the development and/or stabilisation of the wing membrane (how cold shock might have exerted this effect is described below). This is consistent with a number of other cold shock and natural phenomena. The partitioned right hindwing in cold shocked D. sannio male no.4 (section 9.30.v) may have resulted from the veins bordering s0/4 not having stabilised the membrane intervening the anterior and posterior compartments. It is possible that s0 may represent a space between the compartments, its membrane thus serving to bridge the two. Indeed in the many-plume moths (Alucitidae), the wing spaces are each bordered by veins but are discrete and not fused to one another, while in the plume moths (Pterophoridae), division of the forewing into anterior and posterior sections is the usual condition, although the two sections are fused from the wing base to a position corresponding to the discoidal veins (Nováak, 1980). Thus, the discoidal veins may serve to keep anterior and posterior compartments as a unit in the adult wing.

Although it had been asserted that the wing spaces probably do not correspond to compartments (Nijhout, 1978), they do appear to be the fundamental unit of colour pattern formation (Nijhout, 1984), the latter possibility having been closely examined in the Satyrids Cercyonis pegala and Smyrna blomfildia (Nijhout, 1985b). It is most likely that the wing spaces are established through progressive subdivision of the true anterior and posterior wing compartments, the pattern homology among wing spaces simply reflecting the similar role in patterning of the termen and of each of their bordering veins (below).

The means by which temperature shocks mediate changes to wing shape and surface topology have been documented for Drosophila (Waddington, 1950). As in Lepidoptera, the wing anlage comprises two closely apposed cell layers, and each hair arises from a single surface cell (in Lepidoptera each scale arises from a single

scale-forming cell, Nijhout, 1980b). In Lepidoptera, the scale-forming cells degenerate prior to eclosion (in Precis at 120h post-pupation, *ibid.*), while in Drosophila only the wing hairs evidently remain as surface structures on the adult wing. The effects of cold shock in Lepidoptera will therefore be considered by comparison with its known effects in Drosophila.

In the early Drosophila pupa, wing cell number firstly increases by mitoses in the proximo-distal direction (stage 1), which temperature shocks can inhibit so resulting in a broader wing. At this stage genetic mutants can affect the number of divisions and their main orientation. Now in Lepidoptera (Precis), the scale-forming cells are deployed in proximo-distal rows with the epidermal cells interspersed (Nijhout, 1980b). Up to 20h post-pupation and under the influence of ecdysone, these cells undergo mitosis when the scale cells also enlarge (*ibid.*). It is therefore suggested that in the Aglais urticae specimen with short wings (section 9.28.iii), the reduced ecdysone turnover during cold impeded these proximo-distal mitoses. The antennae were also abnormally short, and it is likely that the cold also impeded mitoses along a proximo-distal axis in the antenna discs. This suggests that there may be some global control, or some individual but common *type* of control, of growth in all imaginal discs, including a prior and coordinated induction of mitosis along some proximo-distal polarity. Since in Lepidoptera final antenna length is attained *before* eclosion, and so unrelated to any post-eclosal expansion, the above brachyptery is probably not the result of an effect on adult wing expansion.

In Drosophila, the cells then enlarge and haemolymph accumulates between the two basement membranes (*ie.* in the wing sac) to produce maximum inflation, after which the haemolymph is withdrawn and the epithelia (cell layers) contract (stage 2). Temperature shocks at this stage exaggerate this contraction to produce dumpy wings (Waddington, 1950). In Precis, the scale cells start to extrude a scale process at 26h post-pupation and which by 36h post-pupation can be identified as either the short ground- or longer cover-type scale.

Stage 3 in Drosophila involves mitosis and cell enlargement in the anterio-posterio direction, which can be inhibited by cold shocks so resulting in elongated wings with rounded tips (Waddington, 1950). Since in Precis the scale cells do *not* undergo further mitoses after

26h post-pupation (Nijhout, 1980b), any anterio-posterior mitoses analogous to stage 3 in Drosophila must involve the interspersed epidermal cells. Indeed it may be just such anterio-posterior mitoses that *enable* the epidermal cells to later become more deeply embedded into the membrane (in Precis at 50h-55h post-pupation), resulting in the proximo-distal pleating that allows post-eclosion wing expansion (Nijhout, 1980b).

The left hindwing stump in the cold shocked male Pararge aegeria (section 9.28.ii) and the left and right hindwing stumps in heat-reared D. sannio male no.4 (section 9.30.v) were smaller than the normal unexpanded post-eclosion wing size in these species (pers. obs.), implicating failure of cell mitosis and/or growth; although their venation and surface scale morphology (if any) have yet to be examined.

However, it is postulated that the progressive establishment of the non-bordering veins from the termen carries with it an associated proximo-distal signal, possibly a wave function, that induces the proximo-distal mitoses as it proceeds. In the Arctiidae (Tiger moths and Footmen, Skinner, 1984) there is a proximo-distal alternation of short and long scales (Nijhout, 1980b), which may correspond to points of corresponding signal strength (say minima and maxima). It is also postulated that an anterio-posterior wave is set up between the vein positions, perhaps even while their developing ends are still progressing, whose (say) maxima and minima determine the alternating rows of epidermal and scale-forming cells and induce their mitosis and growth. It is possible that the minimum signal strength required to induce cell growth and differentiation between the anterior and posterior wing compartments is greater than within the compartments.

It is postulated that the latter was diminished in right hindwing stump of heat-reared D. sannio male no.4 so that no A-P connecting membrane developed. That each portion, in particular the anterior, was elongated is consistent with such postulated A-P induction occuring *after* that along the proximo-distal axis. It may be the norm in Lepidoptera (and possibly other insect orders) for proximo-distal mitosis and growth to procede faster in the anterior than posterior compartment, accounting for most species having a typically longer costa than dorsum on both the fore- and hindwings.

In the cold shocked L. coridon female devoid of membrane in s0 in both hindwings, the wings *were* similar in size to the normal unexpanded post-eclosion wing size in this species (pers. obs.). It is suggested that since the s0 membrane requires a stronger threshold signal, it was the first region to suffer cold-mediated failure of anterio-posterior development. It is known that the determination of the size and colour of eyespot rings from their foci (below) is transmitted cell to cell, coincides with other cellular events, and is slower under cold (Nijhout, 1980b), and it is likely that the above proximo-distal (*re* heat-reared A. urticae) and anterio-posterior signals too are transmitted cell to cell and similarly slowed or inhibited by cold. Even if there were partial cell division and/growth, if this prevented pleating of the wing surface then the membrane in s0 might split when the insect attempts to expand the wings on eclosion.

In Drosophila, stage 4 involves cell expansion in a proximo-distal direction when cold shocks result in broad wings, followed by cell expansion in both directions when cold shocks result in smaller wings, and a final cell expansion in an anterio-posterior direction when cold shocks give elongated wings. On eclosion, the ventral surface normally expands faster than the dorsal surface, and the effects of early pupal cold shock manifest at this stage as warping (Waddington, 1950). A comparable (epidermal) cell enlargement in Lepidoptera too might facilitate pleating of the wing surfaces.

The partial wing expansion in the cold shocked L. coridon male and female proved to involve crumpling near the termen of the otherwise largely expanded wings. Since relative expansion may be greater near the termen (where the force of the haemolymph through the veins is dissipated), and since the veins through which the haemolymph is pumped are common to both surfaces, the crumpling most likely reflects a dorso-ventral discrepancy in the degree of surface pleating. Since the wings were otherwise largely expanded suggests that inhibition of early-stage 4 cell enlargement is most likely (especially in the male where the overall effect was to deepen the wing shape).

The relatively small hindwings of cold shocked P. aegeria male 01002019 probably result from a reduction in final epidermal cell enlargement in both directions (mid-stage 4); the rounder forewings in this specimen may result from a similar reduction in the forewing but

affecting the anterior compartment more than the posterior one. Indeed in Precis, putative wing compartments are known to differ in the extent to which cold shock affects eyespot focal activity (Nijhout, 1985a), and the foregoing results suggest that the difference concerns overall wing development. That the onset of cold shock was 12h-24h rather than <12h post-pupation is consistent with the cold having interfered with a later rather than some earlier stage.

The cold shocked T. jacobaeae with creasing of the anterior and posterior forewing edges (section 9.30.vi) most likely represents a reduction in anterio-posterior epidermal cell enlargement in late-stage 4, since the wingspan was normal. Uneven cell enlargement on the two surfaces would have resulted in the fully expanded wing not being pulled flat.

A number of P. aegeria specimens among subsequent generations of STOCK 01 and among other stocks proved to have unexpanded wings of normal size but already noticeably warped (see Chapter Ten). It is possible that these represent an exaggerated contraction of the basement cell layers during stage 2, as their normal pre-eclosion size rules out mitotic failure, while inappropriate pleating would be expected to result in warping after, rather than before, eclosion. Moreover, their onset of treatment was within 12h post-pupation, when the cold would have coincided with the earlier stages of wing development.

