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THE ECOLOGICAL COST OF SEXUAL REPRODUCTION

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ABSTRACT

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The aim of this thesis was to investigate ecological conditions under which the genetic variation inherent to sexual reproduction may confer immediate competitive advantages over asexual reproduction. The model species for these tests were the freshwater crustacean *Daphnia*, with seasonally sexual and obligately parthenogenetic forms, and the littoral enchytraeid worm *Lumbricillus lineatus*, which has sexual diploid and pseudogamous triploid reproductive systems.

Laboratory experiments with *Daphnia pulex* during their parthenogenetic phase set genetically diverse and genetically uniform populations in competition for an impure food mix, simulating sexual–asexual coexistence. Genetically diverse populations had significantly higher birth rates in competition with genetically uniform populations than in competition with themselves. This small competitive release was predicted to be sufficient for immediate coexistence of sexual and asexual populations of *Daphnia pulex*. Further laboratory experiments instigated invasions by small and large groups of genetically diverse *Daphnia obtusa* into large genetically uniform populations of the same species, and vice versa. Genetically diverse invaders of initially large group size increased their representation by more than those of initially small size; in contrast, genetically uniform invaders of large group size diminished on average by more than those of small size, supporting the hypothesis that larger genetically diverse groups, with greater genetic variation, had a greater competitive advantage than smaller groups with less genetic variation.

The population dynamics of the pseudogamous *Lumbricillus lineatus* system were investigated by studying life-history characteristics at different starting ratios of triploids to diploids. At lower temperatures and higher food quality, reproductive outputs of triploids and diploids were each inversely proportional to the abundance of the other. This dynamic appeared to favour eventual reversion to diploid-only populations as a result either of diploids out-performing triploids, or of triploids out-performing diploids and crashing in the absence of diploids. Diploid:triploid ratios of *L. lineatus* on Anglesey were 4:1, suggesting that pseudogamy may crash and arise frequently in diploid-only populations. The geographical distribution and habitat requirements of *L. lineatus* were investigated in a series of surveys and were shown to have become more restricted over the last 40 years. *L. lineatus* populations were found only in areas with good drainage of coarse or pebbly sand, on sheltered beaches experiencing little physical disturbance.

The results of the laboratory experiments and field surveys are discussed in the context of current theories about the role of genetic variation in compensating for the costs of investment in male gametes.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

The cost of sexual reproduction has long been a controversial topic in evolutionary biology (Williams 1975; Maynard-Smith 1978), with numerous theories attempting to explain how sex persists in the face of competition by the more efficient mode of asexual reproduction (Fisher 1930; Muller 1932; Michod 1990; reviewed by Bell 1982). Males generally make up half of a sexual population yet contribute nothing to future generations except through females, leading to an immediate two-fold cost in reproductive capacity in comparison to asexual reproduction. Sex is ubiquitous in the natural world, however, despite this immediate disadvantage (Bulmer 1988), with asexual strategies living alongside sexual strategies and apparently posing little threat to the persistence of sex (Case 1990; Radtkey et al. 1995; Vrijenhoek and Pfeiler 1997). There have been many attempts to explain how sex prevails in nature, but the problem remains as open today as it was 30 years ago. The compensating advantages to sex of genetic variation tend to be expressed only over evolutionary timescales. A recent ecological model proposes an explanation for this complicated problem in terms of the competitive impacts of genetically uniform asexual individuals on genetically diverse sexual individuals under conditions of density dependent resource limitation (Doncaster et al. 2000). The new model differs from previous explanations by explicitly recognising the inter-dependency of inter-specific with intra-specific competition.

The aim of this thesis was to measure empirically the competitive advantages of genetic variation that the ecological model predicts to allow coexistence of sexual and asexual reproductive modes under conditions of density-dependent resource limitation (Doncaster et al. 2000; Pound et al. 2002; Doncaster et al. 2003). The freshwater crustacean *Daphnia* and the marine enchytraeid worm *Lumbricillus lineatus* were used as model species to measure biological parameters, investigate the population dynamics after invasion, and calculate competition coefficients in

order to further our understanding of the costs of sexual reproduction in the presence of an invasion threat from asexual modes. This chapter begins by reviewing the theoretical background to the advantages and disadvantages of sexual reproduction and critically assessing the theories of the cost of sex to date. It then discusses competition and population ecology of relevant systems, and introduces the ecological model to be tested in the thesis. Detailed objectives of the study are set out in Section 1.5.

1.2. THE COST OF SEX

1.2.1. Disadvantages of Sexual Reproduction

It has long been known that sexual reproduction carries substantial costs to fitness in comparison to the more efficient method of asexual reproduction (Maynard-Smith 1978). Males generally constitute about half the individuals in any sexual population (or half the investment in gametes for hermaphrodites: Fisher's sex ratio theory), yet these males (or male gametes) only produce further offspring through females (or female gametes), thereby halving the intrinsic growth rate of a sexual lineage relative to a female-only asexual lineage, unless males can provide resources that increase the fecundity of their mates. An asexual lineage has reduced or absent male reproductive effort, freeing up reproductive resources for the production of offspring and leading to an ecological advantage over asexual reproduction (Peck and Waxman 2000). Effectively, the reproductive capacity of a sexual female is half that of an asexual individual, assuming all else is equal. This is known as the two-fold cost of males (Maynard-Smith 1978) and constitutes the cost of sex that is the focus of this thesis. Similarly, the two-fold cost of meiosis describes how a sexual female passes on a haploid instead of a diploid cell, and the parent-offspring relatedness is reduced from 1 in a parthenogenetic female, to 0.5 in a sexual female (Williams 1975). Related to this cost of meiosis is a segregation load from the continual production of homozygotes, when the heterozygotes are the favoured genotype at a particular locus, and recombination load from the break-up of co-adapted gene complexes through chromosomal assortment or crossing-over during meiosis, resulting in the loss of desired characters from the population (Case and Taper 1986; Bulmer 1988). The effect of these costs depends on the

mechanism of sexual reproduction, for example adopting the process of pre-meiotic doubling avoids them. Most types of parthenogenesis, or asexual reproduction, involve the production of unreduced diploid offspring, as copies of the parent, each able to continue this production of identical genotypes. An asexual individual can fix the heterozygote genotype and preserve superior epistatic gene combinations through generations, carrying superior fitness for as long as the environment continues to favour them (Browne and Halanych 1989).

For dioecious species, and also hermaphrodites unless they can self-fertilise, sexual reproduction suffers the cost of searching for a mate. Sexual populations also endure the risk of suffering a loss of fitness due to a sexually transmitted disease (Peck et al. 1997). On the other hand, genetic uniformity can cause vulnerability to epidemics, for example the corn blight of the 1960s in the U.S.A. (Wilson 1999). Even if a species produces a fraction of its offspring sexually and the majority asexually, it has been shown theoretically that populations where sex is rare will produce poorly adapted offspring when heterozygote advantage is in force, as shown in plants, insects, rats and humans (Peck and Waxman 2000). The problem would be less severe if sexual reproduction was more common in the species, and if everyone suffered the same disadvantage. This suggests that the benefits of sex are greatly reduced in populations where sex is rare, and obligate asexuality would be favoured. Another disadvantage of sexual reproduction is that the mixing of cytoplasm when gametes fuse can allow deleterious intra-cellular parasites (also known as deleterious selfish genomes or selfish cytoplasmic elements) to invade other anisogamous individuals in the population (Hurst 1995; Hastings 1999). The cost of such intra-cellular parasites is likely to be quite high. Low levels of paternal leakage observed in many extant organisms may facilitate the spread of these elements, resulting in declines in population fitness. The cost of selfish elements is decreased in sexual life cycles that undergo periods of asexual cellular divisions to impede the spread to selfish agents. Sexual life cycles that involve a large number of asexual cellular divisions avoid much of the cost of selfish elements, impeding the spread of the elements and reducing the loss of fitness attributable to them. *Chlamydomonas* avoid the two-fold cost of sex through isogamy, having evolved a sophisticated mechanism ensuring uni-parental transmission of mitochondria that prevents the invasion of deleterious mitochondrial elements (Hastings 1999).

In the short term, therefore, sexuality seems to have substantial disadvantages over asexuality. Short-term costs are very important to consider because asexual mutants can arise within sexual populations. Sexual and asexual forms of many species live sympatrically, for example snails (sexual and asexual *Potamopyrgus antipodarum*; Jokela et al. 1997), fish (sexual *Poeciliopsis monacha* and *P. lucida* and unisexual hybridogen *P. monacha-lucida*; Vrijenhoek and Pfeiler 1997), geckos (sexual *Lepidodactylus moestus* and parthenogenetic *L. lugubris*; Radtkey et al. 1995) and lizards (sexual Arizona Desert whiptail lizard *Cnemidophorus tigris* and parthenogenetic Sonoran whiptail lizard *Cnemidophorus sonorae*; Case 1990). It is unclear in many cases, however, whether the coexistence is transient or stable (Lynch 1984a). Many theoretical models predict that such associations should lead to the displacement of the sexual lineage by the parthenogenetic form (Maynard-Smith 1978; Bell 1982; Stearns 1987; Michod and Levin 1988). Despite these theoretical predictions, however, sex persists through generations suggesting that there must be compensatory advantages (Table 1.1).

Table 1.1—The short- and long-term advantages and disadvantages of sexual reproduction and the associated hypotheses

	Advantages of sex	Disadvantages of sex
Short-term	Ability to withstand epidemics Heterozygote advantage leads to poorly adapted offspring Competitive release	Sexually transmitted diseases Deleterious intra-cellular parasites Two-fold cost of males Two-fold cost of meiosis Segregation load Recombination load Searching for a mate
Long-term	Adaptive variation in variable environments DNA and genetic repair **Create favourable mutations:** *Frequency-dependent:* Fisher-Muller / Sib Competition models / Red Queen Hypothesis *Density-dependent:* Tangled Bank / Frozen Niche Variation / Lotka-Volterra models **Purge harmful mutations:** Reduce mutational load / avoid Muller's Ratchet	Slow evolution in constant environments

1.2.2. Advantages of Sexual Reproduction

It is widely recognised that the genetic variation inherent to sexual reproduction provides substantial advantages over evolutionary timescales in changing environments. Sex enables genetic and DNA repair, and the resetting of developmental programmes through recombination (the breakage and exchange of DNA between two homologous chromosomes) and crossing over (where two different homologous chromosomes come together in the same cell; Bernstein et al. 1985). Sex can incorporate favourable mutations and resist the accumulation of unfavourable mutations more efficiently, and respond to spatial heterogeneity more effectively (Fisher 1930; Muller 1964; Bell 1982). Despite these clear long-term

benefits to the capacity of sexual lineages to adapt to changing environments, the problem remains of how sex persists, given the significant short-term disadvantages explained in the last section. There are more than 20 theories that attempt to explain how sex persists in the short term, which can be split into two schools of thought: (1) that sex facilitates the creation of successful genotypes by bringing together beneficial mutations; and (2) that sex purges the genome of harmful mutations. These theories of sorting versus repair do not necessarily constitute mutually exclusive mechanisms, however. For example, sex can facilitate the establishment of beneficial mutations by disengaging them from linked deleterious mutations (Zeyl and Bell 1997). The theories could also be divided into 'genetic' and 'ecological' hypotheses. Genetic hypotheses include mutational load and genetic disequilibrium and attempt to explain sex in terms of the advantages of recombination (Lively 1996). The ecological hypotheses include theories of temporal and spatial environmental heterogeneity (Bell 1982; Vrijenhoek 1979; Case and Taper 1986; Doncaster et al. 2000) and attempt to explain the persistence of sex in terms of the adaptive variation in variable environments. A pluralistic approach combines appropriate assumptions of more than one of these theories to explain the cost-of-sex problem (West et al. 1999). The more popular theories are generally based on the notion that sex is maintained because somehow it improves the rate of evolution by natural selection of the species that adopt it.

I will start by briefly discussing what has been termed the 'scandal' of ancient asexual lineages (Judson and Normark 1996). If sex is advantageous in the long term, and asexual lineages are short-lived as a result of adapting too slowly to environmental change or suffering from the accumulation of deleterious mutations (see below), then the existence of very old, fully-asexual taxa defies explanation. Such taxa exist, however, notably in the class Bdelloidea and the ostracod family Darwinulidae (Butlin 2002). Bdelloid rotifers are especially well known for being 80–100 million years old and having undergone no sexual reproduction. Evolutionary consequences of long-term asexual reproduction include the Meselson effect (the independent evolution of two alleles at any one locus in apomictic lineages), allowing evolution in the absence of recombination (Welch and Meselson 2000).

1.2.2.1. The Creation of Beneficial Mutations

Beneficial mutations that arise in different lineages can be united in the same genome over a single generation by recombination to create novel genotypes, which may enable individuals to disperse and colonise new niches (Fisher 1930; Muller 1932; Crow and Kimura 1965; Felsenstein 1974). Clonally reproducing species, in contrast, have a much slower rate of accumulation of beneficial mutations by a process of progressive evolution (Rice 2002). Slow evolution may be the best strategy in constant environments, but not in variable environments. This explanation for the relative benefit of recombination tends to invoke a group selection argument, unless the population is small and subject to strong selection (Crow and Kimura 1965). Group selection can be defined as a process by which a trait spreads or is maintained in a population because of the differential reproduction of genotypes that arises from the positive association of individuals exhibiting the trait (Nunney 1989). The original group selection argument proposed by Fisher (1930) assumes that individuals abandoning sexual reproduction will lead to the extinction of the resulting parthenogenetic clone as a result of its inability to evolve. Nunney (1989) explains how a simple set of general assumptions results in the maintenance of sexual reproduction through the action of group selection, although it cannot suggest how sex evolved initially. Another study investigates the role of group selection in relation to asexual population changes of a boom and bust nature due to chaos (Manning and Goulding 1991). Other theories that lend the short-term advantages of sexual reproduction to the creation of beneficial mutations by recombination include both frequency- and density-dependent hypotheses.

1.2.2.1.1. Frequency-Dependent Selection. These hypotheses, also described as mechanisms of hard selection, whereby poor competitors die, broadly argue that there is an advantage to being genetically different from the majority genotype, or uncommon. Hard selection has been explained as being more conducive to mutation and/or migration in large populations (Hamilton 1993). There is empirical evidence of an evolutionary advantage of sexual reproduction in producing rare or unique genotypes with a frequency-dependent advantage in grasses of the genus *Anthoxanthum* (Antonovics and Ellstrand 1984; Ellstrand and Antonovics 1985; Kelley 1989). Frequency-dependent theories include those which are classed as

models of 'Sib Competition' (Williams 1975; Maynard-Smith 1978; Bulmer 1980 Young 1981; Bell 1982; Price and Vasser 1982), which broadly show how competition between relatives can lead to the best genotype predominating in a particular habitat (Antonovics and Ellstrand 1984). Sexual females within groups can have a competitive advantage because resource utilisation of genetically diverse siblings results in higher mean fitness for the group of siblings (Maynard-Smith 1978; Ellstrand and Antonovics 1985). More importantly, natural selection will favour the genotype that best matches the environmental conditions of the patch, and genetically diverse families have more than one chance of providing a fitter genotype, unlike uniform families of asexual females (Maynard-Smith 1978; Taylor 1979; Bulmer 1980). This specific Sib Competition hypothesis has been termed the 'Lottery Selection model' by Young (1981). However, in order for sexual reproduction to overcome its two-fold cost of sex, the following assumptions must be met: competition must be very severe; numbers of offspring high and all offspring of a given parent assumed to be located within the same patch; patches must differ in quality; and parents must chose patches randomly. This therefore restricts the likelihood of the persistence of sex (Koella 1988). The theory of Sib Competition was supported by looking at chiasma frequencies and recombination indices of plants, suggesting that recombination is maintained so that the intensity of competition with sibs is reduced (Koella 1993). A similar model was tested in a series of experiments using uniform and genetically diverse half-sib progeny of the grass *Anthoxanthum odoratum*. The observed advantage for genetically diverse individuals fit the assumptions of the model, which assumed that biotic interactions would result in truncation selection (Kelley 1989).

The 'Red Queen hypothesis' is also a frequency-dependent theory of the short-term advantage of sex (Van Valen 1973), getting its name from the chess piece in Lewis Carroll's 'Through The Looking Glass' that takes Alice on a dream-like chase where she finds that no matter how fast she runs, she cannot get anywhere; it is as though the world runs along with her. The theory comprises the idea that evolution is an arms race between species constantly co-evolving to attempt to 'out-run' their competitor, or predator. The evolution of any ecological improvement in one species results in a deterioration in the habitat of species that interact with it, giving rise to selection for counter-adaptations in these species. Thus an individual's

fitness is continually deteriorating with respect to its biotic environment, and it must continually evolve new adaptations to keep pace with the change. The prime example of this is parasite- or pathogen-induced selection pressure (Hamilton 1980). Parasites increase mortality and so can be strong agents of selection, and they evolve much faster than their hosts and so are quick to catch up in an evolutionary arms race. Parasites adapt to the most common host genotypes in order to be maintained in the population, so evolution will favour rare combinations of resistant genes (Morell 1998). The model simulates gene matching between host and parasite and demonstrates a negative frequency dependence in which rare genes become favourable once the parasite has matched to common genes (Hamilton 1980; Bell 1982). Selection therefore favours genetic diversity. The new genotypes arising by mutation and the non-instantaneous reaction of parasites to the genetic changes in their host population results in a time-lagged response (Lively 1996).

Sexual populations gain a relative advantage over asexual populations by having the capability to rearrange the genotype of their offspring, by segregation and recombination. They are therefore able to maintain polymorphisms and rare genotypes among their descendants. The essence of the parasite theory is that sex stores temporarily bad alleles and does not eliminate them. In this respect it contrasts to the mutation theory of Muller's Ratchet (see Section 1.2.2.1.2), that sex facilitates the elimination of unequivocally bad alleles.

The Red Queen hypothesis assumes that the parasites or pathogens have genetic variation for infectivity or virulence and that the host population has genetic variation for resistance to specific strains or parasites (Van Valen 1973; Moritz et al. 1991). Further assumptions are that the parasites have significantly shorter generation times than the hosts (Stearns 1985; Seger and Hamilton 1988) and that infection by the parasites reduces the fitness of individual hosts. This latter assumption has been supported in captive lizards (Moritz et al. 1991), barn swallows (Moller 1990) and freshwater snails (Lively 1996). Isozyme analysis was compared with incidence of parasitic trematode infestations of sexual and asexual variants of the dioecious freshwater snail *Potamopyrgus antipodarum*. It was found

that where there were fewer parasites, snail populations tended to be asexual and where there were more parasites, snails were mostly sexual (Lively 1996).

Support for the Red Queen hypothesis comes from studies of parthenogenetic geckos of the *Heteronotia binoei* complex, which were found to have more haematophagus mites than their sympatric sexual relatives (Moritz et al. 1991); and from non-inbred sexual populations of topminnows, which were found to have lower levels of trematode infestations than sympatric clonal lineages (Lively et al. 1990). A similar effect has been found in plants, with genetically uniform crop cultivars being highly prone to infection by pathogens (Levin 1975). Cereal monocultures are notoriously vulnerable to attack by evolving, clone-specific, fungal diseases than are genetically mixed cultures (Brown 1994). Furthermore, virulence of viruses was suggested to be higher in human populations with low genetic diversity at their major histocompatibility complex than in major histocompatibility complex-diverse populations (Black 1992). Parasite co-evolution has been described as superior to previous models of the maintenance of sex because it applies to populations with very low fecundity and broadly overlapping generations, such as in humans. Also it involves realistic patterns of genotype fitness and changing environment and allows frequent mutation to parthenogenesis, even while sex is paying its two-fold cost (Hamilton et al. 1990).

Problems with the Red Queen theory include the observation that parasites often do not kill or even sterilise their hosts, so they would be unlikely to prevent the replacement of a sexual population by a clone with a two-fold advantage. Also parasites do not select for sexual reproduction, *per se*, but for genetic diversity, allowing a sufficiently diverse set of clones to replace a sexual population, and rare advantage can lead to the accumulation of clonal diversity (Lively and Howard 1994).

A sperm-dependent parthenogen, referred to in animals as a pseudogamous parthenogen, is an asexually reproducing form of a species that requires the sperm of its sexual sibling in order to activate embryogenesis in the unreduced ova, without assimilating any genetic material from the donor sperm (see Chapter 5 for review). Inheritance has been described as clonal and matrilineal (Beukeboom and

Vrijenhoek 1998), and although some studies have suggested a degree of paternal inheritance (for example, Beukeboom et al. 1996a), it is commonly agreed that the sexual hosts 'waste' sperm on their pseudogamous counterparts. Pseudogamy is therefore a type of reproductive parasitism and may be maintained by some mechanism of host–parasite co-evolution consistent with the Red Queen hypothesis. Sperm-dependent parthenogenesis is seen in plants, invertebrates, molluscs, fish and reptiles (Nygren 1954; Case 1990; Vrijenhoek and Pfeiler 1997; Weinzierl et al. 1999; Calame and Felber 2000; Qi et al. 2000; Felip et al. 2001). This special host–parasite relationship is further investigated in the review of Chapter 5 and the experimental Chapters 6 and 7 that look specifically at the biology of the pseudogamous enchytraeid worm *Lumbricillus lineatus*.

'Synergistic Epistasis' is a term used when the mean fitness of sexual populations exceed that of asexual populations because each additional deleterious mutation causes a greater loss of fitness in genomes that already carry greater numbers of deleterious mutations (Kondrashov 1988; Zeyl and Bell 1997). Evidence for the effects of Synergistic Epistasis is available when fitness declines more rapidly with number of deleterious mutations than predicted by a linear decline, and has been demonstrated through studies with *Daphnia magna* (Salathe and Ebert 2003). Synergistic Epistasis is similar in concept to the variation on the Red Queen hypothesis known as rank-order truncation selection (see Section 1.2.2.3), supported in *Chlamydomonas moewusii* (de Visser et al. 1996).

1.2.2.1.2. Density-Dependent Selection. These hypotheses, also known as mechanisms of soft (truncation) selection, whereby individuals are poor competitors for resources, argue that sex is advantageous because competing genetically diverse siblings have different resource requirements and so a greater fitness than competing genetically uniform progeny. This type of selection is more conducive to mutation and/or migration in small populations (Hamilton 1993). A specific example of such a hypothesis is the 'Elbow Room model' of Sib Competition, which predicts that sexually-reproducing parents can have a fitness advantage over asexually-reproducing parents if competition within their diverse, sexually-produced offspring is less intense than that within the more homogeneous, asexually-produced offspring (Young 1981). This model was unsupported by

experiments with *Tribolium* (Garcia and Toro 1992), *Anthoxanthum* (Ellstrand and Antonovics 1985) and *Impatiens capensis* seeds (Schmitt and Ehrardt 1987). The 'Vicar of Bray' hypothesis (Bell 1982) assumes that sex is beneficial due to its ability to create favourable genotypes by recombination, but differs slightly from other density-dependent theories in that it is a group selection argument, rather than one of individual selection. The theory suggests that sex evolved because it infers a benefit on the group, leading to a faster rate of evolution.

The 'Tangled Bank' hypothesis gets it name from the closing paragraphs of *The Origin of the Species* in which Darwin contemplates a roadside bank with its tangle of vegetation, the invertebrates that live amongst it and the birds that feed from them. He remarks on the variation in forms and complex inter-dependencies that permit such a diversity of life (Darwin 1859). This led to the theory of how niche differentiation can allow sexually reproducing types to persist in the face of invasion by asexual clones (Ghiselin 1974; Bell 1982). Specifically, the theory states that in a heterogeneous environment there exist several niches even at a local scale, and so the uniform progeny of an asexual female will compete intensely for space and resources within the same niche (Williams 1975). In contrast, the genetically diverse progeny of a sexual female will reduce competition by exploiting a wider range of niches (Bell 1985). The theory is density-dependent, being less effective at higher population numbers, and no time lags are expected, as in the Red Queen hypothesis (Lively 1996).

The assumptions of the Tangled Bank hypothesis are that resource utilisation and fitness of a genotype depend on the environment in which the genotype is raised; there must be small within-phenotype niche width and a high intrinsic rate of increase. These assumptions have been supported by the model of Koella (1988), an extension of the simple Lotka-Volterra model of density-dependent population growth that describes competitive interactions among phenotypes for a limiting amount of resources (Smouse 1976). This extended model showed that non-equilibrium effects such as those found in changing environments (Bell 1982) should be considered too, in order for competition to explain the maintenance of sex in nature. The concept of the Tangled Bank has only been tested theoretically through simulations by Bell (1982) and Case and Taper (1986), however. Bell

(1982) concluded that sex can be maintained in non-equilibrium conditions, but required changing heterogeneous and coarse-grained environments, and random extinction of resources (Koella 1988; Gaggiotti 1994). The simulation of Case and Taper (1986) allowed sexual populations to out-compete asexual populations even in a fine-grained environment, and considering only populations in equilibrium. Empirical tests of the Tangled Bank model largely failed to show a sufficient increase in Sib Competition within asexual populations to match the two-fold cost of sex (Pound et al. 2002).

The simulation of Case and Taper (1986) is consistent with a slightly different theory: the 'Frozen Niche Variation' hypothesis (Van Valen 1973; Vrijenhoek 1979). This theory specifically explains how each asexual clone has a genotype 'frozen' to that of the sexual individual from which it arose, leading to selection among clones for specialised genotypes having minimal niche overlap with established clones and the sexual ancestors (Jokela et al. 1997). This process generates a limited number of clones that coexist on a local scale with the sexual ancestors, as long as the cumulative niche of the clones does not eclipse the niche of the sexual ancestors. The theory is different to the Tangled Bank, where the dynamic influences on coexistence are solely intra-specific. The Frozen Niche Variation hypothesis is supported by studies that show that asexual clones often represent a limited sample of the genetic variation in the sexual population, constraining the phenotype of each clone to a narrower ecological niche (Vrijenhoek 1979; Honeycutt and Wilkinson 1989; Semlitsch et al. 1997). The theory has been implicated in many studies of coexistence between sexual and asexual forms of a species (Case 1990; Christensen et al. 1992; Barata et al. 1996; Fox et al. 1996; Vrijenhoek and Pfeiler 1997; Negovetic et al. 2001). The outcome of an asexual invasion under this hypothesis depends on the interactions between sexual and asexual competitors (Doncaster et al. 2000). The theory may not be sufficient to allow repeated invasion by asexual mutants (Weeks 1993), but it can significantly influence the course of an asexual invasion (Pound et al. 2002).

Doncaster et al. (2000) developed a Lotka-Volterra model for the coexistence of sexual and asexual reproduction, based on ideas from both the Tangled Bank theory and the Frozen Niche Variation hypothesis (see Section 1.3 below for the model).

Their model investigates competitive interactions in heterogeneous environments and shows that invading asexual individuals may have more of an adverse competitive effect on members of their own population than they will have on those of a competing sexual population. This is due to their uniformity and restricted niche widths. The model differs from previous ecological models of costs of sex in recognising that density-dependent intra-specific competition reduces the differences in reproductive output of sexual and asexual competitors well below the two-fold difference in intrinsic reproductive capacity (the intrinsic two-fold cost of sexual individuals). The small realised differences in reproductive output therefore require a much less than two-fold advantage in competition with asexual individuals to permit coexistence (Pound et al. 2002). Sexual populations may therefore balance their two-fold disadvantage in growth capacity (due to the presence of males) by competing inter-specifically just slightly more effectively for limited resources. Asexual populations may continue to invade and compete with sexual individuals without ever succeeding in eliminating them. The two-fold cost of sex is often used as a measure of the amount by which sex must benefit from its evolutionary advantages of greater genetic variation (Hamilton et al. 1990; Kondrashov 1993; Hurst and Peck 1996), but this ignores the fact that competing species can coexist by trading large differences in growth capacity against small differences in competitive ability (Nee and May 1992). The previous models based on Sib Competition (Ghiselin 1974; Bell 1982; Burt and Bell 1987) or character displacement (i.e., Frozen Niche hypothesis; Case and Taper 1986), do not calibrate Sib Competition against inter-specific competition either in theory or in empirical tests (Pound et al. 2002).

1.2.2.2. The Purging of Deleterious Mutations

Mutational load is defined as the reduction of fitness of a population due to accumulation of deleterious mutations (Rice 2002). Populations that employ meiosis or self-fertilisation, thus involving recombination, have the ability to reduce this mutational load (Kondrashov 1984) and should therefore be able to out-compete asexual lineages, all else being equal. This is supported in calculations of load of mildly deleterious mutations in sexually and asexually reproducing species (Dickson and Manning 1984). However, the cycles of adaptation and counter-

adaptation between males and females and the variation in fitness between the sexes in a sexual population reduce female productivity and therefore competitive ability of sexual populations (Rice 2002).

'Muller's Ratchet' is a theoretical explanation of the maintenance of sex that focuses on the genetic deterioration caused by deleterious mutations that are accumulated by genetic drift in small, asexual populations with high deleterious mutation rates (Muller 1964; Manning and Thompson 1984; Kondrashov 1988; Chao 1997). Since most mutations are deleterious, this effect of intensified genetic drift increases the rate of genetic fixation or extinction, and will lead to a decrease in population fitness (Chao 1997). This leads to the loss of mutation-free individuals in species with a high mutation rate. Each new mutation reduces the fitness of the population as a whole and if the population is haploid asexual, the loss is irreversible and mutations increase in a ratchet-like manner. No asexual individual can produce progeny with fewer mutations than it has itself. Back mutations can occur to recreate a mutation-free genome, but at a much slower rate than that of forward mutations and only occurring at sites on the genome that will restore the original genome. A forward mutation can occur at any site of the genome (Chao 1997). The probability of a mistake by chance alone is therefore much higher than the probability of an appropriate correction.

This process of Muller's Ratchet has been shown to occur in RNA viruses, which have high mutation rates and probably experience genetic drift when their populations are forced through bottlenecks during infection (Chao 1990; Chao 1997). Support was also found in yeast *Saccharomyces cerevisiae*, where sex increased the mean fitness in an environment to which the populations were well adapted but not in one in which a new adaptation occurred (Zeyl and Bell 1997). Sex can be advantageous because it can make up for the low back-mutation rate and escape the grasp of Muller's Ratchet by reconstructing a mutation-free genome through recombination or chromosome re-assortment (Chao 1997). This avoidance of Muller's Ratchet may occur by the facilitation of the repair of such mutations, or by the decrease in frequency of deleterious mutations that would otherwise arise within the population, in a culling effect (Hurst and Peck 1996). Peck (1996) presents a mathematical model that shows that when the frequency of deleterious

mutations in the population increases, dispersal of offspring is limited and so the equilibrium level of genetic load increases. Likewise, as sex involves the dispersal of genetic material (such as the movement of pollen from one site to another), it also enhances competition among adults. Sexual reproduction can therefore lead to a decrease in the equilibrium frequency of deleterious mutations. The mechanism does not involve genetic recombination. Some studies that have purported to demonstrate an avoidance of Muller's Ratchet have been disputed, however. For example, a study of the Amazon molly *Poecilia formosa* was criticised for showing no evidence for the replacement of deleterious mutations with favourable ones and for the potential paternal leakage into asexual strains (Beukeboom et al. 1995).

There are several limitations to the theory of Muller's Ratchet. First, it assumes that all mutations are deleterious. This is unrealistic in finite sexual and asexual populations as it suggests that either mean fitness will decline forever or the species will become extinct (Peck et al. 1999). Fisher's similar model does allow for beneficial mutations, however (Fisher 1930). Also, lethal mutations are eliminated in a single generation and hence do not accumulate. Second, it is unlikely that Muller's Ratchet could operate fast enough to provide a short-term advantage to sexual reproduction (Lively 1996) and it cannot explain the well-known distribution patterns of parthenogenetic plants and animals (Bell 1982). There are also several ways in which Muller's Ratchet can be stopped or avoided. If the mutation rate per locus is low, or if the number of genes in the genome is small, the mutation rate per genome will be reduced (Judson and Normark 1996). If synergistic epistasis selection pressures are sufficiently strong (Kondrashov 1994), Muller's Ratchet might be stopped and an asexual population could persist indefinitely long. Furthermore, the process assumes haploidy, but increasing ploidy of most asexual individuals slows the process because most mutations are recessive (Mogie and Ford 1988); and homogeneity of the environment, but in heterogeneous environments elimination of compound mutants may be slowed. Last, most deleterious mutations are produced in the male germ line, so male gametes may give progeny more mutations than the associated sexual recombination manages to eliminate and asex will therefore have fewer deleterious mutations to accumulate (Redfield 1994).

The 'Mutation-Load-Reduction' hypothesis (Crow 1994) works on the same principles as Muller's Ratchet, but is irrespective of population size allowing it work on larger populations that are not subject to such intensified genetic drift and avoid Muller's Ratchet. Sexual populations are able to remove more mutations over time than even large populations of asexual individuals, and so could reduce their mutation load quicker. If the genomic mutation rate is as much as only one mutation per generation, some mechanism to reduce the load is necessary (Judson and Normark 1996).

1.2.2.3. Pluralism

A third school of thought has been developed by those who believe that different mechanisms from the former two categories may act simultaneously or even synergistically, to provide sex with strong advantages in the face of asex (Hurst and Peck 1996). There are advantages to such pluralistic methods, as multiple selection pressures are common and factors that maintain sexual reproduction may not be the same as those that led to its evolution. Furthermore, different mechanisms may be working at different levels within the same species, for example clonal existence and maintenance of sex across niches are quite different issues. A pluralistic approach allows empirical work to estimate the relative importance of various mechanisms instead of eliminating any theories (West et al. 1999). Theoretical modelling has shown that a synergy between the Frozen Niche Variation hypothesis and mutation accumulation (Muller's Ratchet) may provide a stronger explanation for the ubiquity of sex in nature than either theory alone (Pound et al. 2004 in press).

A further study has shown how an interaction between host–parasite co-evolution (the Red Queen) and mutation accumulation can give sexual populations stability under repeated invasions by asexual mutants, overcoming the theoretical difficulties of the Red Queen hypothesis alone (Lively 1996; Howard and Lively 2002). Parasites prevent the fixation of clones in the short-term, and increase the rate at which clones accumulate mutations through the action of Muller's Ratchet by periodically depressing their population size (Howard and Lively 1994). Indeed, mutation accumulation may become less important as the number of loci increases

and if exposure values are low, as this combination increases the risk of clonal extinction (Howard and Lively 2002).

Empirical evidence for the pluralistic approach comes from data on plants. The greater fitness of sexual progeny of the grass *Anthoxanthum odouratum* compared to asexual progeny can be explained using a synergism of mutation accumulation and environmental mechanisms (Kelley et al. 1988). A theory combining the soft selection of the Tangled Bank model and the frequency-dependent selection of the Red Queen theory suggests a parasite-mediated truncation selection against the most-infected individuals, even if the direct effects of infection are minor (Hamilton et al. 1990). This rank-order truncation selection, similar to Synergistic Epistasis, also prevents the accumulation of clonal diversity.

Michiels et al. (1999) support this pluralistic approach and suggest the addition of individual as well as population-level processes. Such processes might include the extent of paternal care and the strength of sexual selection (Trivers 1972). They term this additional factor 'individual quality-control' and explain how such mechanisms may be manifested at very basic levels, such as spermatogenesis or sperm-egg interactions, therefore being likely to apply to any given system (Michiels et al. 1999).

However, evolutionary ecologists continue to argue about the mechanism of how sexual reproduction persists in the short-term when in competition with asexual reproduction. Dunbrack et al. (1995) failed to support their theoretical hypothesis that sex would be out-competed when they experimentally tested the competitive abilities of sexual and asexual forms of the red flour beetle, *Tribolium castaneum*. Rapid evolution enabled the elimination of an invading asexual competitor, despite its two-fold advantage. There must, therefore, be a short-term advantage that at least balances out the clear advantages of asexual reproduction to enable sexual individuals to coexist with arising asexual mutants. The next section describes in greater detail the model of Doncaster et al. (2000), proposed to counter the theoretical drawbacks to the explanations of the persistence of sex so far discussed in this chapter.

1.3. ECOLOGY OF COMPETITION

Competition can be defined as the interaction between individuals in which one organism has a negative effect on another by consuming, or controlling access to, a resource that is limited in availability (Keddy 1989). The negative effects are ultimately expressed in terms of fitness reduction through fecundity or mortality and proximal measures of fitness are obtained by investigating resource use, somatic growth, survival and reproductive output (MacNally 1983). Competition can occur through a variety of mechanisms, broadly grouped into exploitation and interference. Exploitation competition occurs when other individuals reduce the availability to an individual of resources such as space, food, water or mates as a result of the use of those resources. Each individual's consumption of resource can be described entirely in terms of resource density, although density of resources may be itself a function of consumer density. Exploitation competition thus produces impacts on each individual's resource use that are not directly dependent on the density of its own population (Lessells 1995; Doncaster 1999). In addition to exploitation competition, individuals may interfere directly with each other's access to the resources, by inflicting direct harm or by excluding them physically or behaviourally (Keddy 1989). Each consumer's intake of resource is then a direct function of consumer density as well as resource density. Interference competition thus produces impacts on per capita resource use that increase with the density of the population (Lessells 1995; Doncaster 1999).

In a simple environment where a resource such as food is present in limited supply, one species will always eliminate or displace another species with identical requirements, an outcome that is known as the principle of competitive exclusion (Gause 1934). Essentially, if one species' niche completely overlaps that of the other species in all its dimensions and all of the time, then the first species to arrive will eliminate the second. The stable coexistence of two species is only possible in the presence of some degree of resource partitioning of habitat, food or behaviour, such that the two niches do not overlap completely. The two species may use identical resources but assimilate them at different rates (Nee and May 1992; Doncaster et al. 2003) or at different times (Kelly and Bowler 2002). A particular type of resource partitioning, known as character displacement, involves

modification of the morphological or ecological features of a species as a result of competition. This can occur on a spatial niche axis, for example, the size of two closely related species may be very similar when in isolation from each other, but one may grow significantly bigger than the other when living in sympatry; or, similarly, temporal niche differences can result in character displacement of otherwise sympatric organisms. The occurrence of competitive resource partitioning can be determined by comparing the extent of niche overlap of the two species in sympatry to the overlap in allopatry. Indeed, the true effects of competition on species are revealed when one species is removed from the equation and the other expands its niche, known as competitive release. However, there may be instances where competition is important, but competitive release is not possible. It should be noted that competitive interactions are often asymmetrical, even in the presence of equal mutual impacts, due to age-, size- or species-specific differences in competitive ability (Keddy 1989).

Competitive interactions can be classed as intra- or inter-specific. Intra-specific competition is that between individuals of the same species for resources in short supply and can result in a decrease in the contribution of the individual to the next generation compared to its potential contribution had there been no competitor. This natural force limiting the growth of animal and plant populations has been described by Charles Darwin as a crucial process driving evolution through natural selection (Darwin 1859). As population density increases, individual fitness and individual and population growth rate decline in a negatively density-dependent manner leading to fast population growth at low densities and slow population growth at high densities. Intra-specific competition is often stated to involve reciprocity, assuming that all the individuals involved are equal and have the same effect on each other. However, this is not always the case, as weaker or younger individuals may be out-competed by older or stronger ones.

Inter-specific competition is the negative effect of individuals of different species that share a common resource in limited supply, and is commonly represented by the Lotka-Volterra predator–prey equations (Volterra 1926; Lotka 1932). These equations generally employ a logistic model of population growth under intra-specific competition in order to analyse outcomes of competition between species

with more or less overlapping niches. Niche overlap is expressed by competition coefficients α_{ij} to measure the per capita competitive effect of individuals of species j on those of species i relative to the per capita effect on individuals of its own species. The model that is to be tested in this thesis (Doncaster et al. 2000) investigates these impacts between species relative to the within-species impacts under density-dependent population growth, defining sexually and asexually reproducing species as Species 1 and 2 respectively. The remainder of this chapter sets out the theory of the model, and explains how it is to be tested empirically.

1.4. THE ECOLOGICAL MODEL OF THE COST OF SEX

In the absence of any restraining influences, the growth rate of a population of self-replicating individuals is proportional to the existing population size:

$$\frac{dN_1}{dt} = r_1 N_1 \qquad (1)$$

where N_1 is population size of Species 1, and r_1 is its intrinsic net replacement rate. For positive r_1, this constantly increasing population growth with density describes an exponential increase over time, since integrating equation (1) over time yields the prediction: $N_1(t) = N_1(0)e^{rt}$. A population with this dynamic has a constant doubling time regardless of its current size.

It is safe to assume, however, that a natural habitat will impose limitations in resource supply that set an upper limit to the population size, known as the carrying capacity, k (a constant in the simplest models). We can represent population growth under density-dependent regulation most parsimoniously with a logistic model:

$$\frac{dN_1}{dt} = r_1 N_1 \left(\frac{k_1 - N_1}{k_1} \right). \qquad (2)$$

This equation is the simplest model of density-dependent growth, in the sense that it describes a linear decline in net population growth per capita, from an intrinsic

rate r in a virgin habitat to zero at the carrying capacity k. If population size is very small, then the population will have a dynamic close to that of equation (1), and if the population size is large enough for N to approach k, its growth rate will be close to zero.