Since the pupal stage in P. aegeria as well as in the above L. coridon, A. urticae and D. sannio lasted between one and three weeks, and hence were of similar duration to Precis, it is likely that in all the above species stage 4 is completed within 50h-55h post-pupation. Since the cold shocks were applied within 12h post-pupation and continued for 96h (*ie.* applied between 12h-108h post-pupation), the period of cold exposure would encompass all four pre-eclosion developmental stages. Thus the foregoing effects are most likely due to the decreased ecdysone turnover during shock rather than the post-shock pulse. The cold shocked T. jacobaeae also underwent the <12h,24h onset/duration. That late stage 4 was apparently affected suggests that all four stages were completed before the pupae underwent vernal diapause (*cf.* section 9.30.vi).

It is possible that the reduced ecdysone turnover during cold treatment or the sudden increase immediately following might disrupt the development of the secondary trachaea, so interfering with post-eclosion wing expansion. Indeed, such blockage could also account for cold-induced pigmentation changes, since if the developing wing of Precis is folded during scale development to constrict the veins, then distal to the fold the pigmentation is much paler (Nijhout, 1980b).

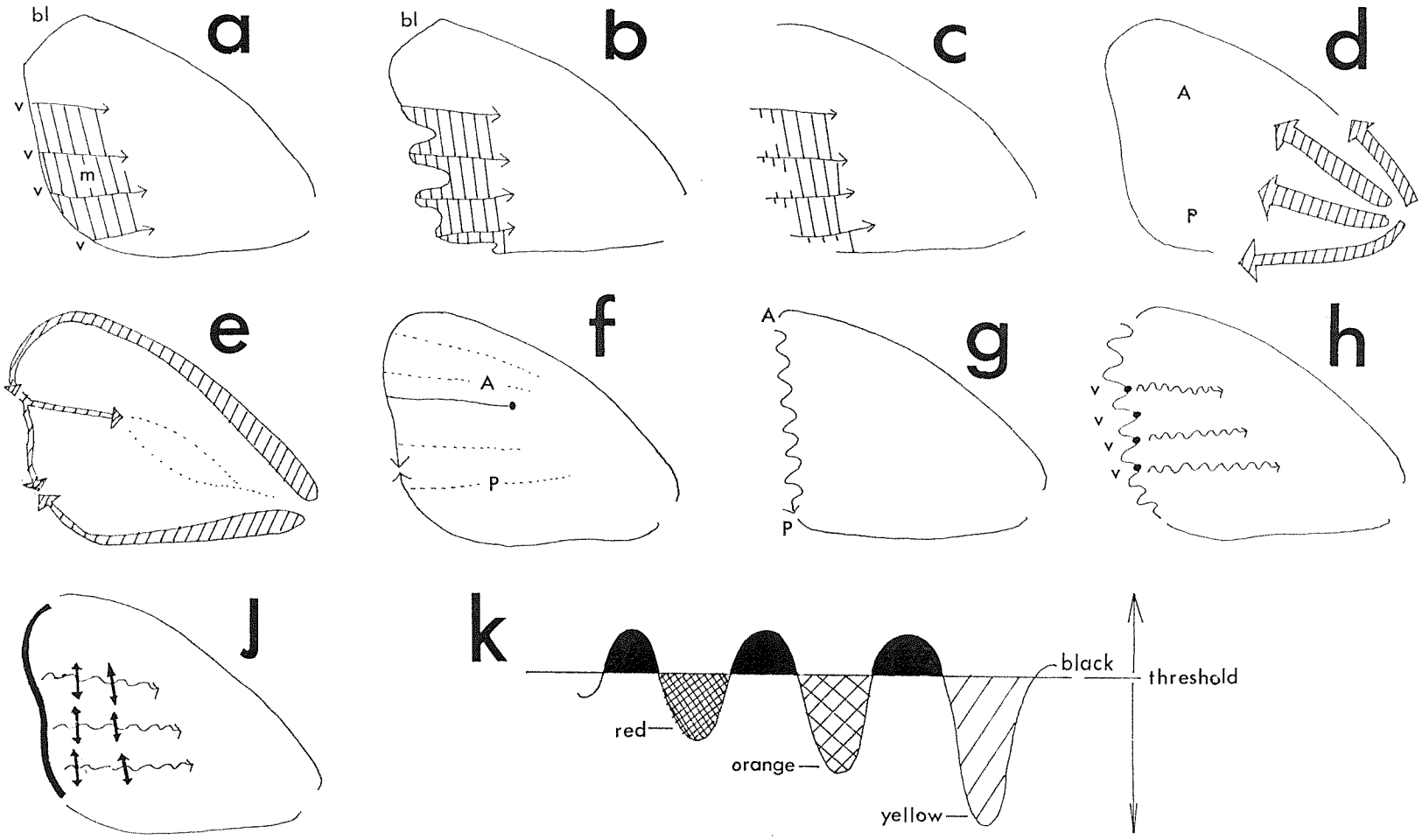
That cold treatment initiated *after* pupation resulted in one P. aegeria developing a left hindwing stump, suggests that in this species the first proximo-distal mitoses commence or are most prevalent within the first 12h post-pupation, while the altered wing shape of specimen 01002019 suggests that the final cell enlargements (here mid-stage 4) are mostly completed between 12h-24h post-pupation. Of course, as with prepharate duration (chapter seven), individual development rates probably differ. Thus, the variety of cold shock effects probably reflect differences in (1) development rate, so that the onset of cold coincides with different developmental stages, (2) individual differences in the canalisation of corresponding stages, (3) *canalisation aside*, differences in the susceptibility of each given stage, and (4) the particular process (*eg.* proximo-distal mitosis or epithelial contraction) ongoing at the time of cold application or of the subsequent ecdysone pulse . Since each stage cannot ensue unless the preceding stage has been adequately completed, the particular change induced by the shock will reflect the first suceptible stage in the respective individual, and indicate that the such stage (or its determination) must have commenced at or after the time of onset of treatment. It is therefore suggested that a variety of onset/duration combinations of cold shock might be used to identify the timings of particular wing developmental stages.

9.33. Venation and pattern

(i) *Groundplan elements*

In section 9.32.i it was suggested that the venation is established from the wing base and progresses distally around the costa and dorsum continuing around the termen, to branch at the A/P compartment border which one fork follows till it reaches the discoidal (D^1) region while the other continues around the termen to circumscribe the wing. Concurrent with the marginal progression from the wing base is a distad progression along the compartment boundaries. Thus, each compartment is fully circumscribed, the two loops closing at D^1. The intra-compartment venation then proceeds proximo-distally from the termen till it meets the compartment border (Fig. 9.30).

It was proposed that the venation determinant loses potential as it proceeds and that this is consistent with the degree of vein development at various positions on the wing. It is now proposed that the progression also carries a parameter of pattern determination, whose local value defines the nature and degree of pigmentation. Firstly, the greater the value, the greater the melanisation. Since the veins serve as a source of the determinant, the value will be maximal at the vein positions, and indeed, in most Lepidopteran species the wing veins are medium brown to black. Secondly, other features of Lepidopteran patterning are concordant with the quality of vein development. Several species have dark scaling near the base of the wings, which often extends along the costa and dorsum. A good example of both the above is the Black-veined White butterfly, Aporia crataegi L. (*cf.* Higgins & Riley, 1975); and in several species, costal and dorsal spaces 1A and 12 are often distinctly coloured even if not the darkest. P. aegeria and T. jacobaeae both exemplify this phenomena (*cf.* Skinner, 1984). The maximal potential near the base could also account for the distinctive colouration of s0 in certain species, in particular the distribution of blue and purple scaling on the dorsal forewing in females of several 'blues' (Lycaenidae: Polyommatini) and of the Hairstreak Quercusia quercus L. (Lycaenidae: Theclini). In these, the iridescent colouring is structural, and it is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possible that maximal parameter values might also influence scale structure (below). It is worth noting that s1A, s0 and s12 can build up a variety of mosaics that may correspond to colour fields that had

FIGURE 9.30 (opposite). Establishment of the wing venation and pattern. The development of wing membrane (m) is understood to depend on the surrounding venation including the border lacuna (bl) an transverse veins (v). The outline of the wing thus respects the border lacuna: (a) normal development of the border lacuna results in a smooth wing outline at the termen; (b) irregular development of the border lacuna may result in the transverse veins originating at indented positions, with the wing membrane displaying a similarly irregular outline. In both cases the border lacuna then degenerates, to leave a smooth or (c) irregular wing margin. The model proposes that the establishment of the venation (d) first progresses from the wing base distally along the costa and dorsum, and along the radian and median veins, then (e) continues round the termen until it meets the anterio-posterior compartment border along which it then returns to the discal region. The anterior compartment is understood to be smaller than the posterior one, so that the anterior component of the progression is the first to reach the compartment border first. Here the anterior component branches to continue along the termen to meet the posterior progression. Transverse veins (f) may then further compartmentalise the wing. The border lacuna can be conceptualised (g) as comprising a wave function between the distal ends of the costa and dorsum, whose nodes initiate the proximo-distal establishment of transverse veins (h). This latter progression is modelled as a reaction-diffusion function (j): black pigment is produced wherever the solution to the function exceeds a critical threshold; below this threshold, whites (unpigmented) through yellows and reds to browns are produced, the type or quantity of pigment produced depending on the precise sub-threshold value. The pattern is manifest by scale cells at corresponding proximo-distal positions within the wing space, typically as a series of dark and variously coloured lighter bands (elements and interspaces respectively). For further explanation see text.

hitherto not fitted into any particular developmental scheme (*cf.* Nijhout, 1978). Such basal maxima could also account for the basal melanisation in P. aegeria.

Patterns that relate to topographical features of the wing are known as dependent patterns, for example intervenous stripes which are always equidistant from the surrounding veins (*ibid.*). In the following paragraphs, a scheme will be developed in which, by viewing *all* patterning as ultimately dependent on reaction-diffusion parameters originating or delimited by the venation, Lepidopteran pattern development, including that of P. aegeria, can be understood within a unifying scheme.

For reaction-diffusion over a rectangular domain, with m and n the substrate and co-substrate, solutions to the field equation take the form:

$$e^{\lambda_{mn}t} \cos(n\pi x/x_o) \cos(m\pi y/y_o)$$

where m and n are diffusion coefficients, x and y are concentrations, t is time and λ, x_o and y_o are constants, the latter two concerning the boundary dimensions of the essentially rectangular domain (Murray, 1981). Murray (*loc. cit.*) showed for the integument of mammalian embryos, that if the domain is too thin only one-dimensional modes can be unstable, while if it is too small then no spatial pattern can be generated. However, if neither x_o nor y_o are too small, then two-dimensional unstable modes are possible. The latter generate spot patterns, although these often degenrate to stripes at thin extremities, the spot size increasing with the field dimensions till they fill the available space. As the domain becomes still larger, the spots fuse until the scale of the intervening pattern becomes so fine that essentially no pattern can be seen.

The dark fringe venosa in P. aegeria appear as a dark striping of the fringe, which appears to be a feature of several Rhopalocera (*cf.* Higgins & Riley, 1975). It is suggested that a reaction-diffusion is established through the border lacuna, striping rather than spotting resulting on account of its narrow proximo-distal dimension. Murray (1981) models the anterior and posterior wing veins as the source of a hypothetical morphogen; hence the border-lacunal striping would be

established from their distal ends (Fig. 9.30g).

The dark venosae may correspond to local maxima which set up a proximo-distal reaction diffusion that follows the basad progression of the venation towards the base (Fig. 9.30h). It was suggested that in P. aegeria both pigmentation and the positions of the dark pattern elements are established from the termen towards the base (sections 9.16 and 9.19.ii). It was found also found that colour became paler the more basal the position within the wing space. The following scheme is proposed. There is an epimorphic reaction-diffusion from the termen along the wing veins whose solution describes a sinusoidal curve. Above a given threshold value, brown/black is always specified, while below this threshold, white (unpigmented) through yellow to red-brown are specified with increasing sub-threshold value. Thus the Schwanwitsch (1924) elements correspond to serial supra-threshold values, the basally lightening interspaces corresponding to the serial and basally decreasing sub-threshold values (Fig. 9.29j).

It is also proposed that as the reaction-diffusion progresses along the veins, it determines the future pigmentation of the cells either side of it, thus generating a corresponding banding pattern in the wing space (Fig. 9.29k). The Schwanwitsch (1924) pattern elements thus link corresponding proximo-distal values. Indeed, in Precis at least, the submarginal band follows the wing margin very closely (Nijhout, 1986), and likely corresponds to the high determinant value at the border lacuna. Moreover, most Rhopalocera have a black rim around the termen (*cf.* Higgins & Riley, 1975) which, since the border lacuna degenerates before pupation (Nijhout, 1985c), must be due to dark pigmentation of the wing scales themselves and not that of an underlying wing vein. P. aegeria too has such a rim, indicating that the determination of pattern elements in this species commences pre-pupation. As in eyespot determination (Nijhout, 1980b), the determination of elements within the spaces probably involves cell to cell communication, which it is suggested progreses anterio-posteriorly from each vein to meet along the wing space midline, the latter so acting as a sink.

Although the equipotentials will arise at similar relative positions along the veins, their exact positions may differ somewhat on account that the initial establishment of reaction-diffusion

systems includes an element of randomness (*cf.* Murray, 1981). Thus bands (elements) homologous between the spaces need not, however, be contiguous between them (*cf.* Nijhout & Wray, 1986), and such lack of contiguity could account for the apparent dislocation of homologous pattern elements between adjacent spaces (*cf.* Nijhout, 1978).

In P. aegeria, the lightest (least pigmented) wing area was the eyespot pupil (cOC element) in forewing s5. Such foci in Lepidoptera always fall on the proximo-distal space midline (Nijhout, 1986), and it is possible that their positions correspond to coincident maximum or minimum values of both the proximo-distal reaction diffusion and the anterio-posterior cell to cell communication. Eyespot determination is examined more closely below.

(ii) *The discal spot*

In section 9.32.i it was proposed that the discal spot (the Discalis I of Schwanwitsch, 1924) functioned as the sink for some venation determinant. However, it might more rightly be regarded as being generated *by* a sink. It is proposed that at the positions where the loop of bordering-vein determination closes (about halfway between the wingbase and termen along the adjacent boundaries of the anterior and posterior compartments), the venation determinant accumulates, and so is dissipated through the generation of an intervening lacuna. The points between which it is dissipated thus correspond to the distal ends of the future median and radian veins. Since the discoidal veins are known to arise as secondary lacunae *without* tracheae (Nijhout, 1985c), this would suggest that pathfinding in general involves the permeation of haemolymph along the prospective vein positions *before* the primary tracheal branches develop. Space 0 includes lacunae that branch basally from the discoidal lacuna, implicating the latter as functionally homologous with the wing-border lacuna. The proximo-distal branches or median lacuna, however, although apparently contiguous with the transverse lacunae of s5 (or hindwing s4), are *not* functionally homologous, since they degenerate pre-pupation whereas the transverse lacunae do not (*cf.*Nijhout, 1985c).

Nonetheless, the median lacunae probably do establish pattern in a similar manner to transverse lacunae, as in P. aegeria, the

distal-basal size and colour relationships within s0 were similar to those within the spaces of sectors 2 and 3 (*cf.* Figs. 9.13 and 9.14). Indeed, in Lepidoptera the discal lacunae atrophy *after* pattern formation (Nijhout, 1978), commensurate with the above hypothesis. As with the transverse lacunae, the determinants of pattern may be carried with those of venation. (Both may in fact involve a common determinant, below). Since the venation determinant reaches s0 last of all, the pattern of s0 too might be expected to be the latest to be determined, and indeed the central symmetry system centred on D^1 *is* the last to be determined in many species (Nijhout, 1978).

(iii) *Eyespots*

Above it was postulated that foci might represent the intersect of the nodes of each a proximo-distal and lateral reaction-diffusion. That Lepidopteran eyespots in general tend to follow the wing margin (Nijhout, 1986) would suggest that the termen at least is involved in establishing focus positions. This most likely ensues in the final instar larva as manipulations on pupal wings and late larval discs cannot modify focal position (Nijhout, 1986), it being the triggering by signal sources of *pigment* production by the wing epidermal cells that ensues in pupa (*ibid.*). The number and position of foci are species specific, and in the Polyommatini foci-like spots are also prominent on the ventral surface (Nijhout, 1978). Similar ventral spots also occur in the ventral hindwing s0 of P. aegeria, which in specimen 01012018 were very prominent (as was the discal spot, D^1) when there appeared to be one per putative intra-s0 space (Plate 9, frame 17A-18).

Nijhout (1978) modelled eyespot pattern as a cone shaped gradient, with the pupil at the real or imaginary projection of its apex, the focus. Cautery inhibited eyespot formation, thus demonstrating the focus to be a physiological entity (Nijhout, 1980a) that produced an inductive signal reminiscent of classical embryonic inducers (Nijhout, 1986). Nijhout (1980b) found that in Precis, eyespot determination moves across the early pupil wing at 0.27mm per day but more slowly under cold, this temperature dependence being consistent with reaction-diffusion. The activity of foci in each cell also varied

independently (Nijhout, 1985b).

Cautery in Precis at sites normally *devoid* of eyespots, however, induced a black circle typical of the outer eyespot ring, suggesting that foci might act as *sinks* rather than sources (Nijhout, 1985b). This would be commensurate with the proposal that, in P. aegeria at least, foci mark the coincidence of proximo-distal and anterio-posterior reaction-diffusion minima, which could also account for the unpigmented white pupils (below). In Precis, eyespot ring formation appeared to have more to do with the hypothetical interpretation landscape (Nijhout, 1980a), a two-dimensional (but not necessarily planar) section through the cone shaped gradient onto which projection of the gradient describes the resultant pattern (Nijhout, 1978).