If a second species is present and competing with Species 1 for a limiting resource, each individual of Species 2 will consume the resource at some fraction, α_{12}, of the rate at which Species 1 consumes it. The competition coefficient α_{12} therefore represents the per capita effect of Species 2 on Species 1, calibrated against the impact of Species 1 on itself (usually taking values between 0 and unity). Likewise, α_{21} is the per capita effect of Species 1 on Species 2 relative to Species 2 on itself. By definition, each species' coefficient of competition upon itself (i.e., α_{11} and α_{22}) is unity. The resources available to Species 1 are therefore a function of the number of individuals of Species 1 present and the resources used up by the individuals of Species 2. The following inferences should be noted: if $\alpha_{12} = 0$, Species 2 does not affect Species 1; if $\alpha_{12} = 1$, Species 2 consumes the resource at the same per capita rate as Species 1; and if $\alpha_{12} = >1$, Species 2 has a greater impact on Species 1 than Species 1 has on itself. The effect on the population growth rate of each species by the other can be incorporated into equation (2). It is shown here with its reciprocal equation, together comprising the Lotka-Volterra model for exploitation competition:

$$\frac{dN_1}{dt} = r_1 N_1 \left(\frac{k_1 - N_1 - \alpha_{12} N_2}{k_1} \right) \qquad \frac{dN_2}{dt} = r_2 N_2 \left(\frac{k_2 - N_2 - \alpha_{21} N_1}{k_2} \right). \tag{3}$$

These classical equations for rates of change in numbers over time $\dot{N}_1$ and $\dot{N}_2$ can be extended to distinguish intrinsic rates of death d_i from birth b_i:

$$\dot{N}_1 = b_1 \left[1 - \frac{N_1 + \alpha_{12} N_2}{k_1} \right] N_1 - d_1 N_1 \qquad \dot{N}_2 = b_2 \left[1 - \frac{N_2 + \alpha_{21} N_1}{k_2} \right] N_2 - d_2 N_2. \tag{4}$$

The carrying capacity of each population (k_i) is measured in isolation from the other and before deaths. With deaths, the carrying capacity of Species i is obtained by setting $dN_i/dt = 0$ and $N_j = 0$, giving $K_i = k_i(1 - d_i/b_i)$. Thus the population carrying capacity K_i has an extrinsic (environmental) component, given by k_i, and an intrinsic (heritable) component, which is a function of the lifetime reproductive output: b_i/d_i. To picture the two-species interaction introduced above, we can use 'zero isoclines' to represent equilibrium states. A zero isocline for a population is derived by setting the population growth rate to zero, giving all possibles sets of conditions for its equilibrium size. With $dN_1/dt = 0$ in equation (4), Species 1 is now in equilibrium, showing no change in growth rate. The growth rate will be zero if either net replacement rate (r_1) is zero, the number in the population (N_1) is zero, or in the less trivial situation of positive r_1, N_1:

$$K_1 - N_1^* - \alpha_{12}N_2 = 0 \quad \text{or} \quad N_1^* = K_1 - \alpha_{12}N_2 \qquad (5)$$

where $K_1 = k_1(1 - d_1/b_1)$. The asterisk indicates no change over time in the number of individuals of Species 1. It is now possible to solve for the intercepts. By setting N_2 to zero in equation (5) we obtain the N_1-axis intercept at: $N_1 = K_1$. Similarly, we set N_1 to zero to obtain the N_2-axis intercept at: $N_2 = K_1/\alpha_{12}$. Equation (5) defines a straight line joining these values for the two intercepts (Figure 1.1).

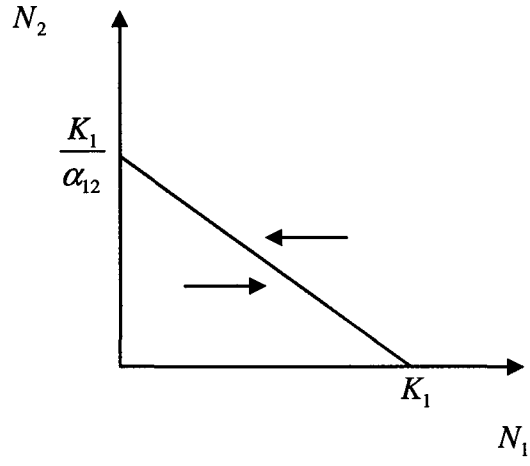


Figure 1.1—Isocline for the population of Species 1, showing all possible combinations of N_1 and N_2 where the growth of N_1 is zero. The arrows show the direction of population change for values of N_1, N_2 lying away from the isocline

Above the zero isocline for Species 1, the population has exceeded carrying capacity and so must decline in size. Below the isocline, the number in the population can increase towards carrying capacity. Using this graph, we are able to explore how N_1 will change under all possible combinations of different sizes of the two populations of Species 1 and Species 2.

To explore this interaction at the equilibrium population size for Species 2, we must also plot the zero isocline of N_2. Setting dN_2/dt to zero in equation (4) yields:

$$K_2 - N_2{}^* - \alpha_{21} N_1 = 0 \quad \text{or} \quad N_2{}^* = K_2 - \alpha_{21} N_1 . \qquad (6)$$

$N_2 = K_2$ when $N_1 = 0$ and $N_1 = K_2/\alpha_{21}$ when $N_2 = 0$. The numbers of each species present at the point of stable equilibrium is the point where the two straight-line isoclines cross, at (putting equation (6) into (5) and (5) into (6)):

$$N_1{}^* = \frac{K_1 - \alpha_{12} K_2}{1 - \alpha_{12}\alpha_{21}}, \quad N_2{}^* = \frac{K_2 - \alpha_{21} K_1}{1 - \alpha_{12}\alpha_{21}} . \qquad (7)$$

Using these lines and their point of intersection we can plot the four possible outcomes of this inter-specific competitive interaction. These outcomes are displayed in the graphs of Figure 1.2.

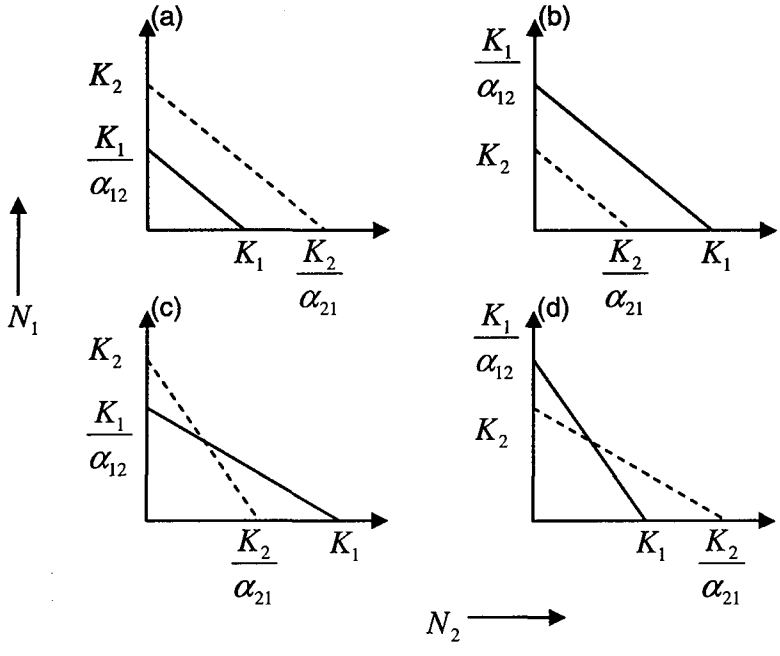


Figure 1.2—Four possible arrangements of the isoclines of Species 1 and Species 2 (taken from Keddy 1989). (a) Competitive exclusion of Species 1; (b) competitive exclusion of Species 2; (c) exclusion of one species or the other depending on starting densities; and (d) equilibrium coexistence

Only one species persists in three of the four cases illustrated by Figure 1.2. This species is classed as the 'dominant' and in the first two cases it is the one with its isocline furthest from the origin. In the third case, the winner is the species with the highest initial density. In the fourth case, the two species coexist, because each has more of a negative effect on members of its own population than on those of the other. In effect, $K_i < K_j/\alpha_{ji}$. This is the case that gives positive values for both N_1^* and N_2^* in equation (7).

It is the distinction of independent birth and death terms in equation (4) that allows us to model explicitly the dynamics of a sexual Species 1 competing with an asexual Species 2 that has twice the intrinsic birth rate. Because of this intrinsic difference, Species 2 also has a larger K, though not necessarily twice as large: see Pound et al. (2002) correcting the assumptions of earlier models including those of Case and Taper (1986), Gaggiotti (1994) and Koella (1993). In effect, we confer an identical intrinsic per capita birth rate on sexual females as on asexual individuals, and a zero birth rate on males which are deemed to constitute half the sexual population, so $b_1 = 0.5b_2$. If the two types differ only in this respect, we can expect $d_1 = d_2$, and $k_1 = k_2$.

Setting both rate equations (4) to zero yields equilibrium solutions N_1^* and N_2^* given by equations (5) and (6). The conditions for invasion by each species are then:

$$\begin{aligned} N_1^* > 0 \text{ if : } \quad & \alpha_{12}K_2 < K_1 \quad \text{i.e., if : } \quad \alpha_{12}(1 - d_2/b_2) < 1 - d_1/b_1 \quad \text{assuming } k_1 = k_2, \\ N_2^* > 0 \text{ if : } \quad & \alpha_{21}K_1 < K_2 \quad \text{i.e., if : } \quad \alpha_{21}(1 - d_1/b_1) < 1 - d_2/b_2 \quad \text{assuming } k_1 = k_2 . \end{aligned} \tag{8}$$

For a sexual Species 1 with fast growth capacity, given by high b_1/d_1 (albeit half the value of b_2/d_2 for the asexual competitor Species 2), coexistence with a single clone is possible even with α_{12} close to unity (Doncaster et al. 2000). This is an expression of the trade-off between competitive impact and intrinsic growth that is

a well-known ecological principle permitting coexistence of slow growing but strong competitors with faster growing species (Nee and May 1992; Doncaster et al. 2003). In the particular case of competitive advantages to the sexual population brought about by its having access to a wider resource base than any one clone, niche breadth has a complicated relation to b_i and K_i in equations (4). Versions of equations (4) expanded for niche breadth, nevertheless, demonstrate how a sexual population will withstand multiple clonal invasions, provided it retains at least a small resource refuge from the clones (Pound et al. 2002). This is because clonal diversity tends to be associated with intense competition amongst the clones, with the result that they can prevent each other from establishing more effectively than they inhibit coexistence with the sexual population.

This adaptation of the Lotka-Volterra model differs categorically from Tangled Bank models of Sib Competition (Bell 1982), which consider influences of genetic variation on the intensity of competition amongst sexual siblings compared to competition amongst identical asexual siblings. Under Sib Competition, advantages of sex are inferred from observing a higher growth rate for sexual populations with lower competition amongst siblings. In contrast, the Lotka-Volterra dynamics consider how genetic variation in a sexual population influences the impact of asexual on sexual competitors, relative to the impact that the sexual individuals have upon themselves. These two consequences of genetic variation, reducing intra-specific competition (Sib models) and reducing inter-specific competition (Lotka-Volterra model), may co-occur but are not necessarily found together (e.g. Maynard Smith 1989). The many empirical tests showing a less than two-fold difference in Sib Competition (e.g. Schmitt and Ehrardt 1987 and Kelley 1989) leave untested models of inter-specific competition which predict coexistence from more subtle differences in niche usage (Case and Taper 1986; Koella 1993; Gaggiotti 1994; Doncaster et al. 2000; Kerszberg 2000, Pound et al. 2002; Doncaster et al. 2003). These models are closer in concept to Vrijenhoek's (1979) Frozen Niche Variation hypothesis: that asexual lineages arise in a sexual population with genotypes frozen to those of the progenitor parent. Several empirical studies present evidence of limited genetic variation in asexual clones relative to sexual populations, constraining the clonal phenotype to a narrower ecological niche (Vrijenhoek 1979; Hebert et al. 1988; Honeycutt and Wilkinson

1989; Jokela et al. 1997; Semlitsch et al. 1997). The literature contains numerous examples of empirical studies on a range of taxa providing evidence that such niche differentiation may result in coexistence of sexual and asexual types, for example: enchytraeid worms (Christensen et al. 1992), crustaceans (Barata et al. 1996), molluscs (Fox et al. 1996), insects (Becerra et al. 1999), fish (Vrijenhoek and Pfeiler 1997), frogs (Negovetic et al. 2001) and lizards (Case 1990). None of these studies, however, have explicitly tested the relative strengths of inter-specific and intra-specific competition. In this study, in contrast, we are able to test a quantitative theoretical model by calibrating the strength of inter-specific competition against intra-specific competition.

The underlying assumptions and limitations of the Lotka-Volterra model are as follows: the competing populations of sexual and asexual species each have a genetically coded rate constant for conversion of resource uptake into new consumer biomass. The resource stock is assumed to renew logistically and at a relatively fast rate compared to the turnover of consumers. In this model, competition coefficients (α) and carrying capacity values (K) are constants, with the equilibrium abundance of the two consumers depending on the equilibrium of the other. Further assumptions of the model are that death rates for sexual and asexual females are identical, and that males have a zero birth rate. The model ignores stochasticity, allows for age-structured competition and assumes full mixing of individuals in the population.

Chapter 3 uses cyclically parthenogenetic *Daphnia pulex* to test the model by measuring competition coefficients of genetically diverse and genetically uniform populations. *Daphnia pulex* is a good model because its aquatic habit and fast growth facilitate measuring competitive impacts, and its parthenogenetic (asexual) phase allows control over genetic variation. The *Daphnia* system suits Lotka-Volterra dynamics for this experimental timescale, involving continuous reproduction from the mixing of individuals and resources in a 3-dimensional habitat. *Daphnia* can respond on fast timescales to the abundance of their food supplies, facilitating the application of *Daphnia* systems to the Lotka-Volterra model.

It must be considered, however, that although the system of this test species may suitably apply to Lotka-Volterra theory, *Daphnia* may be a special case and the theory may not transfer to other species in the real world. This model applies only to population dynamics at or close to density-dependent equilibrium, and so relatively free from fluctuations induced by short-term environmental disturbances. It must also be borne in mind through all competition studies that other factors may result in coexistence and an in-depth knowledge of the biology of the organism is essential. First, predators may target the most abundant prey species, causing fluctuations in relative numbers, but in general allowing and maintaining coexistence. Second, there may develop a dominance ring of species, whereby one species displaces the other, then vice versa, and so on. In addition, coexistence may have resulted from a chance pattern of colonisation, with mature established individuals preventing further colonisation and thus maintaining diversity, or there may be an underlying habitat mosaic causing the observed diversity. Alternatively, the situation may have been observed and recorded whilst mid-dispersal or in transition from stability to the elimination of one species. A further point to note is that species density is also affected by factors other than competition: dispersal, density-independent mortality and resource availability for example. Composites of morphological, behavioural and physiological phenotypic characteristics, such as competitive ability or predator avoidance patterns, can also affect the outcome of such interactions. These are all less parsimonious explanations, however, than that provided by the Lotka-Volterra model, and they may not need invoking if the simple model is sufficient to explain coexistence.

1.5. AIMS AND OBJECTIVES

This Lotka-Volterra model of the cost of sexual reproduction could help to explain how sexually and asexually reproducing populations coexist in the short term, allowing sexual reproduction to eventually express its advantages of genetic mixing over evolutionary timescales. To our knowledge, no previous models have investigated both inter- and intra-specific competitive effects of coexisting genetically diverse and genetically uniform populations. The aim of this thesis is to test the ecological model for the coexistence of sexually and asexually reproducing

species, using species of the freshwater crustacean genus *Daphnia* and the littoral oligochaete genus *Lumbricillus*.

Specifically, the objectives of this thesis are:

1) To investigate the role of genetic variation in conferring an immediate competitive advantage to sexual populations in the presence of genetically uniform asexual populations. *Daphnia* population biology was reviewed, and the level of genetic variation in natural populations of *Daphnia* in ponds of the U.K. was explored to provide baseline descriptive information (Chapter 2). Values of competition coefficients (α) were calculated for genetically diverse and genetically uniform populations of *Daphnia pulex*, in order to quantify the potential for coexistence between sexual and obligate asexual forms (Chapter 3). A subsequent experiment investigated the population dynamics of genetically diverse populations of *Daphnia obtusa* when undergoing invasions of large and small groups of genetically uniform individuals, and the dynamics of reciprocal invasion events (Chapter 4).
2) To investigate the pseudogamous parthenogenetic reproductive system of *Lumbricillus lineatus*. The reproductive biology of this and other pseudogamous species was reviewed, in order to identify the special conditions for coexistence of sexual and asexual forms presented by this reproductive mode. A series of field surveys were conducted in order to obtain life history parameter values for *L. lineatus* in the U.K., to facilitate future modelling of their reproductive system (Chapter 6). A series of experiments was performed to measure the reproductive output, body growth and population dynamics of sexual diploid and parthenogenetic triploid forms of *L. lineatus* under different environmental conditions and at different starting ratios of one to the other (Chapter 7).

CHAPTER 2

REVIEW OF THE REPRODUCTIVE BIOLOGY OF *DAPHNIA* AND FIELD SURVEYS OF GENETIC VARIATION IN *DAPHNIA* IN THE U.K.

2.1. INTRODUCTION

The freshwater crustacean *Daphnia* is an arthropod in the class Crustaceae, the order Cladocera, also including *Gammarus* and *Artemia*, and the family Daphniidae. The genus *Daphnia* consists of three subgenera *Ctenodaphnia*, *Hyalodaphnia* and *Daphnia*. Species of the subgenus *Daphnia* are found mainly in cool temperate and boreal biomes of the Holarctic, living in freshwater habitats ranging from small ephemeral and permanent ponds to large lakes. Arctic ponds and lakes usually contain only a single species, whilst heavy predation by fish and high levels of U.V. radiation reduce *Daphnia* density in tropical ponds. Temperate climates have the greatest species richness and the tropics the lowest. Ponds in North America hold three or four species, and lakes a few more (Hebert 1995), whereas British ponds and lakes contain one or two species, or up to three, respectively.

Daphnia has a number of life-history characteristics that suit it to the testing of the ecological model for the advantage of sexual reproduction introduced in Chapter 1. *Daphnia* have short generation times and exponential population growth and are easily cultured and maintained in the laboratory. *Daphnia* species exhibit seasonal sexuality, reproducing parthenogenetically throughout favourable seasons of the year, and sexually over the unfavourable conditions of winter. Clonal lineages derived from the parthenogenetic phase of their reproduction can be used to create genetically diverse and genetically uniform populations without introducing costs due to male presence. This enables tightly controlled experiments to test for the effects of genetic variation, as provided by sexual reproduction, on competitive ability. Previous experimental studies (Ellstrand and Antonovics 1985; Kelley 1989; Bell 1990; Weeks and Sassaman 1990) provide evidence against the Tangled

Bank model of Sib Competition, but they do not constitute evidence against inter-specific competition as a mechanism for the maintenance of sex. To our knowledge, no previous studies of the maintenance of sex have allowed the calibration of inter-specific competitive impacts against intra-specific impacts to obtain Lotka-Volterra competition coefficient (α) values that are crucial to coexistence, as performed by the model of Doncaster et al. (2000) that is under test in this thesis. This chapter aims to (1) describe the aspects of *Daphnia* biology and ecology relevant to the experiments performed on *Daphnia* in Chapters 3 and 4, with a particular emphasis on *D. pulex* which was the test species for the experiments in Chapter 3; and to (2) present the results of a general survey of *Daphnia*-inhabited ponds in the U.K. carried out by the author, and the genetic variation therein.

2.2. DAPHNIA REPRODUCTION

2.2.1. Cyclical Parthenogenesis

Many organisms reproduce by various parthenogenetic processes, for example ostracods *Cyprinotus* (Turgeon and Hebert 1994), brine shrimp *Artemia salina* (Browne and Halanych 1989), parasitoid wasps *Apoanagyrus diversicornis* (Pijls et al. 1996), the freshwater snail *Potamopyrgus antipodarum* (Lively 1992; Fox et al. 1996; Jokela et al. 1997), fish of the genus *Poeciliopsis* (Schenck and Vrijenhoek 1986), frogs *Rana esculenta* (Semlitsch et al. 1997), the tropical gecko *Lepidodactylus lugubris* (Radtkey et al. 1995) and lizards of the genus *Cnemidophorus* (Congdon et al. 1978). Cyclical parthenogens are populations that reproduce predominantly by parthenogenesis with seasonal bouts of sexuality. They therefore regularly undergo a production of novel genotypes by recombination (during their sexual phase) along with readjustments in the frequency of existing favourable genotypes (during their asexual phase). This suggests that this reproductive strategy allows for a rearrangement of genetic variation that is not available to obligately sexual organisms (Hebert 1974c). Cyclical parthenogenesis, or seasonal sexuality, may have come about due to the requirement to occupy habitats with sudden changes in conditions (Ruvinsky et al. 1986). This

reproductive system has evolved in a small number of invertebrate groups, including trematodes (order Digenea), rotifers, cladoceran crustaceans, aphids, gall wasps (Cynipidae) and gall midges (Cecidomyiidae; Hebert 1974c; Lynch 1984b; MacKay et al. 1993). The most studied of these is arguably the freshwater crustacean *Daphnia.*

For the majority of the year populations of *Daphnia* are predominantly female. An adult female begins to reproduce after four or five adult instars (average of four days old), and will subsequently produce a brood of eggs immediately following each moult (every three days or so). Diploid eggs are produced by ameiotic parthenogenesis, where there is no pairing of homologous chromosomes and usually only a single maturation division occurring in the oocyte during oogenesis, by mitosis. Between 1 and 300 eggs are deposited into the brood chamber, the exact number depending directly on the size of the individual *Daphnia* and its food intake (Hebert 1978b), as well as oxygen concentration and pH of the water, light regime, temperature and endogenous rhythms. An adult in restricted food conditions will produce fewer eggs and their young will be better suited to withstand long periods of starvation than the many young produced by an adult with high food levels (Gliwicz and Guisande 1992). Larger mothers also produce larger offspring, though it has been shown that offspring are not necessarily larger (with higher starvation resistance) when born into low food levels (Boersma 1995). In the brood chamber young develop through a number of distinct stages (for example red-eye stage 24 hours prior to release; pers. observ.). Young are identical to each other and to their mother. These broods are normally single-sexed, either male or female, although mixed broods have been known to occur (Innes and Dunbrack 1993). *Daphnia* have environmental sex determination, whereby environmental conditions, such as temperature, one hour prior to egg deposition are the critical features in sex-determination (Banta 1929; Kleiven et al. 1992; Innes 1997). Favourable conditions usually lead to the development of all-female broods, whereas unfavourable conditions, for example over-crowding (Burns 1995) and limited food supply, lead to the production of a brood of male offspring. Amongst populations in North America non-male-producing genotypes exist (Innes 1997), although the genetic basis behind this is poorly understood (Innes and Dunbrack 1993) and it is very uncommon. Usually one half of all broods held by females in cyclical

parthenogenetic populations are males, and males are detected in approximately one half of all clonal isolates established from single females (Innes et al. 2000).

If favourable conditions immediately resume, the female will continue to produce parthenogenetic broods. However, if poor conditions persist the female will produce two haploid eggs by normal meiosis (Hebert 1978b), and deposit them in her brood chamber. These eggs become encased in a protective ephippium and are subsequently fertilised by a male. In cyclical parthenogens, this sexual reproduction will occur seasonally for several weeks at a time and it has been suggested that the presence of males can stimulate the production of sexual females (Innes 1997). The female will eventually moult, releasing the carapace along with the ephippial eggs. Ephippia can withstand desiccation, freezing and digestive enzymes, making them ideal for over-wintering in frozen English ponds or diapausing in the substrate of dried-up North American ponds in the summer. Ephippia are assumed to play a major role in *Daphnia* colonisation, and although they rely on passive dispersal, there are several adaptations that facilitate their distribution. The dorsal ephippial margin possesses a series of spines enabling them to attach to other aquatic organisms, and the stark contrast of the white ephippial matrix with the black egg chambers attracts the attention of predators, for example migratory waterfowl (Crease et al. 1997), enhancing the likelihood of consumption and subsequent transfer to a new habitat. Populations are commonly founded by a single or at most a few individuals in this way (Hebert 1974c). However, extinction and recolonisation events are suggested to have an impact on mtDNA variation because of its haploid maternal inheritance (Crease et al. 1997). Ephippial development is usually triggered by the occurrence of favourable conditions in terms of light and temperature after a period of diapausing, although some ephippia are known to develop immediately. Ephippial development is difficult to stimulate under laboratory conditions (Innes, pers. comm.). Young that develop from these eggs are invariably female and necessarily genetically distinct.

2.2.2. Obligate Parthenogenesis

Some cyclical parthenogens, such as some species of *Daphnia* (*D. pulex*, *D. pulicaria*, *D. tenebrosa* and *D. middendorffiana*; Zaffagnini and Sabelli 1972,

Colbourne et al. 1997), have populations that have abandoned their sexual phase in favour of obligate parthenogenesis. Populations of obligate parthenogens occur in Canada and to a lesser extent further south in the Americas (Lynch et al. 1989), and have been found cohabiting with cyclical parthenogens in a pond in the U.S.A. (Innes et al. 1986). There is no evidence, to the author's knowledge, of obligate parthenogenesis in the U.K., and no support could be found for its existence in Scandinavia (Palsson 2000). The arrival of new obligate parthenogenetic lines has been suggested to have come about as a result of the males produced by some obligate parthenogens transmitting a dominant gene for sex-limited meiosis suppression to their progeny (Innes and Hebert 1988). The mutation arises and suppresses meiosis during female gamete formation, but not during male gamete formation, enabling males to spread the gene for asexuality to further female lines (Hebert and Crease 1983). This is shown to be the cause of the spread of asexuality in the plant genus *Taraxacum* (dandelions), and possibly other monoecious plants (Richards 1973). It therefore follows that the gene could spread through a cyclically parthenogenetic population resulting in a genotypically diverse group of obligate parthenogens. It is reasonable to assume that this process is continuing and therefore that some obligate parthenogen clones have occurred relatively recently (Innes and Hebert 1988). Allozyme studies have shown that these populations exhibit great clonal variation that possibly results from multiple transitions to this breeding system, for example in *D. pulex* (Hebert et al. 1988; Crease et al. 1989; Lynch et al. 1989). This large amount of variation may be due to a high rate of origin of obligate parthenogens relative to the rate of extinction (Lynch et al. 1989). Polyphyletic origins of obligate parthenogenesis in this species is supported by Crease et al. (1989) and in other species, such as aphids, by Delmotte et al. (2001). Asexual lineages of *D. pulex* have been shown to be relatively short-lived (Lynch et al. 1989).

Obligate parthenogens have the same life stages as the cyclical parthenogens, but without the fertilisation of haploid eggs. They may still produce ephippia under appropriate conditions, which do not require fertilisation and will develop into genetically identical offspring from ameiotic parthenogenesis (Korpelainen 1992). These obligately parthenogenetic populations can generally be characterised by the absence or reduction of males at times of ephippial production, allowing them to

effectively avoid the cost to population growth capacity of producing males and of finding mates (Innes et al. 2000). There has been some evidence of male production, however, in obligate parthenogens of *D. pulex*, *D. cephalata* (Hebert 1978a) and *D. middendorfianna* (Tash 1964) possibly stimulated by high population densities (Innes et al. 2000). These parthenogenetically produced males provide the asexual individuals with a means of exchanging genes with closely related sexual species. However, these males may cause some of the progeny to make the transition to obligate asexuality (Innes and Hebert 1988) and many exhibit abnormal spermatogenesis, suggesting that the ability to produce males is eventually lost due to selective processes or accumulation of deleterious mutations (Innes and Hebert 1988). Non-male-producing females have been found by crowding in the laboratory, and their existence is confirmed by the study of natural populations and their broods (Innes 1997). The variation among clones for male production suggests the potential for evolutionary shifts in the pattern of sex allocation in *Daphnia* (Innes and Singleton 1994).

A third group of cladocerans include those species that have both cyclic and obligate parthenogenesis, for example *D. pulex* (Hebert and Crease 1980; Innes et al. 1986) and *D. cephalata* (Hebert 1981). *D. pulex* populations in Canada consist of a few obligately parthenogenetic genotypes, with cyclical parthenogens becoming more common south of the Great Lakes. The occurrence of obligate and cyclical parthenogenesis varies greatly further south, with mixtures of the two types also being common (Lynch et al. 1989), presenting a latitudinal gradient in the incidence of sex. It is unclear as to why the spread of obligate parthenogenesis by sex-limited meiosis suppressor genes has not yet been completed in the south. The coexistence of obligate parthenogens with a reduction or elimination of males, and cyclical parthenogens still burdened with males, suggests a greater fecundity of the cyclicals (Innes et al. 2000). As bisexuality is the only way of transmitting genetic information from one year to the next, clones that lack this ability should be eliminated (Ruvinsky et al. 1986), so it is unclear as to why more species are not facultatively sexual. It should be considered, however, whether the supposed ecological and genetic advantages of cyclical parthenogens are fulfilled in natural populations (Lynch 1984b). Also, great cytogenetic barriers and stringent requirements for the transition may prevent the widespread evolution of cyclical

parthenogens. Due to these rigid cytogenetic requirements, cyclically parthenogenetic species are probably founded by single individuals, and the early ecological and evolutionary success of the species would depend on the suitability of the genetic structure of the founder to the environment (Lynch 1984b). Once established, cyclical parthenogens then face the problem of displacement by secondarily-derived obligate parthenogens by genetic disruption, sperm robbing, and competition for resources, which has occurred in *Daphnia*, rotifers and aphids (Lynch 1984b).

A period of parthenogenetic reproduction is widely accepted to be evolutionarily adaptive in conditions where rapid population growth is advantageous, as a population doubles its intrinsic capacity for increase as compared to a population that is reproducing sexually. In addition, parthenogenesis allows any favourable genotype to replicate without dilution by recombination and dominant alleles as in sexual reproduction. An obligate parthenogen has to have such favourable genotypes in the first instance, however, and would accumulate a load of recessive deleterious genes, as a finite population can never have a load less than its least-loaded clone (Muller's ratchet, see Chapter 1) and this limits their lifespan (Innes and Hebert 1988; Innes 1989). The possibility of this genetic load became apparent when the relative survival of individuals of self-mated clones was found to be significantly lower than that of out-crossed clones (Innes 1989). This is not necessarily the case in plants, however, as hermaphrodites are often advantaged over sexual females (Graff 1999). Cyclical parthenogens are advantaged, therefore, as they can create favourable genotypes by recombination and then multiply them by parthenogenesis. There is a reduction in fitness as a result of sexual reproduction, however, and a loss of favourable genotypes by recombination and the production of deleterious genotypes (Banta 1939). Overall, however, reproducing asexually most of the time with infrequent bouts of sex allows cyclical parthenogenesis to extend the short-term benefits of asexuality over the long-term (Lynch 1984b; Hurst and Peck 1996). This maintains the genetic structure of a sexual population and decreases the long-term costs of accumulation of harmful mutations and a lack of new gene combinations (Taylor et al. 1999). Cyclical parthenogenesis provides an effective mechanism for coping with environmental instability (Lynch 1984b). Sexuality in *Daphnia* is advantageous for its role in

adaptation to each local environment when founding populations but the complete elimination of sexuality may be prevented by ecological distinctions between the two types of individuals (Lynch et al. 1989).

2.3. MORPHOLOGY AND FEEDING

Maximum carapace lengths can range from 0.5–3.5 mm in the subgenus *Daphnia*, depending on food supply, with *D. pulex* and *D. obtusa* averaging 2 mm in length. The ratio of head:body area also differs greatly between species, for example between *D. cephalata* (1.4) and *D. nivalis* (0.1) of the *D. carinata* group. It has been shown, however, that environmental conditions such as temperature, food supply, water turbulence and chemical signals from predators can directly affect head size of some species (Brooks 1947; Jacobs 1961; Hebert 1978a). Cyclomorphosis can involve the production of spike- or blade-like extensions to the head, known as helmets, or thorny spines on the neck, known as neck teeth (Colbourne et al. 1997). All *Daphnia* possess spines on the external margin of their ventral carapace, but only a number of North American species, including *D. pulex*, possess elongate setae on the internal margin (see Figures 2.1 and 2.2).

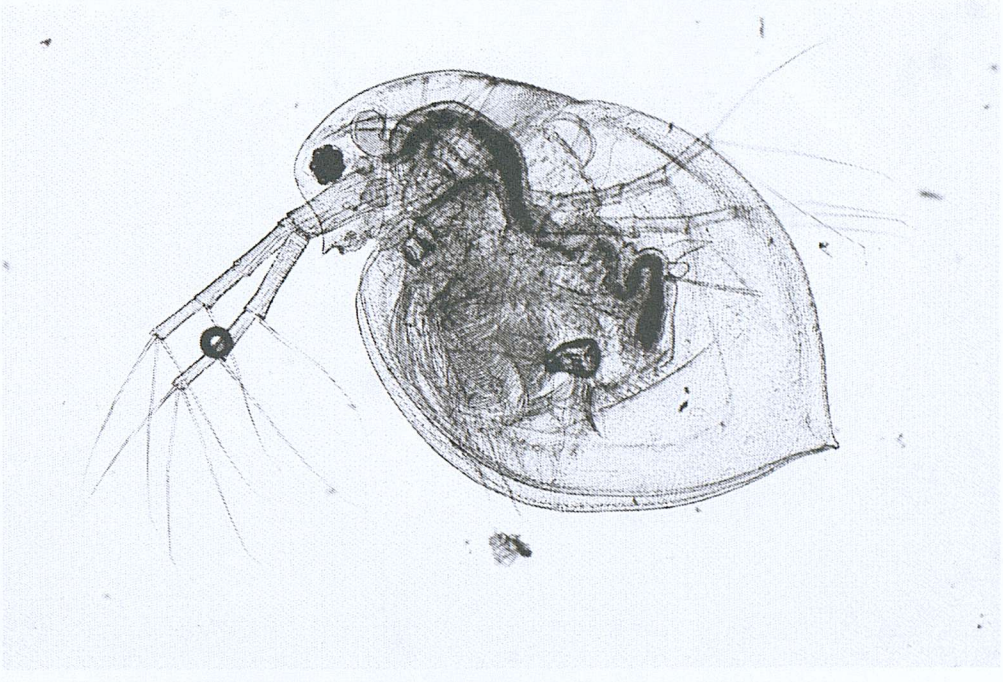


Figure 2.1—Photograph of adult *Daphnia*, lateral view (magnification x 1200)

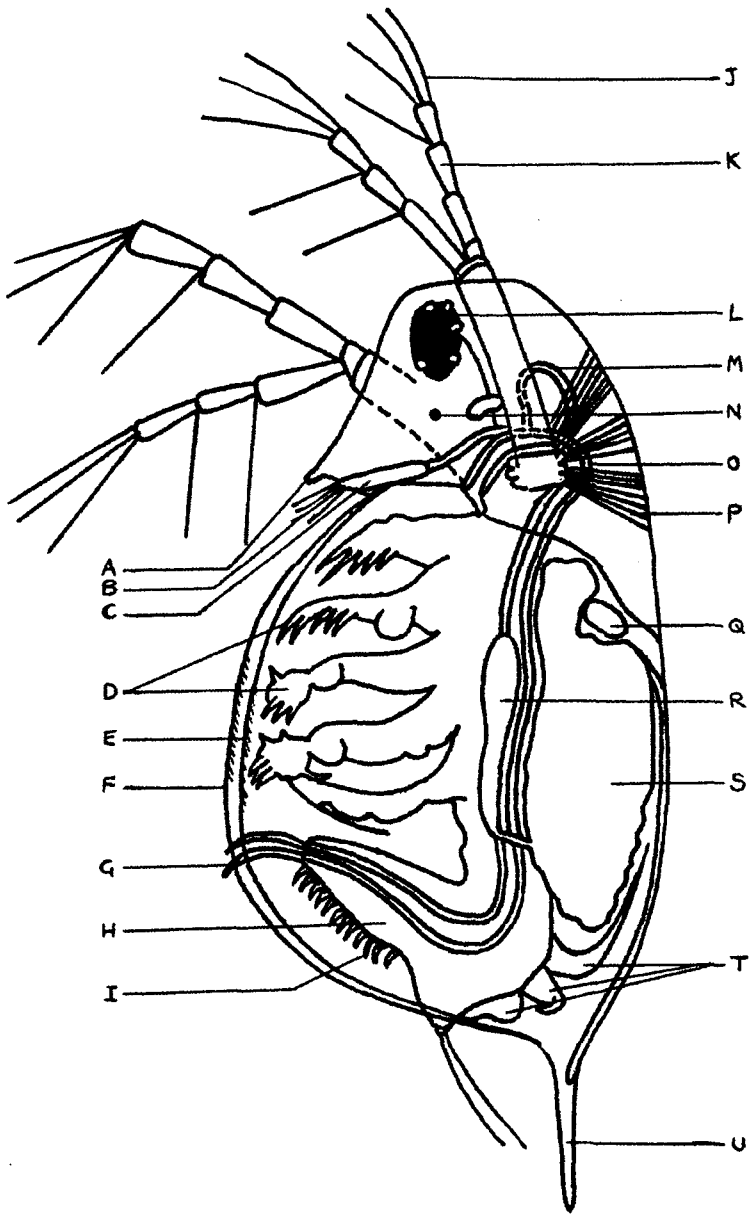


Figure 2.2—Adult female *Daphnia*, lateral view (magnification x 1200). A = rostrum; B = aesthetes; C = antennule; D = thoracic appendages; E = plumose setae; F = carapace; G = pecten; H = postabdomen; I = anal spines; J = swimming hairs; K = secondary antennae; L = eye; M = enzyme gland; N = ocellus; O = oesophagus; P = swimming muscles; Q = heart; R = ovary; S = brood chamber; T = abdominal appendages; U = tail spine

Daphnia use these fine setae to filter small particles out of the water that is constantly flowing past the thoracic appendages. All *Daphnia* are herbivorous or detritivorous, and can survive on algae, protozoa, bacteria, fungi and organic debris. In English reservoirs, organic debris seem to be the staple diet, with algae being important during blooms (Nadin-Hurley and Duncan 1976). There is great variation in the assimilation efficiency of different types of algae, however, with flagellate *Cryptomonas* and green alga *Ankistrodesmus* being 90–100% assimilated, while other green algae such as *Gloeocystis* and *Oocystis* and blue-green algae are <15% assimilated (Schindler 1970; Arnold 1971). Assimilation efficiency can depend on abundance of the food source, as over-ingestion may lead to a reduced efficiency of digestion. The feeding efficiency of *Daphnia* is measured as food intake divided by metabolic demand, and *D. pulex*, for example, has a maximum feeding efficiency of approximately at 20°C (Lynch 1978). The feeding rate of *D. magna* is proportional to the concentration of food up to a certain level (Rigler 1961). Also affecting the assimilation of ingested food is the action of enzymes such as proteases, lipases and amylases. Preliminary feeding trials confirmed the poor quality of populations fed on pure algal cultures or on yeast alone. Indeed, there is much evidence to suggest that diets of a single alga are inadequate for normal reproduction (Taub and Dollar 1968). Ecological theory states, however, that nutrient enrichment can cause large amplitude cycling and increased periods within populations, known as the 'paradox of enrichment' and leading to instability. This has not been supported in empirical studies of *Daphnia* populations, however (McCauley and Murdoch 1990). It is suggested that inedible bacteria dampen *Daphnia* population cycling due to the absorption of nutrients required by the edible algae (Murdoch et al. 1998; McCauley et al. 1999). The food supplies that we used for *Daphnia* in the experiments described in Chapters 3 and 4 were derived from aquaria-cultured algae-water. This is produced using goldfish to fertilise the algae in the water of the aquarium, and constant lighting to ensure its maintenance. Such aquaria-cultured algae-water contains inedible algae and bacteria as well as edible algae.

2.4. DEVELOPMENT

Juvenile development is fairly uniform throughout *Daphnia* species. There are four pre-adult instars (though there can be as many as six in *D. pulex* and eight in *D. magna*), and as many as 28 subsequent moults throughout adult life has been recorded (Lei and Clifford 1974). Frequency of moulting depends on temperature, as does the longevity of individuals, but at a particular temperature the mean life spans of different species are very similar (for example 25–40 days at a temperature of 20°C). *Daphnia* are also known to live longer at lower food concentrations (Banta 1939) and on particular types of algae (Arnold 1971). A table of vital rates for *D. pulex* is given below (Table 2.1).

Table 2.1—Vital rates for *Daphnia pulex*. Average lifespan, generation time, and brood size of *Daphnia pulex* are given.

Rate	Measure
Life span	~50 days at 20ºC
Generation time	~5 days at 20ºC
Brood sizes	1–300

2.5. ECOLOGY AND COMPETITION

Large brood sizes and increasing population density are evident during spring and towards early summer in temperate regions. Population sizes increase over the summer and brood sizes diminish. Brood sizes tend to be very constant between individuals, suggesting that *Daphnia* are limited by, and also in equilibrium with, their food supplies, facilitating the application of *Daphnia* systems to the Lotka-Volterra model. Death is caused by both endogenous and exogenous mortality factors, such as senescence and predation respectively (Hebert 1978b).