9.34. Pattern determination and realisation

Pattern within each wing-space is understood to depend on (1) focus position, (2) the shape of the landscape and (3) the kinds of pigments used. In some spaces the ocelli may not be expressed at all (Nijhout, 1978).

However, subsequent mitoses or cell growth, as well as post-eclosion wing expansion, could also influence pattern. Not only might these processes distort an already determined pattern, but they might also be determining events in their own right. Determination could equally depend, say, on some state of cell excitation (as in nervous conduction) or its position in the cell cycle, behaviours which too can be described in terms of wave or reaction-diffusion functions (*cf.* Ho *et al.*, 1983b). Such modes of pattern determination could account for the elusive nature of suspected chemical morphogens (*cf.* Nijhout, 1980a). Indeed the determination of the size and colour of eyespots is known to coincide with cellular events (Nijhout, 1980b). Since each wing scale is of a single colour only (Nijhout, 1981), mitosis and cell growth could influence pattern determination and adjust its subsequently visible geometry *concurrently*. Indeed, it is very likely the latter which in its *effect* constitutes the interpretation landscape. It is therefore suggested that the concepts of morphogen gradient and interpretation landscape might be more

appropriately understood in terms of pattern determination and pattern realisation respectively. The above model can resolve the paradox of epimorphic pattern determination (section 9.16.i) yet the pattern being adjusted to the size of the wing space (and indeed the size and shape of the wing overall): the progression of proximo-distal mitoses effects epimorphic pattern determination, while the *growth* of these cells scales the pattern to the wing shape and size (just as pattern is so scaled up and distorted during final wing expansion). Indeed the Aglais urticae with brachypterous wings and the male with an indented right forewing appear to bear this out. That in the Pararge aegeria and Diachrysia sannio males with hindwing stumps the usual pattern was visibly absent, would suggest that cell division indeed effects a role in pattern determination.

9.35. A unified model of pattern establishment

(i) *Eyespots and bands*

As the unit of pattern propogation is the cell, it would be more accurate to consider *effective* rather than absolute field dimensions at the time of laying down. The reaction diffusion model predicts that below a critical field width spots degenerate to stripes (Murray, 1981). It is proposed that bands and spots differ only in the effective width of the inter-vein domain, bands indicating a thinner domain (as with the extensive nigrism that typifies P. aegeria), spots indicating a broader one (*eg.* the dark markings of Argynnis spp. or the dots in s0 on the ventral hindwing of P. aegeria). Any bands can fuse and often bend to do, so they all involve physiologically compatible processes (Nijhout & Wray, 1986). In Cynthia cardui, for example, the parafocal elements (externa[3]) often elongate into the focus and are absorbed (Nijhout, 1984), a less extreme manifestation present in P. aegeria cold shocked specimen 01002008 (Plate 9, frame 7).

Since foci simply reflect the intersect of minima rather than sites of distinct physiological activity *per se*, it is contended that bands and spots, including those with foci, simply reflect a progressive change in spot size with effective field size. This could

explain why symmetry systems appear to originate from point sources (*cf.* Nijhout, 1986), and why foci are not found in all spaces (*cf.* Nijhout, 1978). The impression that eyespots are physiologically distinct could thus be a false one (their *apparently* autonomous behaviour is explained below).

In support of this hypothesis is the behaviour of longitudinal stripes (Nijhout, 1978). Here it the domain might be limited to the cell midline, perhaps to yet unmitosed or in some other way still immature cells. When present, these stripes are the only pattern in the space (Nijhout & Wray, 1986), in accord with the prediction (*cf.* Murray, 1981) that as the domain becomes too thin pattern tends to uniformity. Yet the midline stripe often degenerates into series of evenly spaced points (Nijhout, 1986), which probably reflects a wider domain so that striping can start to appear along the cell midline (such stripes would appear as spots relative to the entire space). Indeed, spots and longitudinal stripes appear to be extremes of a morphocline (Nijhout, 1986), and that they also appear mutually exclusive (*ibid*) again implicates them each as alternative but overall field solutions.

The above scheme can accommodate the addition or loss of bands (elements) within spaces (Nijhout & Wray, 1986), when if two elements correlate fusion rather than division is implicated (*ibid.*). It would also explain why space patterns appear as distortions of a common theme (Nijhout, 1984, 1985b), as well as certain homologies between particular spaces. For example, the Satyridae show a strong association between forewing s2 and s5 in the occurence of eyespots (Schwanwitsch, 1930), and it is possible that these spaces possess similar field parameters at the time of pattern determination. The model so far enables the following morphocline to be constructed: (1) uniform longitudinal stripes, (2) midline stripes (appear as spots), (3) space bands, (4) space spots (can include foci), and nigrism as these further enlarge with increasing field dimension. Before using the model to explain cold shock effects, however, it is necessary to examine ripple patterns.

(ii) *Ripple patterns*

These appear as fine ripples that resemble the wave pattern created when a stone falls into water. They occur naturally in several species, particularly on the ventral surface of the Vanessini (Nyphalidae) including the British Peacock butterfly, Inachis io L.

In the moth Chrysiridia madagascarensis (Uranidae), the green ripples appear to interact with the central symmetry system, and it was proposed that they involve a qualitatively similar mechanism (Nijhout, 1978). Now the reaction-diffusion equation predicts that as the domain gets still larger, the pattern becomes increasingly fine and eventually invisible (Murray, 1981). It is therefore suggested that ripple patterns represent the next progression in the morphocline of reaction-diffusion patterns. They are always oriented perpendicular to the transverse veins, suggesting that they originate from the termen rather than these (Nijhout, 1978); and they probably describe the field solution when more cell divisions have been completed and hence the domain is effectively larger. Indeed these patterns typically cover most of the wing, although absent at the wing margin (Nijhout, 1978), and tend to be better defined in larger species (including P. aegeria) where the embryonic wing domains are possibly larger. Nonetheless, ripple patterns often stop or change at the transverse veins (Nijhout, 1985b), which, as with spot and banding patterns, probably represents simple dislocation between spaces of the vectors of equal field strength (*cf.* section 9.33.i).

It is proposed that as the domain becomes yet larger, the pattern becomes so fine that it is resolved by individual wing scales. This could account for fine irrorations such as in the dorsal hindwing U:M^1 interspace of male P. aegeria (section 9.23.i). Here, the scales might resolve local determinant values as either sub-threshold (light) or supra-threshold (dark), but the *particular* sub-threshold (supra-threshold) value resolved by each light scale might produce a more complex irroration, say yellow and orange with dark brown; as indeed was present in several secimens. The general lack of ripple patterns near the termen (Nijhout, 1978) might result from the high determinant value close to the border lacuna overriding or absorbing any adjacent rippling.

(iii) *Reaction-diffusion and cold shock*

The morphocline from longitudinal stripes to nigrism thus continues with ripple patterns and irroration, eventually yielding uniform colour fields as the domain increases in size. One implication of reaction-diffusion is that slight parameter changes could have a drastic effect on pattern (Nijhout, 1986), and it is proposed that cold mediates its effects by changing the parameter values.

Above it was proposed that in normal development, adult wing pattern reflects the pattern of early pupal cell division and growth, and that this involved a progressive change in the size of domains (these being the wing spaces). Indeed, the information for [non-circular] patterns does appear to lie in the *response* mechanisms, when pattern differences reflect *quantitative* differences in the rates or timings of the relevant processes (Nijhout, 1986). Firstly, as cells divide and grow, the domain becomes geometrically larger, so that a continuously propogated signal (*eg.* a chemical morphogen) would have further to travel. Secondly, in the case of signals propogated cell to cell, the increasing cell number will render the domain functionally larger. Pattern determination does in fact seem to be propogated in this way (Nijhout, 1980b), probably via gap junctions (Nijhout, 1986).

It is therefore proposed that cold shock modifies pattern by effectively enlarging the domain. Firstly, cold might slow the rate of reaction between a substrate and co-substrate. Secondly, it would reduce metabolic rate, so that propogation of a signal mediated through progressive cell divisions or via gap junctions too would be slowed. Since this increases the *time* taken for the signal (chemical or functional) to traverse the field, this is equivalent to enlarging the domain given a constant temperature. In Aglais urticae, two black forewing spots (corresponding in position to the s2 and s3 M^1 element) among *untreated* samples were smaller in those reared at the warmer temperature, which might be understood as faster wing development effectively reducing the size of the domain.

The effective increase in field dimensions under cold could account for the pattern changes illustrated on Plate 9. Widening (enlargement) of the dorsal forewing OC spot in specimen 01012019 would eliminate the pupil, the yellow scales within the dorsal forewing OC spot of specimen 01004021 probably an intermediary stage in this process at which the sub-threshold value (*cf.* section 9.33.i) fluctuates above its nodal minimum. The missing eyespot pupils and reduced E^3:U on the dorsal hindwing of the latter specimen again suggest a widening (nigrism) of the dark intra-space bands (Ξspots). Although not cold shocked, cool-reared, assimilated specimen 01012019 provides convincing evidence for increased field dimensions at cooler temperatures. The diminished and absent dorsal hindwing eyspot pupils suggest enlargement of the OC elements (bands), similar enlargement of the other elements here accounting for the narrower E^3:U interspaces. On the posterior ventral forewing, the bands have enlarged to fill the field. That the dark spots within ventral hindwing s0 (and the D^1) are particularly prominent in the specimen suggests that they too have started to enlarge, while the smooth appearance of the ventral hindwing ground colour is explained by the usual ripple pattern having become so fine that it is no longer visible as such. In other words, each kind of patterning normally present in this species has shifted to the next position in the morphocline. The marbled appearance of cold shocked female 01004016 proved to comprise a dark grey brown mottling within the black interspaces, this mottling on close inspection having a rather rough appearance reminiscent of the male 'sex brand' (section 9.29). The reaction-diffusion may have resulted in local determinant strength superceding the upper limit for melanisation and so effecting a change in scale ultrastructure. In Aglais urticae, heat shock induced dark grey mottling within the black markings, and its *total absence* from the lighter areas would appear to confirm the above explanation. Excessive heat, like cold, could interfere with cell growth and division, possibly by denaturing enzymes (even irreversibly).

In Precis, the time at which cautery became ineffective was earlier at higher temperatures, and this was explained as the susceptible stages already being completed by the time cautery was applied (Nijhout, 1985a). In Aglais urticae reared at 15°C - 25°C, cold shock resulted mainly in minor indentations of the termen

(especially in forewing sector 3) and dark scaling along the transverse veins, whereas in those reared at ≥30°C, the darkening induced by cold shock extended to within the spaces and the wing deformities were more severe. It is likely that while those reared at the higher temperature developed more quickly, their pattern determination was not yet completed at onset of cold, which therefore interfered with a later stage (cell division and growth) rather than an earlier one (vein positioning). This would also explain why cold shock affected wing size and shape more severely in heat-reared animals.

Nijhout (1986) proposed that cold delays the processes that establish the landscape so that at the time of pattern determination the landscape is not fully developed, and that gene mutations might act in this way. Since Lepidopteran wing development appears generally similar to that in Drosophila (section 9.32.ii), where mutations and temperature shocks are known to influence particular stages of cell division and growth, the notion that the landscape describes such cellular processes would also corroborate with Nijhout's contention.

Natural genetic variation could influence the composition (via DNA base sequence) of substrate and co-substrate and hence their reactivity and diffusion coefficients, their concentrations (via gene activity), and the rates and timings of cellular processes (genetic periodicity as in the cell cycle) . Genetic variation could also influence the precise structure of gap junctions (and hence their efficacy) or metabolic rate (and hence cell growth). Genetic variation would also relate to the sensitivity of these processes to extrinsic parameters such as temperature. Intrinsic irregularities in the putative landscape could explain the wide *variety* of individual cold shock effects (Nijhout, 1985a), as in P. aegeria (and other species, sections 9.25 and 9.26).

Nijhout (1985a) proposed that the landscape could be conceptualised as having values ('levels') of say 1500, 1000, or 500, the eyespot foci a value of say 300, and that eyespot rings were induced wherever the source/landscape ratio was say 0.5(±0.2):1. Cold lowered the level of the landscape, which for Precis eyespots might already be near the critical level. Cold might lower the level over a large area (Nijhout, 1985a), and mitosis and cell growth are just the sorts of processes that might be susceptible to perturbation on a

global scale.

9.36. Pigment acquisition and compartmentalisation

(i) *Pigment acquisition*

The wing pattern is essentially a mosaic of independent units as each scale is of a single colour only (Nijhout, 1981). Yet although pigments are synthesised only in the scales, it is not known if pattern *determination* involves just the scales or all cells in the wing (Nijhout, 1980b). The pigment synthetic enzymes are active and lodged in the scale cuticles, their substrates understood to be produced sequentially in the haemolymph, and two models have been postulated (*ibid.*). The first is that all substrates have access to the scales at all times, but each scale has only one enzyme and can use only one substrate. The second is that scale maturity determines which substrates have access to the scales, since in the pupal wing corresponding to prospective black areas proved to be less mature than those destined for a lighter colour (Ford, 1957a: Pieris; Nijhout, 190b: Precis).

In Pararge aegeria, colouring up involves first a yellowing of the entire wing, after which the eyespot becomes visible as a small rust-coloured dot. The wing then becomes orange where the future elements will be, and the eyespot appears black. The other elements then darken as the pattern becomes visible through the pupal cuticle. Melanins, of which the P. aegeria pattern would appear to be composed (section 9.4), however, pass through red and brown stages during their synthesis (Ford, 1957a). The *uniform* initial yellowing suggests some common interaction between an enzyme and substrate, and it is suggested that only the initial substrate is produced in the haemolymph. This then enters the all the scale cells, where the pigment pathway proceeds. However, it is proposed that maturation of the scale renders the enzymes inactive, perhaps simply the result of their final spatial geometry (just as in mitochondria electron transport depends on the appropriate deployment of enzymes and cofactors within their

membrane, Hinkle & McCarty, 1978). This is commensurate with reaction-diffusion as establishing the pattern of cell division and growth. Subsequent to perfusion with the initial substrate, the less mature cells have yet more growth to complete, so that by the time their growth is completed the pigment pathway is further progressed. This might also explain why abnormal (as suggested cold-induced mottling) or anomalous scale ultrastructures (as in hair-scales or androconia) tend to be associated with dark colouration; these cells not only are the last to start maturing but might remain in a somewhat (structurally) immature condition. Thus hair-scales might represent an ontogenetically more primitive condition, suggesting that scale-forming cells are ultimately derived from hair cells similar to those on the wing surface of, for example, Drosophila.

Alternatively, reaction-diffusion initiates the synthesis of enzymes in scale forming cells. If their respective coding sequences function as an operon (*cf.* Goodenough, 1978), then the earlier determined cells will include enzymes appropriate to further progression through the pathway. Reaction-diffusion with maximal values at termen (the origin) and in the proximo-distal centre, lesser values in other supra-threshold regions, and with its lowest values in sub-threshold regions, could explain why the eyespot blackens first, and then the elements. Both these models are more parsimonious than those implying the production of unecessary substrates (*ie.* that all are present in the haemolymph) or enzymes (*ie.* that only one substrate, however, enters the cell).

(ii) *Compartmentalisation*

The lack of colour concordance between the ventral and dorsal forewing s5 in P. aegeria suggests that colour is determined after the establishment of some dorso-ventral (D/V) distinction. Indeed, the dorsal and ventral surfaces of most Lepidoptera are known to be independent (Murray, 1981), and that homoeotic conversions are limited to one surface suggests that in Lepidoptera, unlike Drosophila, the segregate very early (Sibatani, 1980). In C. madagascarensis they are apparently determined at different times post-pupa (Nijhout, 1978).

P. aegeria exhibits Oudemans' phenomenon of the Argynnis type (section 9.1; Plates 1, 2 and 10). It is therefore proposed that prior to folding to form the wing anlage, the disc unilayer comprises four compartments - the anterior-ventral (A/v), anterior-dorsal (A/d), posterior-ventral (P/v) and posterior-dorsal (P/d), which fold along the anterio-posterior axis to produce the two surfaces (Fig. 9.29d). While Oudemans'phenomenon of the Polygonia type (where the entire dorsal and ventral surfaces differ, Schwanwitsch, 1935) might be explained as differences subsequent to (or resulting from) folding of the anlage, the similarity between the basal ventral and dorsal forewing in the Argynnis type implies that they are determined similarly and independently of D/V separation. Since venation and pattern appear to be established from the termen, and since in Lepidoptera no communication between the surfaces has been reported, it is possible that the primary determination of pattern (and venation) occurs *prior* even to folding of the anlage. The procession of determination of each surface would still be similar to that outlined in the foregoing discussions, except that it might originate from two poles at each end of an imaginary axis perpendicular to the anterior-posterior axis (Fig. 9.39e), and if proven to be the case, would call for radical revision of current theories of Lepidopteran pattern formation. It is interesting to observe that the putative alignment of inter-compartment axes, transverse veins, and hypothetical foci on such a surface bear a remarkable resemblance to the axes of holoblastic cleavage (Fig. 9.30f, *cf.* Goodwin, 1984a).