In freshwater habitats there is often coexistence between species, with great seasonal or annual fluctuations in relative abundance. It is argued that species in natural environments display niche segregation as each species only utilises a fraction of the range of food sizes available, and cohabiting species tend to favour

different food sizes. As brood size is directly affected by energy intake, competing species that assimilate different foods will show varying degrees of brood production, and so this could be a measure of niche overlap. Laboratory experiments have suggested that there is an overlap in the food sizes favoured by different species of *Daphnia*, and therefore that competition must occur frequently. Specialisation may not be possible if food size and composition is unpredictable; and also it would be too costly to be choosy at times of low food supply.

Oscillations in *Daphnia* populations may be due to the accumulation of energy reserves that leads to time lags in the response to decreased food densities, as shown in laboratory populations of *D. galeata mendotae* (Goulden and Hornig 1980). This time lag means that the density equilibrium is exceeded leading to the starvation and death of many individuals. External factors, such as temperature deviations, food quality changes or light shock, may also cause fluctuations in *Daphnia* populations (Kreutzer and Lampert 1999).

In terms of mating behaviour, there is no evidence that males use chemical cues to increase their probability of finding females, but only that they made contact at random. There is evidence, however, that males can determine the sex of encountered individuals as contact periods with females lasted longer than with males, possibly due to chemical cues (Winsor and Innes 2002). Male *Daphnia* may also be able to assess female attributes such as species and reproductive status (Brewer 1998).

2.6. GENETICS AND CLONAL VARIATION

Most work on *Daphnia* genetics has focussed on populations that persist all year round and not the intermittent populations found in ponds that are seasonally habitable. Such *Daphnia* populations generally contain little genetic variation, although there are large differences in gene frequency among populations in close proximity. This is in part due to the founder effect of populations being re-established each year from a relatively small number of diapausing eggs, which results in inbreeding. It is therefore hard to assess levels of intra-specific variation. In those populations, however, gene frequencies at enzyme loci generally fit the

assumptions of mating being random, population size large and natural selection weak. Such frequencies are stable from year to year, confirming that these populations are re-established from diapausing eggs, and that gene flow is low. Clonal variation has been shown in rotifers and aphids (Loaring and Hebert 1981) and the grasshopper *Warramaba virgo* (Honeycutt and Wilkinson 1989), and is thought to result from multiple hybridisations between sexual individuals. Natural populations of *D. pulex* have been shown to include a number of clones, with differences in intrinsic rates of increase, competitive abilities, rates of ephippial egg production and life spans (Loaring and Hebert 1981) and in fecundity (Innes and Singleton 1994). On average, only about two clones of obligate parthenogens ever exist in the same pond. Sexual reproduction tends to lead to Hardy-Weinberg equilibrium, but heterozygote excesses rapidly develop at loci during subsequent parthenogenesis. A clone can persist through several seasons or annual cycles in permanent populations. Molecular studies have found a low level of mitochondrial DNA sequence divergence between cyclic and obligate *D. pulex*, suggesting that the obligate parthenogens are of relatively recent origin (Crease et al. 1989; Lynch et al. 1989).

The fitness components of parthenogenetic and ephippial egg production can be used in natural populations to study genotypic variation. The extent of parthenogenetic egg production of a particular genotype is determined by the proportion of adult females in the population that are carrying a brood, and the mean brood size carried by those females. Genotypic differences in egg production are found both in sparse populations, where brood size varies but reproductive ratio does not, and in crowded populations, where both brood size and reproductive ratio can vary, but more so in the latter. The favoured genotype often has a higher reproductive ratio and a larger brood size, but differences between genotypes differ with time (Hebert 1974a; Hebert and Ward 1976). Ephippial egg production can be quite sporadic, and not all females in a population will produce these eggs at any one time. Genotypic differences have been observed between ephippia-producing and non-ephippia-producing females, although different genotypes may be expressed to greater degrees at different times throughout the period of ephippial egg production. This suggests that genotypes differ in their mean threshold to the factors that induce ephippia production. Also, there are often a small number of

ephippial females present in any large parthenogenetic population, and the genotype frequencies of these females often differ from the rest of the population, in which they are usually declining in frequency. A general decline in frequency suggests that the genotype is unfavourable for the particular environment experienced, and so it makes sense that those individuals expressing the genotype should be less fit and therefore producing ephippia. However, other fitness components such as age to first reproduction, probability of survival and longevity must also be considered when attempting to correlate genotypic frequencies with egg production.

2.7. GENERAL SURVEY OF GENETIC VARIATION IN *DAPHNIA* IN PONDS OF THE U.K.

2.7.1. Introduction

It was once believed that clones possessed little or no genetic variation (Williams 1975). The competitive exclusion principle specifically suggests that competitive interactions should prevent the coexistence of closely related species or clones (Williams 1975) and has been demonstrated in several studies, including those on *Paramecium* (Hairston and Kellerman 1965) and Cladocerans (Frank 1957; Allan 1973). Recent genetic analysis, however, has demonstrated much genetic variation for life-history characters in aphids (MacKay et al. 1993), and clones of obligately parthenogenetic *Daphnia pulex* (Lynch 1984c; Weider et al. 1987; Lynch et al. 1989). Hebert and Crease (1980) found an average of 2.4 clones per pond in 11 ponds, with certain clones being restricted to woodland ponds, and others to ponds in grassland habitats, suggesting ecologically determined fitness differences. Ecological differences between genotypes has been demonstrated in the pea aphid *Acrythosiphon pisum* (Frazer 1972), which showed variation in intrinsic rates of increase, as well as the rotifer *Euchlanis dilata* (King 1972) and *Daphnia magna* (Hebert 1974a), suggesting that populations of cyclical parthenogens consist of an array of ecologically differentiated genotypes. Such variation is likely to be the result of mutations and of parthenogenetic clones arising on independent occasions from the cyclically sexual populations (Lynch 1984d; Lynch et al. 1989). Crease et al. (1989) showed that the high clonal diversity present amongst the obligately

parthenogenetic clones of *D. pulex* in the Great Lakes region was due to their polyphyletic origin.

Genetic variation in life-history traits of individuals of *Daphnia* populations can have important consequences. For example, the ability of a genetically diverse population to balance its two-fold cost in competitive superiority will depend upon the life-history traits of the genetically uniform population against which it is competing (investigated in Chapter 4). Also, studies have shown homozygote fecundity to be lower than heterozygote fecundity (Young 1979b). In brief, different clones exhibit differing measures of intrinsic rates of increase, competitive abilities, rates of ephippial egg production and life spans (Loaring and Hebert 1981). Furthermore it has been shown that clones with high rates of increase tended to be better competitors than clones with low rates of increase (Loaring and Hebert 1981).

This survey by the author attempted to answer questions about U.K. *Daphnia* populations, such as whether obligate parthenogens exist and how *Daphnia* populations persist over time, in relation to the degree of genetic variation present. A previous survey of *Daphnia magna* populations in East Anglia found very limited gene flow (Hebert 1974b). Furthermore, previous surveys of ponds in the U.K. have investigated the distribution and ecological and habitat requirements of *Daphnia* species and have found coexistence of more than one species in the same pond (Hebert 1974b; Fryer 1985; Innes et al. 1986). The present survey attempted to offer some support as to whether different species of *Daphnia* coexist. The specific aims of this survey were to locate and sample *Daphnia*-inhabited temporary ponds across England in order to (1) investigate the inherent genetic variation of *Daphnia* populations and (2) build up a picture of the fluctuation in *Daphnia* numbers across the seasons.

2.7.2. Methods

Ponds in Hampshire, Kent, Cambridgeshire and Devon were sampled during 2002 (Table 2.2). One temporary pond in the New Forest in Hampshire, Pig Bush pond, was regularly sampled for 17 months.

Table 2.2—Locations, Ordnance Survey maps and grid references for each of the ponds sampled in the present survey.

Pond	OS map	OS map reference
HAMPSHIRE		
Pig Bush	Out 22/195	364049
King's Hat	Out 22/195	387054
Holmsley	Out 22/195	222011
Thornyhill	Out 22/195	203999
Ivy Wood	Out 22/195	314026
Stockley	Out 22/195	342023
Ferny Crofts	Out 22/195	366057
Ladycross	Out 22/195	335027
Lyburn	Out 22/195	248173
Nomansland	Out 22/195	254173
KENT		
Graveney 1	Exp 149	-
Graveney 2	Exp 149	-
CAMBRIDGESHIRE		
Longstowe Moat	Exp 208/153	-
Toft	Exp 209/154	365562
Landbeach	Exp 226/154	476653
DEVON		
Whitchurch Down	Out 28/191	512736
Balmer Lawn	Out 22/195	304030

Daphnia were collected using a plankton net (mesh size 300 μm) towed immediately below the water surface and sampling approximately 100 l. Clones were cultured from individual females carrying a brood. Each female was placed in a plastic cup with 80 ml zooplankton media (Appendix 1; Lynch et al. 1986) and fed *ad libitum* with algae-water from an aquarium culture system. Aquaria-cultured algae-water contains bacteria and inedible algae as well as edible algae (in this case predominantly *Scenedesmus quadricauda*). The cups were kept in a temperature-controlled cabinet at 15°C for a 20L : 4D hr photoperiod to encourage good levels of health and reproduction. *Daphnia* were identified using an electronic identification key from the U.S.A. (Hebert 1995). It should be noted that *Daphnia*

of North America differ from those of the U.K.; indeed Hebert has previously attempted to specifically describe the European *D. magna* (Hebert 1978a), but the U.S.A. key provided sufficiently accurate identifications. Abundance of allelic variation in *Daphnia* populations from each pond was determined using a method of cellulose acetate electrophoresis (Appendix 2; Hebert and Beaton 1989), staining for mono- or polymorphism of AD, AO AMY, GPI, FUM, LDH and MDH. A higher proportion of polymorphism in a pond suggests a greater degree of allelic variation and that populations were reproducing sexually. A high level of monomorphism indicates that certain clones have risen disproportionately in the population by asexual reproduction. Each application was scored as homozygous slow (SS) or fast (FF), or as heterozygous (SF). Alleles that do not differ much in mobility can be difficult to distinguish through electrophoresis, so it is possible that some of the allozymes tested actually had more than two alleles. The seasonal survey of one temporary pond, Pig Bush pond, involved testing samples of *Daphnia* for allozyme GPI each month throughout 2002 and into 2003. Whether or not the population was reproducing sexually at the time of sampling was determined by testing the electrophoretic results for Hardy-Weinberg equilibrium using a Chi-squared test.

2.7.3. Results

2.7.3.1. Ponds of the U.K.

Daphnia identification is shown in Table 2.3. All ponds sampled in Hampshire and one pond in Devon contained populations of *D. obtusa*. The two ponds sampled in Kent contained populations of either *D. magna* alone, or both *D. magna* and *D. obtusa*. The species of *Daphnia* present in the remaining four ponds was not recorded. The status of each of the seven allozymes stained for is shown in Table 2.3. Some allozymes failed to stain and some stained more reliably than others, resulting in some ambiguous results.

Table 2.3—The allozyme status and the species of *Daphnia* present in the ponds surveyed. M = monomorphic; P = polymorphic; ns = no stain. LDH can stain as two separate sets of bands, indicated by numbers in brackets.

Pond	Species present	Allozyme						
		AD	AMY	AO	FUM	GPI	LDH	MDH
HAMPSHIRE								
Pig Bush	*D. obtusa*	M	M	M	M	P	M(1 & 2)	M
King's Hat	*D. obtusa*	M	ns	M	M	M	M(1 & 2)	M
Holmsley	*D. obtusa*	M	ns	M	M	M	M(1 & 2)	M
Thornyhill	*D. obtusa*	M	ns	M	M	M	M(1 & 2)	M
Ivy Wood	*D. obtusa*	M	ns	M	M	M	M(1 & 2)	M
Stockley	*D. obtusa*	M	ns	M	M	M	M(1 & 2)	M
Ferny Crofts	*D. obtusa*	M	ns	M	M	P	M*	M
Ladycross	*D. obtusa*	M	ns	M	M	M	M(1 & 2)	M
Lyburn	*D. obtusa*	P*	ns	P	M	M	M	ns
Nomansland	*D. obtusa*	P*	ns	P	M	M	M	ns
KENT								
Graveney 1	*D. magna*	M	ns	M	M	M	P	M
Graveney 2	*D. magna & D. obtusa*	M	ns	M	M	M	P	M
CAMBRIDGESHIRE								
Longstowe Moat	Not recorded	P	ns	P	M	M	ns	ns
Toft	Not recorded	P	ns	P	M	P	M	P
Landbeach	Not recorded	M	ns	M*	M	M	M	M
DEVON								
Whitchurch Down	*D. obtusa*	M	ns	M	M	M	M	M
Balmer Lawn	Not recorded	P	ns	M	M	M	M	M

*Gel was partially unclear or ambiguous and result is therefore unreliable.

Eight of the ponds sampled (King's Hat, Holmsley, Thornyhill, Ivywood, Stockley, Ladycross, Landbeach and Whitchurch Down) displayed monomorphism for all allozymes tested. This suggests that the *Daphnia* populations in these ponds are comprised of a limited number of clones with low genotypic diversity, and could even suggest that populations are reproducing by obligate parthenogenesis (Hebert et al. 1988). Eight ponds displayed polymorphism for one or two of the seven allozymes (Pig Bush, Ferny Crofts, Lyburn, Nomansland, Graveney 1, Graveney 2, Longstowe Moat and Balmer Lawn), suggesting the presence of more than one clone. One pond (Toft in Cambridgeshire) exhibited polymorphism in more than

half of the allozymes tested. This suggests a high level of genetic variation in a number of different clones of the *Daphnia* population present in this pond, and the occurrence of cyclical parthenogenesis (Hebert et al. 1988). The overall percentage of monomorphism and polymorphism displayed by all *Daphnia* samples is shown in Figure 2.3 and the average percentages of monomorphism and polymorphism for each allozyme is shown in Figure 2.4.

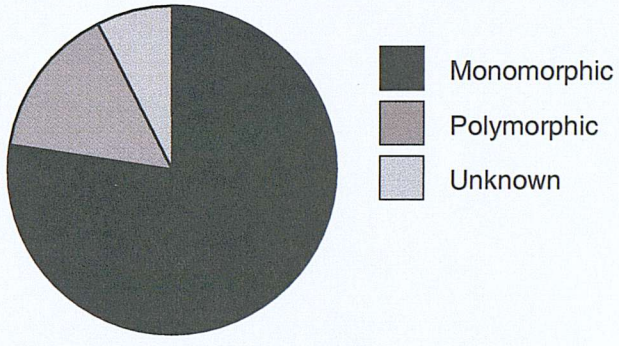


Figure 2.3—Average percentage monomorphism and polymorphism of the six allozymes tested in the *Daphnia* samples collected from all 17 ponds.

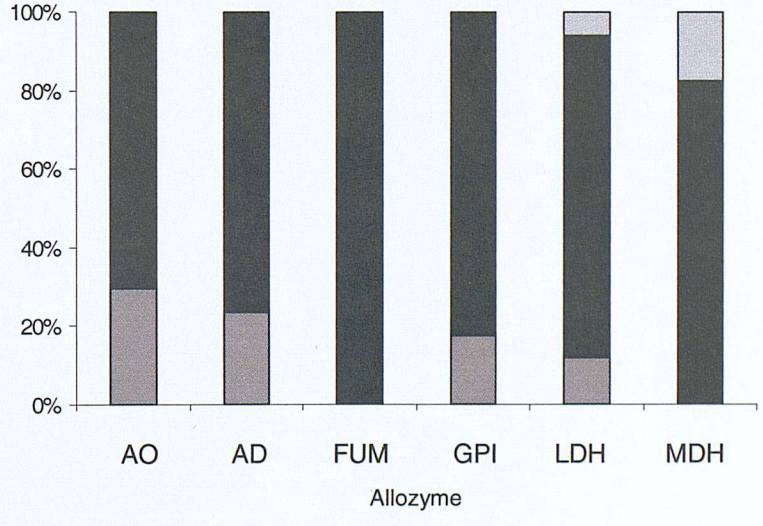


Figure 2.4—The percentage of monomorphism and polymorphism of allozymes in the 17 ponds sampled. Black = monomorphic; dark grey = polymorphic; light grey = unknown.

It is evident from these data of 17 temporary ponds in the U.K. that monomorphism of enzymes is high (over 75% of ponds sampled). This suggests a common predominance of only a limited number of clones of *Daphnia* in U.K. temporary pond populations. FUM (and possibly LDH) was monomorphic for all *Daphnia* populations sampled, suggesting that the allozyme may be linked to favourable character traits that have led to a disproportionate increase of a certain status of the allozyme in the population. In contrast, allozyme AO exhibited polymorphism in 30% of the populations of *Daphnia* sampled, suggesting that no particular status of this allozyme is linked to favourable life-history traits and that there may be several clones of *Daphnia* present in these populations.

2.7.3.2. Seasonal Survey of Pig Bush Pond

Table 2.2 shows the change in *Daphnia* abundance and genetic variation over time in Pig Bush pond. The pond contained large populations of *D. obtusa* in the first two months of 2002, while the weather was generally dry and cold and the pond was large (12 × 6 m and 0.4 m deep at the centre). As the weather became milder, but with more rain and wind, the pond began to reduce in size and *Daphnia* became less abundant (March 2002). The pond and its *Daphnia* population continued to reduce in size until late April, when the pond was finally completely dry. For the rest of the spring and through summer, until November 2002, the pond remained dry and no *Daphnia* were present. Average weather patterns during this period ranged from rainy and cool to very hot. This extended period of dryness may be specific to the year studied, however, as it was a hot summer. When the pond refilled in late October/early November 2002, the weather was becoming once again wet and cold. For the rest of the year and into early 2003, the pond once again held large populations of *D. obtusa.*

Table 2.4—Abundance of *Daphnia* and the degree of genetic variation determined by the allozyme GPI Pig Bush pond in the New Forest, Hampshire, throughout 2002–2003.

Month	Average weather	Pond size	Presence of free swimming *Daphnia*	SS,SF,FF	χ^2 value*	Hardy-Weinberg equilibrium
2002						
January	Dry, cold	12 × 6 m	Abundant	18,21,9	0.41*	Yes
February	Dry, cold	12 × 6 m	Abundant	29,38,7	121.56	No
March	Rainy	6 × 4 m	Abundant	34,12,9	11.02	No
April	Rainy, windy	3 × 2 m	Some	-	-	-
May	Changeable	-	None	-	-	-
June	Hot, sunny	-	None	-	-	-
July	Overcast, hot	-	None	-	-	-
August	Very hot	-	None	-	-	-
September	Still, humid	-	None	-	-	-
October	Dry, cool	-	None	-	-	-
November	Rainy, cool	8 × 6 m	Abundant	19,18,3	0.20*	Yes
December	Cold	8 × 6 m	Abundant	203,190,63	2.93*	Yes
2003						
January	Dry, cold	12 × 7 m	Abundant	18,20,9	0.64*	Yes
February	Dry, cold	12 × 6 m	Abundant	24,19,5	0.18*	Yes
March	Cold, windy	8 × 6 m	Abundant	36,8,4	7.53	No
April	Dry, cool	3 × 2 m	None	-	-	-
May	Mild	-	None	-	-	-

*Population in Hardy-Weinberg equilibrium (calculated χ^2 value does not exceed tabulated χ^2 value of 3.84).

During the months of November 2002–February 2003, when the pond had refilled, the observed proportions of GPI alleles were shown to be significantly similar to the expected proportions (Chi-squared tests), and therefore the populations of *D. obtusa* were in Hardy-Weinberg equilibrium, indicating random mating and the occurrence of sexual reproduction (Lynch 1983). In March 2003, as the pond was beginning to diminish once again, *Daphnia* populations were no longer in Hardy-Weinberg equilibrium (allele proportions were significantly different to expected proportions). The population no longer reflected that of a sexually reproducing

population with the number of heterozygotes in the population declining, suggesting that selection and evolutionary stochastic forces were acting on the traits governed by the alleles. The allele SS had risen in proportion in this population more than SF or FF, suggesting that it is linked to favourable character traits that better equip certain clonal lineages to the local conditions enabling them to rise disproportionately in the population.

2.7.4. Discussion

Only one pond surveyed in this study was found to contain more than one species of *Daphnia.* The presence of more than one *Daphnia* species living in the same pond has been recorded previously in Yorkshire (Fryer 1985), East Anglia (Hebert 1974b) and the mid-west U.S.A. (Innes et al. 1986) but does not necessarily tell us anything about their ability to coexist, as different habitats and different foods even in a small pond ensure that there are few demands on a common resource. However, some indication of similarity of preferences can be obtained (Fryer 1985). The distribution of *Daphnia* in Yorkshire shows *D. obtusa* to be the most ecologically isolated, although it has been known to coexist with other species (Hebert 1974b; Innes et al. 1986).

2.7.4.1. Monomorphism and Polymorphism

The *Daphnia* populations in many of the ponds sampled were monomorphic for the allozymes examined suggesting that they are comprised of a limited number of clones with low genotypic diversity. These factors, along with large Hardy-Weinberg deviations, characterise *Daphnia* populations reproducing by obligate parthenogenesis (Hebert and Crease 1983; Hebert et al. 1988). There is no other evidence, however, for the existence of obligate parthenogens in the U.K., and so an alternative explanation is suggested. The *Daphnia* samples analysed were collected over the space of 12 months, resulting in the possibility of *Daphnia* populations reproducing asexually for a sufficient length of time for certain better-adapted clones to have risen disproportionately in the population, therefore reducing the overall degree of genetic variation present in the population. Furthermore, it has commonly been found for ponds to hold only a small number of

clones (e.g., as few as 3, Hebert et al. 1988) perhaps due to limited colonisation opportunities or competitive interactions between superior and inferior clones (Hebert et al. 1988). A long-term study of a polluted lake in Northern Italy found no genetic variation within the resident population of *D. obtusa*, possibly because the level of toxicity of the lake was poisonous to other populations of the same species (Bachiorri and Menozzi 1991). The staining of a greater number of allozymes and at different times of the year would help to confirm or dispute this suggestion. In contrast, the polymorphism detected in some ponds suggests the presence of a greater number of clones and therefore greater genotypic diversity. A temporary pond in Cambridgeshire (Toft) exhibited polymorphism in more than one half of the allozymes tested, suggesting a high level of genetic variation in a number of different clones within the *Daphnia* population of the pond, and strong evidence for the occurrence of sexual reproduction (Hebert et al. 1988). Furthermore, allozymic molecules with the same observed electrophoretic banding might comprise variations in their DNA content, therefore under-estimating the genetic variation present in the populations sampled. The large genotypic variability of *Daphnia* may be maintained by the selective forces of high reproduction efficiency of the parthenogenetic life stage and the short-term advantage of high resistance to crowding and unfavourable conditions of the sexual individuals (Ruvinsky et al. 1986). Clonal diversity in natural habitats must be maintained by spatial or temporal heterogeneity of the environment. Understanding of these factors can be achieved by undergoing temporal studies of clone frequencies in natural habitats and continuous monitoring of physico-chemical parameters in the environment (Loaring and Hebert 1981).

2.7.4.2. Sexual Reproduction

Populations of *D. obtusa* were found to be in Hardy-Weinberg equilibrium during the favourable months of the year in the present survey, which indicates random mating and the occurrence of sexual reproduction (Lynch 1983). The investment in sexual reproduction in temporary pond habitats very soon after re-establishment from resting eggs is shown by the presence of males (Schwartz and Hebert 1987; Innes 1997). All ex-ephippial *Daphnia* are female, so this early incidence of males suggests that they are produced during the first few broods of the ex-ephippial

females (Innes 1997). Males are also produced under harsh conditions such as crowding, when females subsequently produce two haploid eggs that require fertilisation by the males. The resting eggs that are deposited in the substrate for the duration of the unfavourable season eventually hatch out to establish a new genetically diverse population. Unless affected by selection or evolutionary stochastic forces, the newly established population will maintain this level of genetic variation and be found to agree with Hardy-Weinberg ratios. The high degree of genetic variation inherent in sexually reproducing populations in newly filled temporary ponds at the end of the harsh season should allow the population to adapt to any subtly changing conditions in the following year.

Daphnia populations in the present survey were no longer in Hardy-Weinberg equilibrium (allele proportions were significantly different to expected proportions) once unfavourable conditions occurred. The population no longer reflected that of a sexually reproducing population, suggesting that selection was acting on the traits governed by the alleles, and asexual reproduction was taking place, with certain clones rising disproportionately in the population. The allele SS had risen in proportion in this population more than FF, and in turn FF had risen more than the heterozygote SF. This suggests that certain alleles or loci may be linked to favourable character traits that better equip particular clonal lineages to the local conditions and enable them to persist and thrive. A study on populations of *D. pulicaria* in a lake in Minnesota showed the homozygous slow (SS) allele for PGI to increase with time over the summer, an annual pattern and apparently coincident with decreasing concentrations of dissolved oxygen (Ross et al. 1996). Under a different mechanism, asexual populations of the parasitoid wasp *Venturia canescens* were found to consist only of homozygous individuals, whereas sexual populations consisted of both homozygotes and heterozygotes (Malmberg et al. 2000). Studies on *Daphnia* have shown that heterozygotes are more stable and less sensitive to deteriorating ecological conditions, however (Yampolsky and Kalabushkin 1991). Evidence from studies on the grasshopper *Warramaba virgo* (Honeycutt and Wilkinson 1989) and *Daphnia* (Hebert et al. 1982) have shown that heterozygotes are more common in long-standing asexual populations, and it cannot be attributed to hybridisation events due the occasional bouts of sexual reproduction (Peck and Waxman 2000). Differences in tolerance to low oxygen

have been noted, and are suggested to be due to differences at the haemoglobin locus (LaBerge and Hann 1990), although the effects of oxygen levels may be more evident in permanent habitats such as lakes, rather than temporary ponds. However, the same study suggested clone-specific responses to temperature, very likely to be a factor of temporary ponds. Seasonal changes in clone frequency have been shown to be related to differences in thermal tolerance of certain genotypes (Carvalho 1987). The relatively shallow and small pond in the present study would most likely experience marked changes in temperature throughout the year.

Established *Daphnia* clones have been shown to be prone to a particular mode of reproduction. For example, one clone may resort to sexual reproduction at the earliest sign of food shortage, whereas another clone may persist asexually until much later in the season. Certain clones will therefore prevail at different population densities. For example, in populations of *D. magna* from a temporary pond in Cambridgeshire, genotype GOT FF prevailed at increasing population densities and GOT SF at decreasing ones (Young 1979a). As clones are unable to transmit genetic information to the following generation, it would be expected for genetic variation to be depleted in this case, homozygosity to be increased and evolutionary flexibility to be lost (Ruvinsky et al. 1986). Possible mechanisms counteracting these events are the disconnection of physiological processes giving rise to males and ephippial eggs, making mating events between different clones more likely. Also, there may be low mating efficiency between clones. Patterns of clone frequency may develop due to genotypically distinct clones inhabiting certain regions that suit their genetically based physiological tolerances (LaBerge and Hann 1990). Indeed it has been suggested and supported that a population of *Daphnia* may consist of ecological generalists and seasonal specialists (Carvalho and Crisp 1987; Ross et al. 1996). Furthermore, heterozygous clones are generalists, having high growth and reproduction rates under a variety of conditions, and homozygous clones are specialists, suited only to a narrow range of conditions (Yampolsky and Kalabushkin 1991). It has been argued that the success of asexual taxa may be due to consisting of a large number of genotypes with narrow ecological preferences, rather than few broadly adapted genotypes (Hebert et al. 1988). There is evidence from the earthworm *Octolasion tyrtaeum* that successful clones have general-purpose genotypes allowing persistence despite

environmental changes (Jaenike and Selander 1979). Spatial and temporal components of the changing environments of a pond or lake must be considered simultaneously in order to provide a better understanding of the mechanisms that influence genetic patterns (Weider 1985). It would be interesting to study the changing abundances of alleles at more loci in order to distinguish between obligate parthenogens and new parthenogenetic lineages from season to season.

CHAPTER 3

RESOURCE COMPETITION BETWEEN GENETICALLY DIVERSE AND GENETICALLY UNIFORM POPULATIONS OF *DAPHNIA PULEX* (LEYDIG): DOES SEXUAL REPRODUCTION CONFER A SHORT-TERM ECOLOGICAL ADVANTAGE?

[This chapter is in revision with Evolution, under the same title and authored by Tagg, Doncaster and Innes.]

3.1. ABSTRACT

Small competitive advantages may suffice to compensate for a large disadvantage in intrinsic growth capacity. This ecological principle has recently been applied to the evolutionary question how sexual reproduction can persist in the face of invasion by female-only parthenogens. Small competitive advantages resulting directly from sexual reproduction are predicted to cancel a two-fold disadvantage in intrinsic growth capacity caused by half the sexual population comprising males that do not themselves produce offspring. In this paper we test the principal assumption of this theory, that the genetic diversity inherent to sexual populations confers a competitive advantage over self-identical asexual invaders. We set up competition between a diverse clonal assembly of *Daphnia pulex* and genetically identical populations from single clones. At young ages, the population comprising genetically diverse *Daphnia* had significantly higher birth rates in competition with populations of genetically uniform *Daphnia* than in competition with itself. This result indicates competitive release and a Lotka-Volterra competition coefficient $\alpha_{12} < 1$. No such difference was apparent under greater food stress, possibly due to individuals channelling more energy into survival, or for old-aged populations possibly as a result of reduced selective pressures for high reproduction in old females. Mean birth rates differed between the clones at all ages in the presence of competition, providing evidence of variation in life history traits between clones. A

Lotka-Volterra model calculated empirical estimates of $\alpha_{12} = 0.896$ (genetically uniform on diverse) and $\alpha_{21} = 1.010$ (diverse on uniform), which permit immediate coexistence of a sexual population of *D. pulex* even with an asexual lineage having twice the intrinsic population growth capacity.

3.2. INTRODUCTION

Why sex prevails in nature remains one of the great puzzles of evolution. Sex has an immediate cost relative to asexual reproduction, since males only express their contribution to population growth through females. With no males to sustain, asexual mutants can double their relative representation in the population in successive generations. This is the widely accepted 'two-fold cost of males' for anisogamous species in which half the population comprises males (Williams 1975; Maynard-Smith 1978). Recent theoretical studies by Gaggiotti (1994), Doncaster et al. (2000), Kerszberg (2000) and Pound et al. (2002) have shown that the presence of males can incur a considerably less than two-fold cost on population growth for sexual populations at density-dependent carrying capacity. Small advantages in competition for the sexual population are sufficient to halt the invasion of asexual mutants. The asexual competitors then exert a weaker inhibitory effect on the carrying capacity of the sexual population than they exert on their own carrying capacity through intra-specific competition. The stable outcome is coexistence on a depleted resource base, both locally and regionally (Doncaster et al. 2000; Doncaster et al. 2003). Under these ecological conditions we expect the sexual population eventually to drive out the asexual competitor by virtue of the longer-term benefits to its inherent genetic variation (for example, Kondrashov 1993). This is a general treatment of ideas present in earlier models of Sib Competition (Bell 1982) and niche differentiation (the Frozen Niche Variation hypothesis of Vrijenhoek 1979). The recent theory differs from those models by calibrating the competition between sexual types against competition within each type, using the conceptual framework of classical Lotka-Volterra dynamics (see Pound et al. 2002, in response to West and Peters 2000).

The objective of this paper is to test the underlying assumption of Lotka-Volterra models for competition between sexual and asexual forms, that genetic diversity confers a competitive advantage. We experimentally measured the birth rate achieved by a genetically diverse population of *D. pulex* (Leydig) in competition with a genetically uniform population, and compared this to the birth rate under intra-specific competition alone. By using *D. pulex* during asexual phases of their life cycle, we could measure competitive impacts on population growth rates due to differences in genetic variation without introducing confounding influences of male presence.

3.2.1. Theoretical Context

Doncaster et al. (2000) and Pound et al. (2002) analysed the cost of sex using Lotka-Volterra equations for two competing species with populations of sizes N_1 and N_2. The classical equations for rates of change in numbers over time ($\dot{N}_1$ and $\dot{N}_2$) were extended to distinguish intrinsic rates of death d_i from birth b_i:

$$\dot{N}_1 = b_1\left[1-\frac{N_1+\alpha_{12}N_2}{K_1}\right]N_1 - d_1N_1 \qquad \dot{N}_2 = b_2\left[1-\frac{N_2+\alpha_{21}N_1}{K_2}\right]N_2 - d_2N_2 . \quad (1)$$

The parameters K_i represent the carrying capacity of each population in isolation from the other and before deaths. The competition coefficients α_{ij} describe the per capita impact of species j on the population growth rate of species i, relative to the impact from intra-specific competition of species i on itself. Thus $\alpha_{12} = 1$ means that Species 2 has the same impact on Species 1 through inter-specific competition as Species 1 has on itself through intra-specific competition. Although density-dependent impacts are modelled on the birth term in equation (1) the dynamics are not changed by applying them to the death term, or to survival up to and including the first fecund age in a population with age-structured fecundity (Doncaster, in press).

It is the distinction of independent birth and death terms in equations (1) that allows us to model explicitly the dynamics of a sexual Species 1 competing with an asexual Species 2, such that Species 2 has twice the intrinsic growth capacity (and consequently a larger carrying capacity, though not necessarily twice as large, see Pound et al. 2002; cf assumptions of the earlier models of Case and Taper 1986; Koella 1993; Gaggiotti 1994). In effect, we confer an identical intrinsic per capita birth rate on sexual females as on asexual individuals, and a zero birth rate on males which are deemed to constitute half the sexual population, so $b_2 = 2b_1$. If the two types differ only in this respect, we can expect $d_1 = d_2$.

Setting both rate equations (1) to zero yields equilibrium solutions N_1^*, N_2^*. The conditions for invasion by each species are then:

$$N_1^* > 0 \text{ if : } \quad \alpha_{12} K_2 \left(1 - \frac{d_2}{b_2}\right) < K_1 \left(1 - \frac{d_1}{b_1}\right)$$

$$N_2^* > 0 \text{ if : } \quad \alpha_{21} K_1 \left(1 - \frac{d_1}{b_1}\right) < K_2 \left(1 - \frac{d_2}{b_2}\right). \qquad (2)$$

For a sexual species 1 with fast growth capacity, given by high b_1/d_1 (albeit half the value of b_2/d_2 for the asexual competitor 2), coexistence with a single clone is possible even with α_{12} close to unity (Doncaster et al. 2000). This is an expression of the trade-off between competition and growth that is a well-known ecological principle permitting coexistence of slow growing but strong competitors with faster growing species (Nee and May 1992; Doncaster et al. 2003). In the particular case of competitive advantages to the sexual population brought about by its having access to a wider resource base than any one clone, niche breadth has a complicated relation to b_i and K_i in equations (1). Versions of equations (1) expanded for niche breadth, nevertheless, demonstrate how a sexual population will withstand multiple clonal invasions, provided it retains at least a small resource refuge from the clones (Pound et al. 2002). This is because clonal diversity tends to be associated with intense competition amongst the clones, with the result that they can prevent each

other from establishing more effectively than they inhibit coexistence with the sexual population.

This adaptation of the Lotka-Volterra model differs from Tangled Bank models of Sib Competition (Bell 1982), which consider the advantage of genetic variation on the intensity of competition amongst sexual siblings compared to competition amongst identical asexual siblings due to a higher growth rate (Schmitt and Ehrardt 1987; Kelley 1989). They are closer in concept to Vrijenhoek's (1979) Frozen Niche Variation hypothesis: that asexual lineages arise in a sexual population with genotypes frozen to those of the progenitor parent and the limited genetic variation in asexual clones relative to sexual populations constrains the clonal phenotype to a narrower ecological niche (Vrijenhoek 1979; Hebert et al. 1988; Honeycutt and Wilkinson 1989; Jokela et al. 1997; Semlitsch et al. 1997; see review in Chapter 1). None of these studies, however, have explicitly tested the relative strengths of inter-specific and intra-specific competition. In contrast, our Lotka-Volterra dynamics consider how genetic variation in a sexual population influences the impact of asexual on sexual competitors, relative to the impact that the sexual individuals have upon themselves. This allows the prediction of coexistence from more subtle differences in niche usage (Case and Taper 1986; Koella 1993; Gaggiotti 1994; Doncaster et al. 2000; Kerszberg 2000; Pound et al. 2002; Doncaster et al. 2003). These two consequences of genetic variation, reducing intra-specific competition (Sib models) and reducing inter-specific competition (Lotka-Volterra model), may co-occur but are not necessarily found together (e.g. Maynard Smith 1989). In this study, therefore, we are able to test a quantitative theoretical model by calibrating the strength of inter-specific competition against intra-specific competition.

3.2.2. Experimental Context

The quantitative ecological model described by equations (1) and conditions (2) has yet to be tested experimentally in the context of competition between reproductive modes. This paper reports first tests with *D. pulex*, which has both obligately and cyclically parthenogenetic forms in North American ponds (Hebert et al. 1988). Laboratory experiments were designed to estimate competition coefficients α_{ij} from

controlled observations of birth and death rates for populations alone and in competition with each other. We used cyclically parthenogenetic *D. pulex* as a model organism because its aquatic habit and fast growth facilitate measuring competitive impacts, and its parthenogenetic (asexual) phase allows control over genetic variation. The *Daphnia* system suits Lotka-Volterra dynamics for this experimental timescale, involving continuous reproduction from the mixing of individuals and resources in a 3-dimensional habitat. Several different clones were established in the laboratory by isolating individual females from natural populations. By allowing populations comprising several clones to compete against populations comprising single clones, we were able to measure directly the influence of genetic variation on competitive ability. We tested two hypotheses: (a) net growth of a genetically diverse population is higher in competition with a genetically uniform population than in competition with itself ($\alpha_{12} < 1$ in the first of equations (1)); and (b) net growth of a genetically uniform population is higher in competition with a genetically diverse population than in competition with itself ($\alpha_{21} < 1$ in the second of equations (1)). By estimating the values of α_{12} and α_{21} we could then use the equilibrium conditions (2) to test whether coexistence would be possible in the presence of a two-fold cost of males (i.e. with $b_1 = 0.5 \cdot b_2$). This prediction assumes that the males are identical to females in all respects except the zero production of offspring. For example, it assumes that they have the same resource requirements as females, and that they neither reduce the fecundity of sexual females by harassment or inefficiency in finding mates, nor do they reduce the fecundity of asexual females by harassment. These and other constraints on the use of *Daphnia* as a model organism are evaluated in the Discussion.

3.2.3. Reproductive Biology of *Daphnia*

All-female populations of cyclically parthenogenetic *Daphnia* reproduce asexually until unfavourable conditions arise (for example over-crowding, reduced food or a change in temperature) and males are produced parthenogenetically. Sexual females then produce haploid eggs that are fertilized by these males, resulting in 'resting eggs' protected by the ephippium (modified carapace) that are able to withstand extreme conditions of freezing or desiccation. The resting eggs hatch

upon resumption of favourable conditions, and so permit *Daphnia* populations to occupy temporary pond habitats. Some species of *Daphnia*, including forms of *D. pulex*, have abandoned the sexual phase of their life cycle in favour of obligate parthenogenesis. The arrival of obligate parthenogenetic lines has been suggested to result from the males produced by some obligate parthenogens transmitting a dominant gene for sex-limited meiosis suppression (Innes and Hebert 1988). Evidence that these males can pass such genes to their progeny suggests that the gene could spread through a cyclically parthenogenetic population resulting in a genotypically diverse group of obligate parthenogens. It is reasonable to assume that this process is continuing and therefore that some obligate parthenogen clones have originated relatively recently (Innes and Hebert 1988). These populations occur in Canada (Hebert et al. 1988) and to a lesser extent further south (Lynch et al. 1989). Obligate parthenogens have the same life stages as cyclical parthenogens, but without the fertilisation of haploid eggs. They produce resting eggs that do not require fertilisation and will hatch into genetically identical offspring.

3.3. MATERIALS AND METHODS

3.3.1. Synchronisation of Clonal Genotypes

Ten random clonal genotypes were established from individual females collected from a temporary pond population (Long Point 8A) near Port Rowan in South-western Ontario, Canada, in early May. The 50-m^2 woodland pond is less than 1 m deep (Innes 1997). Long Point 8A is inhabited by genetically diverse cyclically parthenogenetic *Daphnia pulex* during April and May each year (Innes et al. 2000a) with polymorphic loci displaying Hardy-Weinberg equilibria (Innes 1991). *Daphnia* were collected using a plankton net (mesh size 300 μm) towed immediately below the water surface, and sampling approximately 100 l. Clones were cultured from individual females carrying a brood. Each female was placed in a plastic cup with 80 ml zooplankton media (Appendix 1; Lynch et al. 1986) and fed *ad libitum* with algae-water from an aquarium culture system. Aquaria-cultured algae contain bacteria and inedible algae as well as edible algae (in this case predominantly *Scenedesmus quadricauda*). The cups were kept in a temperature-

controlled cabinet set at 15°C for a 20L : 4D hr photoperiod to encourage good levels of health and reproduction. Neonates of the second-generation females were used in the experiment, all aged between 4 and 10 days old, and pre-reproductive maturity.

3.3.2. Experimental Design

Plastic cups containing 120 ml zooplankton media were divided in two by a central mesh of 200 μm gauge, preventing passage of *Daphnia* but allowing free mixing of their food (confirmed by a preliminary experiment). For the experimental treatments, ten immature females all from the same clone were placed on one side of the mesh (genetically uniform population), and one immature female from each of the ten clones were placed together on the other side (genetically diverse population) as shown in Figure 3.1. This treatment was repeated 20 times to allow each of the clones 1–10 to act as the genetically uniform population twice. For the 'genetically diverse' control treatment (ten replicates), one female from each of the ten clones was placed each side of the cup. For the 'genetically uniform' control treatment, ten individuals from the same clone were placed each side of the cup. This treatment was replicated for each of the clones 1–10. Cups were kept in a temperature-controlled cabinet, set at 15°C for a 20L : 4D hr photoperiod. Reserve stocks of each of the ten clones were held in similar conditions.

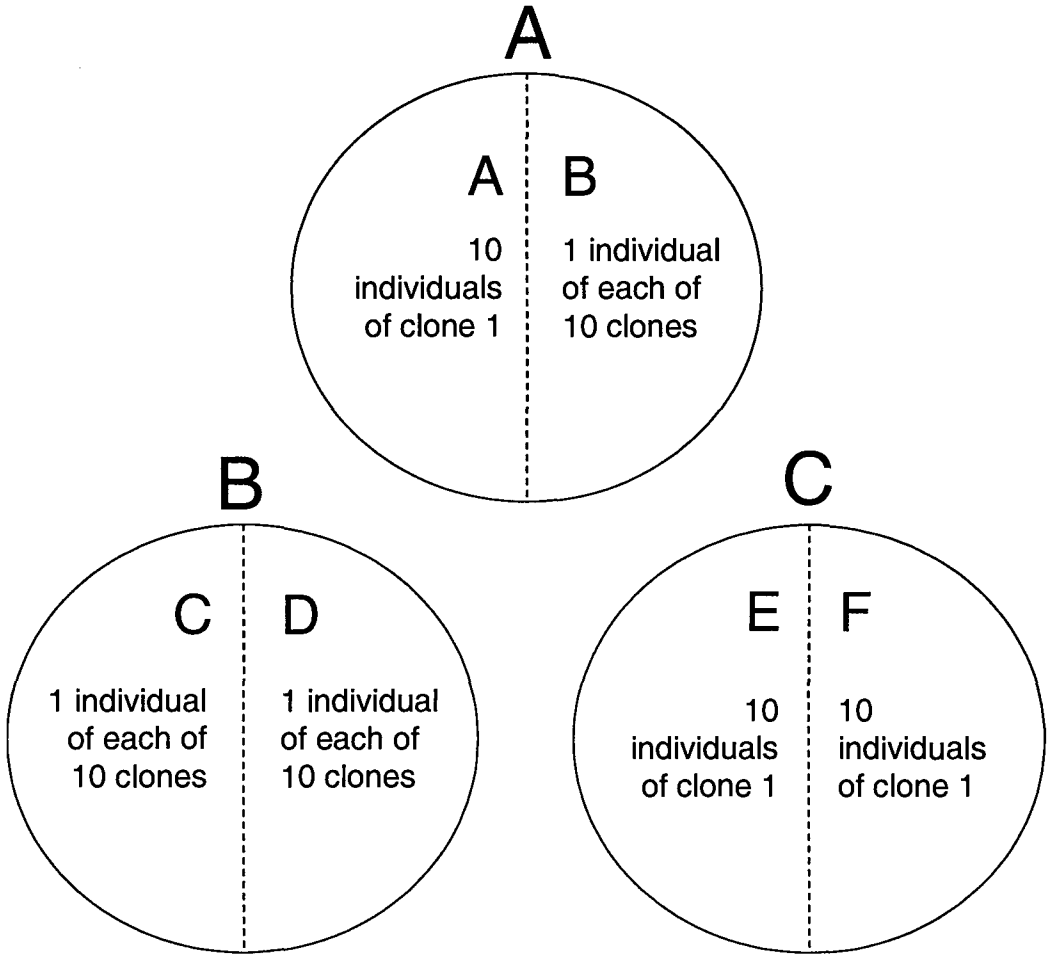


Figure 3.1—The experimental design. Cups were divided into two halves by a 200-μm mesh. (A) Experimental treatments in which a genetically diverse population competed with genetically uniform populations (two replicates for each of clone 1 through 10, here showing clone 1); (B) first control condition in which the genetically diverse population competed against itself (10 replicates) and (C) second control condition in which genetically uniform populations competed against themselves (two replicates for each of clone 1 through 10, here showing clone 1)

3.3.3. Feeding and Monitoring

Conditions of limited food supply were sustained by providing all cups each day with 2.0–2.4 $\times$ 10^6 algal cells, or 100,000–120,000 cells per *Daphnia* (haemocytometer counts), divided into twice daily feeds for 9 days (*Daphnia* aged 4–20 days old). Preliminary feeding trials had established that this regime provided sufficient food for reproduction, with brood sizes of about 10 per female per day, whilst clearly inducing some food stress since well-fed females can produce on the order of 50 or more eggs per brood, but avoiding adult starvation. The food was administered by pipette and mixed into the media by swivelling the cup. For the next 9 days (days 10–18 of the experiment; *Daphnia* aged 13–29 days old), food supplies were reduced to 1.5–2.0 $\times$ 10^6 algal cells, or 75,000–100,000 cells per *Daphnia*, in order to test the hypothesis that with increased food stress, exploitative competition would reveal subtle competitive advantages. For the last 11 days of the experiment (days 19–29; *Daphnia* aged 24–40), the higher food allowance was resumed. Throughout the experiment, levels of zooplankton media were maintained at 120 ml, and any settled inedible algae or detritus was removed. Cups were examined twice daily and newly released young immediately removed and counted, to prevent prolonged changes in population size. Reproductive success was measured in terms of the number of live young produced per adult per day. The competitive impacts of different treatments were measured from variation in this number, which could result from variation in brood size or in survival of offspring immediately *post partum* (since only live young were counted as contributing to population growth). Ephippial females were counted, and this allowed for a rough conversion to the number of ephippia released (total number of ephippial females per population every 4 days) and subsequently to reproductive units (1 ephippia = 9 reproductive units: Lynch 1983; Korpelainen 1992), in order to include the reproductive effort of these females in the analysis. Of the 200 test adults in the genetically uniform populations, 44 died during the experiment. Each of these individuals was immediately replaced with a fresh individual from the reserve stock of that clone. Reserve individuals were of the same age as the experimental individuals, and only non-brood carrying individuals were used in order to minimise differences in physiological state. Of the 200 test adults in the genetically

diverse populations, 20 died during the experiment. For each of these individuals, its genotype could not be identified and therefore it could not be replaced without changing the genetic variation by an unknowable amount. Instead, an individual from the other side of the cup (i.e., a random genetically uniform individual) was removed to balance the population sizes on either side. In the case of decreasing population sizes, food supply was reduced accordingly.

3.3.4. Statistical Analysis

The null hypothesis H_0: $\alpha_{12} = 1$ was tested with a one-way general linear model and planned contrasts (SAS v. 6.12). The model analysed the hypothesis that the per capita birth rate of the genetically diverse population depended on Treatment with 11 levels, accounting for the genetically uniform clones 1–10 and the single genetically diverse control. The planned contrasts were made between the 10 treatments of genetically diverse-on-uniform and the one treatment of diverse-on-diverse. These contrasts are numerically equivalent to a two-factor general linear model that partitions the variation due to Treatment into a Competitor effect (genetically uniform versus genetically diverse) and a Clone effect (the 10 clones nested within the genetically uniform Competitor):

$$\text{Births}_{\text{diverse}} = \text{Competitor} + \text{Clone (Competitor)} + \text{Replicate' (Clone (Competitor))}.$$

The impact of the genetically uniform population was measured from the B-side of the Figure 3.1A cup, with two replicates for each of the 10 treatments; the control impact of the genetically diverse population was calculated from the mean value of the two sides of each Figure 3.1B cup, with 10 replicates.

The null hypothesis H_0: $\alpha_{21} = 1$ was tested with a two-factor analysis of variance (Minitab v. 13.1). The model analysed the hypothesis that the per capita birth rate of the genetically uniform population depended on an interaction of Competitor with Clone:

$$\text{Births}_{\text{uniform}} = \text{Competitor} + \text{Clone} + \text{Competitor} \times \text{Clone} + \text{Replicate' (Competitor} \times \text{Clone)}$$

with two levels of Competitor (genetically diverse and genetically uniform) and 10 levels of Clone (representing clones 1–10). The impact of the genetically diverse population was measured from the A side of the Figure 3.1A cup, with two replicates per Clone; the control impact of the genetically uniform population was calculated from the mean value of the two sides of each Figure 3.1C cup, also with two replicates per Clone.

3.3.5. Competition Coefficients

The intrinsic birth rate, b, of each clone was required in order to calculate competition coefficients. Estimates of b were obtained by culturing five females from each clone individually in plastic cups kept under optimal conditions with food and space provided *ad libitum*. All live offspring were removed immediately after release, and counted.

Carrying capacities, K_i, were estimated from the experimental controls of intra-specific competition alone, in which values were known for the population growth rate, $\dot{N}_i$, the rate constants, b_i and d_i, and population size, N_i in the growth equations:

$$\dot{N}_1 = b_1\left[1-\frac{N_1}{K_1}\right]N_1 - d_1 \qquad \dot{N}_2 = b_2\left[1-\frac{N_2}{K_2}\right]N_2 - d_2. \qquad (3)$$

This model assumes the simplest form of density-dependent growth, given by a linear decline in births per capita with increasing density, and density-independent deaths. The predictions may be biased if density-dependence is non-linear, although this appears unlikely for these laboratory populations of *Daphnia* which have dynamics controlled by their algal food and not by interactions with other components of the environment (McCauley and Murdoch 1987; Gurney and Nisbet 1998). Their algal food generally has self-limiting logistic growth in the absence of predation (e.g. Nisbet et al. 1991), and *Daphnia* have a linear response of food intake to food availability up to saturation levels (a Type I functional response:

Rigler 1961), and of birth rate to food intake (Richman 1958). Equations (3) use rates of instantaneous population growth ($\dot{N}_i$) that were obtained from continuous reproduction by a constant number of adults of known age. The design therefore avoided the complications of age structure and discrete reproductive events present in wild *Daphnia* populations, which require more sophisticated models (McCauley et al. 1999). Although laboratory populations are susceptible to cycle (McCauley et al. 1999), we assume that wild sexual populations produce males at K_1 during a period when this carrying capacity is gradually declining as the environment deteriorates.

Competition coefficients were calculated from equations (1), given values of b_i, d_i, and K_i. For the genetically uniform populations deaths were replaced and therefore $d_2 = 0$. Competitive impacts between populations were assumed to have the same density-dependent profile as the impacts within populations given by equations (3). Finally we tested the hypothesis that a genetically diverse population with a sex ratio of 1M:1F could coexist with a genetically uniform population of female-only parthenogens. Condition (2) was tested with lifetime estimates of b_2, d_1, d_2 provided by Paloheimo and Taylor (1987). We set $b_1 = 0.5 \cdot b_2$, on the parsimonious assumption that sexual populations invest equally in both sexes (Fisher's sex-ratio theory), and the presence of males has no other effect than to halve the intrinsic growth capacity of the sexual population.

3.4. RESULTS

At young ages, the genetically diverse *Daphnia* population had a higher mean birth rate in competition with all genetically uniform populations, compared to competition with itself (4–20 day-olds over first 9 days of experiment, Competitor contrast: $F_{1,19} = 15.44$, $P = 0.001$). Within the genetically uniform group, clones diverse little in their competitive impact (4–20 day-olds, Clone contrast: $F_{9,19} = 0.48$, $P = 0.868$). Figure 3.2 illustrates the relatively lower impact of all the genetically uniform populations on the genetically diverse population, from which we conclude $\alpha_{12} < 1$ for young individuals. No differences were observed for the second 9 days under food stress (13–29 day-olds, Competitor contrast: $F_{1,19} = 0.16$, $P = 0.690$; Clone contrast: $F_{9,19} = 0.87$, $P = 0.565$) or for the last 11 days after the period of food stress (24–40 day-olds, Competitor contrast: $F_{1,19} = 0.90$, $P = 0.345$; Clone contrast: $F_{9,19} = 0.52$, $P = 0.840$).

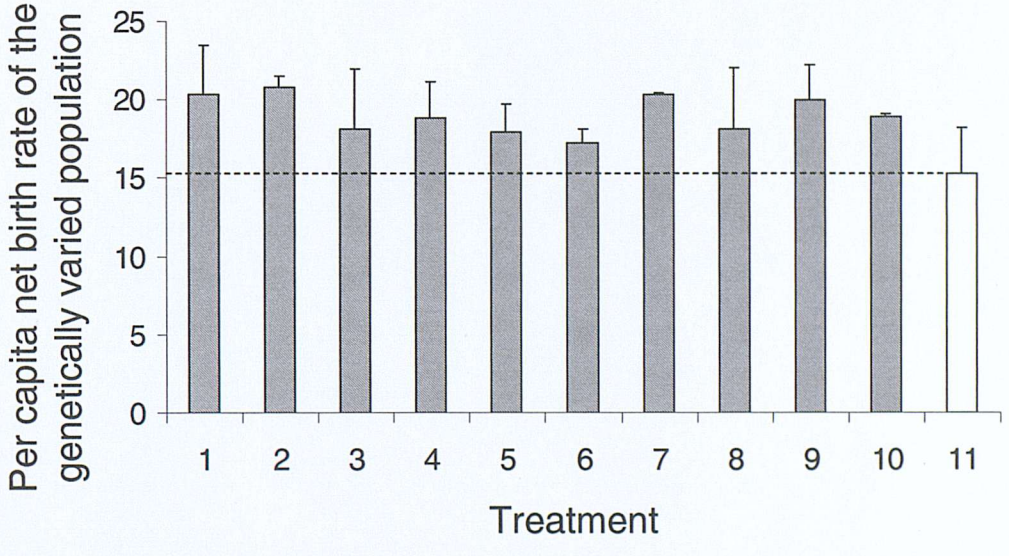


Figure 3.2—Birth rate of the genetically diverse population over the first 9 days, in competition with genetically uniform populations (hashed bars), and with itself (clear bar). Values are young produced per individual by the genetically diverse *Daphnia* population over the period (scaled per day), measured from the B side of Fig. 3.1A cups for competition with each clone (treatments 1–10 showing mean ± s.e. of two replicates), and from the mean of both sides of Fig. 3.1B cups for competition with itself (treatment 11 showing mean ± s.e. of 10 replicates)

The genetically uniform populations of *Daphnia* showed no systematic difference in competition with the genetically diverse population, compared to competition with themselves. The impact of the genetically diverse population on birth rates depended on the clone that made up the uniform population only at old ages (24–40 day-olds over last 11 days, Competitor × Clone interaction: $F_{9,20} = 4.68$, $P = 0.002$). Inter-specific competition had no main effect on birth rates of genetically uniform populations either at young ages (4–20 day-olds over first 9 days of experiment, Competitor main effect: $F_{1,20} = 0.04$, $P = 0.839$), or under food stress (13–29 day-olds over days 10–18 of experiment, $F_{1,20} = 0.01$, $P = 0.917$), or at old ages (24–40 day-olds over last 11 days of experiment, $F_{1,20} = 0.03$, $P = 0.870$). Figures 3.3A and B illustrate the absence of an overall pattern with respect to competitor, from which we conclude $\alpha_{21} = 1$. The greater degree of variation in birth rates between clones at 24–40 days old suggests a general reduction in young production, and increased mortality.

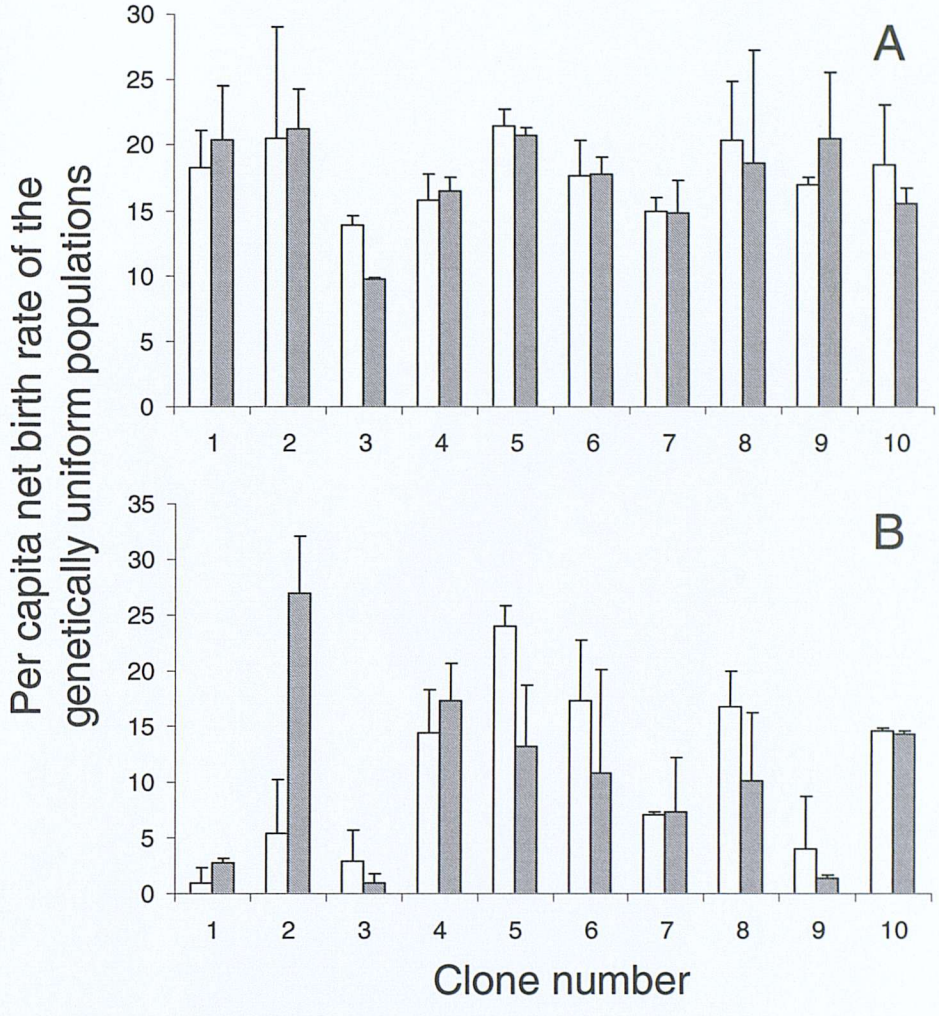


Figure 3.3—Birth rate of the genetically uniform populations when in competition with the genetically diverse population (hashed bars) and in competition with themselves (clear bars), (A) at 4–20 days old and (B) at 24–40 days old. Values are young produced per individual by each of the ten genetically uniform *Daphnia* populations over the period (scaled per day), measured from the A side of Fig. 3.1A cups for hashed bars (mean ± s.e. of two replicates), and from the mean of both sides of Fig. 3.1C cups for clear bars (mean ± s.e. of two replicates)

3.4.1. Clonal Variation

Figures 3.3A and B show a significant overall variation in birth rate between clones in the presence of competition, both at young ages (4–20 day-olds, Clone main effect: $F_{9,20} = 2.40$, $P = 0.049$) and old ages (24–40 day-olds, $F_{9,20} = 10.44$, $P < 0.001$), but not during food stress (13–29 day-olds, $F_{9,20} = 1.75$, $P = 0.143$). The mean number of young produced each sampling day differed for each of the clones throughout the experiment. Figure 3.4 shows the difference in mean young production between the 10 clones when competing with other genetically uniform populations (continuous lines) and when in competition with a genetically diverse population (broken lines) for young and old reproducers pooled. Most births occurred in the first 9-day period for most clones, and food stress from days 10–18 markedly reduced birth rates. Young production displays short-term cycling with the majority of young being produced every few days in all clones. The production of young shows no pattern of difference between clones competing against themselves and clones competing against a genetically diverse population.

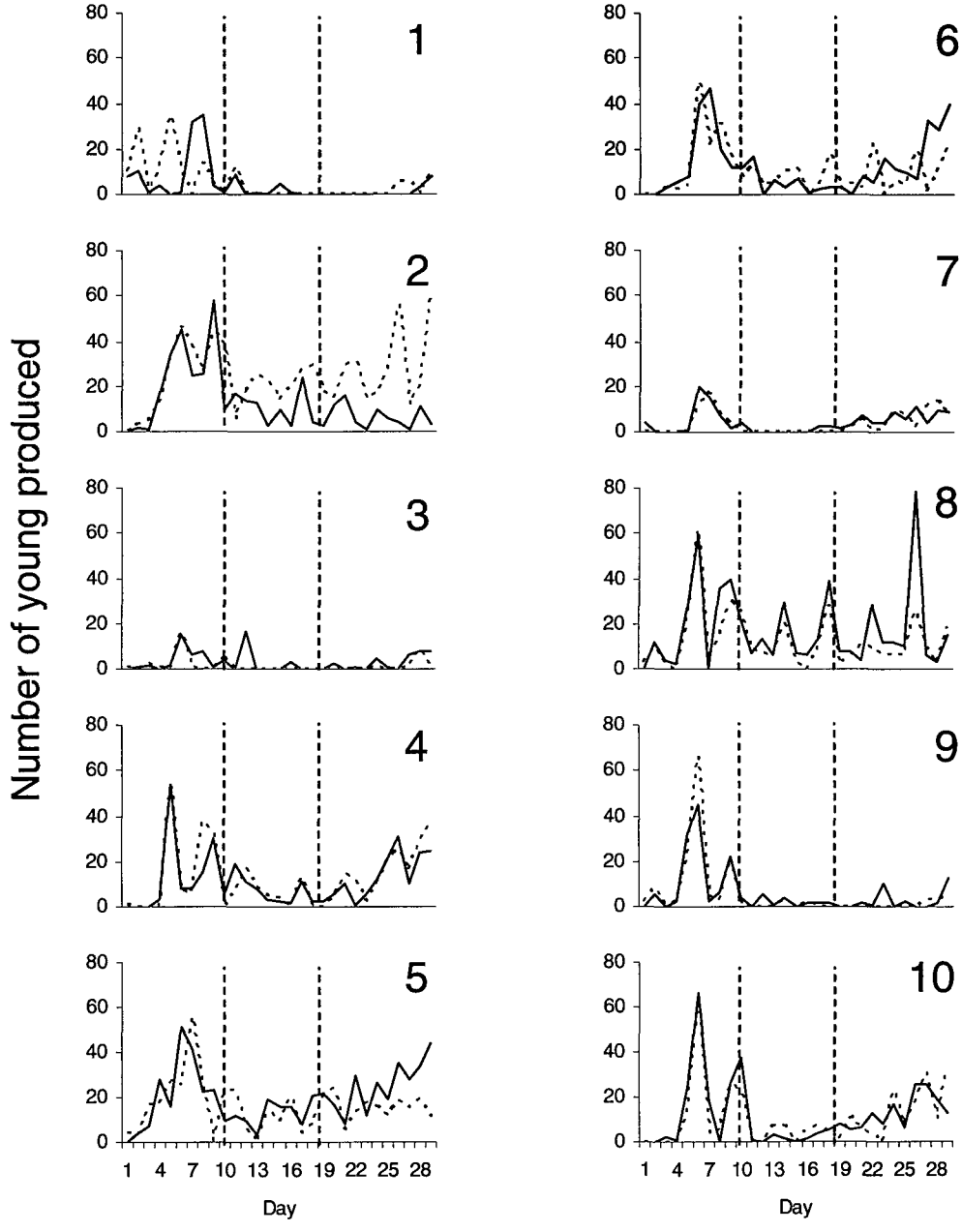


Figure 3.4—Young production per day by clones 1 to 10 when in competition with themselves (continuous line, mean of four replicates) and with the genetically diverse population (broken line, mean of two replicates). The period of food stress over days 10–18 is delimited by vertical broken lines

3.4.2. Competition Coefficients

Table 3.1 shows estimates of intrinsic birth rates for each of the ten clones, representing the ten genetically uniform populations in the study. Mean production did not differ between clones ($F_{9,40} = 1.14$, $P = 0.359$). The combined average for the group of clones provides an estimate of the intrinsic birth rate for the single genetically diverse population created from these 10 clones.

Table 3.1—Intrinsic birth rates of each of the 10 clones, calculated per day from the mean number of young produced per isolated female over a 19-day period

Clone	Production per individual over 19 days (mean ± s.e. of 5 females)	Intrinsic birth rate, *b* (per individual per day)
LP8A 1-2	170.75 ± 22.87	8.99
LP8A 1-5	213.20 ± 17.40	11.22
LP8A 1-6	158.20 ± 19.78	8.33
LP8A 1-8	173.00 ± 7.03	9.11
LP8A 1-11	172.20 ± 3.17	9.06
LP8A 1-16	205.60 ± 13.55	10.82
LP8A 1-20	202.80 ± 16.94	10.67
LP8A 1-21	191.20 ± 20.66	10.06
LP8A 1-27	204.40 ± 26.71	10.76
LP8A 2-1	176.00 ± 7.40	9.26
Pooled	186.74 ± 8.47	9.83

Table 3.2 shows the calculation of carrying capacities for genetically diverse and genetically uniform populations from equations (3), using estimated rates b_i, d_i and $\dot{N}_i$, for given N_i. With these estimates of K_i, competition coefficients were calculated from equations (1) as: $\alpha_{12} = 0.896$ and $\alpha_{21} = 1.010$, as shown in Table 3.3. These values are conservative estimates since they apply to the full 29 days of the experiment, even though the value of α_{12} was consistently below unity only for the first 9 days (as described by Figure 3.2). The values of the coefficients may be biased by the slightly higher value of d_1 that was obtained in competition compared to isolation (cf. Table 3.2 with 3.3), and by these deaths forcing $N_i < 10$ over the course of the experiment since it was not possible to replace them. If the competition between populations was responsible for increasing the death rate per capita, however, as well as decreasing the birth rate, then the value of α_{12} given by the first of equations (1) in Table 3.3 is larger than the true value.

Table 3.2—Calculation of the pre-death carrying capacities, K_i, for the genetically diverse (i = 1) and genetically uniform (i = 2) populations in isolation. Values were obtained from inputs into equations (3) of the empirical estimates of intrinsic birth and death rates per capita and population growth for given size. Parameter b_i = mean birth rate per capita per day (from Table 3.1); d_i = mean death rate per capita per day; $\dot{N}_i$ = mean number of young produced per population per day over the 29 days of the experiment (population growth rate); N_i = number of individuals in population. The value of N_1 in parentheses is the mean number by the end of the experiment, and using this in equations (3) gives the K_1 value in parentheses

Genetically diverse population in isolation		Genetically uniform populations in isolation	
Parameter	Value	Parameter	Value
Inputs to equations (3)			
b_1	9.830	b_2	9.830
d_1	0.00179	d_2	0
$\dot{N}_1$	33.590	$\dot{N}_2$	27.234
N_1	20 [19.60]	N_2	20
N_2	0	N_1	0
Output from equations (3)			
K_1	24.127 [23.744]	K_2	23.217

Table 3.3—Calculation of the competition coefficients α_{12} and α_{21} for the populations experiencing inter-specific competition. Values were obtained from inputs into equations (1) of the empirical estimates of intrinsic birth and death rates per capita and realised population growths for given sizes. Parameter definitions as in Table 3.2. Values of N_i in parentheses are the mean numbers by the end of the experiment, and using these as inputs to equations (1) gives the α_{ij} values in parentheses

Genetically diverse population in competition		Genetically uniform populations in competition	
Parameter	Value	Parameter	Value
Inputs to equations (1)			
b_1	9.830	b_2	9.830
d_1	0.00278	d_2	0
$\dot{N}_1$	21.017	$\dot{N}_2$	13.210
N_1	10 [9.52]	N_2	10 [9.52]
N_2	10 [9.52]	N_1	10 [9.52]
K_1	24.127 [23.744]	K_2	23.217
Output from equations (1)			
α_{12}	0.896 [0.933]	α_{21}	1.010 [1.094]

Condition (2), testing for coexistence of sexual and asexual types, was quantified with the numbers N_i, K_i and the calculated parameter values for α_{ij} given in Table 3.3, along with lifetime estimates of $b_2 = 0.194$, and d_1, $d_2 = 0.011$ per individual per day given by Paloheimo and Taylor (1987). Setting $b_1 = 0.5 \cdot b_2$, the prediction for $N_1^* > 0$ was met, with $19.623 < 21.391$ in the first of conditions (2). The prediction for $N_2^* > 0$ was also met, with $21.605 < 21.901$ in the second of conditions (2). The first of conditions (2) was also satisfied by using end-of-experiment values for N_i (parenthetical values in Tables 3.2 and 3.3), although the second was not. We therefore conclude that the observed competitive advantage conferred by genetic variation suffices to permit persistence of young sexual populations with or against asexual invaders, even if the growth capacity of the sexual population is half that of the asexual competitors due to the presence of up to 50% males.

3.5. DISCUSSION

The principal result of the experiment is that genetic variation can contribute significantly to competitive release in the presence of genetically uniform populations. Further work will be needed to elucidate the mechanism of competitive release, but we expect it is a combination of responses to the impure mix of edible and inedible algae and bacteria, expressed in the fertility of females and the survival of their newborn offspring (dead newborns were not counted amongst the live offspring from which we measured population growth). The different mean growth rates of the genotypes (Figures 3.3 and 3.4) suggest that the heterogeneous food supply provided sufficient habitat variation against which different genotypes could express variation in ecological advantages. An even greater release from competition may occur in natural settings, presenting a Tangled Bank of microhabitats to populations. Our calculations indicate that an estimated competitive impact even as high as $\alpha_{12} \sim 0.9$ can provide sufficient release to cancel the two-fold disadvantage in growth capacity that would be incurred by a sexual population comprising 50% males. This is a standard prediction for coexistence from Lotka-Volterra theory which, surprisingly, has never previously been tested on the question of maintenance of sex (Doncaster et

al. 2000). Although we did not include males in our experiments, copepod males are known to express aggressive mating behaviour (Brewer 1998). This may extend to harassment of asexual females in particular, since they gain no compensating benefit of mating, which would further increase the competitive advantage of the sexual population. In regions where sexual and asexual populations of *Daphnia pulex* coincide (Hebert et al. 1988) we can at least expect that the genetic variation inherent to the sexual populations may cancel the immediate advantage to asexual populations of greater growth capacity to allow coexistence. Sex then has time to express its longer-term advantages of genetic variation in meeting the demands of environmental change, resulting in eventual displacement of asexual invaders.

In heterogeneous habitats, such as the temporary ponds favoured by *Daphnia*, adapting to new environments is essential to long-term survival. Cyclical parthenogens confront seasonally changing environments by creating new genotypes in sexual recombination, and then multiply the favourable ones by parthenogenesis. Obligate parthenogens can only create genetic diversity in a stepwise accumulation of mutations. Moreover, they carry an ever-increasing load of recessive deleterious genes, with a finite population never having a load less than its least-loaded clone, and the resulting accumulation of deleterious mutations may limit their life span (Innes and Hebert 1988). Evidence for this genetic load has been found in a decreased relative survival of offspring from self-mated clones (parthenogenesis) as compared to those from outcrossed clones (Innes 1989). Parthenogenesis is widely accepted to be advantageous, however, under conditions of uninhibited population growth, when an asexual population can double its representation each generation relative to an otherwise identical sexual population. In addition, parthenogenesis allows any favourable genotype to be replicated without costs of meiosis in segregation and recombination load (Maynard-Smith 1978). Once populations reach carrying capacity, our study has shown that these short-term advantages to parthenogenesis may be cancelled by small competitive advantages to a genetically diverse sexual population.

Short-term advantages of sexual reproduction have also been recorded for other taxa. New World sexual populations of the brine shrimp *Artemia salina* out-competed sibling asexual populations at high food levels (Browne 1980; Browne

and Halanych 1989). Amongst dipterans, genetically diverse populations of *Drosophila melanogaster* evolving under natural selection developed more pupae than homogeneous populations whose larvae were unable to evolve (Becerra et al. 1999). Similarly for coleopterans, Dunbrack et al. (1995) set two populations of *Tribolium castaneum* in competition, with the population prevented from responding to selection for competitive ability being eliminated within a few generations. Although those experiments pre-date the quantitative framework for ecological costs of sex that we use here, they all suggest levels of competitive release that may permit coexistence of sexual with asexual types.

The competitive release that we observed for genetically diverse *Daphnia* populations in competition with genetically uniform populations, giving $\alpha_{12} < 1$, arose only in the first part of the experiment for young competitors before food deprivation. The subsequently increased food stress may have stimulated females to channel their energy more into survival than fecundity. Browne (1980) observed a similarly reduced competitive ability in sexual populations of *Artemia salina* when held at low food availability. In our experiment, the absence of competitive release in old-aged genetically diverse *Daphnia* (24–40 days old) may be explained by reduced competitive effort at older age. The experiment was repeated in its entirety using individuals from different ponds to increase the degree of genetic variation for life-history traits present in the populations. However, cultures were maintained in the laboratory for a longer period prior to the experiment and individual *Daphnia* were not as healthy as the ex-ephippial females used in the previous experiment. Ex-ephippial *Daphnia* grow faster, have bigger body sizes at maturity and higher fecundity than parthenogenetic generations (Arbaciauskas and Gasiunaite 1996). No competitive release was observed for the genetically diverse populations in the repeated experiment, suggesting a reduced competitive effort of old lineages as well as old-aged individuals.

In previous tests of Sib Competition, several experimental studies found that the difference in growth between isolated populations of sexual and asexual types became less apparent at higher growing densities (Ellstrand and Antonovics 1985; Kelley 1989). Experimental studies of genetic mixtures in laboratory and

agronomic settings have shown that their yield is not much greater than that of the average of their pure components, and is rarely greater than that of the best component (e.g. Bell 1990; Weeks and Sassaman 1990). Although such studies showing a less than two-fold reduction in the impact of intra-specific competition within a genetically diverse type, compared to intra-specific competition within a uniform clone, have provided evidence against the Tangled Bank model of Sib Competition, they do not constitute evidence against inter-specific competition as a mechanism for the maintenance of sex. The theory and experimental test described in this chapter demonstrate how a very modest competitive release for a genetically diverse population competing with a uniform clone can be sufficient to compensate for the two-fold disadvantage in intrinsic growth capacity that is the cost of obtaining genetic variation from male production. To our knowledge, no previous studies of the maintenance of sex have attempted to measure competitive release specifically in the form of a lower inter-specific impact (from the clone) compared to the impact of intra-specific competition on growth of the genetically diverse population. Of the few other studies directly competing sexual or genetically diverse types against asexual clones, however, most have found that the latter performs relatively poorly (e.g. review in Lynch 1984b; Michaels and Bazzaz 1986) although this is not always the case (e.g. Jokela et al. 1997).

The choice of *D. pulex* as an empirical model for testing cost of sex theory has some potential disadvantages to set against the advantages of measurable genetic variation and testable competitive impacts. Although wild populations have long-term stability (Gurney and Nisbet 1998; Murdoch et al. 1998), they undergo seasonal changes in abundance and can cycle at least under laboratory conditions (McCauley et al. 1999). In sexual forms, males tend to be produced as environmental conditions deteriorate and the products of sexual reproduction hatch from diapausing eggs. Sexual and obligate asexual forms may therefore compete out of phase with each other. Furthermore, the size of young may vary depending on competitive ability of individuals and is a factor that could be taken into account in future studies. Nevertheless, for the short period when sexual forms start to produce males, the experiment is designed to show whether genetic variation confers competitive advantage on a population at its carrying capacity.

Our experiment replicated the genetically uniform populations with 10 different clones from a genetically diverse population. It was once believed that clonal species had little or no genetic diversity (Williams 1975), but more recent genetic analysis has shown much genetic diversity, for example, among clones in *D. pulex* (Weider et al. 1987), the prosobranch *Potamopyrgus antipodarum* (Dybdahl and Lively 1995; Fox et al. 1996) and the dandelion *Taraxacum officinale* (Lyman and Ellstrand 1984). In addition Lynch (1984c) and Lynch et al. (1989) have demonstrated much genetic variation for life-history characters in *D. pulex*. Any associated inter-clonal differences in niche requirements or life history traits may affect average competitive impacts. In our experiment, birth rates differed significantly between clones, particularly at older ages, and the late-age birth rates of some clonal genotypes were improved by competition with the genetically diverse population (Figure 3B). The relative growth rates of clones will depend particularly on differences in age at first brood, size of first brood and size of subsequent broods. In obligately parthenogenetic *D. pulex*, such variation in life-history traits is likely to be derived both from mutation (Lynch 1984d) and the polyphyletic origin of obligately parthenogenetic clones from the sexual cyclical parthenogens (Lynch et al. 1989). *Daphnia* are known to exhibit age and maternal effects and clones may be prone to early or late reproduction, although our experiment attempted to control for these factors by using similarly aged and experienced females in the starting populations.

Whether or not sexual reproduction can balance the two-fold cost of sex in competitive superiority will certainly depend upon the life-history traits of the genetically uniform population against which it is competing. The clonal genotypes of *D. pulex* that we used were derived from a single population of cyclical parthenogens. Obligate parthenogens from different ponds would be expected to have an even greater degree of variation in life-history traits (for example, filtering efficiency) due to their spatial isolation over generations, and may therefore present a more formidable competitive challenge to sexual populations of *D. pulex*. Chapter 4 tests whether sexual populations of *D. pulex* can resist invasion by obligately parthenogenetic *D. pulex*.

CHAPTER 4

GENETICALLY UNIFORM AND GENETICALLY DIVERSE POPULATIONS OF *DAPHNIA OBTUSA* (KURZ): POPULATION DYNAMICS AFTER INVASION

4.1. ABSTRACT

We test the assumption from ecological theory that the genetic diversity inherent to sexual populations can provide them with sufficient competitive advantage both to facilitate invasion by sexual groups into asexual populations and to withstand invasion by asexual competitors. In particular, we explore the hypothesis that larger genetically diverse groups, with greater inherent genetic variation, have greater competitive advantage when invading into genetically uniform populations than do smaller groups with less genetic variation. We set up small and large groups of diverse clonal assemblies of *Daphnia obtusa*, representing sexual invaders, and injected them into populations of genetically uniform individuals from single clones, representing asexual populations. We then reversed the procedure, injecting small and large genetically uniform groups into genetically diverse populations. The proportionate change in size of invading group within the population after 13 weeks depended on an interaction between mode of invader and its initial group size. Genetically diverse invaders of initially large group size increased their representation by slightly more than those of initially small size; in contrast, genetically uniform invaders of initially large group size diminished on average by considerably more than those of initially small size. These results demonstrate an advantage to genetic variation, both in invasion and resisting invasion. We attribute this advantage to the competitive release experienced by genetically diverse individuals when competing against genetically uniform individuals, due to a wider niche and reduced relative intra-specific competition. The effect is enhanced for a larger genetically diverse group with greater inherent genetic variation. The allozyme glucose-6-phosphate isomerase (GPI) was used as a genetic marker and genetically diverse and genetically uniform invading groups of genotype SS

increased in proportion in the recipient population more than groups of genotype SF. This result suggests a heterozygote disadvantage for the enzyme.

4.2. INTRODUCTION

Sexual reproduction has an immediate cost relative to asexual reproduction, since males only express their contribution to population growth through females. With no males to sustain, asexual mutant invaders can double their relative representation in the recipient population in successive generations, all else being equal. This is the widely accepted two-fold cost of males for anisogamous species in which half the population is comprised of males (Williams 1975; Maynard-Smith 1978). Recent theoretical studies by Doncaster et al. (2000), Kerszberg (2000) and Pound et al. (2002) have shown that the presence of males can impose a considerably less than two-fold cost on population growth for sexual populations at density-dependent carrying capacity. Small advantages in competition for the sexual population are sufficient to halt the invasion of asexual mutants. The asexual competitors then exert a weaker inhibitory effect on the carrying capacity of the sexual population than they exert on their own carrying capacity through intra-specific competition. The stable outcome is coexistence on a depleted resource base both locally and regionally (Doncaster et al. 2000; Doncaster et al. 2003). Under these crowded conditions the sexual population may eventually drive out the asexual competitor by virtue of the longer-term benefits to its inherent genetic variation (e.g. Kondrashov 1993). The recent theory of Doncaster et al. (2000) differs from previous ecological models by calibrating the competition between sexual types against competition within each type, using the conceptual framework of classical Lotka-Volterra dynamics (see Pound et al. 2002, in response to West and Peters 2000). Chapter 3 has demonstrated empirical evidence using *Daphnia pulex* in support of the theory that small competitive advantages resulting directly from sexual reproduction can cancel a two-fold disadvantage in growth capacity caused by half the sexual population comprising males that do not themselves produce offspring. It was found that, at young ages, Population 1 consisting of genetically diverse *Daphnia* had significantly higher birth rates in competition with Population 2 of genetically uniform *Daphnia* than in competition with itself ($\alpha_{12} <$

1), indicating competitive release. The calculations indicated that the estimated Lotka-Volterra competition coefficient $\alpha_{12} = 0.896$ is sufficient to prevent the exclusion of sexual types even if sexual populations have half the growth capacity of asexual populations.