In Precis, the superposition of the dorsal and ventral forewing foci is simply coincidental (Nijhout, 1980a). In P. aegeria the relative shift of the eyespot within the umbral interspace clearly indicates some difference in their realisation, although the overall similarity between dorsal and ventral forewing s5 would suggest some common parameter in their determination. It is clear that the venation (in the larval anlage including the border lacuna) is common to both, again corroborating with the thesis that pattern is established from the margin in concert with the venation. The shift in eyespot position might result from differential cell division and growth between the surfaces, and that this shift involved the eyespot rather than surrounding elements further points to greater lability at the proximo-distal centre of the field. Differential cell division and

growth could also explain why some markings where similar and/or concordant in colour between the surfaces while others were not. In Precis, differences between forewing and hindwing in their response to cautery probably relate to asynchronous development (Nijhout, 1985a).

In Satyrids, post-bithorax homoeotic conversions (where the posterior meta-thorax converts to posterior meso-thorax) are the commonest (Sibatani, 1980), suggesting that posterior hindwing is the most labile compartment. It also suggests that the hindwing might have originated through conversion of some haltere-like structure to the forewing, as in bithorax conversions in Drosophila. (Contra-bithorax, where the anterior forewing converts to anterior hindwing, are also frequent in the Satyridae, Sibatani, 1980).

Figure 9.16 shows that the rank size and colour of posterior hindwing spaces 2 and 3 are greater than those of anterior hindwing spaces 4 and 5, and that the rank values of forewing space 5 were the greatest. This would suggest that the hindwing indeed comprises two compartments (anterior and posterior), with their border between s3 and s4. The similarity here between anterior forewing and posterior hindwing may reflect the path of determination, which loses strength as it proceeds, introducing a mirror symmetry about the meso-/metathoracic axis. Hence, this mirror symmetry might be rather an outcome of wing geometry rather than necessarily any genealogical homology. Such geometry effects might explain the mirror duplications occasionally found naturally in Lepidoptera, some of which have involved the entire forewing (*cf.* Sibatani, 1980).

The dorsal hindwing of cold shocked P. aegeria specimen 01004021 (Plate 9, frame 36A) shows a difference between s2-s3 and s4-s5 in the reduction of pattern, this being more severe in the anterior pair. The ventral forewing of assimilated and cool-reared specimen 01012018 (Plate 9, frame 14A-15) shows a difference between s1b-s2 and the remaining anterior, the extensive nigrism being confined to the posterior pair. These results indicate a difference between anterior and posterior wing compartments in their response to cold shock, suggesting that each wing comprises anterior and posterior compartments. That the effects of cold shock on pattern were also confined to one surface, or differed between the surfaces when both were affected, too implicates the dorsal and ventral surfaces as distinct compartments. Similar results have been reported for Precis

(Nijhout, 1985a). Thus, it appears that each wing does indeed comprise the four compartments (A/v, A/d, P/v and P/d) postulated above. That cold shock effects were limited or differed between them suggests they are independent and/or canalised to different extents.

Fluctuating asymmetry on the P. aegeria hindwing was limited to s3 and s4. These spaces coincide with the anterior-posterior compartment boundaries, which if the two compartments are independent, and so not fully coordinated, could be expected to be more unstable. On forewing s5, characters differing between samples were confined exclusively to within the U:U element (except for the U:M^1 interspace which fluctuated more in males than females, Fig. 9.17B and 9.18B). This is in accord with the notion that changes to the reaction-diffusion parameters alter the pattern most severely near the proximo-distal centre of the wing space. Indeed, Murray (1981) found that the domain of non-zero value within reaction-diffusion fields is constant for different field dimensions.

That differences between samples in fluctuating asymmetry were confined to size characters (except for the forewing s5 distal U:U), might be explained by changes to the *width* of bands of supra- and sub-threshold value rather than to changes in value within these bands. The eyespot ring on dorsal hindwing s4 differed between all sample comparisons, and its particular lability is explained by it lying at the intersect of the compartment boundary *and* the proximo-distal centre of s4 component of s0/4, each of which is already unstable.

That variabilities were greater in untreated samples than under foil (Fig. 9.24b) probably reflects a greater incidence of mitosis and cell growth under the higher ecdysone levels associated with summer daylength, so leaving development more open to random fluctuations and perturbation. These involved forewing s5 and hindwing s2, suggesting that their instability might relate to strength of the hypothetical determination, which would be similar for both these spaces.

The characters which (in males) were more variable under foil than cold treatment (Fig. 9.26b) may similarly relate to the greater incidence of cell division and growth under the higher ecdysone levels. Indeed, the affected spaces were again forewing s5 and hw s2. Colour characters more variable under cold than foil suggest lability to the post-shock ecdysone pulse. In males, characters so affected

were present in forewing s5 and s0 and hindwing s4, suggesting that the anterior compartments are more unstable (less canalised) to cold *shock* than the posterior ones.

In females, variability was always greater under cold than foil treatment (Fig. 9.26b), when the only hindwing character affected was the size of hindwing s5 pupil. That the forewing s5 pupil too was affected, again points to a greater lability to cold shock of the anterior compartments, and homology between them. Of course, differences in variability could also reflect individual differences in the extent and timing of susceptibility to the particular treatments.

So far, we have seen how the dynamics of larval growth render the early pupa particularly susceptible to perturbations, and the way in which darkness and cold mediate their effect through their influence on ecdysone turnover. The genetic assimilation of the effects of cold treatment on various stages of the life cycle has been demonstrated, and the mode of this inheritance proposed. We have now seen that cold treatment can also modify the wing pattern, and a model of wing development has been proposed that can account for these effects. The occurence of natural phenocopies of temperature shock phenotypes was also discussed. In the concluding chapter, the ways in which these findings might relate to the evolution and ecology of Pararge aegeria are considered, and their more general evolutionary implications are outlined. The use of digital analysis as a means of investigating overall wing morphology is introduced here.

CHAPTER TEN

Summary

The assimilation of wing phenotypic cold shock effects is explored. The expressivity, but *not* frequency (about 1-2 in every 20 animals), of pattern abnormalities increased with A^i. Affected animals had darker wings which might result from their developing more slowly and so being less mature at the time of substate perfusion. Cooler control range temperature would act similarly. The unaltered frequency of phenocopies may reflect the susceptible stage being reached only *after* cold shock has been completed. The Hampshire stock became more orange with inbreeding. Orange is probably the norm for the species: inbreeding breaks down canalisation of the 'atypical' cream colour. The frequency of abnormalities in the British hybrid stock was again 1-2 in 20, implicating heterosis: most of its F^2 showed wing deformities. The Doncaster stock showed several deformities: under southern conditions it may be more advanced at the time of cold shock.

Lepidopteran wing pattern evolution is discussed including similarities between ecologically distinct groups (pseudo-mimicry). Effective shifts in reaction-diffusion parameters can account for the range of cold shock transformations and, with venation, of pattern features typical of Lepidoptera.

Moment invariant analysis is introduced. This uses statistical parameters of the x and y coordinates of positions of light or dark. Sets of replicated wing photographs, to which various kinds of damage and fading were applied, showed that they could resolve anterior-posterior and dorsal-ventral differences and detect fading. The problems attendant with such artifical samples are discussed.

The protocol is assessed and deemed a realistic reconstruction of natural aegeria populations, which too can originate from isolated strays, suffer little harm from inbreeding, and rapidly recover from falls in number. The chapter concludes by considering the origin, spread, and adaptiveness of the subspecies' life cycles and phenotypes. It would appear that its pale and orange facies, and its uni- and multi-voltinism, are rather interchangeable, this contributing to the species' success.

CHAPTER TEN

EVOLUTION AND ECOLOGY

Preamble

This concluding chapter opens by considering the genetic assimilation of the wing-morphological effects of cold shock and their inheritance. The implications of these effects and their genetic assimilation for Lepidopteran wing pattern evolution in general is then outlined. The use of digital analysis as a means of investigating overall wing shape and pattern is introduced, with particular emphasis to the efficacy of the method in resolving morphological differences. Ways in which the technique might be applied are also presented. The occurrence of genetic assimilation in P. aegeria in nature is considered in the light of its known population biology and ecology, and a model for the evolution of its voltinism strategies is presented. Finally, the role of genetic assimilation in the evolution of the wing phenotype is considered, with particular reference to cold shock phenocopies and known wild aberrants. The chapter closes with an assessment of the contribution of the present findings to evolutionary theory.

10.1. Genetic assimilation and the wing pattern

In Chapter Eight it was shown that cold shock slowed pupal development in the generations subsequent to treatment, and it was proposed that cold influenced the development rate of the maturing ova within the parent female, resulting in adjustments to their subsequent life cycle stages in an attempt to regulate the linear growth dynamic. It might therefore be expected that pupal wing development too would be slower in animals derived from cold shocked parents, from female parents in particular.

Slower mitotic cell division and growth would mean the wing scales being less mature at the time of pigment-substrate perfusion, so that even in the absence of repeated cold they might darken further. The

slower cell development might also result in the pattern as determined by reaction-diffusion progressing through the morphocline. In this way, cold shock phenotypes might occur without treatment in the subsequent generation.