These theoretical and empirical studies of the ecological costs of sex give rise to a new hypothesis that we test in this chapter. If genetic variation confers competitive advantage, a larger group of genetically diverse individuals, with more genetic variation, should have a greater advantage than a smaller group of genetically diverse individuals. We explored the effects on a genetically uniform recipient population of the invasion of a small or large number of genetically diverse individuals of *Daphnia obtusa* Kurz (Crustacea: Cladocera). We also explored the reciprocal effects on a genetically diverse recipient population of the invasion of a small or large number of genetically uniform individuals. We tested two hypotheses: (1) a genetically diverse group invading into a genetically uniform population will increase within the population and perhaps even eliminate the genetically uniform recipient population; whereas a genetically uniform group invading into a genetically diverse recipient population will decrease within the population and perhaps even be eliminated by the genetically diverse recipient individuals; and (2) larger genetically diverse invading groups will increase in the population more than the smaller genetically diverse invading groups, due to their greater genetic variation.

The experiment used cyclically parthenogenetic *D. obtusa* during parthenogenetic growth. The aquatic habitat and fast growth of *Daphnia* facilitate measuring competitive impacts, and its parthenogenetic (asexual) phase allows control over genetic variation without the confounding effects of the presence of males or the cost of searching for a mate and mating. We are therefore able to test for competitive advantages of the genetic variation inherent in sexual reproduction in isolation from the growth capacity disadvantage of producing males. All-female populations reproduce asexually until unfavourable conditions arise (for example over-crowding, reduced food or a change in temperature) and males are produced parthenogenetically. Sexual females then produce haploid eggs that are fertilized by

these males, resulting in resting eggs protected by the ephippium (modified carapace) that are able to withstand extreme conditions of freezing or desiccation. The resting eggs hatch upon resumption of favourable conditions, enabling *Daphnia* populations to persist in temporary habitats. Some species of *Daphnia* have abandoned the sexual phase of their life cycle in favour of obligate parthenogenesis. These populations occur in Canada (Hebert et al. 1988) and to a lesser extent further south (Lynch et al. 1989). Obligate parthenogens produce diapausing eggs that do not require fertilisation and will hatch into genetically identical offspring. The arrival of obligate parthenogenetic lines has been suggested to result from the males produced by some obligate parthenogens transmitting a dominant gene for sex-limited meiosis suppression (Innes and Hebert 1988). Evidence that these males can pass such genes to their progeny suggests that the gene could spread through a cyclically parthenogenetic population, resulting in a genotypically diverse group of obligate parthenogens. It is reasonable to assume that this process is continuing and therefore that some obligate parthenogen clones have originated relatively recently (Innes and Hebert 1988). Such arrival of these obligate parthenogen mutants perfectly demonstrates a natural invasion event into a genetically diverse population as explored in this experiment.

4.3. MATERIALS AND METHODS

4.3.1. Establishing Cultures

Laboratory cultures of asexually reproducing *Daphnia obtusa* were established and acclimatised, each derived from single post-ephippial females taken from Pig Bush Pond in the New Forest, Hampshire immediately after the pond had refilled in the late October (favourable season for *Daphnia* being October–May, see Chapter 2). Females were of a large healthy size, with brood. Cellulose acetate electrophoresis was performed on a random sample of *Daphnia* (Appendix 2; Hebert and Beaton 1989) for the allozyme glucose-6-phosphate isomerase (GPI). This confirmed the population to be in Hardy-Weinberg equilibrium (SS = 203, SF = 190, FF = 63; χ^2 = 2.975). Electrophoresis was subsequently performed on one female from each monoclonal culture, to determine its GPI status. All cultures displaying FF

homozygosity were discarded, and those displaying SS homozygosity or heterozygosity (SF) were maintained. Therefore, all individuals used in the study were known to belong to one of these two groups. Each culture was labelled with the relevant 'genotype group' SS or SF accordingly and numbered. This continued until 177 healthy cultures of each genotype had been distinguished. Cultures were then maintained in plastic, open-mouthed containers, each holding approximately 1.5 l zooplankton media (Appendix 1; Lynch et al. 1986), and were fed *ad libitum* on aquaria-cultured algae, containing bacteria and inedible algae as well as edible algae. The containers were kept in a temperature-controlled room set at 19°C for a 16L : 8D photoperiod, until they became healthy populations of at least 100 individuals. Four random clones from each genotype were housed in several containers in order to achieve numbers approaching 1000.

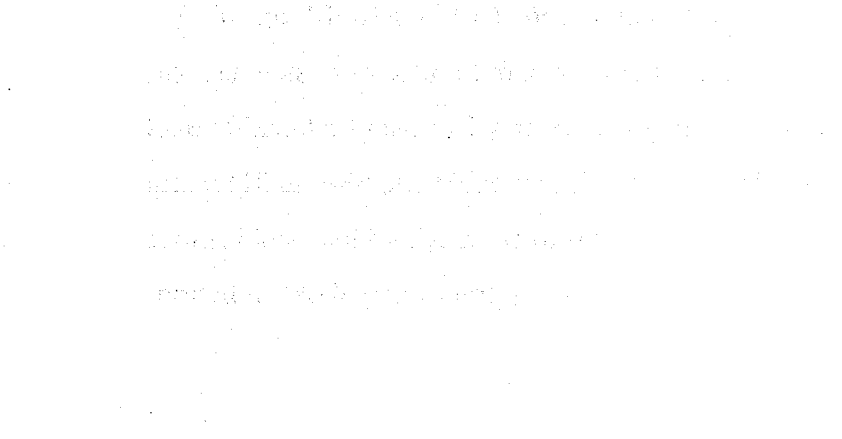
4.3.2. Assembling Genetically Diverse and Uniform Populations and Groups

Treatments comprised recipient populations of 200 individuals and invading groups of 10 or 100, with both genetically diverse and genetically uniform individuals alternately comprising populations and groups. The status (SS or SF) of the allozyme glucose-6-phosphate isomerase (GPI) was used as a genetic marker to distinguish between the genetically diverse and genetically uniform populations in the experimental treatments. Eight different genetically uniform recipient populations were assembled using four different cultures of genotype SF and four different cultures of genotype SS. The switching of the GPI marker allowed us to control for any possible life history trait differences between genotypes. Genetically diverse invading groups were assembled by taking one female from each of 10 cultures, chosen at random, for the smaller invading group, and one female from each of 100 cultures for the larger invading group. Eight genetically diverse invading groups were assembled, four assembled from cultures of genotype SF and four of genotype SS. Similarly, eight genetically diverse recipient populations were created by taking one female from each of the 177 cultures of the same genotype, plus another female from each of 23 of the cultures chosen at random to make the numbers up to 200 (four assembled from cultures of genotype SF and four of genotype SS). Genetically uniform invading groups were made up

of either 10 or 100 individuals from the same culture and genetically uniform recipient populations were created by taking 200 females from one culture. Again, eight invading groups and eight recipient populations were assembled, in each case four from cultures of genotype SF and four of genotype SS. Invasion events were then set up, with the four genetically diverse invading groups of genotype SF injected into the four genetically uniform populations of genotype SS and vice versa, and the four genetically diverse invading groups of genotype SS injected into the four genetically uniform populations of genotype SF and vice versa. For clarity, invasion events 1–4 represent invading groups of genotype SF and invasion events 5–8 represent invading groups of genotype SS in all figures.

4.3.3. Experimental Design

There was a total of 32 experimental treatments, paired into two conditions: the invasion of a genetically diverse group into a population of genetically uniform individuals and the reciprocal invasion of genetically uniform groups invading into genetically diverse populations, as shown in Figure 4.1. Each invasion event for both small and large invading group sizes was replicated eight times.



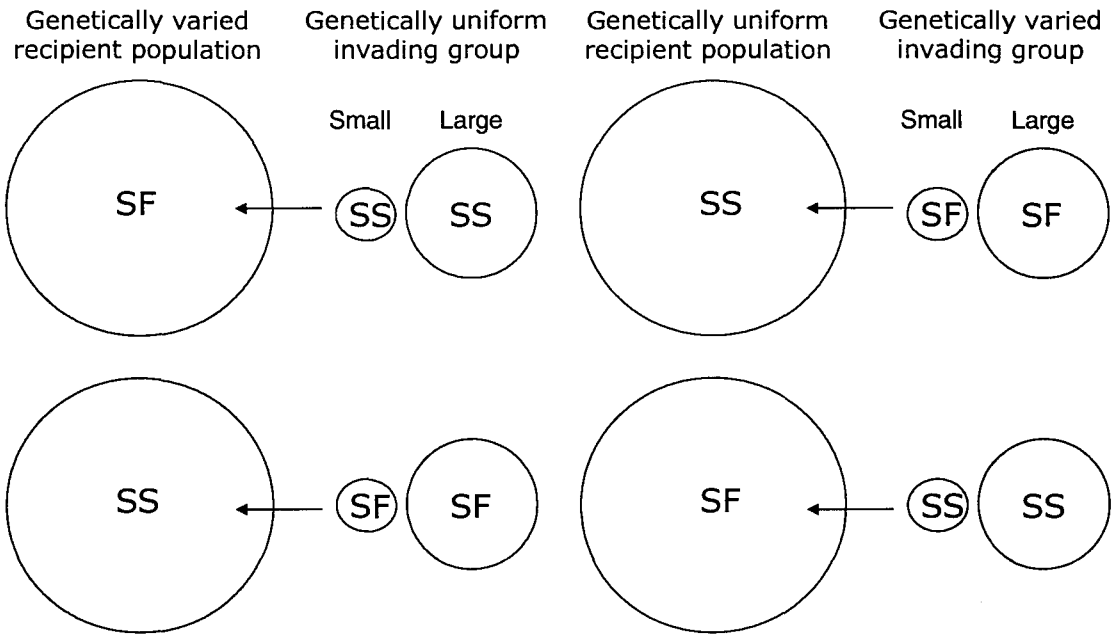


Figure 4.1—Experimental design. Small and large genetically diverse and genetically uniform invading groups were injected into recipient genetically uniform and genetically diverse recipient populations respectively. All genetically diverse and uniform invading groups in invasions 1–4 were of genotype SF and all genetically diverse and uniform invading groups in invasions 5–8 were of genotype SS

4.3.4. Statistical Analysis

A balanced ANOVA design (Minitab v. 13.1) was used to test the null hypothesis that any proportionate change in invading group within the population was independent of Mode of invader (genetically diverse or genetically uniform), initial Size of invading group (10 or 100) and Genotype (SF or SS). Table 4.1 shows how each of 16 Populations, identified by the clone of the genetically uniform component, were nested in Mode and Genotype and cross-factored with Size, in the model:

$$\text{Response} = \text{Size} \mid \text{Population}' (\text{Genotype} \mid \text{Mode}).$$

The factor Population was treated as random, so Mode, Genotype and their two-way interaction (Mode × Genotype) were tested against the error term Population' (Mode × Genotype), with 12 error degrees of freedom. Size of invading group and its interactions were tested against the residual variation, also with 12 error degrees of freedom.

The response was the proportion of the invading genotype originally injected into the recipient population subtracted from the proportion of the invading genotype sampled in the population at the time of the analysis (at 3, 6, 9 and 13 weeks). A proportionate increase in invading group gave a positive value, and a decrease gave a negative value. Due to the difference in initial proportion of small and large invading groups in their recipient populations, they had specific limits of change, but over the same range. Small invading groups were restricted between an increase in proportion of 0.952 and a decrease in proportion of –0.048, whereas large invading groups were restricted between 0.667 and –0.333 (both groups therefore occupying ranges equal to 1). A main effect of size was expected due to these different limits within which small and large invading groups can gain and lose in proportion. The hypothesised effects of genetic variation were sought in a mode by size interaction. As the response was proportionate, it was subjected to an arcsin root transformation prior to statistical analysis.

Table 4.1—The ANOVA design used for analysis at each stage in the study. M = mode of reproduction of invading group; G = genotype of invading group; S = size of invading group; P = population identified by the clone of the genetically uniform component; O = observation

S|P'(G|M)	$M_{diverse}$								$M_{uniform}$							
	G_{SF}				G_{SS}				G_{SF}				G_{SS}			
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	P_{13}	P_{14}	P_{15}	P_{16}
S_{10}	O_1	O_3	O_5	O_7	O_9	O_{11}	O_{13}	O_{15}	O_{17}	O_{19}	O_{21}	O_{23}	O_{25}	O_{27}	O_{29}	O_{31}
S_{100}	O_2	O_4	O_6	O_8	O_{10}	O_{12}	O_{14}	O_{16}	O_{18}	O_{20}	O_{22}	O_{24}	O_{26}	O_{28}	O_{30}	O_{32}

An inherent assumption of this design is that there is no significant interaction of Size with the error term Population' (Mode × Genotype). The model cannot test for this interaction because it has no estimable residual error. Other assumptions of ANOVA were checked: the variances were homogeneous (Homogeneity of Variances test, Minitab v. 13.1) and the residuals normally distributed (Normal Probability Plot of the Residuals, Minitab v. 13.1).

4.3.5. Feeding and Monitoring

Each recipient population was housed in 1.4 litres zooplankton media (7 ml per individual *Daphnia*) in a plastic, open-mouthed, 2.5-litre container, to which the appropriate invading group was added. The volume of media reflected the total recipient population at the start of the experiment, and was maintained at this level regardless of invading group size or any fluctuations in population size during the experiment. Zooplankton media was topped up when necessary and all populations had their media changed when required. Any settled inedible algae, detritus or dead *Daphnia* was removed daily. Treatments were fed 3–5 times a week with 2.0–2.4 × 10^6 algal cells, or 100,000–120,000 cells per *Daphnia* (haemocytometer counts), grown in an aquarium housing a goldfish. Such aquaria-cultured algae-water contains inedible algae and bacteria as well as edible bacteria, which provides a multi-dimensional niche in which we would expect genetic variation to have an advantage. A pure strain of one edible alga would encourage the success of a certain clonal genotype and the demise of all others. An impure food supply also prevents booms and crashes in the population as cycles are dampened by inedible algae utilising the nutrients required by the edible algae (McCauley et al. 1999). All treatments were housed in a temperature-controlled room at 19°C for a 16L : 8D hr photoperiod.

4.3.4.1. Population Counts and Cellulose Acetate Analysis

Analyses were performed after 3, 6, 9 and 13 weeks. At each stage, population counts were taken and cellulose acetate electrophoresis was performed on a sample of 48 individual *Daphnia* from each treatment (Appendix 2; Hebert and Beaton

1989). Analyses at the earlier stages were expected to reflect starting proportions of invading group genotypes. Non-brood-carrying females, approximately one week prior to sexual maturity, were used in the experiment. Once offspring were produced, the limited food supply meant that many would not survive into adulthood and progressive generations would only have a chance to make an impact on the population once the original adults began to age and die. Population estimates of each treatment were taken prior to each cellulose acetate electrophoretic analysis. One hundred millilitres of the agitated media were extracted, along with any *Daphnia* in the sample, and all adult individuals in the sample were counted. The number of *Daphnia* present in the 100-ml sample was multiplied by 14 to give an estimate of the total number of *Daphnia* present in the 1.4-l container. *Daphnia* were then returned to their respective container. Population counts were required in order to detect any population crashes or booms throughout the experiment. Samples of 48 randomly selected individuals were extracted from each container after 3, 6, 9 and 13 weeks and cellulose acetate electrophoresis for the allozyme GPI was carried out on all individuals in the sample (two applications or 24 individuals per plate). This resulted in two gels being run per treatment and 64 gels in total for each analysis. Individuals in the sample were then classed as GPI genotype SS or SF and the proportion of the recipient or invading GPI genotype in the sample was then extrapolated to that of the whole population, and the proportionate change in invading groups within the populations could be calculated.

4.4. RESULTS

4.4.1. At 3 Weeks

Mode of invader (diverse or uniform) had an effect on the proportionate change in invading group within the population at 3 weeks (Mode effect: $F_{1,12} = 9.58$, $P = 0.009$, Figure 4.2). At this stage genetically uniform invading groups had increased in proportion in the genetically diverse recipient populations more than genetically diverse invading groups had increased in the genetically uniform recipient populations, although the changes in proportion are all by <10%. Invading group size and genotype had no effect on the proportionate change in invading group and there were no two- or three-way interactions between mode of invader, invading group size and invading group genotype.

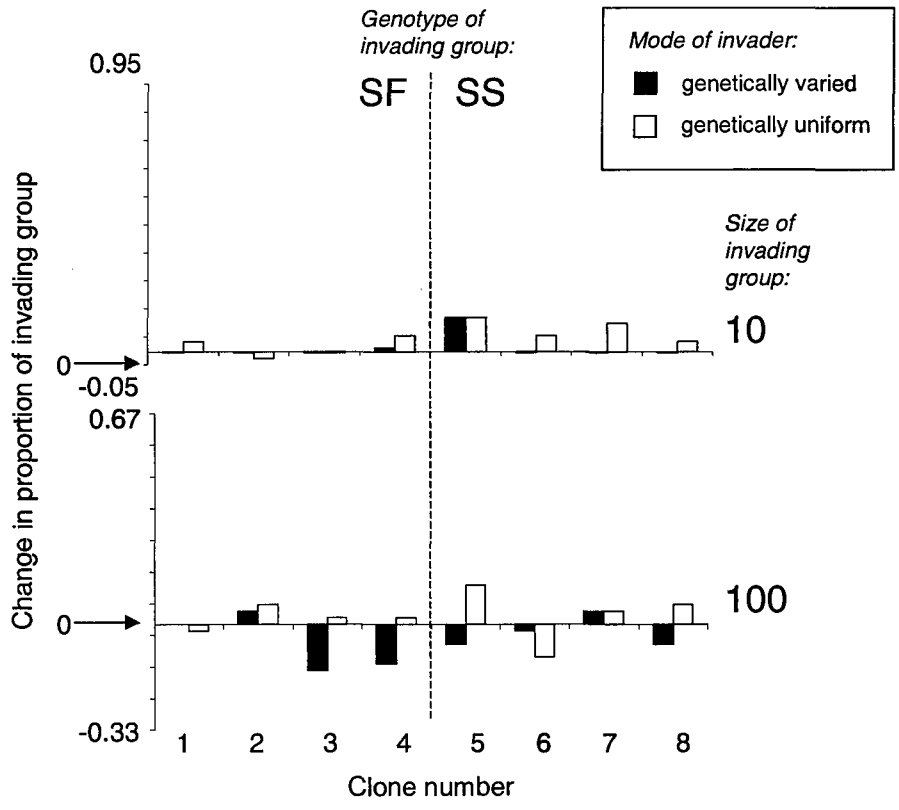


Figure 4.2—The proportionate change in invading group in the population at 3 weeks, depending on mode of invader. Top graph: small invading groups of initially 10 individuals; bottom graph: large invading groups of initially 100 individuals; black = genetically diverse invading groups; grey = genetically uniform invading groups; invasion events 1–4, genotype of invader = SF; invasion events 5–8, genotype of invader = SS

4.4.2. At 6 Weeks

Mode of invader (diverse or uniform), invading group genotype (SF or SS) and invading group size (10 or 100) all interacted in their effect on the proportionate change in invading group within the population at 6 weeks (Mode × Genotype × Size interaction: $F_{1,12} = 5.28$, $P = 0.040$; two-way interactions and main effects all non-significant). Figure 4.3 shows large genetically diverse invading groups of genotype SS appearing to increase in proportion more than small genetically diverse invading groups of genotype SS.

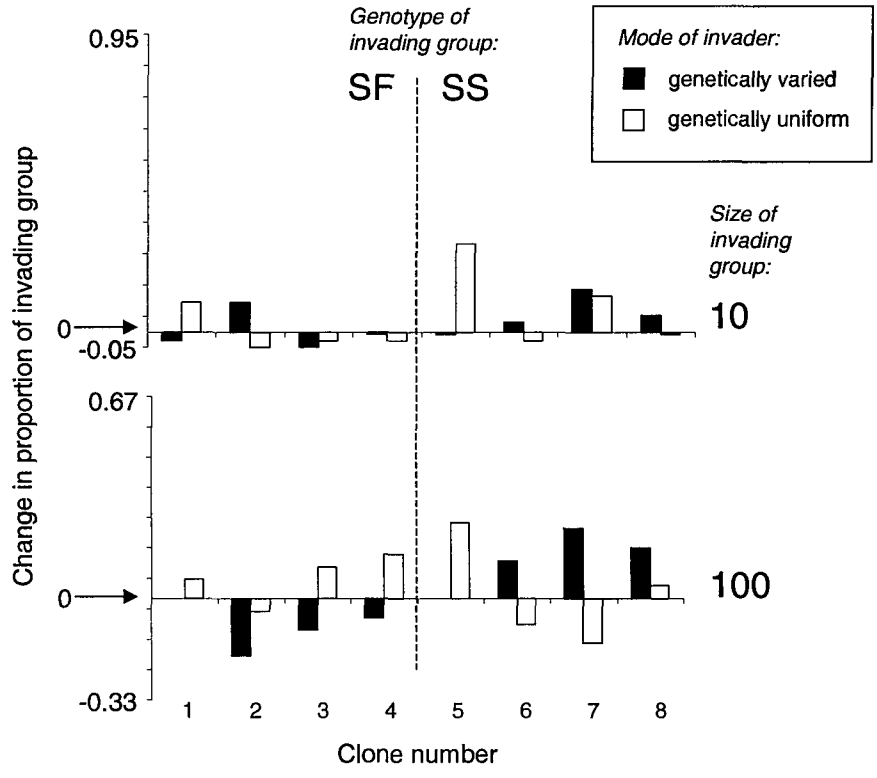


Figure 4.3—The proportionate change in invading group in the population at 6 weeks, depending on mode of invader, invading group genotype and invading group size. Top graph: small invading groups of initially **10** individuals; bottom graph: large invading groups of initially **100** individuals; black = genetically diverse invading groups; grey = genetically uniform invading groups; invasion events 1–4, genotype of invader = **SF**; invasion events 5–8, genotype of invader = **SS**

4.4.3. At 9 Weeks

Invading group genotype (SF or SS) had an overall effect on the proportionate change in invading group within in the population at 9 weeks (Genotype effect: $F_{1,12} = 5.03$, $P = 0.045$). Figure 4.4 shows invading groups of genotype SS having a greater proportionate change within the population than invading groups of genotype SF. Invading group size also had an overall effect (Size effect: $F_{1,12} = 5.56$, $P = 0.036$), with large groups (100), which could fall further than small groups (10), tending to do so. Mode of invader (diverse or uniform) had no effect on the proportionate change in invading group, although Figure 4.4 suggests that the larger genetically diverse invading groups of genotype SS again increasing more than the smaller genetically diverse invading groups of genotype SS. There were no two- or three-way interactions between mode of invader, invading group genotype and invading group size.

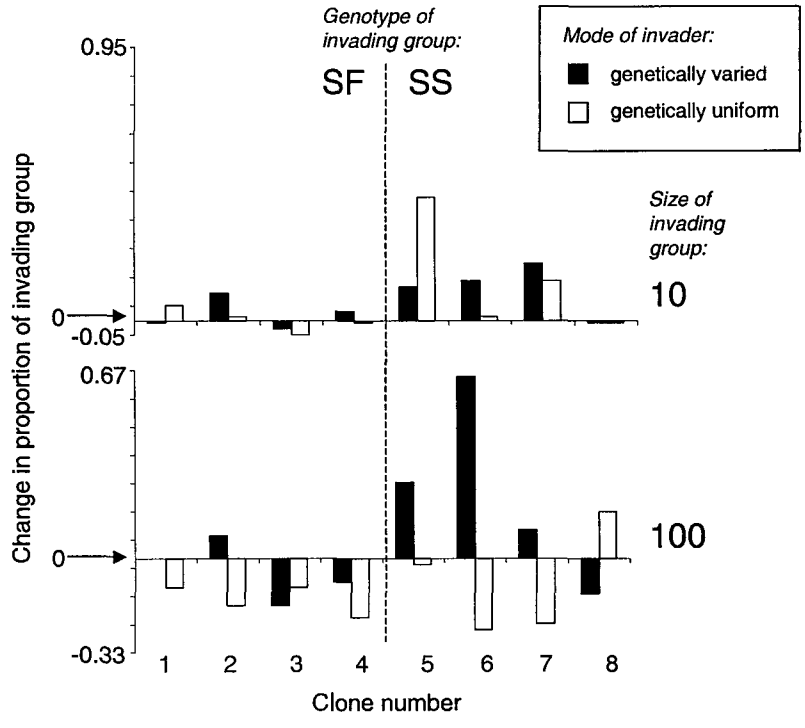


Figure 4.4—The proportionate change in invading group in the population at 9 weeks, depending on mode of invader and invading group size. Top graph: small invading groups of initially **10** individuals; bottom graph: large invading groups of initially **100** individuals; black = genetically diverse invading groups; grey = genetically uniform invading groups; invasion events 1–4, genotype of invader = **SF**; invasion events 5–8, genotype of invader = **SS**

4.4.4. At 13 Weeks

Mode of invader (diverse or uniform) had an effect on the proportionate change in invading group within the population, depending on invading group size (10 or 100) at 13 weeks (Mode × Size interaction: $F_{1,12} = 11.27$, $P = 0.006$; Mode effect: $F_{1,12} = 14.61$, $P = 0.002$; Size effect: $F_{1,12} = 8.11$, $P = 0.015$). Figure 4.5 shows that the most obvious contribution to this interaction is the genetically diverse invaders of initially large group size increasing their representation by slightly more than those of initially small size; in contrast, genetically uniform invaders of initially large group size are diminishing on average by considerably more than those of initially small size. Genotype also had an effect (Genotype effect: $F_{1,12} = 6.09$, $P = 0.030$) with invading groups of genotype SS increasing in proportion in the population more than invading groups of genotype SF. There were no other two-way or three-way interactions affecting proportionate change in invading group.

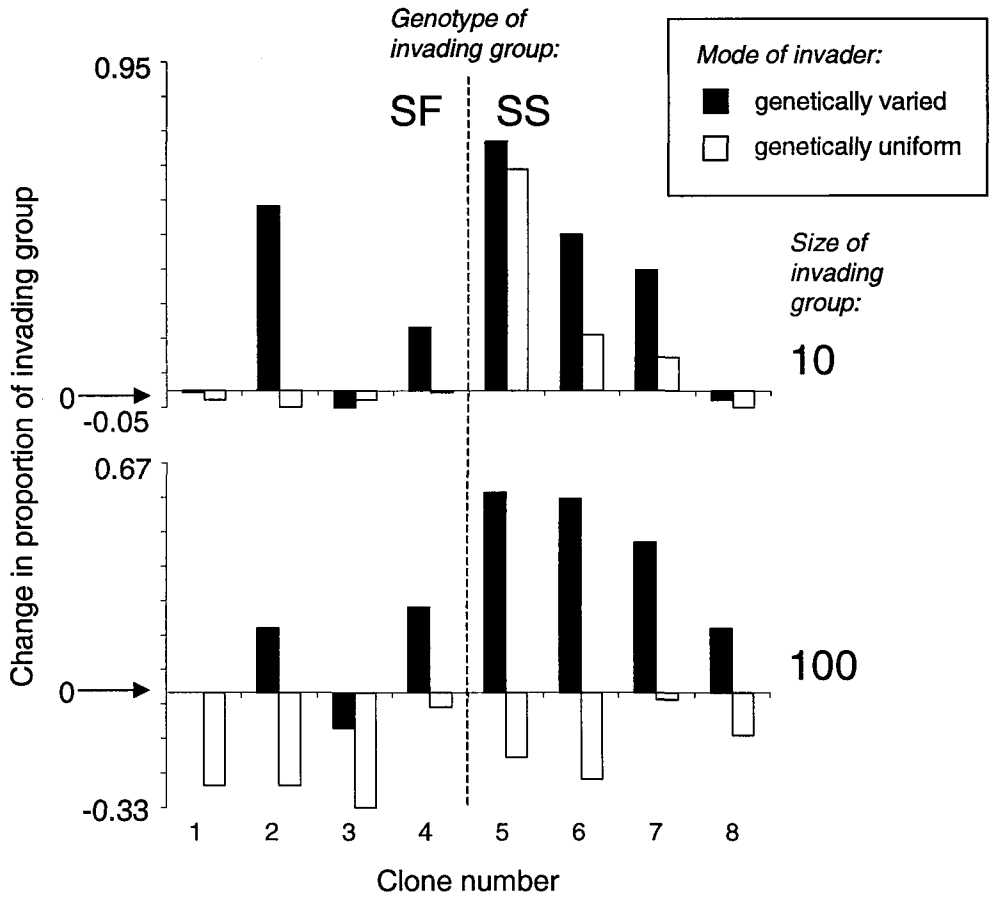


Figure 4.5—The proportionate change in invading group within the population at 13 weeks, depending on mode of invader, invading group size and invading group genotype. Top graph: small invading groups of initially 10 individuals; bottom graph: large invading groups of initially 100 individuals; black = genetically diverse invading groups invading into genetically uniform recipient populations; grey = genetically uniform invading groups invading into genetically diverse recipient populations; invasion events 1–4, genotype of invader = SF; invasion events 5–8, genotype of invader = SS

4.4.5. Rise and Fall of Invading Groups

At 13 weeks, almost half of the invading groups had risen in proportion relative to their starting ratios (14 out of 32; 11 of the genetically diverse invading groups and three of the genetically uniform invading groups). No invading groups entirely ousted their recipient population. Thirteen of the 16 genetically uniform invading groups had decreased in proportion relative to the starting ratios, whereas only four of the 16 genetically diverse invading groups had fallen in number (and one stayed the same). Table 4.2 shows that the rise or fall of invading groups depended on their mode ($G_1 = 9.384$, $P < 0.005$). Three of the genetically uniform invading groups were wiped out by the recipient population compared to only one of the genetically diverse invading groups. For the genetically diverse invading groups, the majority of the groups that decreased in relative proportion were of small invading group size (three out of four), and the majority of the groups that stayed the same or increased in relative proportion were of large invading group size (seven out of 12). With the genetically uniform invading groups, eight of the 13 invading groups that decreased in proportion within their recipient populations were of large invading group size, but all three of the invading groups that increased in relative proportion were small invading groups.

Table 4.2—Contingency table for the fate of the invading population in relation to its mode

		Population change for invader		
		Rise	Fall	Total
Mode of invader	Genetically diverse	11	4	15
	Genetically uniform	3	13	16
	Total	14	17	31

4.4.6. Population Counts

Populations fluctuated in size throughout the course of the experiment (Figure 4.6). There were no remarkable crashes or booms in any of the populations. There appears to be no common trend between the cycling of populations that were invaded by each genotype or by each mode of reproduction, but the populations comprising larger invading groups appeared to fluctuate more than the populations comprising smaller invading groups (black versus grey lines in Figure 4.6).

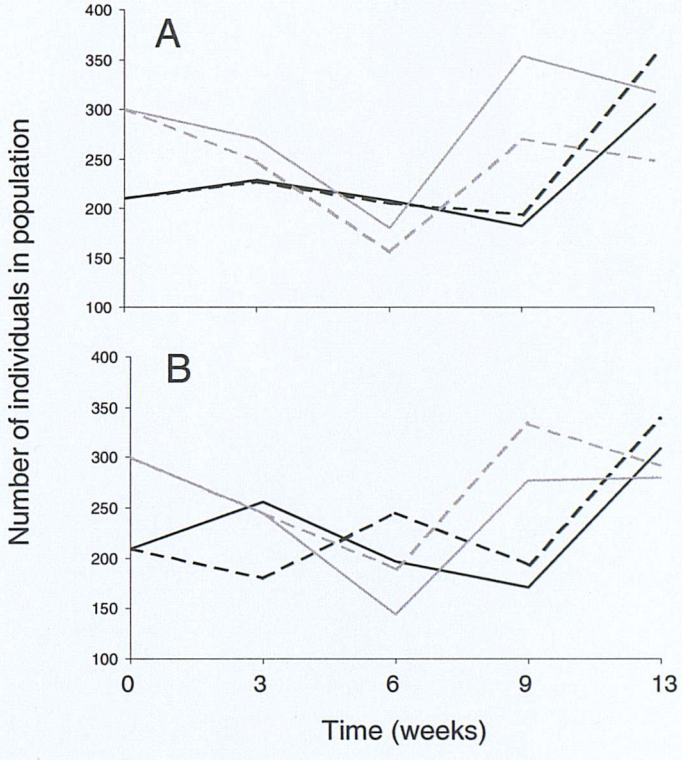


Figure 4.6—Estimated number of *Daphnia* in each population at each sampling stage (3, 6, 9 and 13 weeks). (A) Populations invaded by genetically diverse invading groups; (B) populations invaded by genetically uniform invading groups; black lines = populations invaded by invading group sizes of 10 (each a mean of four populations); grey = populations invaded by invading group sizes of 100 (each a mean of four populations); unbroken line = genotype of invading group is SF; broken line = genotype of invading group is SS

4.5. DISCUSSION

Males were not present in any experimental population, although they would arise in natural populations of sexually reproducing *D. obtusa* as environmental conditions begin to deteriorate. Any advantage of genetic variation in natural populations of *Daphnia,* as measured here, will be counterbalanced to some degree by the cost of producing males to sustain the genetic variation. Since the design did not impose any carrying capacity on populations, we were not able to estimate competition coefficients, as had been possible in Chapter 3. The response to competition between genetically diverse and genetically uniform populations and groups was instead measured in terms of proportionate change in invading group within the population. It is beyond the scope of this experiment to know whether the proportionate changes in invading groups were due to higher fecundity of the better competitor or greater mortality of the worse competitor. Because this design had repeated measures on Population, we must assume no significant interaction of invading group size with the genotype that defined each population. Perusal of the data at 13 weeks, however, shows that the four pairs of observations within any mode by genotype (for example, O_1 to O_8 in Table 4.1) tended to differ in different directions, suggesting an interaction.

Despite these design limitations, we have been able to show that proportionate increases in the genetically diverse invading groups within their genetically uniform recipient populations were greater than those of the genetically uniform invading groups in the reciprocal invasions after 13 weeks of growth (black versus grey bars in Figure 4.5). Chapter 3 identified a small competitive release for genetically diverse individuals in the presence of a genetically uniform population ($\alpha_{12} < 1$), sufficient to cancel a two-fold disadvantage in growth capacity incurred by a sexual population that comprises 50% males. This competitive release may have been present in this experiment also, enabling the genetically diverse invading groups to increase in number within the recipient populations more than the genetically uniform invading groups. Theoretical models have demonstrated how a sexual population could withstand invasion of multiple asexual clones, provided the sexual population retains a resource refuge (Pound et al. 2002). The underlying

assumption is that the genotype of an asexual clone arising from a sexual population will be 'frozen' to that of its progenitor parent, restraining it to a narrower ecological niche (Vrijenhoek 1979), causing competition amongst the clones and therefore providing the sexual competitors with their resource refuge. Our empirical results are consistent with this assumption.

In addition, we have shown a big disadvantage for large compared to small genetically uniform invading groups, contrasting with a small advantage for large compared to small genetically diverse invading groups (Figure 4.5). The observed disadvantage to larger uniform invaders reflects their having more individuals to lose and consequently doing so in the presence of the genetically diverse recipient population. The observed advantage to large genetically diverse invading groups matches our expectation that their greater inherent genetic variation (compared to small groups) confers a competitive advantage. This result cannot be directly extrapolated to sexual and asexual populations as sex would later on have to pay the price of producing males to sustain the genetic variation, but the competitive release may be enough to provide the time required for sex to express its long-term advantages of genetic variation in meeting the demands of environmental change. An extended invasion experiment may reveal the eventual displacement or stabilisation of recipient populations and invading groups.

The effect of reproductive mode of invader and invading group size on the proportionate change in invading group within the population was not evident until the experiment had run for 3 months. At 3 weeks there was a significant effect of mode of invader (Figure 4.2) where genetically uniform invading groups were increasing in proportion in the population significantly more than genetically diverse invading groups. However, the proportionate changes of both invading groups were by <10% and at such an early stage a reliable effect of mode of invader would not yet be expected. At 6 weeks mode of invader, size of invading group and genotype of invading group had a significant interaction effect on proportionate change in invading group within the population (Figure 4.3) and the pattern of difference between large and small genetically diverse invading groups was emerging. At 9 weeks invading groups of genotype SS began to increase in proportion more than invading groups of genotype SF, and small invading groups

(with more to gain) increased more than large invading groups (with more to lose) overall. Figure 4.4 also suggests, however, that for invading groups of genotype SS, large genetically diverse groups were beginning to show a greater proportionate change than small genetically diverse groups, as became apparent at 13 weeks. All populations and groups in the experiment comprised young non-brood-carrying female *Daphnia*, requiring the first week to mature and begin to produce young. Food supply was limited to maintain approximately the same number of *Daphnia* in the original population, so many of the young produced may not have survived into adulthood before the original adults began to age and die. The fluctuations in population sizes seen over the course of the experiment can be partly explained by these dynamics (see Figure 4.6). Once sexually mature, *Daphnia* can produce as many as 30 identical young every few days for their 40-day lifespan. Generation times are therefore short and population growth rapid, but the effects of population turnover were not expected to become visible until about the third month of the experiment.

The invasion events manipulated in this experiment may reflect those that occur in nature. Obligately and cyclically parthenogenetic forms of *D. pulex* exist in North American and Canadian ponds (Hebert et al. 1988) and to a lesser extent further south (Lynch et al. 1989). An event involving large numbers of one reproductive form invading into an established population of the other may occur when neighbouring ponds overflow and mix in the rainy season. Invasion events involving a smaller number of individuals may occur in nature as diapausing eggs are naturally dispersed by mammals or migratory waterfowl (Crease et al. 1997). The experiment realistically reflects these natural occurrences as the genetically diverse and genetically uniform individuals were not physically divided and the limited but impure food supply allowed the competitors to forage on their own resource base. The population counts taken at every stage in the experiment (Figure 4.7) served to chart the rise and fall of each population and to monitor any booms or crashes. No dramatic increases or decreases of overall population size were found for any treatment. The limited food supply provided enough only for the original population of 200 individuals, preventing the population from increasing dramatically. The impure mix of edible and inedible algae as well as bacteria in the food may have dampened population cycling as inedible algae utilise the nutrients

required by the edible algae in nutrient-rich conditions (Murdoch et al. 1998; McCauley et al. 1999). Fluctuations in population size were apparent, however. It is well documented that *Daphnia* populations cycle under laboratory conditions (McCauley et al. 1999), although natural populations are known to achieve long-term stability (Gurney and Nisbet 1998; Murdoch et al. 1998). The invasion event of genetically diverse into genetically uniform reflects the evolutionary origin of sexual reproduction, where sex (meiosis) evolved in the Cambrian era in primitive eukaryotes of the Kingdom Archezoa (Cavalier-Smith 1995). The reciprocal invasion event, where genetically uniform groups invaded into genetically diverse populations, is also informative as it reflects the ecological pressure on sexual populations in nature wherever faster reproducing asexual mutants arise by spontaneous mutation. Three genetically uniform invading groups (two small, one large) were eliminated from their genetically diverse recipient population and all but three other invading groups decreased in proportion in the recipient population after 13 weeks. This demonstrates the potential advantage a genetically diverse population has both for invading a genetically uniform population and resisting invasion by a genetically uniform population.

4.5.1. Effect of Genotype

The potential for sex to balance its two-fold cost in competitive superiority will of course depend upon the traits of the genetically uniform population against which it is competing. The experiment involved the use of eight different clones of *D. obtusa*, each randomly established from post-ephippial adult females obtained from the same temporary pond. These large, healthy females with big, early-season broods were collected immediately after the pond refilled in the autumn, and while the population was in Hardy-Weinberg equilibrium and reproducing sexually under the renewed favourable conditions of the cooler season. Genetic variation between clones should therefore have been suitably high. The experiment has found that such clonal variation may be reflected in the status of certain allozymes polymorphic in *Daphnia* populations. As the nature of the experiment required a pre-determined status of the allozyme glucose 6-phosphate isomerase (GPI) in all individuals, 'genotype' was factored into the analysis. Figures 4.3–4.5 show the greater proportionate increase in invading groups of genotype SS within the

populations compared to that in invading groups of genotype SF. Genetically diverse invading groups of genotype SS had almost all increased in proportion in the population at 13 weeks (Figure 4.5), whereas more than half of all genetically diverse invading groups of genotype SF had decreased in proportion, including one that was eliminated. This effect was emerging as early as 6 weeks (Figure 4.3), where it is clear that invading genotypes of SS were beginning to increase in proportion in the population more than invading genotypes of SF, and where this effect was dependent on mode of invader and invading group size. Previous work has shown homozygote fecundity to be lower than heterozygote fecundity (Young 1979b), suggesting that the enzyme GPI must be closely linked to certain life history traits relevant to competition, and those with traits linked to GPI SS are more successful competitors than those with traits linked to GPI SF, compensating for their reduced fecundity. Lynch (1984c) and Lynch et al. (1989) demonstrated genetic variation for life history characters in *D. pulex*, and Weider et al. (1987) used genetic analysis to show much genetic diversity among clones in *D. pulex*, despite the previous belief that clonal species had little or no genetic diversity (Williams 1975). Such variation is likely to be the result of mutations and of parthenogenetic clones arising on independent occasions from the cyclically sexual populations (Lynch 1984d; Lynch et al. 1989). Crease et al. (1989) showed that the high clonal diversity present amongst the obligately parthenogenetic clones of *D. pulex* in the Great Lakes region was due to their polyphyletic origin. The result in this experiment might suggest a form of heterozygote disadvantage. In Chapter 2, half of all samples of *D. obtusa* collected from the natural populations in Pig Bush pond in the New Forest, Hampshire during the period of study of the pond throughout 2002–2003 revealed the homozygote SS status of the allozyme GPI to outnumber the heterozygote (SF) status (see Table 2.2 in Chapter 2). The deviation from Hardy-Weinberg equilibrium evident in natural populations over time (Table 2.2 in Chapter 2) suggests that evolutionary forces are acting, and that certain clones may become better adapted to the conditions and increase in number disproportionately compared to other clones. In this case, it appears that the clones with GPI status SS are better suited to the particular conditions of the experiment than those of GPI status SF. Further work could quantitatively describe potential relationships between life history traits and allozyme variation in temporary pond *Daphnia* populations in the U.K.