In STOCK 01, there did not appear to be an evident increase in the frequency of cold-shock phenocopies. It is possible that slower wing development means the shock ensues prior to the onset of a susceptible stage. However, a cooler *control* range temperature might further slow the retarded cell division and growth, which could account for the markedly altered phenotypes of assimilated F_1 specimens 012018 and 012019. Of course, natural variability among individuals in the timing of susceptible stages might mean that in those whose susceptible stages *do* coincide with the cold shock, the phenotypic modification may be more severe.

The photographs were therefore perused to examine the possible effects of assimilation. In STOCK 01, there indeed was no evident increase in the frequency of pattern phenocopies, although only one generation of assimilation *per se* was achieved before hybridisation proved necessary to continue the lineages. However, the F_3 adults did appear to have a distinct overall pale orange tinge to the light markings (interspaces). Colonies from south Hampshire are known to sport a particularly high frequency of naturally orange-tinted phenotypes (form intermediana Lempke) among generation 1 part i (Russwurm, 1978), and it is possible that the increase in this trait among the F_3 above reflects increasing homozygosity at the loci concerned. These loci might interact with vernal pupal scotophase (the F_3 controls were dark-treated). It is speculated that orange coloration might be the norm for P. aegeria as a species (this colour typifies other species of the genus and the related genus Lasiommata, Higgins, 1975; Higgins & Riley, 1975), when the cream coloration of subspecies tircis would be atypical. It is therefore possible even that the increased level of inbreeding in the above F_3 resulted in a breakdown of canalisation of the usual - but atypical - cream colour.

Cold shock was then examined in STOCK 02 (family 001) from Bardney Forest, Lincolnshire. In male no. 023 the ventral hindwing ground colour appeared uniform and the eyspots were reduced to points. Again this is explained as the ripple pattern becoming so fine as to be invisible, with expansion of elements resulting in their fusion with

the eyespot (the remaining pupils still representing some lower value). On the ventral surface the elements are typically ochre rather than black, at first sight belying the fusion. (The latter might also account for the diminished ventral eyespots in assimilated STOCK 01 specimen 01012019 (Plate 9) In male no. 021 the unexpanded wings were of normal size but crumpled, suggesting that the cold interfered with stage 2 of wing development, resulting in a 'dumpy'-type phenotype. The frequency of cold shock phenotypes was similar to that in the STOCK 01 F_1 (1-2 per 20 specimens).

Hybrid STOCK 03, derived from STOCK 01 assimilated and cool-reared male 012018 and STOCK 02 cold shocked female 001015, was then examined. Of the F_1 family 002, cold shocked female 005 had expanded but crumpled wings, suggesting uneven expansion on each surface. The latter phenomenon too points to dorso-ventral compartmentalisation and independence. Yet despite this family being more assimilated (A_i = 0.750) than STOCK 02 family 002 (A_i = 0.000) and nearly as assimilated as STOCK 01 family 012 (A_i = 1.000) the frequency of cold-modified individuals was still only of the order 1-2 per 20. As suggested for the apparent lack of assimilation of prolonged pupal development (*cf.* Chapter Eight), it is possible that heterosis canalised the family against the effects of cold shock and of underlying assimilation.

When the STOCK 03 F_2 were examined, however, it was found that in the further assimilated families 004 and 007 (A_i = 1.750), the majority of specimens had small, misshapen, or crumpled but otherwise fully expanded wings, indicative of pathological development at stage 4. Cold shocked male no. 004008, for example, had an obvious wing shape asymmetry, and the light markings were suffused with darker scaling. The greatly increased frequency of cold-induced modifications over the F_1 might be expected given the further assimilation. However, two further phenomena were apparent among the F_2. The first was that in F_2 family 003 whose parents had *not* been cold shocked, the frequency of modifications resulting from immediate cold treatment was similar to that in the further-assimilated families 004 and 007. As with pupal development times again, it is possible that the level of assimilation in family 002, through its genealogical connection with assimilated STOCK 01 family 012, had been higher than estimated, the greater level of inbreeding in family 003 (F_i = 0.250) than in 002 (F_i = 0.250) beginning to break down the heterosis so allowing the

underlying assimilation to become manifest. The second, which supports this hypothesis, is that in assimilated STOCK 03 F_2 families 004 and 007, wing deformities were prevalent even among the foil treated specimens reared at the warmer temperature (17.6°C): male no. 004002, for example, had crumpled wings (possible expansion discrepancy between the surfaces), while male 03004010 had small forewings (possible reduced cell enlargement in mid-stage 4).

STOCK 06 from Doncaster, Yorkshire was then examined. Although the parental family 001 was unassimilated (A_1 = 0.000) the majority of cold shocked specimens exhibited shape deformities. These tended to involve distortions rather than crumpling, implicating abnormalities of cell enlargement during stage 4.

A number of these specimens did exhibit pattern phenotypic modifications under cold shock. In male no. 027, the eyespot on the dorsal left forewing was diminished and lacked the pupil. Female no. 018 had patches of lighter coloration (each ranging from cream to pale orange yellow) around the umbral region on both wings. Although irregular in shape, these patches were almost identical in position on the dorsal and ventral surfaces, suggesting that they resulted from some obstruction to the veins during pattern determination; the wing shape was essentially normal in this specimen.

Two of the foil-treated specimens also exhibited wing shape abnormalities. In male no. 046 the left forewing was completely unexpanded but otherwise normal. Here, the veins may have become obstructed, although this would have occurred after pattern realisation had been completed. In male no. 047 there were symmetrical indentations along the forewing and hindwing costa on both sides.

Of the four adults comprising assimilated STOCK 06 F_1 family 003, cold shocked female no. 004 had the usual dark brown ground colour replaced by an ochre/light grey brown suffusion over most of both dorsal hindwing surfaces. An assimilated slowing of wing development may have resulted in poorer vein development, so that the value of the pattern determinant at the veins was lower than normal. This may have lowered the supra-threshold values of the reaction-diffusion pattern (elements) so resulting in the paler colour.

STOCK 06 may be adjusted to develop normally under cooler climatic conditions than STOCK 01, so that when reared at similar temperatures, STOCK 06 animals develop faster. This would mean their

wing development being more advanced at the time of cold application, thus accounting for their high incidence of cold shock abnormalities in unassimilated animals.

That in most shape abnormalities the pattern was simply distorted to 'fit' the wing but otherwise normal, strongly supports the hypothesis that shape and pattern are established concurrently. It was also observed that the specimens exhibiting *pattern* modification, whether by itself or together with shape deformity, tended to be rather small. It is speculated that in such individuals, developmental processes in general proceed at a normal rate, but that cell *division and growth* is slower, resulting in a smaller but otherwise normal animal in which the reaction-diffusion domains, therefore, are already effectively tending towards larger relative sizes.

10.2. Lepidopteran wing pattern evolution

Fusion of the ocellar and parafocal elements is the norm for wild type patterns in the Nymphalid genera Boloria, Clossiana and Chlosyne (Argynnini), while the wild type pattern of Vanessa tameamea resembles cold-shock phenotypes of the related Cynthia cardui (Nijhout & Wray, 1986). Thus the Argynnini and C. cardui may exemplify the genetic assimilation of progressive stages in a morphocline. Indeed, the morphoclines within each respective species group show similar ranges of effects suggesting that they do involve a similar process (Nijhout & Wray, 1986).

That each wing comprises four independent compartments, and that changes in the parameters of reaction diffusion can account for the wing space morphocline: (1) longitudinal stripe, (2) midline spots, (3) spots, (4) bands, (5) nigrism, (6) fusion of bands, (7) ripples, (8) irroration and (9) large uniform colour fields, means that that the range of possible patterns that can be *explained* in terms of the parameters of reaction diffusion is much greater than might be expected from simple serial homology among the wing spaces (*cf.* Nijhout, 1981, 1984; Nijhout & Wray, 1986). For example, it could account for iterative homology which describes patterns apparently built up as a mosaic of pattern elements, such as those typical of the Nymphalid genus Limenitis (Nijhout & Wray, 1986). It is interesting to

note, therefore, that the extensive nigrism on the posterior ventral forewing of P. aegeria specimen 01012018 (Plate 9, frame 14A-15), gives this wing surface a striking resemblance to iterative homology. It is thus likely that iterative homology results simply from broadening and fusion of the dark bands to leave rather square regions of paler colour within the wing spaces of the particular compartment(s).

Indeed, the European Satyrid genera Chazara, Pseudochazara, Hipparchia and Neohipparchia produce white forms very similar in appearance to Limenitis, a resemblance perhaps most strikingly illustrated by the Great Banded Grayling, Brintesia circe (see Higgins and Hargreaves, 1983, for illustrations)[1]. With colour fields now understood as mosaics of distinctive venation-dependent patterning in regions of high determinant value (these being the basal costa, basal dorsum and s0); with the fringe and parafocal elements, as well as vein patterns, now understood as resulting from their proximity to the source of reaction-diffusion (which may involve physiological rather than chemical mediation); and with eyespots now understood as a particular manifestation of spot patterning, virtually all known Lepidopteran patterns can be constructed.