CHAPTER 5

REVIEW OF PSEUDOGAMY AND BIOLOGY OF *LUMBRICILLUS LINEATUS* (MULLER)

5.1. INTRODUCTION

The previous chapters investigated the problem of how sexual reproduction can pay the cost of producing males that do not themselves produce offspring (Williams 1975; Maynard-Smith 1978; Bell 1987; Hurst and Peck 1996). These studies contribute to our understanding of the role of genetic variation in the persistence of sexual reproduction in the face of invasion by asexual reproduction. When considering theories of costs of sex, pseudogamous parthenogenetic (sperm-dependent) species present a special case. Pseudogamy is the name given to a type of asexual reproduction in animals in which clones require spermatozoa of a related sexual individual to initiate egg development, but do not incorporate any of the genetic material of the sperm (Christensen and O'Connor 1958; Christensen et al. 1978). Pseudogamous clones are therefore reproductive parasites on sexual individuals, causing obligatory coexistence (Christensen and O'Connor 1958). Such systems retain some of the disadvantages of sex, such as the cost of finding a mate. They do not benefit from the advantages of variation due to recombination of parental genotypes. Despite these apparent handicaps, pseudogamy has been widely recognised in a diverse range of organisms, prompting many questions as to how these systems evolve and persist. Theoretical models to predict the population dynamics of such reproductive systems require knowledge of the geographical distribution, habitat requirements, biological parameters and population dynamics of pseudogamous species. This chapter aims to review the relevant aspects of the biology of pseudogamous parthenogens, and specifically the case of the littoral enchytraeid worm *Lumbricillus lineatus* Muller (Oligochaeta, Annelida), and set the foundations to the surveys and experiments performed on *L. lineatus* in Chapters 6 and 7.

5.2. PSEUDOGAMOUS PARTHENOGENESIS

Several terms have been used interchangeably to explain the reproductive system of pseudogamy that will be defined and distinguished here. The term 'pseudogamous parthenogenesis' is used for both plants and animals but has important genetic differences. In plant biology it describes cases where embryo formation occurs without fertilisation, but pollination is still required to induce endosperm formation (Asker and Jerling 1992). In its strict zoological sense, relevant to this thesis, however, it refers to the requirement of sperm to fertilise the egg followed by the exclusion of the sperm chromosomes, and it thus describes 'sperm-dependent parthenogenesis'. In this sense, pseudogamous parthenogenesis is very rare in plants, only occurring in the potato hybrid *Solanum tuberosum/phureja* (Clulow et al. 1991) and hybrids of *Tripsacum dactyloides* (Tsunewaki et al. 1976) and maize *Zea mays* (de Wet et al. 1984). Pseudogamous parthenogenesis is more cryptic in plants, however, possibly affecting the estimate of prevalence. The term gynogenesis is sometimes used to describe pseudogamy in animals, but its strict translation is 'female descent'; it therefore does not accurately describe processes in hermaphrodites such as *L. lineatus*, which are not strictly female (Beukeboom and Vrijenhoek 1998). It seems to be the case that each system of pseudogamous parthenogenesis is unique, with its own specific ecological, genetical and evolutionary characteristics. This review will look in more detail at the particular pseudogamous case of the littoral enchytraeid *L. lineatus*.

Most pseudogamous parthenogenetic systems are thought to have evolved relatively recently and frequently from sexual ancestors (Sanderson and Jacob 1957; Uzzell and Goldblatt 1967; Triantaphyllou and Moncol 1977; Turner et al. 1983; Moritz et al. 1989; Pongratz et al. 1998). This view is supported by the discovery of genetic variation among pseudogamous parthenogenetic taxa (Beukeboom and Vrijenhoek 1998). Some clonal lineages, however, have been reported to have achieved a substantial evolutionary age (Quattro et al. 1992). Pseudogamous parthenogenesis is usually associated with inter-specific hybridisation (Maslin 1971). Proof for parthenogens arising through hybridisation comes from karyotype and allozyme studies, skin transplants and protein studies on several species of insects (Suomalainen 1948; Mitter and Futuyma 1977; Parker et

al. 1977; Pongratz et al. 1998), salamanders (Uzzell and Goldblatt 1967), fish (Wetherington et al. 1989; Vrijenhoek and Pfeiler 1997) and lizards (Maslin 1971; Parker 1979; Sites et al. 1990). Hybridogenesis is restricted to a few animal taxa, however, and no hermaphroditic hybridogens have been described (Beukeboom and Vrijenhoek 1998). Pseudogamous parthenogenesis is also associated with polyploidy, and can be initiated by polyploidisation in invertebrates. This occurs when an unreduced ovum is fertilised to produce triploid progeny, and the likelihood of these triploids stabilising as a pseudogamous sibling species has been discussed by Lanier and Kirkendall (1986). The 'genome addition' hypothesis, whereby triploid and other polyploid lineages have arisen multiple times by fertilisation of parthenogenetic eggs or addition of sperm genomes to diploid asexual ancestors has been supported through genetic studies of freshwater fish complexes including *Poeciliopsis* (Cimino 1972) and in some invertebrates, including the freshwater planarian *Schmidtea polychroa* (Beukeboom et al. 1996b). The genetic basis for reproductive systems is polygenic in many species, suggesting that the genes for pseudogamy are present in diploid sexual individuals, but that parthenogenesis will not immediately be stabilised in new polyploid lineages: such genes need to increase in number or expression in order to maintain pseudogamous parthenogenesis (Beukeboom et al. 1996b). The stability of these newly evolved systems, however, is still uncertain. An all-female lineage should rapidly replace its sexual host that pays to produce males, assuming all else is equal (Williams 1975) and as the sexual host becomes scarce, sperm-limitation will reduce the reproductive advantage of the all-female lineage (Moore and McKay 1971; Kirkendall 1990). Competition between sexual and asexual individuals will be asymmetrical because all asexual females are derived from the sexual population and so their genotypes are similar; whereas there should be at least some unique sexual genotypes (Kirkendall and Stenseth 1990). This has been demonstrated between juvenile sexual and pseudogamous salamanders (Wilbur 1971). Despite these theoretical obstacles to coexistence, however, studies have shown that niche separation between sexual and clonal forms of a species can promote stable coexistence (Vrijenhoek 1978; Kirkendall and Stenseth 1990). Density-dependent conditions relating to birth and death rates can also allow coexistence (Stenseth et al. 1985; Doncaster et al. 2000; Pound et al. 2002; Doncaster et al. 2003). Sexual

Cnemidophorus lizard species were shown to have a higher between-individual niche width than sympatric asexual species (Case 1990).

The sexual parasitism of pseudogamy is exceptional in that it may affect reproductive success alone and not the life cycle of the parasitised individual (Schley et al. submitted). Pseudogamous forms should evolve into fully parthenogenetic individuals unless there is compensation for their dependence on their host. It is suggested that the pseudogamous type may have an inferior competitive ability (Schley et al. submitted). Pseudogamy has, however, arisen independently in 24 genera of seven phyla in the animal kingdom including: invertebrates such as the freshwater planarian *Schmidtea polychroa* (Weinzierl et al. 1999), molluscs (Qi et al. 2000), fish such as the triploid top-minnow *Poeciliopsis monacha-lucida* (Vrijenhoek and Pfeiler 1997; Felip et al. 2001), reptiles (Case 1990) and higher plants (Calame and Felber 2000), including both monocotyledons and dicotyledons (Nygren 1954). Identification of several cryptic characteristics is required to detect pseudogamy, suggesting that its incidence is more widespread than has been described. More are expected to be discovered with the advancement of molecular and cytological methods (Bullini 1994). This common occurrence of pseudogamous reproductive methods raises many questions about how such lineages arise and how they coexist with sexual individuals. Due to their similarity in all but reproductive strategy, intensive competition for resources may lead to competitive exclusion, but this principle is complicated for pseudogamous species, as the asexual form will impede its own ability to reproduce in the event that it out-competes the sexual form. The presence of males in sexual populations of gekkonid lizards of the genus *Hemidactylus* increases the competitive advantage of the population, as males are more aggressive than sexual females and more likely to negatively interfere with asexual females (Bolger and Case 1992). A further complication to understanding pseudogamous parthenogenesis is the possibility of biparental transmission. A study of the B chromosomes (dispensable elements in addition to the usual chromosomal complement) of the hermaphroditic flatworm *Polycelis nigra* with both sexual and pseudogamous parthenogenetic lineages, found an unexpectedly high frequency of B chromosomes in the pseudogamous types (Beukeboom et al. 1996a). The most likely explanation involves paternal inheritance of the element during fertilisation

and the subsequent avoidance of elimination from the egg, despite the elimination of paternal autosomes. This could be achieved either through expulsion of the sperm chromosome with a cytoplasmic bud or degeneration of the sperm nucleus in the egg cytoplasm. B chromosomes have been demonstrated as being detrimental to the fitness of their host, by reducing cocoon production and slowing adult growth of *P. nigris* (Beukeboom et al. 1998). Fertility and fecundity were not negatively affected, however. A similar paternally-inherited element is that of the parasitic paternal sex ratio chromosome discovered in the wasp *Nasonia vitripennis*, which appears to survive beyond fertilisation, after actively destroying the sperm chromosomes (Werren 1991; Beukeboom and Werren 1993).

5.2.1. Geographical Parthenogenesis

True parthenogens can escape direct competition with their sexual ancestors by colonising a new habitat. Parthenogenetic lineages tend to be more frequent in extreme latitudes, higher altitudes and in marginal or regularly disturbed habitats (Beukeboom and Vrijenhoek 1998). One-third to one-half of all angiosperms exhibits polyploidy, though this could be higher as polyploidy is cryptic in plants so. Approximately 20% of these known polyploids are pseudogamous (Nygren 1954), the most successful derivatives occupying extreme habitats (Wright and Lowe 1968). This common geographical distribution of polyploids is known as the weed hypothesis. It has been suggested that the presence of such habitats is crucial to the establishment of parthenogenetic species. This is also shown in some insects such as planthoppers (Booij and Guldemond 1984). Pseudogamous parthenogens are constrained to coexist with their sexual hosts, however. Chapter 6 looks at the present day geographical distribution of a pseudogamous littoral oligochaete (*Lumbricillus lineatus*), as compared to a survey of its distribution made 40 years ago.

5.2.2. Size Differences of Diploids and Triploids

Previous studies of the relative survival, growth and physiological competence of diploid and triploid forms of fish species have revealed a variety of results depending on age and species (Ihssen et al. 1990). Unisexual fish have been well

studied for their applications to aquaculture. Generally, juvenile diploid fish have an improved growth performance over juvenile triploids (Benfey 1999), for example in common carp *Cyprinus carpio* (Cherfas et al. 1994), yellow perch *Perca flavescens* (Malison et al. 1993), Atlantic salmon *Salmo salar* (Carter et al. 1994) and tilapia (Mair 1993). This can be attributed to differences in competitive behaviour for shared resources: triploids are less aggressive than male diploids, lowering their position in the hierarchy and therefore reducing their success at competing for food (Carter et al. 1994). Juvenile diploid and triploid brook trout displayed differences in competitive feeding success measured in terms of dominance ranks of the individual fish (O'Keefe and Benfey 1997). Some studies have shown an equal growth performance of juvenile diploid and triploid fish (Benfey et al. 1989; Hussain et al. 1995; Felip et al. 2001), and others have shown juvenile triploid fish to have a growth advantage over juvenile diploids in terms of rates of growth (Benfey and Sutterlin 1984) or final body weight (Kim et al. 1994). In these studies, however, diploids and triploids were housed in separate tanks, making it hard to relate growth performances to competitive efforts.

Any growth advantage of diploid fish tends to lessen as they reach sexual maturity and adult sterile triploids grow faster and often attain larger sizes than diploids. This has been shown in Pleuronectid hybrids (Lincoln 1981), channel catfish *Ictalurus punctatus* (Wolters et al. 1982), rainbow trout *Oncorhynchus mykiss* (Guo et al. 1990) and Asian catfish *Clarius macrocephalus* (Fast et al. 1995). Differences in competitive feeding success of diploid and triploid brook trout diminished as the fish grew (O'Keefe and Benfey 1997). Other studies have revealed equal growth performances of diploid and triploid adults, however (Benfey et al. 1989; Felip et al. 2001). In fish, triploids are not necessarily larger than their diploid relatives possibly due to the reduced cell numbers associated with the condition of triploidy (Benfey 1999). Felip et al. (2001) found that gonadal development in diploid sea bass during the reproductive season led to an increase in body weight in diploids. The reduced gonadal growth and an impaired steroidogenesis in the triploids therefore lead to slower growth than that of the diploids. Felip et al. (2001) argued, however, that the growth advantage to triploids of not directing energy into gonadal growth may be offset by the unfavourable environmental conditions of the

spawning season such as low temperatures. Triploid advantage may also be offset by the deficiency of sex hormones in adults (Benfey et al. 1989).

Proposed hypotheses for differences in growth between diploids and triploids have been put forward by Malison et al. (1993) and Yamazaki and Goodier (1993). They suggested that the procedures associated with the induction of triploidy may have negative effects on growth due to cytogenetic aberrations or to the biochemical actions of specific intra-cellular proteins. The lack of the anabolic effect of sex steroids due to reduced gonadal development in triploids may disadvantage growth (Benfey et al. 1989). Differences in cell size and number may affect basic physiological processes and reduce performance in triploid fish (Benfey 1999). Suresh and Sheehan (1998) found that triploid rainbow trout have larger hyperplastic fibres than diploid individuals, due partly to the effect of increased nuclear size in triploids. These larger fibres may be less favourable to cellular metabolic exchange because of their smaller surface area to volume ratios, and may account for the reduced viability and growth observed in immature triploids (Suresh and Sheehan 1998). The triploid form of *L. lineatus* is larger than the diploid. This could be due to an increase in cell size in a number of tissues of triploids (Felip et al. 2001), expected due to the presence of an extra set of chromosomes. Chapter 7 investigates the relative development times of diploid and triploid forms of a littoral oligochaete *Lumbricillus lineatus*, and the size differences of diploids and triploids depending on their starting ratio and the physical conditions.

5.3. *LUMBRICILLIS LINEATUS*

Worms from the family Enchytraeidae have been well studied in general, particularly in terms of their life-cycle and population dynamics (Birkemoe et al. 2000), interactions (Huhta and Viberg 1999) and self-fertilisation (Dozsa-Farkas 1995) and nutrient cycling and soil formation (Didden 1993). This is due to their potential applications in testing for oil contamination (Filimonova and Pokarzhevskii 2000) and decomposition of waste (Marinissen and Didden 1997; Edsberg 2000). The enchytraeid *Lumbricillus lineatus* has been well studied over the last 50 years. This species has been proven to exhibit a pseudogamous

parthenogenetic reproductive system as described (Christensen 1960). Its common form is the hermaphroditic (sexual) diploid ($2n = 26$) that can exist alone, but often coexists with the parthenogenetic (asexual) triploid form ($3n = 39$) of the same species that can be viewed as a reproductive parasite. Triploids are created by a mutation that prevents synapsis (the formation of bivalents) in the male tissue. The cells follow the normal pattern when they enter meiosis, but the presence of three homologues results in a mix of trivalents and a total breakdown of spermatogenesis (Christensen 1980a). *L. lineatus* can also exhibit tetra- and pentaploids ($4n = 52$ and $5n = 65$) which show the same asynapsis as triploids, but due to the less intense competition between homologues there will be bivalents and multivalents present, resulting in the production of some morphologically normal spermatozoa. The sperm is genetically unbalanced, however, sufficient to stimulate egg development in the polyploids, but inadequate for sexual reproduction. Tetraploids and pentaploids are relatively rare (Christensen et al. 1978).

The four different genotypes are not classed as separate species because they are identical in the morphological features usually used in taxonomy and they copulate with each other (Christensen 1980a). *L. lineatus* polyploids have been described as weed species, those commonly found in extreme conditions (Christensen et al. 1978). Indeed, the tetra- and pentaploids have been found, in the absence of diploids, in the littoral zone along two canals connecting brackish fjords with the saline North Sea, characteristic of strong variations in salinity (Christensen et al. 1978). The fact that the diploids are not present in such habitats would at first appear to be due to their ecological inferiority, thus supporting the weed hypothesis. However, as the unbalanced sperm that these polyploids produce is fatal to the sexual diploids they may act as sterile males, effectively eliminating coexisting diploids that would otherwise be just as well suited to the habitat. The polyploids appear unable to exist outside of restricted geographical areas, however, preventing the sexual diploid from being wiped out of its entire geographical area (Christensen et al. 1978).

It has been suggested that polyploidy in oligochaetes such as *L. lineatus* evolved recently and frequently from sexual diploid populations as a result of a number of mutational events, perhaps including some failure in meiosis or fertilization

(Christensen et al. 1976). Multiple occurrences of unisexual lineages have been shown for hybridogenetic *Poeciliopsis* fish clones by analysing restriction sites of mitochondrial DNA (Quattro et al. 1991). On the basis of such molecular genetic studies it has been concluded that unisexual vertebrates are recently evolved (Densmore et al. 1989). Parthenogens in existence for a long time would be expected to show much variability due to the accumulation and fixation of mutations, leading to divergence between them and their ancestral lineage (Maslin 1971). In particular, a high degree of genetic similarity has been found between sympatric diploid and triploid individuals of *L. lineatus* (Christensen et al. 1978), that is unlikely to be due to genetic exchange among polyploids because of their pseudogamy (Coates 1995), and therefore supporting the suggestion of recent origins.

5.3.1. Identification

Lumbricillus lineatus is approximately 10–15mm long with 38–42 segments and is reddish in colour (Figure 5.1). The female reproductive parts of this hermaphrodite are included within the spermathecae in segment V (Figure 5.2). The ectal duct is short and narrow and widens entally into a distinctly rounded ampulla, which contains received spermatozoa in its tissue. The male sexual parts are located in segments VIII–XII: the sperm funnels, on the rims of which produced sperm is stored as sperm collars, and the penial bulbs, which are inserted into the spermathecae of another worm during mating. The triploid form of *L. lineatus* lacks the sperm-producing organ, the seminal vesicles and so cannot produce sperm (Figure 5.3). The tetraploid and sometimes the pentaploid forms do possess seminal vesicles, although they are only about half the size of those of the diploids and may produce genetically imbalanced if morphologically normal sperm (Coates 1995). Segments XII and XIII comprise the thickened glandular area of the body wall known as the clitellum, and within these protective walls lay the ovaries and developing eggs. Developed eggs awaiting fertilisation float in the coelomic space of segment XIV.

Figure 5.1—Photograph of sexually mature adult hermaphrodite diploid *Lumbricillus lineatus* (magnification x 500). Photograph by Barry Lockyer

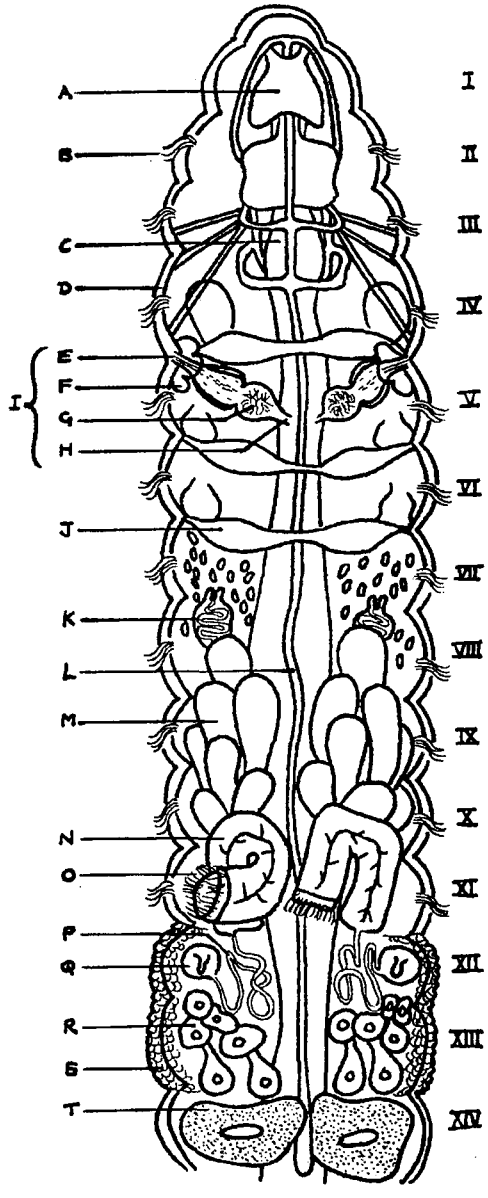


Figure 5.2—Anterior section of sexually mature adult hermaphrodite diploid *Lumbricillus lineatus* (magnification x1000). A = brain; B = setae; C = oesophagus; D = body wall; E = ectal duct; F = glandular lobes; G = ampulla; H = ental duct; I = female reproductive organ, spermatheca; J = septal gland; K = nephridium; L = dorsal blood vessel; M = seminal vesicles; N = sperm funnel; O = sperm; P = sperm canal; Q = penial bulb; R = ovaries; S = clitellum; T = developed egg. Numerals I–XIV on right hand side of diagram indicate segment numbers. (Adapted from Nielsen and Christensen 1959)

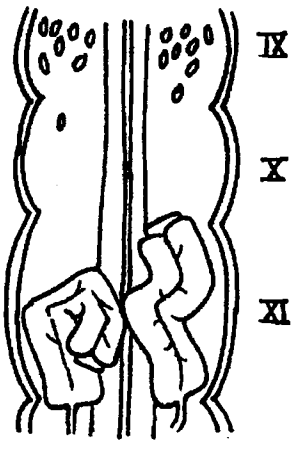


Figure 5.3—Section of sexually mature adult parthenogenetic triploid *Lumbricillus lineatus* (magnification x1000) showing the lack of seminal vesicles in the segments anterior to the sperm funnels, and the absence of sperm around the collar of the sperm funnels (Adapted from Nielsen and Christensen 1959)

There are other species of *Lumbricillus* that may cause identification problems and should be noted at this stage. In particular *L. rivalis* with similar habitat requirements as *L. lineatus* is generally larger (20–35mm long with 50–60 segments) and more robust than *L. lineatus* and of a more intense red colour. In general distinctions between species are made on the structure of the female reproductive organ, the spermathecae. *L. rivalis* possess larger, longer and looser individual cells (or glands) around the ectal openings, generally appearing much less compact than those of *L. lineatus*. There is reduced swelling of the ampulla as it extends the length of the spermatheca, therefore causing the structure to appear more spindle-like than the distinct curves and bulges of *L. lineatus* (Figure 5.4.2.). Due to the positioning of the ampulla, sperm is gathered throughout the spermatheca after mating, therefore colouring the entire length of the organ a deep brown. *L. rivalis* encompasses freshwater forms (possible sibling species), including those that are found in the gravel of water purifying systems. It is also found in seaweed high on the shore, and more saline lower shores. *L. viridus* is larger and fatter than *L. lineatus*, measuring approximately 20–25 mm long and consisting of around 40–50 segments (Nielsen and Christensen 1959). Due to its alimentary canal, the colouring of the worm is distinctly green, clearly setting it apart from *L. lineatus* and *L. rivalis*. The spermathecae of *L. viridus* are also different with clearly defined ampulla (Figure 5.4.1), and the glands around the ectal opening very compact (like *L. lineatus* and unlike *L. rivalis*) but better developed. None of these other species of enchytraeids exhibit polyploidy or sperm-dependent parthenogenesis.

Figure 5.4—Sperm receptacle organs (spermathecae) of (1) *Lumbricillus viridis*: better developed glands around ectal opening and more defined ampulla and (2) *Lumbricillus rivalis*: ectal opening possesses larger and less compact glands and there is less distinction of the ampulla as sperm gathers throughout the spermatheca. Numerals represent segment numbers (magnification x 500; adapted from Nielsen and Christensen 1959)

5.3.2. Reproduction

Hermaphroditic *L. lineatus* are unlikely to self-fertilise in nature, although it has occurred in laboratory cultures (Dozsa-Farkas 1995). Mating is usually mutual, and involves the insertion of the penial bulb of one individual into the seminal receptacle (spermathecae) of the other allowing a transfer of sperm. A cocoon forms a few days later that protects the eggs released into the body coelom. This cocoon is formed when the clitellum secretes a chitin-like material, and the presence of albumin and mucus aid in the development of this protective structure. The cocoon is slowly shed as the worm pulls backwards and the cocoon moves forwards over the anterior sections of the worm, and the eggs are fertilised by the sperm from the seminal receptacles as the cocoon passes over this section of the worm. The triploids receive sperm in the same manner, which penetrates the unreduced ovum and stimulates embryogenesis by fusing with the egg pronuclei to produce functioning nuclei of the embryo, without contributing genetic material and hence information on to the zygote (Christensen 1980a). Syngamy (fusion of egg and sperm pronuclei) and expression of paternal genes do not occur

(Beukeboom and Vrijenhoek 1998); inheritance is clonal and matrilineal. Unactivated eggs can still go through the early cleavage divisions but mitotic divisions become irregular and an imbalance in chromosome number occurs as their development is soon arrested (Christensen 1980a). Why sexual individuals waste sperm on asexual individuals is not clearly understood. It has been suggested that donating sperm to a parthenogenetic individual enables the 'disposal' of unfavourable or unbeneficial genotypes, as a form of group selection. Mate discrimination against pseudogamous parthenogens has been shown in the salamander *Ambystoma laterale* (Uzzell and Goldblatt 1967), the bark beetle *Ips accuminatus* (Loyning and Kirkendall 1996), the planthopper *Ribautodelphax pungens* (den Bieman 1988), and the freshwater fish *Poeciliopsis lucida* (McKay 1971). Although in the latter example males did mate with the asexual hybrids if given limited access to the preferred conspecific females, perhaps providing them with valuable mating experience. Indeed, males of the sailfin molly *Poeciliopsis latipinna* do not appear to discriminate against asexual females, and mating with them actually increases their attractiveness to sexual females (Schlupp et al. 1994). There is no evidence for mate discrimination in the freshwater planarian *Schmidtea polychroa*, nor through laboratory experiments with planthoppers of the genera *Delphacodes* or *Muellerianella*. In this case the stabilising mechanism was suggested to be differences in micro-distribution of sexual and asexual individuals in the field, in relation to males, leading to the easier insemination of sexual females (Booij and Guldemond 1984; den Bieman and Vrijer 1987). The implication here is that sexual females can discriminate where males are better than asexual females, but it is unclear as to whether this is unique to the species or whether it applies to a larger taxon. It has been noted that indiscriminate male care could actually help parthenogens to out-compete sexual forms (Bednekoff 1996). In *L. lineatus* it is not known whether mate discrimination occurs.

Tri-, tetra- and pentaploid *L. lineatus* individuals reproduce by a process of asynaptic meiosis. This is the term for the type of meiosis where at the first meiotic division no bivalents are formed, and undivided univalents are distributed to opposite poles in approximately equal numbers without undergoing an equational division (Christensen 1980a). This first division is therefore numerically reductional. Each chromosome attaches to one of the poles in the spindle apparatus,

but no true metaphase stage occurs; here, the chromosomes are arrested in a mid-anaphase stage. The spindle begins to elongate when the eggs are laid and the daughter nuclei move further apart, and by some 'unknown mechanism' each surprisingly receives approximately half of the chromosome complement. As the spindle bends into a V-shape, the chromosomes divide equationally and a second anaphase stage follows where chromosomes move along the continuous spindle. This results in two of the chromosome complements derived from each of the first-division daughter nuclei moving towards the apex of the V, and fusing near the cell membrane, and the other two moving towards the tips of the arms of the V, where they uncoil and fuse in the interior of the egg. The egg therefore now contains two nuclei each with the complete somatic chromosome number irrespective of the numerical distribution of the chromosomes during the first meiotic division (Christensen 1980a).

CHAPTER 6

FIELD SURVEYS OF *LUMBRICILLUS LINEATUS* (MULLER)

6.1. ABSTRACT

The littoral enchytraeid oligochaete *Lumbricillus lineatus* exhibits a hermaphroditic sexual (diploid) form and a pseudogamous parthenogenetic asexual (triploid) form that requires the sperm of the diploid to stimulate oogenesis. This occurs without incorporating any genetic information of the diploid. The geographical distribution and habitat requirements of both forms of *L. lineatus* were investigated by performing a series of surveys at different coastal locations within the U.K. Beaches in Anglesey, North Wales, which had been sampled 40 years previously, were revisited and other suitable sites in southern England were sampled for the first time. The geographical distribution of *L. lineatus* was found to have considerably reduced over the 40-year period, and *L. lineatus* was absent from many sites on the south coast of the U.K. that appeared to offer a suitable habitat. Contributing factors may be erosion and pollution of beaches and changing global temperatures. Habitat use by *L. lineatus* was also more restricted than 40 years ago. Its present habitat was found to include only coarse or pebbly sand and it was no longer reliably found in fine sand or mud, as previously. *L. lineatus* was most abundant on medium sized rough-textured pebbles. Percentage cover of seaweed, organic content and particle size of the substrate did not influence the abundance of *L. lineatus*. The majority of worms were found living 20–35 m above the low tide line (41–73% of the distance from the low tide line to the strandline). The key requirements for *L. lineatus* colonisation appeared to be the locally steeper and more sheltered beaches that provided areas of well-drained sand frequently washed over by tides, suggesting that the degree of moisture and the low level of physical disturbance of the sand are the most important factors determining the abundance of *L. lineatus*. Diploid:triploid ratios in natural populations were on average 4:1, having reversed from the previously recorded ratio of 1:2.

6.2. INTRODUCTION

Sexually reproducing populations have an immediate disadvantage in population growth over asexually reproducing populations. This is because half of the investment in sexual reproduction generally goes into the production of males (for a gonochoristic population) or male gametes (for hermaphrodites), which can only express their contribution to population growth through females. With no males or male gametes to sustain, otherwise identical asexual parthenogens can double their relative representation in the recipient population in successive generations, at least under density-independent conditions.

Further complications arise, however, for pseudogamous parthenogens. Pseudogamous parthenogenesis is a form of asexual reproduction that requires the spermatozoa of a sexual sibling species to initiate egg development without incorporating the genetic information of the donor sperm (Christensen and O'Connor 1958). A pseudogamous parthenogen is therefore a reproductive parasite of its sexual host. This type of obligatory coexistence has some of the disadvantages of sexual reproduction, such as the cost of searching for a mate, without the compensating advantage of genetic variation from recombination of parental genotypes. If the pseudogamous population out-grows its obligate sexual host, it will diminish its own mating opportunities. The question therefore arises as to why pseudogamous parthenogens do not evolve into full parthenogens, releasing both forms from this enforced relationship. Evidence for this evolution does exist in some triploid unisexual salamanders of the genus *Ambystoma* (Uzzell 1969). In certain areas in Indiana, diploids are rare, possibly due to effects of the environment or man's activities, but triploids are numerous, evidently having escaped the environmental hazards as well as the dependence on males for ova development. However, 24 genera from seven phyla in the animal kingdom are known to exhibit pseudogamy, including insects (Weinzierl et al. 1999), molluscs (Qi et al. 2000), fish (Vrijenhoek and Pfeiler 1997; Felip et al. 2001) and reptiles (Case 1990). Pseudogamy is also widespread among higher plants (Calame and Felber 2000) including monocotyledons and dicotyledons (Nygren 1954), but plants constitute a hugely different case that can only be touched upon in this thesis. Many more pseudogamous species of animals and plants are expected to be

discovered with the advancement of molecular and cytological methods (Bullini 1994). In fact, no truly parthenogenetic fish or amphibia are known (Vrijenhoek 1994). The widespread existence of pseudogamous reproduction suggests the presence of some compensatory mechanisms, such as competitive inferiority of the pseudogamous type (Schley et al. submitted). Few pseudogamous parthenogens are well studied and very few theoretical models exist to predict the population dynamics of such reproductive systems (Schley et al. submitted). Future modelling of this system will require knowledge of the geographical and habitat distribution of pseudogamous test species. This chapter aims to provide such information on the littoral enchytraeid oligochaete *Lumbricillus lineatus* (Muller).

The common form of *L. lineatus* is the hermaphroditic (sexual) diploid, which can exist alone, but is often parasitised by the related parthenogenetic (asexual) triploid form. The sperm-producing seminal vesicles are absent or reduced to mere traces by asynapsis in the triploid form, and so no spermatozoa are produced (Nielsen and Christensen 1959). The triploid form of *L. lineatus* has never been found in the absence of diploids. The fixing and staining of the chromosomes of mature eggs of hermaphroditic sexual and parthenogenetic forms has confirmed the ploidy levels of these morphologically distinct forms (Christensen 1980a). *L. lineatus* also exhibits tetra- and pentaploids which produce unbalanced sperm, sufficient to stimulate egg development in the polyploids, but inadequate for sexual reproduction. These forms are often found in coexistence with triploids both at high and low relative densities (Christensen et al. 1976). Tetra- and pentaploids are commonly found in the absence of diploids, and in extreme conditions (Christensen et al. 1978; see the review in Chapter 5).

L. lineatus has been reported from estuarine and intertidal sites worldwide (Giere and Hauschildt 1979; Christensen 1980b; Coates 1990), being shown to be largely abundant in disturbed habitats outside Europe (Coates and Ellis 1980; Coates and Ellis 1981). A study of the coastlines of Anglesey and the Menai Strait in North Wales found *L. lineatus* to be widely abundant in a variety of different substrates (Tynen 1972). Such studies suggest that the worm lives in sand, on the wet underside of pebbles or debris, or within wrack beds (pers. observ; Tynen 1972). The overall aim of the present study was to gather information on the specific

habitat requirements and geographical distribution of *L. lineatus* in the U.K. A series of surveys was carried out on beaches in Anglesey, North Wales, from where *L. lineatus* had been recorded in 1962–1964 (Tynen 1972) and southern England, where it was suspected that it might occur. The aims of the study were to test whether the abundance of *L. lineatus* was similar to that recorded by Tynen (1972), and specifically to seek coexisting populations of triploid with diploid forms of *L. lineatus*. The effects of ecological variation between and within beaches were investigated, including the slope, degree of exposure, dimensions and main substrate of the beach and the properties of the substrate and occupied pebble.

6.3. MATERIALS AND METHODS

6.3.1. General Survey

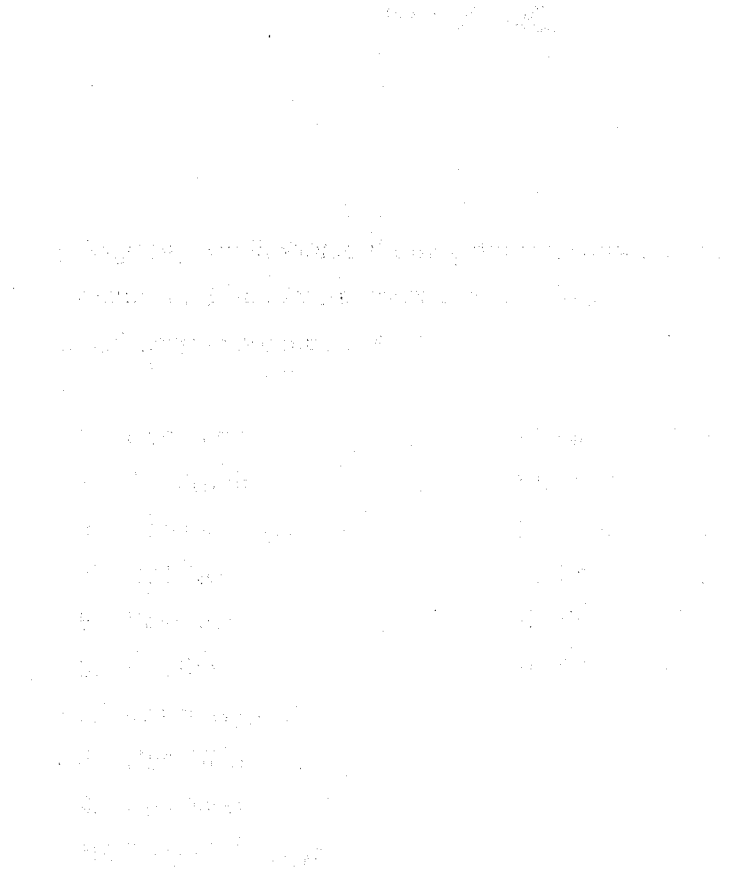
6.3.1.1. Geographical Distribution

Beaches on Anglesey and the Caernarvonshire shore of the Menai Strait, North Wales, held populations of *Lumbricillus lineatus* in all seasons during 1962–1964 (Tynen 1972). A number of these beaches were revisited during 2000–2003 (Figure 6.1) for the present study. One particular beach (Porth Eilian) was sampled many times throughout this period enabling an average proportion of diploids:triploids per sample to be investigated. In addition, a number of other beaches were sampled along the south coast of England (Devon, Cornwall, Dorset and Hampshire, Figures 6.2 and 6.3) for the first time, in habitat that was judged suitable for *L. lineatus* on the basis of descriptions given in Tynen (1972).

6.3.1.2. Habitat Requirements

The collection method of Tynen (1972) was not quantitative, involving taking samples of substratum to a depth of up to 15 cm, concentrating efforts in those areas expected to hold enchytraeids, and taking larger samples where worms appeared to be scarce. I surveyed *L. lineatus* abundance by sampling many sites across the width of the beach and from the strandline to the low tide line. Sampling was performed *in situ* by extracting substrate to a depth of 5 cm, transferring it to a

plastic container and swilling it around with a small amount of seawater. The water, along with any worms present, was then poured off into a glass dish and worms were clearly visible in the water. Where substrate was found to contain worms, larger samples were collected in polythene bags and transferred to the laboratory for identification of species and reproductive form. Diploids and triploids are clearly distinguished by the respective presence or absence of seminal vesicles (ploidy level of these forms confirmed by Christensen 1980a). Tynen (1972) described six different habitat types: freshwater run off, sand, gravel, shingle, stabilised mud and soil. The present study concentrated on substrates present between the low tide line and the strandline, with habitat types classified as mud, muddy sand, fine, medium or coarse sand, gravelly sand, shingly sand, shingle, pebble or seaweed.



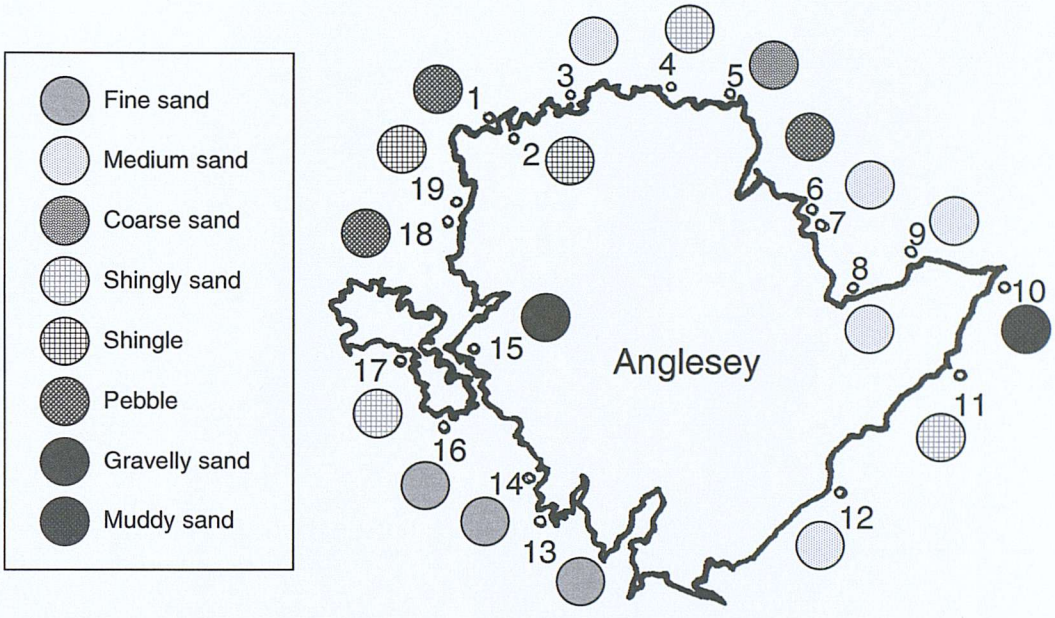


Figure 6.1—Map of Anglesey, North Wales showing the locations of the sites sampled for the presence or absence of *Lumbricillus lineatus* in 2000–2003. Modified from Tynen (1972). 1 cm = 5 km. OS Landranger map 114

1. Hen Borth
2. Cemlyn Bay
3. Caemaes Bay
4. Bull Bay
5. Porth Eilian
6. Moelfre
7. Traeth Bychan
8. Red Wharf Bay
9. Pentrellwyn
10. Trwyn y Penrhyn
11. Gallow's Point
12. Moel-y-don
13. Porth Cwyfan
14. Porth Trecastell
15. Four Mile Bridge
16. Borthwen
17. Trearddur Bay
18. Porth Trewyn
19. Church Bay

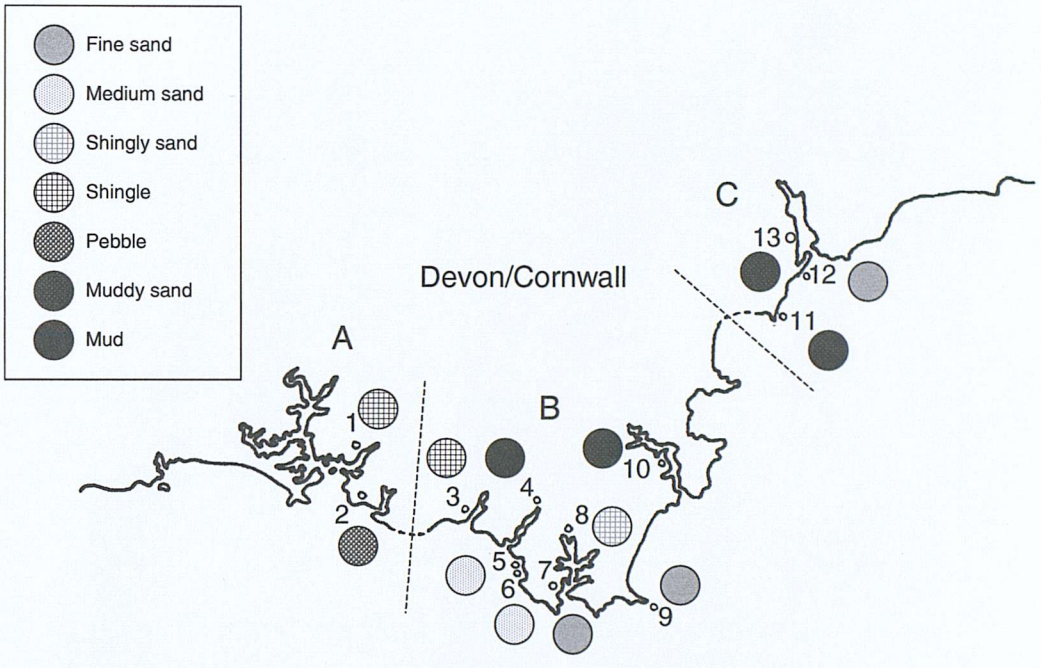


Figure 6.2—Map of the Devon and Cornwall coastline showing sites that were sampled for the presence or absence of *Lumbricillus lineatus* in 2000–2003. 1 cm = 8 km. A = OS Landranger map 201; B = OS Landranger map 202; C = OS Landranger map 192

1. Plymouth
2. Wembury
3. Wonwell Beach
4. 'Tidal Road'
5. Challaborough
6. Bigbury-on-sea
7. Salcombe
8. Kingsbridge
9. Start Bay
10. Dittisham
11. Teignmouth
12. Exmouth
13. Starcross

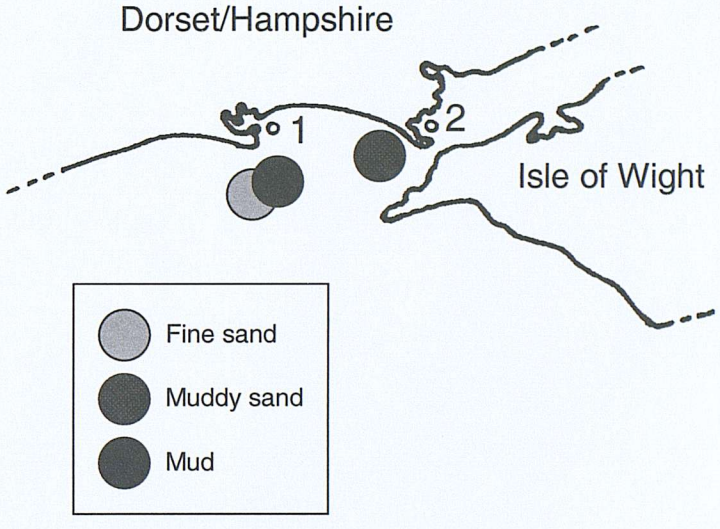


Figure 6.3—Map of Hampshire and Dorset showing the sites that were sampled for the presence or absence of *Lumbricillus lineatus* in 2000–2003. 1 cm = 7 km. OS Landranger maps 195 and 196

1. Christchurch Harbour/Estuary
2. Keyhaven

6.3.2. Between-Beach Survey

Visits were made at low tide in April 2003 to the beaches of Caemaes Bay, Porth Eilian, Traeth Buchan, Gallow's Point, Porth Cywfan and Trearddur Bay (sites 3, 5, 7, 11, 13 and 17 in Figure 6.1) in Anglesey, North Wales. The slope of the beach from the strandline to the sea was measured using a clinometer; the length and span of the beach were measured and recorded and the main substrate type was categorised. The degree of exposure of the shore was estimated. This degree of exposure was estimated qualitatively for comparative purposes, without specific reference to wave climate, width of surf zone and other factors usually required to quantify exposure (Little 2000). In the area thought most likely to support *L. lineatus*, based on previous observations, a 1-m^2 quadrat was placed at least five times within each of three suitable sites, each covering an area of 5 m^2 and situated at least 5 m apart. A 2-minute timed search was carried out within each quadrat, with any sand, pebbles or seaweed containing worms being bagged for later analysis in the laboratory. For all beaches, the presence or absence of *L. lineatus* was noted, as well as the ratio of diploid:triploid forms found in the samples analysed in the laboratory, to allow comparisons to be made between beaches and habitat types.

6.3.3. Within-Beach Survey

Local variation in abundance was studied within the beach at Porth Eilian (site 5 in Figure 6.1) in Anglesey, North Wales. This beach was selected for its large population of *L. lineatus* all year round (see General and Between-Beach Surveys). In April 2003 belt transects were laid from the strandline to the line of low tide, every 7 m across the span of the beach. A 1-m^2 quadrat was randomly placed three times every 5 m along each transect from the strandline to the low tide line. In each quadrat a 1-minute timed search for *L. lineatus* was carried out, with all worms found in the sand, pebbles or seaweed in this time period collected for later analysis in the laboratory. The presence or absence of *L. lineatus* and the relative abundance of diploids:triploids was later recorded. The percentage cover of seaweeds within the quadrat was estimated. In addition, a sample of substrate (approximately 200 g) was taken from each quadrat for laboratory analysis of organic content and particle-

size range. The organic content of the substrate was measured by drying and weighing a small amount of the substrate, then subjecting it to 400°C in a muffle furnace overnight to burn off all organic matter. Samples were reweighed and organic content of the samples calculated. The size range of particles of the substrate was determined by passing the substrate through a series of sieves (aperture sizes: 2400, 1680, 850, 600 and 300 μm). The separated sections of the sample were individually weighed and the percentage of each particle size category was calculated.

6.3.4. Pebble-Size Survey

The area thought most likely to support *L. lineatus*, based on previous observations, was selected at Porth Eilian, Anglesey. Seventy-six pebbles were randomly removed from the sand. Any worms present on the pebbles were washed off with a pipette of seawater into a labelled pot for later identification in the laboratory. Length and width of the pebble and the distance the pebble was buried into the sand were measured. From these data, a value of worms per 100 cm^2 of pebble surface area available to the worm was obtained and pebbles were allocated into one of seven size categories: (1) <5 cm^2; (2) 6–15 cm^2; (3) 16–25 cm^2; (4) 26–35 cm^2; (5) 36–45 cm^2; (6) 46–55 cm^2; (7) 56–65 cm^2; (8) >66 cm^2 surface area available to the worms. Pebbles were also categorised on a scale of rough to smooth for comparison and suggestion of preferred pebble texture: very rough, pitted/jagged surface; quite rough, pitted, but more even surface; very fine pits, overall smooth surface; completely smooth surface.

6.3.5. Statistical Analysis

6.3.5.1. General Survey

The hypothesis that the number of diploids per sample would be similar to the number of triploids per sample in 86 samples of *Lumbricillus lineatus* taken from Porth Eilian beach in Anglesey during 2000–2003 was investigated using a paired t-test (Minitab v. 13.1). The numbers of diploids and triploids were transformed

using the equation ln (number) in order to meet the assumption of a normal distribution of residuals.

6.3.5.2. Within-Beach Survey

The hypothesis that the abundance of worms and the ratio of diploids:triploids found in the sand are influenced by the position on the beach was investigated qualitatively. The numbers of worms present depending on distance from the low tide line to the strandline, and across the span of the beach were mapped graphically. Chi-squared analyses of association (Minitab v. 13.1) were used to test the null hypotheses that the presence or absence of *L. lineatus* did not depend on the presence or absence of seaweed and the level of organic content of the substrate (high or low). Particle-size range of the substrate was examined graphically and compared to worm abundance.

6.3.5.3. Pebble-Size Survey

A two-way ANOVA (Minitab v. 13.1) was used to test the null hypotheses that pebble Surface area available to the worm (covariate) and pebble surface Texture, with four levels, do not affect worm abundance. The test model for this survey was:

Abundance = Surface area | Texture.

The response of worm abundance was measured in terms of the number of worms present per 100 cm^2 of pebble surface area, and numbers were transformed using the equation ln (0.5 + number) in order to meet the assumption of a normal distribution of residuals.

6.4. RESULTS

6.4.1. General Survey

The general survey of beaches in the U.K. revealed a more restricted distribution of *Lumbricillus lineatus* compared to the study carried out 40 years previously (Tynen 1972), with only one beach in Anglesey, North Wales, being a reliable source of *L. lineatus* today (i.e., always present and not mixed with the morphologically similar *L. rivalis*). There also appeared to be limited abundance of the worm in the newly sampled sites in the U.K. where the worm was expected to be found: indeed only three (Kingsbridge, by the river Plym and Keyhaven) of 16 locations were found to hold populations of *L. lineatus* on the south coast of England. The presence or absence of *L. lineatus* in each of the 37 beaches sampled is summarised in Table 6.1.

Table 6.1—The beaches and their main substrate types sampled in 1962–1964 and in 2000–2003 for presence or absence of *Lumbricillus lineatus*. Bold-type indicates beaches surveyed in the between-beach survey of Section 6.4.2.

Beach	OS map - grid reference	Main substrate	Presence of *L. lineatus* if sampled	
			1962–1964	2000–2003
ANGLESEY				
Borthwen	114 - 273748	Fine sand	–	N
Bull Bay	114 - 427944	Shingly sand	Y	N
Caemaes Bay	114 - 373937	Medium sand	Y	N
Cemlyn Bay	114 - 345933	Shingle	–	N
Church Bay	114 - 301894	Shingle	Y	N
Four Mile Bridge (South)	114 - 280783	Gravelly sand	Y	N
Four Mile Bridge (North)	114 - 280785	Gravelly sand	Y	N
Gallow's Point	114 - 597752	Shingly sand	Y	Y*
Hen Borth	114 - 319931	Pebbles	Y	Y**
Moel-y-don	114 - 519679	Medium sand	–	N
Moelfre	114 - 513862	Pebbles	Y	N
Pentrellwyn	114 - 574812	Medium sand	–	N
Porth Cwyfan	114 - 337683	Fine sand	–	N
Porth Eilian	114 - 477930	Coarse sand	Y	Y

Porth Trecastell	114 - 332707	Fine sand	Y	N
Porth Trewyn	114 - 296878	Pebbles	–	N
Traeth Bychan	114 - 515846	Medium sand	Y	Y*
Treardurr Bay 1	114 - 248792	Shingly sand	N	N
Treardurr Bay 2	114 - 245792	Shingly sand	N	Y**
Trwyn y penrhyn	114 - 628797	Muddy sand	–	N
Red Wharf Bay	114 - 530805	Medium sand	Y	Y*
DEVON/CORNWALL				
Bigbury-on-Sea	202 - 650450	Medium sand	–	N
Challaborough	202 - 649448	Medium sand	–	N
Dittisham	202 - 867550	Muddy sand	–	N
Exmouth	192 - 981785	Fine sand	–	N
Kingsbridge	202 - 738435	Shingly sand	–	Y*
River Plym	201 - 505555	Shingle	–	Y*
Salcombe	202 - 732382	Fine sand	–	N
Starcross	192 - 977818	Muddy sand	–	N
Start Bay	202 - 830370	Fine sand	–	N
Teignmouth	192 - 934724	Muddy sand	–	N
'Tidal Road'	202 - 690472	Mud	–	N
Wembury	201 - 517485	Pebbles	–	N
Wonwell Beach	202 - 615475	Shingle	–	N
HAMPSHIRE/DORSET				
Christchurch Estuary	195 - 180910	Mud	–	N
Christchurch Harbour	195 - 182910	Fine sand	–	N
Keyhaven	196 - 315915	Muddy sand	–	Y**

Y = *L. lineatus* present; N = *L. lineatus* not present; *sometimes present; **L. *rivalis* also present; – not sampled.

Twenty-six of the 37 beaches sampled did not hold any *L. lineatus* and mostly comprised fine or muddy sand as their main substrate. Of the remaining 11 beaches found to hold *L. lineatus*, 10 of them comprised sand, coarse sand, shingly sand, shingles or pebbles as their main substrate and the other comprised muddy sand, but also contained the morphologically similar *L. rivalis*. Figure 6.4 shows the percentage of main substrate types associated with beaches that held *L. lineatus*.

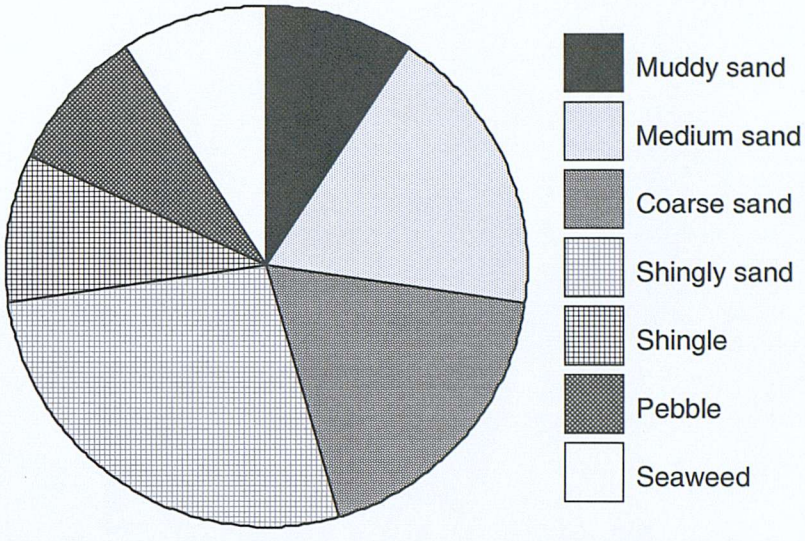


Figure 6.4—The percentage of main substrate types of beaches in North Wales, Devon, Hampshire and Wiltshire found to hold *Lumbricillus lineatus* populations in 2000–2003. n = 9

Of the 12 beaches in Anglesey found to hold populations of *L. lineatus* in the 1960s, only five were found to house the worm in the present study, with three being unreliable sources (i.e. not found at every visit), and one also containing the morphologically similar *L. rivalis*, leaving only one reliable source of *L. lineatus* (Porth Eilian, 5; Figure 6.5). The main substrate types of these five beaches were medium, coarse or shingly sand, or pebbles, with Porth Eilian comprising coarse sand. Habitat occupation is more restricted today in comparison to the presence of *L. lineatus* in gravelly, fine, medium, coarse and shingly sand, and in shingle and pebble in the 1960s (Tynen 1972). This suggests that once suitable habitats are no longer being colonised. Of the two beaches found not to hold *L. lineatus* in the 1960s, one does today (Treardurr Bay 2, 17, comprising shingly sand). This population also contained *L. rivalis*, making it difficult to identify individuals. Seven additional beaches that were not sampled in the 1960s were sampled in the present study and found not to hold any populations of *L. lineatus*.

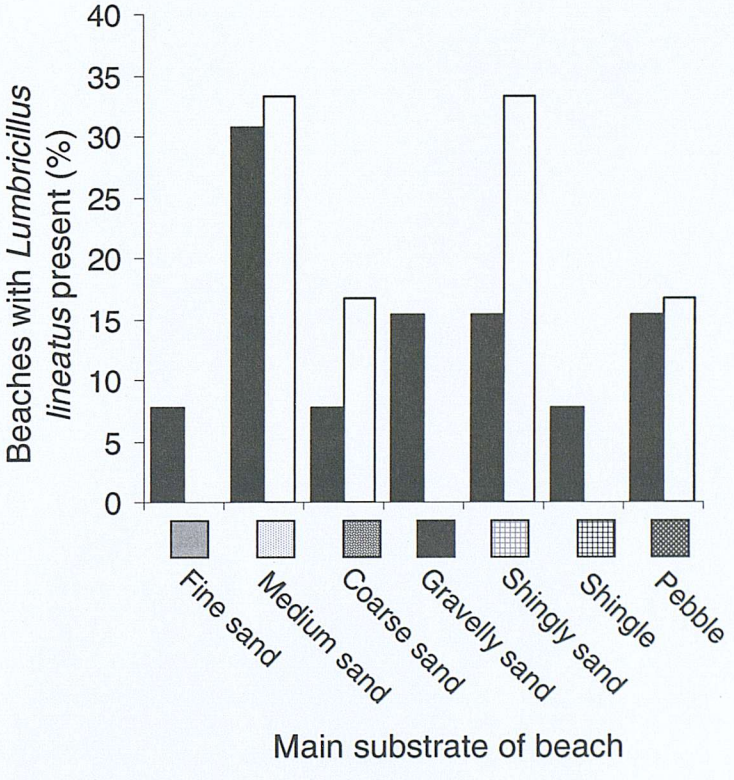


Figure 6.5—Percentage of beaches in Anglesey, North Wales, holding *Lumbricillus lineatus* under each main substrate category in 1962–1964 (black bars) and in 2000–2003 (clear bars)

The average ratio of diploids to triploids found for all 86 samples taken at Porth Eilian during this survey was 4:1 (Figure 6.6). Number of diploids per sample (ranging from 1–37, with an average of 10.2) was greater than the number of triploids per sample (ranging from 0–9, with an average of 0.9; paired t-test: T_{84} = 18.36, P = <0.001, Figure 6.6).

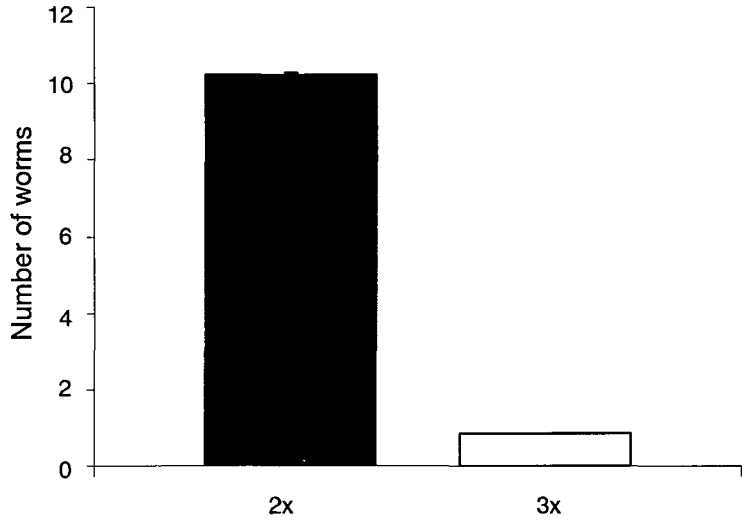


Figure 6.6—Number of diploids (black bar) and triploids (white bar) present in samples taken from Porth Eilian, Anglesey throughout 2000–2003. n = 86; SE bars are shown

6.4.2. Between-Beach Survey

Six beaches were compared in this survey, with three found to hold populations of *L. lineatus* and three not (Table 6.2). Beaches with *L. lineatus* present (Gallow's Point, 11, Porth Eilian, 5, and Trearddur Bay 2, 17, in Figure 6.1) were more steeply sloping than beaches with *L. lineatus* absent (average of 6.8° compared to 4.2°; Slope effect: T_4 = 2.83, P = 0.047), suggesting that *L. lineatus* requires good drainage of seawater after high tide.

Table 6.2—The presence or absence and diploid:triploid ratio of *Lumbricillus lineatus* at six beaches in Anglesey, North Wales, in 2003 and related ecological factors. Span of beach refers to distance from east to west side of the shore; length of beach refers to distance from low tide line to strandline

Beach	Slope (°)	Span (m)	Length (m)	Main substrate	Exposure	*L. lineatus* present (*N*)	2x:3x
Gallow's Point	6.5	100	22	Pebbles	Exposed	15	7:1
Porth Eilian	6.0	44	58	Coarse sand	Sheltered	51	4:1
Treardurr Bay	8.0	78	22	Pebbles	Sheltered	3	1:0
Traeth Bychan	5.5	50	30	Fine sand	Sheltered	0	-
Porth Cywfan	3.0	86	120	Fine sand	Exposed	0	-
Caemaes Bay	4.0	34	76	Medium Sand	Exposed	0	-

Beach span and length did not differ between those beaches that held *L. lineatus* and those that did not. *L. lineatus* was present on beaches that comprised coarse sand or pebble as their main substrate, and conversely where the substrate was fine or medium sand there were no *L. lineatus* present, suggesting a more suitable microhabitat offered by larger particles. Two out of three of the beaches with *L. lineatus* present were sheltered, while two out of three of the beaches with no *L. lineatus* were exposed, suggesting that *L. lineatus* survives better when conditions imposed by the action of the sea and the weather are less harsh. Diploid:triploid ratios of the samples collected were 2:1 for all three beaches pooled. Actual numbers were very small (average of 3.63 worms in 13 samples in which worms were present; average of 0.76 worms in all 45 samples), so absolute presence or absence is of more interest in this survey.

6.4.3. Within-Beach Survey

The pattern of worm abundance was plotted on a map of the beach (Figure 6.7) and appeared to suggest that position on the beach influenced worm abundance. The majority of the worms found were living between 20 and 35 m from the low tide line (41–73% of the distance from the low tide line to the strandline). This distance from the sea may provide the correct level of moisture for the worms to survive, with sufficient drainage and minimal waterlogging of the substrate. The worms also

appeared to be more concentrated on the west side of the shore, perhaps due to being nearer to a rock face and therefore more sheltered. The proportion of diploids ranged from 0.7–1, indicating a very low incidence of triploids in all areas of the beach.

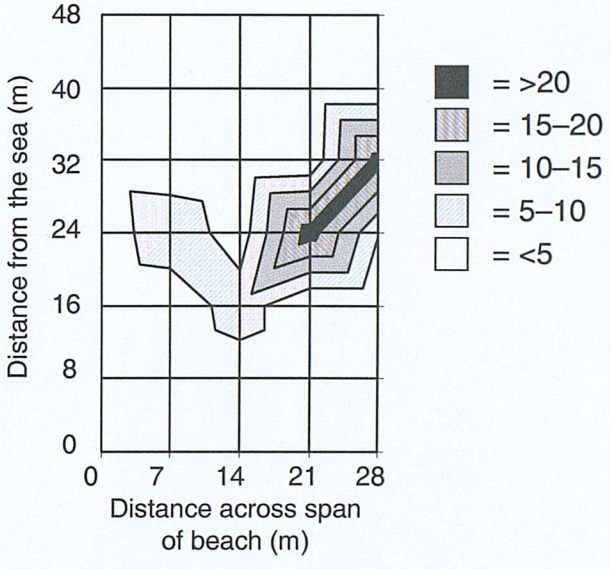


Figure 6.7—*Lumbricillus lineatus* abundance on the beach of Porth Eilian, Anglesey during sampling survey of April 2003. Key refers to the number of worms found in each 1-m^2 quadrat in each area of the beach during a 1-minute timed search. 0 m from the sea represents the low tide line and 48 m from the sea represents the strandline

The presence or absence of *L. lineatus* did not depend on the presence or absence of seaweed at high and low levels of organic content of the substrate (Chi-squared: organic content high $\chi^2_1 = 0.105$, $P = 0.746$, organic content low $\chi^2_1 = 0.989$, $P = 0.320$). Organic content and seaweed percentage cover were plotted on maps of the beach (Figure 6.8). Both variables appeared to be higher along the line of low tide, along the strandline and also along the west side of the shore, suggesting an accumulation of organic debris in these areas, but having no effect on *L. lineatus* presence or absence.

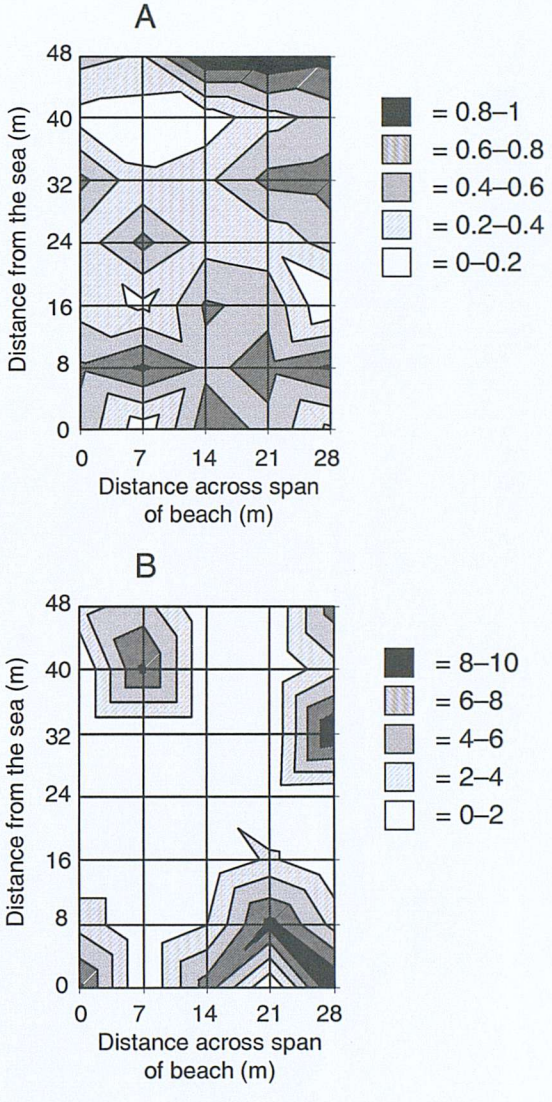


Figure 6.8—(A) organic content (key refers to proportion of substrate being organic); (B) seaweed percentage cover on the beach (key refers to percent seaweed percent cover)

There was a suggestion that worms were more abundant in areas of finer substrate particles and less of the substrate consists of large particles nearer the sea (Figure 6.9A). The majority of *L. lineatus* individuals found were within 20–35 m from the sea and on the west side of the beach. Across the span of the beach, however, relative representation of different particle-size ranges did not vary considerably (Figure 6.9B).

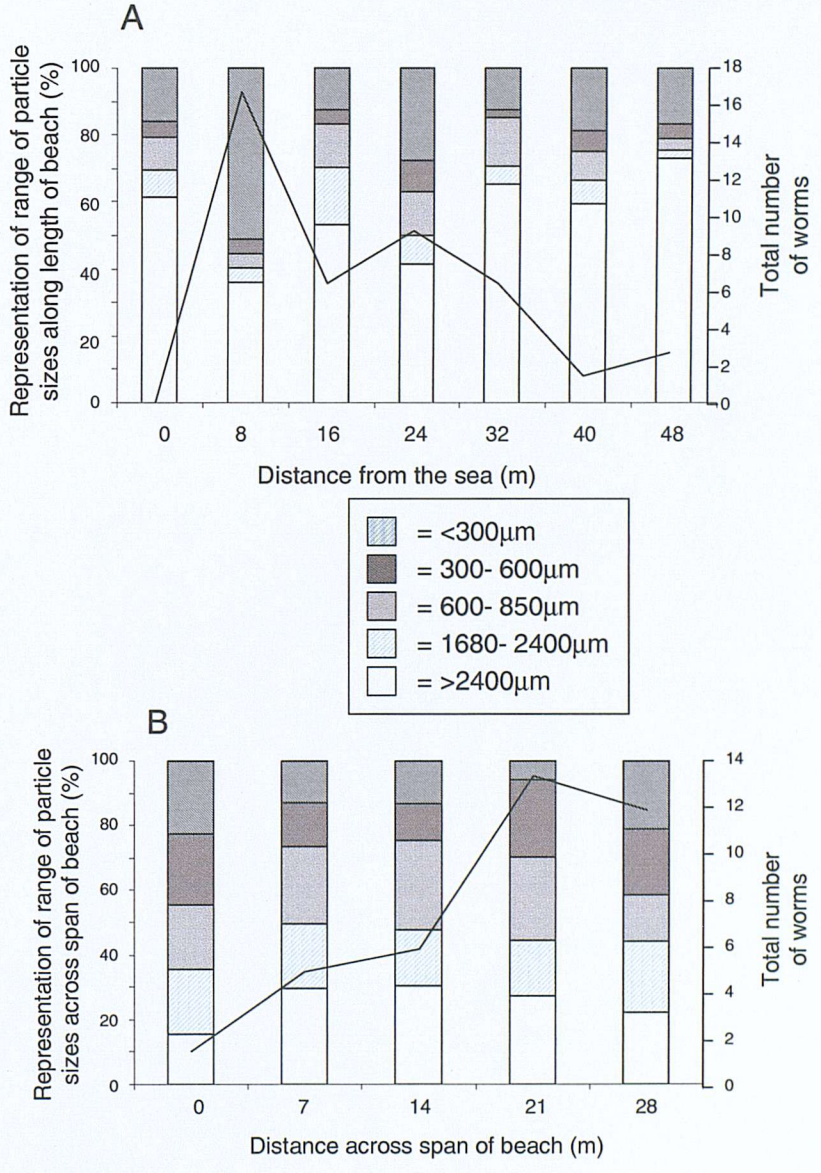


Figure 6.9—The representation of ranges of particle sizes (left axes and bars) (A) along the length of the beach from the sea to the strandline, and (B) across the span of the beach from left to right, and compared to total number of worms present (right axes and lines)

6.4.4. Pebble-Size Survey

The number of worms present per 100 cm^2 of pebble surface area was affected by overall pebble surface area available to the worms (i.e., the section of the pebble buried in the sand) depending on pebble surface texture (Surface area × Texture interaction: $F_{3,68} = 4.79$, $P = 0.004$, see Figure 6.10). On all pebbles apart from the roughest, worms were more abundant on those that provided less than 30 cm^2 of pebble surface area overall. On the roughest pebbles, surface area available was not important, whereas large smooth pebbles were found to be unsuitable for *L. lineatus*.

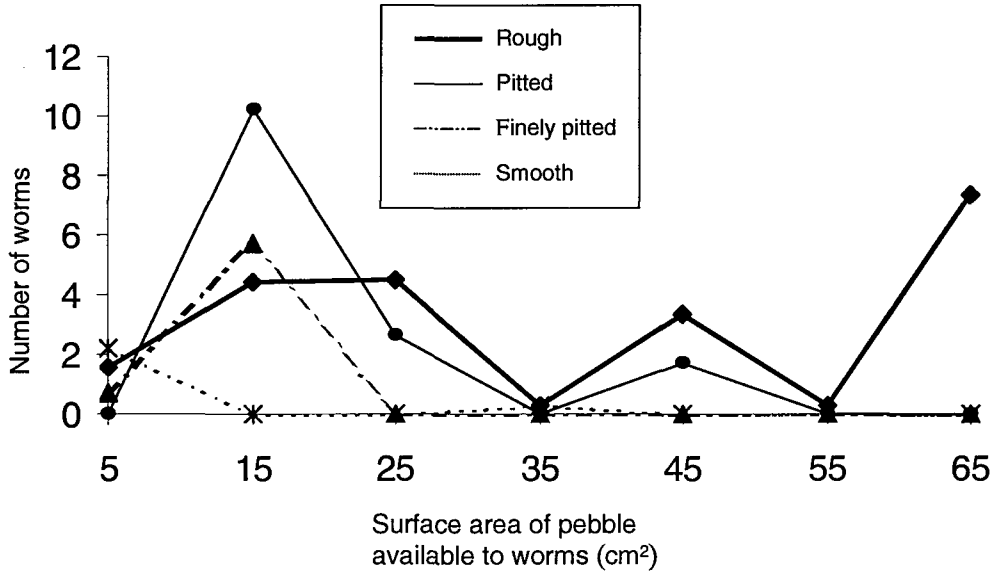


Figure 6.10—Interaction plot of the mean number of worms present per 100 cm^2 pebble surface area available to *Lumbricillus lineatus*, depending on surface area available to the worms and pebble surface texture

6.5. DISCUSSION

6.5.1. Geographical Distribution

The geographical distribution of *Lumbricillus lineatus* appears to be more restricted than it was 40 years ago (Tynen 1972). Few studies have been performed on enchytraeids outside of northern Europe, North America and Asia (Healy and Rota 1992), and the high Arctic where enchytraeids are smaller than their temperate counterparts (Birkemoe et al. 2000). Southern climes have rarely been studied. Perhaps *L. lineatus* is a northern species and not able to survive in the warmer temperatures of the south of the British Isles. In this study, only 16 locations in southern England were sampled for the presence of *L. lineatus*, and some of these consisted of what is evidently an unsuitable habitat type under these climatic conditions. Further surveys of more locations with a suitable habitat type would likely reveal more occurrences of *L. lineatus*.

6.5.2. Habitat Distribution

Tynen (1972) reported *L. lineatus* to be common and eurytopic (of a broad range), as it occurred in 4–6 of his classified habitat types. In contrast my study found *L. lineatus* to occur in only two of Tynen's classified habitat types, making it stenotopic (of a narrower range) by his criteria. Long-term studies of temperate zone enchytraeids have shown density fluctuations to be common, driven perhaps by drought (Springett 1970), abiotic factors such as temperature (Nurminen 1967; Klungland 1981) and depending on species and habitat. *L. lineatus* has been found to be abundant in polluted habitats, however (Suchanek 1993), and Giere and Hauschildt (1979) explained how they are highly adapted to resist stresses such as these due to their flexibility of cocoon development and growth. It has been suggested, however, that this resistance to stress may be due to the polyploidy and clonal diversity present in the experiments and not representative of field populations (Coates 1995). Therefore, pollution may be contributing to the restricted habitat distribution we see today, as well as increase in global temperature and rising tide levels, and increased utilisation and erosion of beaches. In winter storms, 'reflective' beaches (those with larger particle sizes and steeper

slopes that reflect the energy of the waves) can be eroded and partly flattened, possibly built up again as storms subside, but in the long term transformed to some degree to flatter 'dissipative' beaches (where the wave energy dissipates across the wide shallow beach, Little 2000). These shallower beaches are characteristically finer-grained, which evidently constitutes unsuitable habitats for *L. lineatus* and may prevent establishment or colonisation. Beaches are inherently unstable, due to the lack of cohesion between particles, and this has resulted in a high diversity of species of enchytraeids compared to terrestrial habitats (Tynen 1972), but may also contribute to the restricted distribution. Such physical disturbance of the substrate, also caused by the action of the waves and the weather, provides an unfavourable microhabitat for *L. lineatus*. Indeed, the more sheltered beaches surveyed in the present study were found to hold populations of the worm and exposed ones were not. *L. lineatus* and *L. rivalis* habitats are known to overlap, but the latter also exists higher up the tideline and in less favourable *L. lineatus* habitat (for example, muddy sand; pers. observ.) and so is not suggested to be competing with *L. lineatus* or playing a role in its reduced distribution.

L. lineatus abundance was found to be higher half to three-quarters of the way up the shore from the low tide line. This could be attributed directly to the action of the sea, as the worms require constant moisture without stagnating in waterlogged sediments. The reflective aspect of the beach, whereby the steeper slopes and larger particle sizes lead to less effective irrigation of the interstitial spaces below the sand surface, causes the sediment to dry out faster at low tide (Little 2000), so a shorter time between low tides is desired. Constantly renewing supplies of oxygen and nutrients are provided at lower tide levels, although beaches with more shelter tend to accumulate higher levels of substrate organic content (Little 2000), providing a uniform saturation of accessible nutrients, and perhaps explaining the independence of *L. lineatus* distribution. In addition, particle size of the substrate was found to be smaller nearer to the sea (see also Tynen 1969), which may lead to the capture of more seawater than larger particles such as shingle with cavity sizes too large for good capillary action (Tynen 1969). Enchytraeids are thought to have evolved from freshwater species, where the osmoregulatory mechanisms developed. As littoral species they are exposed to prolonged and wide extremes of salinities and so require osmoregulatory mechanisms; poikilosmotic worms such as polychaetes

which are primitively marine do not require such mechanisms and show no means of controlling the passage of water in and out of the body (Tynen 1969). *L. lineatus* has been shown to have no preference over a range of salinities up to 1.5× that of seawater (Tynen 1969). Oxygen requirements have been shown to limit the ecological distribution of enchytraeids in hot climates (Healy and Coates 1999), being absent where oxygen is depleted by high temperatures and may also have an effect in temperate climes. It is interesting to note that triploids have been shown to require less oxygen than diploids in brook trout *Salvelinus fontinalis* that would suggest the likelihood of a different ratio of diploids to triploids in such conditions (Stillwell and Benfey 1996).

A previous study of *Lumbricillus* abundance within a shore, however, found *L. lineatus* to be distributed widely along the length and width of the beach from the low tide line to above the high tide line (Tynen 1969), a range unmatched by any other species of enchytraeid found on that shore. Most studies of oligochaetes have concentrated on sediments or wrack beds in temperate zones. A few papers have reported species from rocky shores (Healy 1996) or from shores in the tropics, but those few have reported littoral enchytraeid fauna to be more widespread than previously thought. Availability of food would also be an important habitat determinant, along with the requirement for a substrate which provides protection against wave action and predators and is appropriate for locomotory activities (Healy and Coates 1999). Colonisation of new habitats relies on dispersal of at least one individual of a self-fertilising hermaphroditic species, but as enchytraeids do not have free larval forms and the adults are not known to be active swimmers, dispersal must be passive if it occurs at all (Coates 1995). Other reproductive information that would indicate the rate and likelihood of colonisation of new habitats are not well understood for diploids or polyploids, such as development times, production rates of embryos, viability of embryos and responses to environmental factors (Coates 1995). Population dynamics of diploid and triploid *L. lineatus* will be further investigated in the next chapter. *L. lineatus* commonly lives on the underside of pebbles resting on the beach, and the present study found medium-sized but rough-textured pebbles to be the most heavily colonised. Worms may move over small smooth pebbles to rougher surfaces by a process of stereotaxis (Tynen 1969).

6.5.3. Polyploids

The present study found an average diploid to triploid ratio of 4:1, compared to the almost 1:2 ratio found in the 1960s by Tynen (1972). It is unclear as to why the ratio of diploids:triploids may have reversed over the last 40 years, either the number of diploids has increased dramatically or triploid numbers have decreased. No tetra- or pentaploids were found in the present study. The presence of polyploids on the beaches of Anglesey was not mentioned by Tynen (1972), and none were found in field sites in England or Wales in a survey by Coates (1995), where they were otherwise found to be as geographically widespread as diploids. Triploids were noted as completely absent from field sites in North America, suggesting that they do not occur in all locations where diploids occur, possibly due to differences in responses to extrinsic factors (Coates 1995).

CHAPTER 7

LIFE-HISTORY CHARACTERISTICS OF COEXISTING SEXUAL DIPLOID AND PARTHENOGENETIC TRIPLOID FORMS OF *LUMBRICILLUS LINEATUS* (MULLER)

7.1. ABSTRACT

The pseudogamous asexual (triploid) form of the littoral enchytraeid worm *Lumbricillus lineatus* requires the sperm of the hermaphroditic sexual (diploid) form to stimulate oogenesis, although no genetic recombination takes place. We investigated the population dynamics of this form of sexual parasitism by studying life-history characteristics of *L. lineatus* at different starting ratios of diploids to triploids. Cultures of diploid and triploid individuals were initiated with ratios ranging from 9:1 to 1:9 and the stability of the ratios was monitored over a 6-month period. Temperature and food quality were manipulated in a series of trials to ascertain their effects on life-history characteristics and relative abundance over time. Relative competitive ability was estimated from somatic output measured in terms of the body length of each adult worm, and from reproductive output measured in terms of the number of developed, free-floating eggs present within the coelom of each worm and scaled for body length. In all conditions, diploids and triploids each tended to have relatively larger adult body sizes than the other where they started with numerical superiority. At lower temperatures and higher food quality, egg production was also relatively higher for the form that started with numerical superiority. This dynamic appears to favour eventual elimination of triploids, as a result either of diploids out-performing triploids or of triploids out-performing diploids and then themselves crashing in the absence of sperm. If triploid *L. lineatus* do not arise frequently from sexual ancestral individuals in nature, stabilising mechanisms must act to limit triploid frequency. Mate discrimination and other possible stabilising mechanisms are discussed.