The model could also account for the nature of alternative phenotypes, as in seasonal polyphenism. For instance, enlargement of the spot pattern of the spring form (levana) of Araschnia levana would result in their apparently fusing into bands at the veins and in their widening proximo-distally so leaving the intervening orange 'bands' very narrow, while a reduction in the absolute sub-threshold value of the reaction-diffusion that defines these orange band positions, could result in these remaining white (unpigmented) rather than synthesising the usual pigment. The result would be the summer form prorsa which, interestingly, resembles the iterative homology typified by Limenitis (cf. Higgins & Hargreaves, 1983). Of course, the relationship between local field values of the reaction-diffusion might result in qualitative differences, so that pigmentation is not necessarily darkest at the termen. In Nymphalis antiopa, the wing margins are pale yellow, while the remainder of the dorsal surface is dark purple-black except for a row of iridescent blue spots that correspond to the position of border ocelli (*cf.* Fig. 9.2). Since the blue is structural, it is postulated that in this species, the positions of

[1] Similarity in developmental process is a pre-requisite for phenotypic resemblance; which in the case of ecologically-unrelated taxa is known as pseudomimicry (Ho *et al.*, 1986)

'foci' correspond to some maximum while the termen corresponds to some minimum value, a quantitative but *not* qualitative reversal of the situation hypothesised above.

That the cold shock phenotypes of species in a morphocline are more alike than their wild types suggests that the former may be ancestral (Nijhout, 1984). Now cold shock phenotypes are generally characterised by darker and rather ill-defined patterning. Since dark pigmentation and irroration are also associated with slower cell division and enlargement, it is postulated that the Lepidopteran wing arose through increased cell division and enlargement (hence their large relative size), while its scales are derived from primitive hair cells whose coloration is an outcome of their increased development rate. This would corroborate with the postulate that the hindwings might have originated as a bithorax-type conversion of some haltere-like structure, although four (or even six) is generally regarded as the primitive insect wing number, with the Diptera derived *from* primitive Mecoptera-type ancestors (related to Lepidoptera, R.J. White, pers. comm.). However, while the foregoing results do indeed demonstrate that P. aegeria (and other species; sections 9.28 and 9.30) can - under temperature shock at least - effectively 'go on' to develop haltere-like structures, the capacity to produce *Lepidopteran*-type wings in the first place, wings from which halteres might have evolved, would still require explanation.

In Charaxes, the ventral hindwing pattern is the most complex, suggesting that it is the most primitive (Nijhout & Wray, 1986). In P. aegeria, the ventral hindwing too is most complex, displaying most of the Schwanwitsch (1924) pattern elements (Plates 1, 2 and 10). It is suggested that the ventral pattern of both the fore- *and* hindwings represents the ancestral condition, and that this was followed by a general darkening of the forewing, perhaps the origin of Oudemans' phenomenon of the Polygonia type. This darkening may then have spread to the hypothetical P/v compartment, resulting in Oudemans' phenomenon of the Argynnis type. The pattern then became more complex in each compartment giving rise to the present condition. Indeed, the assimilated cool-reared P. aegeria specimen 01012018 exhibits an essentially black dorsal surface and an ochre ventral surface on which the dorsal coloration reappears as Oudemans' phenomenon.

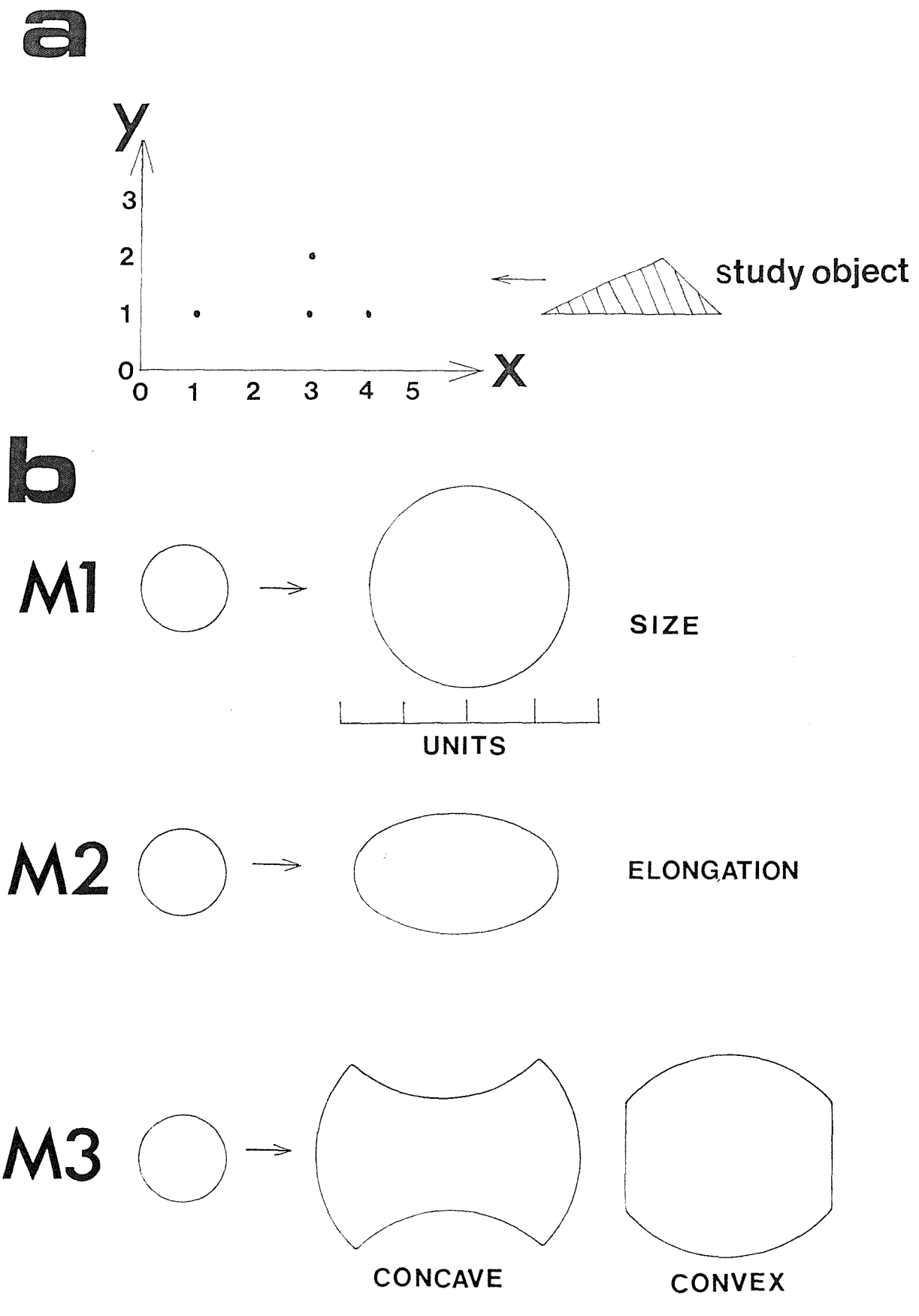
10.3. Introduction to digital pattern analysis

(i) *Overview*

The foregoing investigations of Pararge aegeria wing morphology used manual (microscopic) and photographic methods of analysis. The results discussed in Chapter Nine would suggest that manual analysis, although time-consuming, can reveal pattern differences that would not be recovered by naked-eye inspection. The perusal of specimen photographs, on the other hand, enables the ready detection of larger-scale differences as well as contingent effects that might be overlooked in a statistical analysis. The relevant characters arising from such perusal could, of course, then form the basis of a more detailed statistical study.

Photograpic perusal also revealed variation and abnormalities of wing shape and size. While such differences are readily amenable to non-parametric analyses (for example the G-test to compare, say, the frequencies of 'normal' and 'unexpanded' wings), quantitative descriptions of wing shape and overall size have yet to be furnished. Moreover, Lepidopteran wings may express more subtle variations in size and shape that might pass undetected or be otherwise difficult to classify. Yet statistical descriptors of shape have been furnished for use in identifying aircraft in flight (Dudani *et al.*, 1977), and, more recently, have been applied to biological systems (see White *et al.*, 1988, and references therein). The present investigation explores the use of statistical descriptors to analyse wing shape, with particular reference to the digital analysis of moment invariants.

In Moment Invariant Analysis the shape of the object under study is described in terms of the distribution of Cartesian (x and y) coordinates that coincide with its outline or silhouette (White *et al.*, 1988). Central moments give statistical descriptions of the distributions of such coordinates; the first three central moments give the variance of x, the variance of y, and the covariance of x and y respectively. The computation of the second central moment for a simple shape is shown in Figure 10.1a. The first three moments are called second order moments because the powers of x and y in the variance and covariance formulae total two. Higher order moments also


a
y
3
2
1
0
0 1 2 3 4 5
x
study object
b
M1
SIZE
UNITS
M2
ELONGATION
M3
CONCAVE
CONVEX