7.2. INTRODUCTION

A pseudogamous parthenogen is a species that requires the spermatozoa of a sexual sibling species to initiate egg development without incorporating the genetic information of the donor sperm (Christensen and O'Connor 1958). In effect, it is a reproductive parasite of the sexual form. This type of obligatory coexistence has some of the disadvantages of sexual reproduction, such as the cost of searching for a mate, without the compensating advantage of genetic variation from recombination of parental genotypes. Even the advantage to the parthenogen, of a faster capacity for population growth in the absence of investment in male gametes, has an associated disadvantage for pseudogamous parthenogens. The pseudogamous form will diminish its own mating opportunities if its population out-grows that of its obligately sexual host. It is unclear as to why pseudogamous parthenogens do not evolve into full parthenogens, releasing both forms from this forced relationship. The widespread existence of pseudogamous reproduction suggests the presence of some compensatory mechanism, such as competitive inferiority of the pseudogamous type under density-dependent conditions (Schley et al. submitted). Few pseudogamous parthenogens are well studied and very few theoretical models exist to predict the population dynamics of such reproductive systems (Schley et al. submitted). Future modelling of such pseudogamous systems will require knowledge of the reproductive biology and population dynamics of the species. This chapter aims to provide such information on the pseudogamous reproductive system in the littoral enchytraeid oligochaete *Lumbricillus lineatus* (Muller).

The common form of *L. lineatus* is the hermaphroditic (sexual) diploid, which can exist alone, but is often parasitised by the related parthenogenetic (asexual) triploid form. The sperm-producing seminal vesicles are absent or reduced to mere traces in the triploid form, and so no spermatozoa are produced (Nielsen and Christensen 1959). The obligatory coexistence of pseudogamous species with their sexual hosts prevents direct measurement of intrinsic birth rates. It is therefore impossible to directly calculate competition coefficients to attempt to predict and explain coexistence of the two forms, as was achieved with *Daphnia* in Chapter 3. A series of trials was devised to test for advantages of genetic variation by measuring the

somatic fitness and reproductive effort of out-crossing hermaphrodites and genetically identical clones of *L. lineatus* over time and under varying conditions of temperature and food quality. Competitive ability was estimated indirectly from comparisons of the rate of somatic growth. Competitive ability may also be evident through egg production, measured from the number of developed free-floating eggs in the coelom of an adult worm. Body length and egg production were measured simultaneously, as competition for mates may prevent a large worm (that is, with high somatic success) from achieving a high reproductive output (that is, low reproductive success). A stricter measure of reproductive output was given by egg production per unit body length of adult worm, which enabled us to investigate the direct effects of competition on egg production without the confounding relationship between size of worm and the coelom capacity for developed eggs. The general hypotheses for the population dynamics of this system were (1) that competition for mates will favour the diploid form of *L. lineatus* in terms of both somatic and reproductive output as a result of its inherently greater genetic variation conferring a wider resource niche; and (2) that a higher ratio of triploids to diploids will lead to a reduced absolute reproductive output of triploids as a result of reduced mating opportunities.

7.3. MATERIALS AND METHODS

7.3.1. Identification of Diploids and Triploids

Lumbricillus lineatus were collected from beaches of Copenhagen, Denmark and Porth Eilian, North Wales, throughout 2001–2003. Extraction was performed by removing sand to a depth of 5 cm, transferring it to a plastic container and swilling it around with a small amount of seawater. The water, along with any worms present, was then poured off into a glass dish and worms were clearly visible in the water. Sampling of seaweed was performed by washing off the vegetation into a glass dish to remove any worms present on the surface, or by a wet funnel extraction method (Christensen 1980a) in the laboratory for any worms which may have been living within the seaweed. Worms were then transported to the laboratory, identified and distinguished as diploids or triploids. Polyploidy is evident at early developmental stages on the basis that triploids have little

(irregular) or no seminal vesicles, whereas the diploids have regular, prominently lobed structures (Nielsen and Christensen 1959). Diploids and triploids were then housed in separate Petri dishes with seawater-moistened sand and decomposing *Fucus* as food.

7.3.2. Experimental Design

Five experimental conditions consisted of a range of starting ratios of diploids to triploids (ratios of 9:1, 4:1, 1:1, 1:4 and 1:9, making a total of 10 individuals per population) each replicated at least five times. Starting ratios of 9:1 and 1:9 provided treatments where the diploids and triploids, respectively, had maximal numerical superiority and minimal competition from the other form, whilst still providing capacity for triploid reproduction. This design was used in three experiments, to test influences on diploid:triploid ratios of (A) time (in 3-month and 6-month cultures); (B) temperature (cultures held at either a favourable 15°C or an unfavourable 25°C for 9 weeks); and (C) food quality (cultures provided with either 2-week old seaweed high in accessible nutrients, 1-week old seaweed, or fresh seaweed low in accessible nutrients for a 9-week period). Each of the experiments (A)–(C) examined the effects of starting ratio and polyploid type on the body length and reproductive output of the adults in each culture.

A fourth trial involved measuring time taken to reach reproductive maturity for 10 replicate diploid and five replicate triploid *L. lineatus* individuals. Individual adults with developed free-floating eggs and enlarged clitella (i.e., egg-laying imminent) were monitored in individual cultures until eggs were laid. Adults were then removed and the cultures monitored for the presence of young and subsequently of new reproductively mature adults. Elapsed time was calculated from birth to young and from young to reproductively mature adult.

7.3.3. Maintaining and Monitoring

Each replicated unit consisted of a 9-cm Petri dish containing clean coarse sand. Sand was kept moist with a seawater:distilled water (2:1) medium and decomposing *Fucus* was supplied *ad libitum* as food. Cultures were maintained at

19°C (except in the temperature trial) with a 14L : 10D cycle. Adult worms were extracted from the sand of all treatments at the end of each of trials (1)–(3), counted and identified as diploid or triploid *L. lineatus*. Body length of each adult worm was measured and recorded and all developed free-floating eggs in the coelom were counted to estimate somatic and reproductive output. The number of eggs produced divided by the body length in millimetres provided a measure of reproductive output for each worm without confounding constraints of coelom capacity.

7.3.4. Statistical Analysis

Analyses of variance were used to test the alternative responses of somatic output (body length), reproductive output (developed free-floating egg production), and egg production per unit body length of reproductively mature adult diploid and triploid *L. lineatus,* to interacting effects of Polyploid type (with two levels), starting Ratio of diploids:triploids (with five levels), and a Treatment of either (A) Time (with two levels); (B) Temperature (with two levels); and (C) Food quality (with three levels). The same test model applied to all these three experiments:

$$\text{Response} = \text{Polyploid} \mid \text{Dish}' (\text{Treatment} \mid \text{Ratio}).$$

The Time trial was unbalanced, with more replicates per treatment combination in the second time period (see Table 7.1A), and so was tested with a cross-factored (three-way) General Linear Model using adjusted sums of squares (Minitab v. 13.1); both the other trials were balanced designs and were tested with standard ANOVA (Minitab v. 13.1). The design necessarily involved repeated measures on the random variable Dish, in order to obtain responses on both polyploidy types in competition with each other. The response variables were the means per dish of adult body length in millimetres, to measure somatic output; the number of developed, free-floating eggs in the coelom of each adult diploid and triploid *L. lineatus*, to measure reproductive output; and the number of eggs per millimetre body length of the worm, to measure reproductive output without the constraint of coelom capacity. Table 7.1 shows the arrangement of factors in these models.

Table 7.1—The ANOVA design used for the analyses in each trial of the study: (A) time trial; (B) temperature trial; and (C) food quality trial. S = replicate petri dishes

(A)

		Polyploid type	Ratio of abundance at start				
			$R_{9:1}$	$R_{4:1}$	$R_{1:1}$	$R_{1:4}$	$R_{1:9}$
Time	$T_{3months}$	P_{2x}	S1–3	S4–7	S8–11	S12–15	S16–19
		P_{3x}	S1–3	S4–7	S8–11	S12–15	S16–19
	$T_{6months}$	P_{2x}	S20–24	S25–29	S30–35	S36–41	S42–46
		P_{3x}	S20–24	S25–29	S30–35	S36–41	S42–46

(B)

		Polyploid type	Ratio of abundance at start				
			$R_{9:1}$	$R_{4:1}$	$R_{1:1}$	$R_{1:4}$	$R_{1:9}$
Temperature	$T_{15°C}$	P_{2x}	S1–3	S4–6	S7–9	S10–12	S13–15
		P_{3x}	S1–3	S4–6	S7–9	S10–12	S13–15
	$T_{25°C}$	P_{2x}	S16–18	S19–21	S22–24	S25–27	S28–30
		P_{3x}	S16–18	S19–21	S22–24	S25–27	S28–30

(C)

		Polyploid type	Ratio of abundance at start				
			$R_{9:1}$	$R_{4:1}$	$R_{1:1}$	$R_{1:4}$	$R_{1:9}$
Food Quality	F_{2weeks}	P_{2x}	S1–3	S4–6	S7–9	S10–12	S13–15
		P_{3x}	S1–3	S4–6	S7–9	S10–12	S13–15
	F_{1week}	P_{2x}	S16–18	S19–21	S22–24	S25–27	S28–30
		P_{3x}	S16–18	S19–21	S22–24	S25–27	S28–30
	F_{0weeks}	P_{2x}	S31–33	S34–36	S37–39	S40–42	S43–45
		P_{3x}	S31–33	S34–36	S37–39	S40–42	S43–45

A two-way General Linear Model (Minitab v. 13.1) was used to test the null hypothesis that the End proportion of diploid *L. lineatus* (with 46 replicates in an unbalanced design, see Table 7.1A) would not be affected by the Starting ratio of diploids to triploids, with five levels, and Time, with two levels. The test model for this experiment was:

End proportion = Start ratio | Time.

A two-way General Linear Model (Minitab v. 13.1) was used to test the hypothesis that the development times of *L. lineatus* in days (with five replicates) would not be affected by development Stage, with two levels, and Polyploid type, with two levels. The design necessarily involved repeated measures on the random variable Subject, in order to obtain responses for each polyploid type in both development stages. The test model for this experiment was:

Development time = Stage | Subject' (Polyploid).

7.4. RESULTS

7.4.1. Stability Over Time

Body length of adult worms decreased over time, with average body length of all worms after 6 months being 8.0 mm, compared to 11.5 mm after 3 months (Time effect, $F_{1,36} = 13.99$, $P = 0.001$, Figure 7.1). Deterioration of laboratory cultures may explain the smaller size of all worms after 6 months. The relative size of diploids to triploids depended on starting ratio (Starting ratio × Polyploid type 2-way interaction, $F_{4,36} = 6.13$, $P = 0.001$), with each tending to be bigger than the other where they started with numerical superiority. This effect is most noticeable in the 9:1 and 1:9 treatments, suggesting that numerical superiority is an important contributor to competitive success.

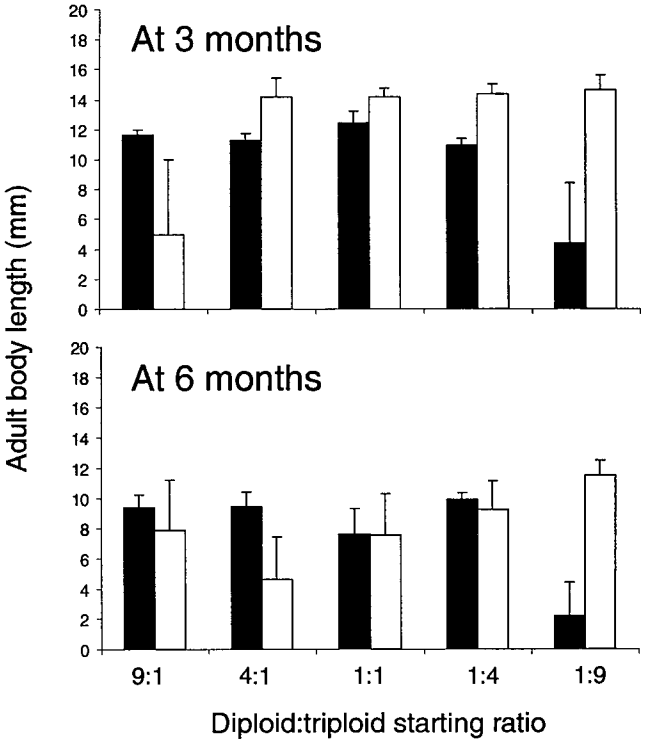


Figure 7.1—Mean and s.e. of body length for reproductively mature adult *Lumbricillus lineatus*, as a function of time, starting ratio and polyploid type (black = diploids, white = triploids)

Egg production and egg production per millimetre of body length by adult worms decreased over time by amounts that depended on polyploid type and starting ratio (egg production: Time × Starting ratio × Polyploid type 3-way interaction: $F_{4,36}$ = 4.00, P = 0.009; Starting ratio × Polyploid type 2-way interaction: $F_{4,36}$ = 4.23, P = 0.007; Time × Polyploid type 2-way interaction: $F_{1,36}$ = 4.73, P = 0.036; Time effect: $F_{1,36}$ = 40.54, P < 0.001; egg production per unit body length: Time × Starting ratio × Polyploid type 3-way interaction: $F_{4,36}$ = 4.01, P = 0.009; Time × Polyploid type 2-way interaction: $F_{1,36}$ = 5.77, P = 0.022; Time effect: $F_{1,36}$ = 20.07, P < 0.001, Figure 7.2). At 3 months, diploids and triploids each tended to have relatively higher outputs than the other where they started with numerical superiority, with the effect being more marked for triploids. Cultures maintained for 6 months produced an average of 0.95 eggs per worm (or 0.08 eggs per millimetre body length), compared to 6.43 eggs per worm (or 0.44 eggs per millimetre body length) after only 3 months, perhaps due to deterioration of laboratory cultures.

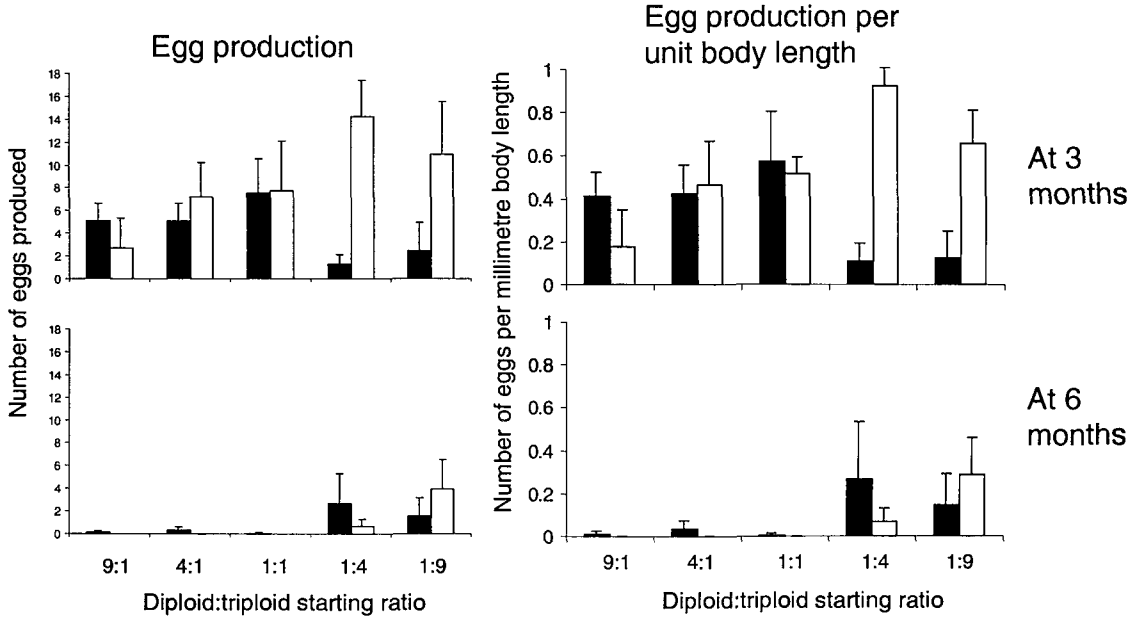


Figure 7.2—Mean and s.e. of egg production (left) and egg production per millimetre of body length (right) of reproductively mature adult *Lumbricillus lineatus* as a function of time, starting ratio, and polyploid type of worm (black = diploids; white = triploids)

7.4.2. Temperature Effects

Relative sizes of diploids and triploids depended on starting ratios regardless of temperature (Starting ratio × Polyploid type 2-way interaction: $F_{4,20} = 22.16$, $P < 0.001$; Starting ratio main effect: $F_{4,20} = 3.69$, $P < 0.021$, Figure 7.3). Diploids and triploids each tended to be bigger than the other where they started with numerical superiority, and in the 9:1 and 1:9 treatments the greater competitive ability of the form of the worm in numerical superiority led to the extinction of the other.

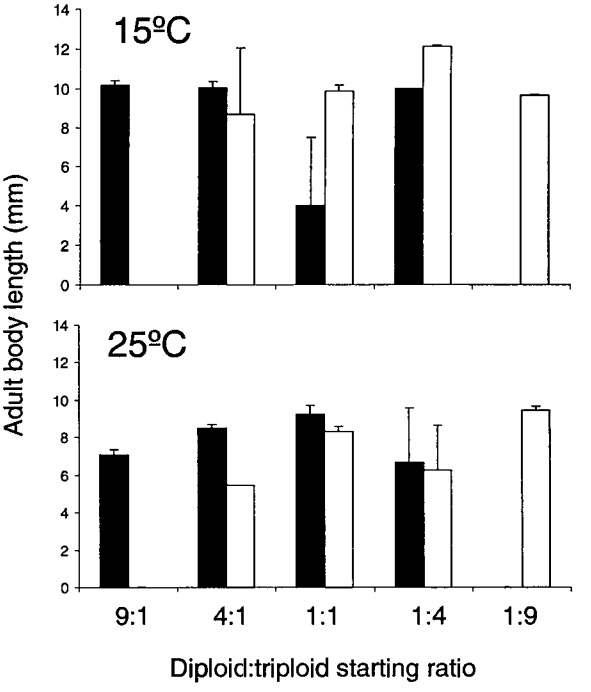


Figure 7.3—Mean and s.e. of adult body length of diploids and triploids *Lumbricillus lineatus* as a function of temperature, starting ratio, and polyploid type (black = diploids, white = triploids)

Egg production was reduced at higher temperatures, particularly at high ratios of one form of the worm to the other, with an average of 8.23 eggs per worm (or 0.77 eggs per millimetre body length) being produced at 15°C, compared to only 0.39 eggs per worm (or 0.04 eggs per millimetre body length) at 25°C, suggesting that the higher temperature is unfavourable for egg production (egg production and egg production per unit body length respectively: Temperature × Starting ratio × Polyploid type 3-way interaction: $F_{4,20} = 4.31$, $P = 0.011$, $F_{4,20} = 5.15$, $P = 0.005$; Starting ratio × Polyploid type 2-way interaction: $F_{4,20} = 4.86$, $P = 0.007$, $F_{4,20} = 5.87$, $P = 0.003$; Temperature main effect: $F_{1,20} = 41.04$, $P < 0.001$, $F_{1,20} = 49.31$, $P < 0.001$; Starting ratio main effect: $F_{4,20} = 3.39$, $P = 0.028$, $F_{4,20} = 3.03$, $P = 0.042$, see Figure 7.4). Diploids and triploids each tended to have relatively higher outputs than the other where they started with numerical superiority, and both types had zero egg production when starting from its lowest ratio.

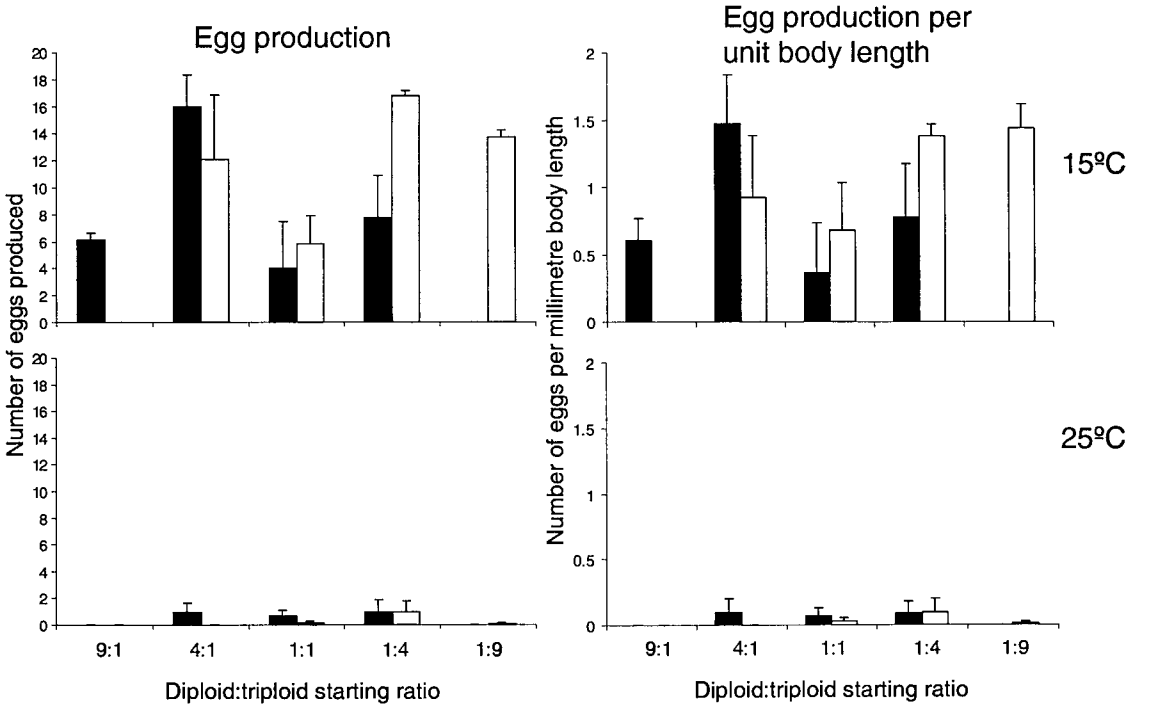


Figure 7.4—Mean and s.e. of egg production (left) and egg production per millimetre body length (right) of reproductively mature adult *Lumbricillus lineatus* as a function of temperature, starting ratio of diploids:triploids, and polyploid type of worm (black = diploids, white = triploids)

7.4.3. Food Quality Effects

Relative sizes of diploids to triploids depended on starting ratio regardless of food quality with a general trend of larger size for the form that started with numerical superiority (Starting ratio × Polyploid type 2-way interaction, $F_{4,30} = 3.81$, $P = 0.013$, Starting ratio effect, $F_{4,30} = 2.98$, $P = 0.035$, Figure 7.5). There were several triploid extinctions, occurring at high diploid:triploid ratios, and one diploid extinction occurring at a low diploid:triploid ratio, but no other apparent trend was seen.

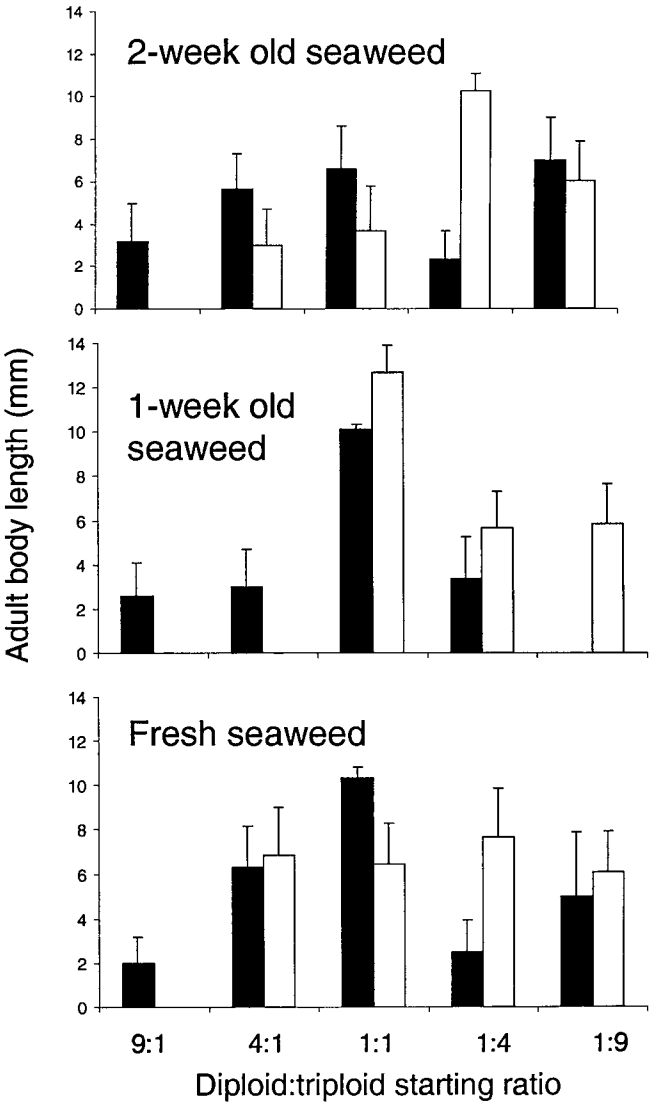


Figure 7.5—Mean and s.e. of body length of reproductively mature adult *Lumbricillus lineatus* as a function of food quality (highest at top to lowest at bottom), starting ratio and polyploid type (black = diploids, white = triploids)

Egg production was reduced with poorer food quality, and egg production per unit body length of adult diploids and triploids was affected by starting ratio. Average egg production for cultures provided with 2-week old, 1-week old and fresh seaweed was 6.27, 3.07 and 2.62 eggs per worm, or 0.16, 0.10 and 0.04 eggs per millimetre body length, respectively (egg production: Food quality effect: $F_{2,30} = 4.20$, $P = 0.025$; egg production per unit body length: Starting ratio effect: $F_{4,30} = 4.23$, $P = 0.008$, Figure 7.6). The suggestion in the top graph of egg production that diploids and triploids each tended to have relatively higher outputs than the other where they started with numerical superiority was not corroborated by any significant interactions with starting ratio and was not evident in the graphs of egg production per unit body length. There were several cases of zero triploid egg production, mainly in treatments of high diploid:triploid starting ratios, reflecting the extinctions of these worms shown in the body length analysis of Figure 7.5. Egg production per unit body length was very low in all treatments, suggesting that absolute reproductive output was not affected by food quality.

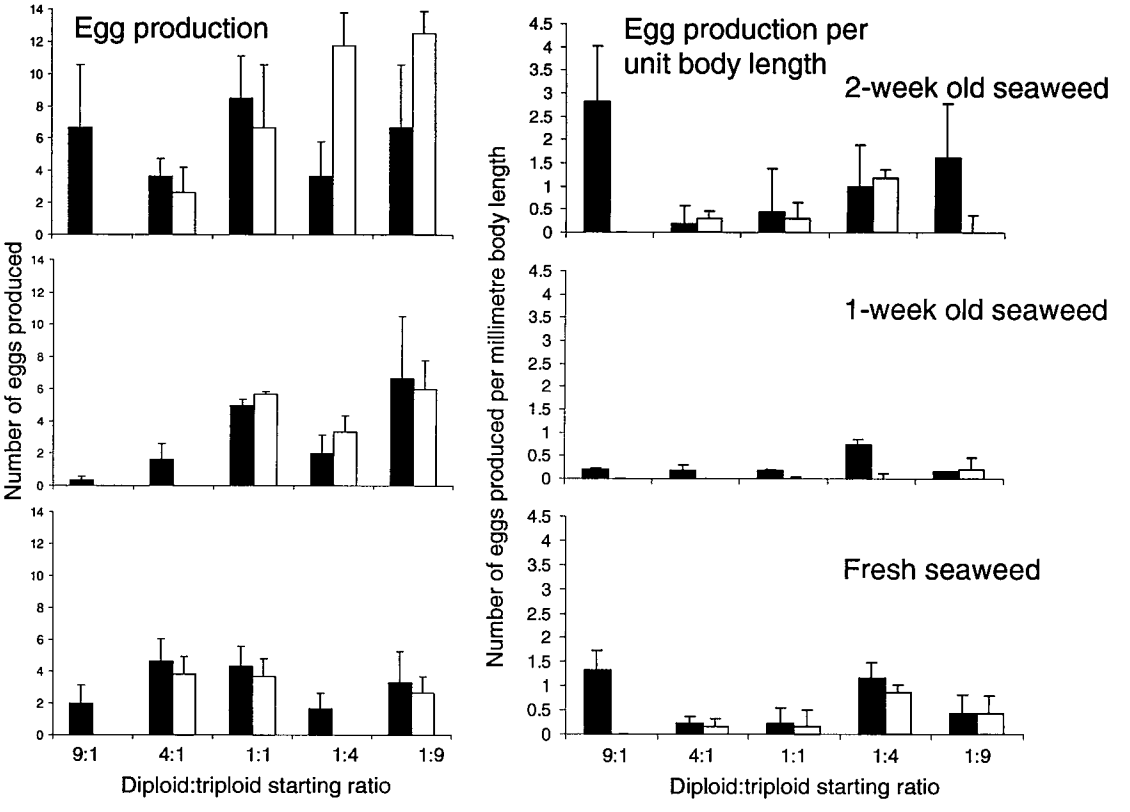


Figure 7.6—Mean and s.e. of change in egg production (left) and egg production per millimetre body length (right) of reproductively mature adult *Lumbricillus lineatus* as a function of food quality (highest at top to lowest at bottom), starting ratio and polyploid type (black = diploids, white = triploids)

7.4.4. Diploid:Triploid Ratios Over Time

The end proportion of diploids in each population was different overall to the starting proportion of diploids (Start proportion effect: $F_{4,36} = 23.70$, $P < 0.001$, Figure 7.7). Time (3 or 6 months) had no effect on end proportions and there was no 2-way interaction of starting proportion and time. After 3 months, diploid:triploid ratios appeared to be merging towards an equal ratio. After 6 months, however, all diploid:triploid ratios had risen in favour of the diploids, appearing to be merging towards a ratio of about 4:1, apart from the 9:1 and 1:9 treatments, which had both fallen in favour of the triploids.

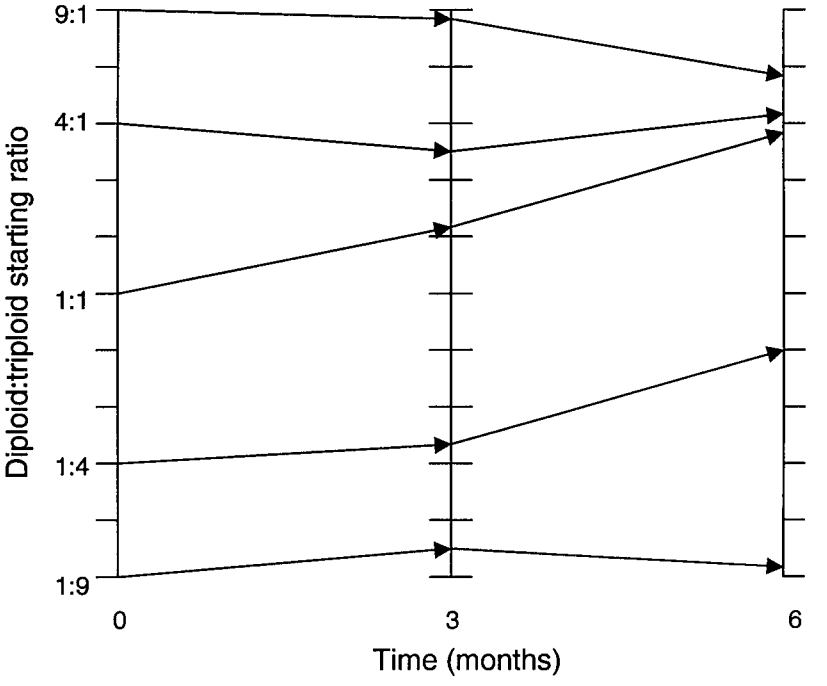


Figure 7.7—Changing ratios of reproductively mature adult diploid and triploid *Lumbricillus lineatus* after 3 months and after 6 months. 0 months indicates starting ratios

7.4.5. Development Times

The development time of *L. lineatus* was found to vary from 1 to 5 weeks between shedding of eggs (birth) and subsequent reproductive maturity. On average, triploid total development times were shorter than those of diploids (18.8 days as compared to 25 days, Table 7.2). The time from shedding the egg-filled clitellum to the appearance of young was shorter for triploids than for diploids (Figure 7.8) but the average time from the appearance of young to the appearance of reproductively mature adults differed by less than one day. Overall, there is no difference between the development times of diploids and triploids (Development stage × Polyploid type 2-way interaction: $F_{1,8} = 0.03$, $P = 0.872$).

Table 7.2—Time in days from shedding of eggs (birth) to the appearance of young and from the appearance of young to the appearance of reproductively mature adults diploid and triploid *Lumbricillus lineatus*. Rows represent replicates

Subject	Diploid		Triploid	
	Shed–Young (d)	Young–Adult (d)	Shed–Young (d)	Young–Adult (d)
1	22	14	16	13
2	7	18	3	14
3	14	7	4	4
4	4	9	14	5
5	8	22	5	16
Mean	**11**	**14**	**8.4**	**10.4**
± s.e.	**±1.4**	**±1.2**	**±1.2**	**±1.1**

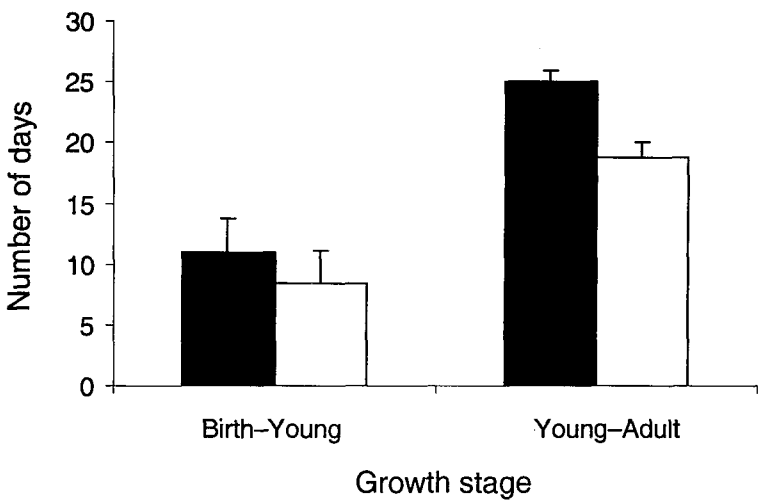


Figure 7.8—Mean and s.e. of time between shedding of eggs (birth) and appearance of young, and between appearance of young and appearance of reproductively mature adult *Lumbricillus lineatus* (black = diploids, white = triploids)

7.5. DISCUSSION

The greatest limitation of the experiments in this chapter is that any investigation of the complex and specific pseudogamous reproductive system of a particular species is applicable only to the species in question, having its own specific ecological, genetical and evolutionary aspects, and cannot be directly related to other pseudogamous species or suggested to provide an explanation to the paradox of the cost of sex. Other unexplained phenomena of the genetics of pseudogamous parthenogenesis, such as the potential of biparental inheritance (Beukeboom et al. 1996a), further complicate our understanding of the systems and the subsequent implications to the cost of sex. Nonetheless, the better understanding of such systems, however specific, allows extensive modelling to continue to predict and demonstrate ways in which sexual and asexual forms of a species coexist in nature, contributing to our overall understanding of the cost of sex.

This investigation into the competitive efforts of *Lumbricillus lineatus*, depending on starting ratios of diploids to triploids, found that the somatic output of the triploid form of the worm was higher with smaller original number of diploids. Likewise, the somatic output of the diploid form of the worm was higher with larger original number of triploids. Although food was provided *ad libitum*, individuals with greater competitive ability were expected to obtain and assimilate more food and display greater somatic success, measured in terms of body length. In terms of reproductive output, measured by counting the number of developed free-floating eggs in the coelom of an adult worm, this competitive ability was evident only at a lower temperature (15°C) and with high food quality (seaweed in an advanced stage of decay). Low temperature and high food quality reflect preferred *L. lineatus* habitats, suggesting that a higher temperature (25°C), or the provision of minimally decomposed seaweed providing an inadequate level of nutrients, prevented somatically successful worms from achieving their potential level of reproductive success. A measure of reproductive output per unit body size enabled us to investigate the direct effects of competition on egg production without the confounding relationship between size of worm and the coelom capacity for developed eggs. The effect of increased success at higher relative frequency mirrored the effects on egg production over time and at low temperatures

when scaled by unit body length of the worm, but was not evident at any level of food quality, suggesting that absolute reproductive success was not affected by food quality. It should be noted that hatching success of triploids might be lower than that of diploids, regardless of their relative measure of egg production. This has been shown to be the case in triploid forms of the freshwater planarian *Schmidtea polychroa* (Beukeboom and Vrijenhoek 1978) and could be further investigated in *L. lineatus* through future studies.

The suggestion that triploid *L. lineatus* perform relatively poorly from a starting point of numerical disadvantage, and relatively successfully from a starting point of numerical advantage, implies diploid–triploid coexistence is finely balanced. A relatively low triploid frequency tends to lead towards a triploid extinction, whereas a relatively high triploid frequency tends to lead towards diploid extinction, from which triploid extinction must necessarily follow. A flexible sex allocation of the hermaphroditic diploids may allow them to assume a 'male role' at low diploid:triploid ratios, whereby they serve to inseminate the 'female' triploids without receving sperm in return and therefore leading to their demise. This liability to crash suggests that the pseudogamous lineage may experience frequent extinctions in nature. Indeed several extinctions were observed in the present study, usually of triploid populations when starting with a numerical disadvantage. Few extinction events of diploid populations were observed. The observed abundance of triploids in nature (average of 25% of all *L. lineatus* collected in field surveys in Chapter 6) then implies that new triploid lineages may arise frequently from diploid populations. In the case of *L. lineatus*, it has been suggested that triploids arise from their ancestral sexual diploid populations relatively rarely as a result of a number of mutational events, perhaps including some failure in meiosis or fertilization (Christensen et al. 1976). However, multiple occurrences of unisexual lineages have been shown for hybridogenetic *Poeciliopsis* fish clones by analysing restriction sites of mitochondrial DNA (Quattro et al. 1991). In these cases, clonally reproducing, all-female lineages are products of hybridisation between congeneric sexual species that resulted in disrupted recombinant processes during meiosis, for example the gynogenetic fish *Menidia clarkhubbsi* (Echelle et al. 1989), the common grey treefrog *Hyla versicolor* (Espinoza and Noor 2002) and possibly the hybridogenetic waterfrog *Rana esculenta* (Spolsky and Uzzell 1986). Many

parthenogenetic lizards are known to have originated through hybridisation followed by further hybridisation leading to triploidy (Maslin 1971). Further support for frequent asexual occurrences comes from molecular genetic studies concluding that unisexual vertebrates are recently evolved (Densmore et al. 1989). Parthenogens in existence for a long time would be expected to show much variability due to the accumulation and fixation of mutations, leading to divergence between them and their ancestral lineage (Maslin 1971). The long-term establishment of hybrids would be ecologically, geographically and phylogenetically constrained (Densmore et al. 1989). In particular, a high degree of genetic similarity has been found between sympatric diploid and triploid individuals of *L. lineatus* (Christensen et al. 1978), that is unlikely to be due to genetic exchange among polyploids because of their pseudogamy (Coates 1995), and therefore supporting the suggestion of recent origins.

The findings of the present study may help to explain the existence of pseudogamous parthenogens in nature. Clanton (1934) was the first to demonstrate the problem with this system: using the salamander of the genus *Ambystoma*, he explained how numbers of pseudogamous females, with their inherent advantage over sexual females, would increase in a population until the number of sexual individuals is diminished. This will eventually lead to the insemination of only pseudogamous forms and the subsequent demise of the sexual population. This advantage does not appear to act to out-compete sexual individuals in nature, as field population ratios of pseudogamous to sexual females have been found to remain fairly stable on average from year to year in planthoppers (Booij and Guldemond 1984; den Bieman and Vrijer 1987; den Bieman 1988), salamanders (Wilbur 1971) and moths (Mitter et al. 1979). Similarly, field studies on the abundance of *L. lineatus* throughout 2001–2003 suggest an average ratio of diploids:triploids of 4:1 (see Chapter 6). The present study shows ratios appearing to begin to merge in favour of diploids after 3 months, with an increase in number beyond the naturally observed limit (Figure 7.7), and certainly not falling in relative abundance, as the cost of sex would predict. Perhaps the decreased success of triploid individuals at lower triploid frequencies displayed in the present study can be extrapolated to the success of triploid populations coexisting amongst diploid populations, suggesting that triploid numbers are limited preventing the diploids

from being completely wiped out in natural populations. Cultures of *L. lineatus* were maintained for longer in the present study to follow the relative rise or fall of the diploids and triploids, but the natural build-up of toxic gases in the old laboratory cultures resulted in markedly reduced egg production and increased mortality, masking any effect of competitive ability.

A suggested mechanism that may work to stabilise the coexistence of diploids and triploids is mate discrimination, demonstrated in *Poeciliopsis* (McKay 1971), the salamander *Ambystoma jeffersonianum* (Uzzell and Goldblatt 1967), the bark beetle *Ips acuminatus* (Loyning and Kirkendall 1996) and the planthopper *Ribautodelphax pungens* (den Bieman 1988). Males of *R. pungens* actively discriminated against triploid females in choice experiments, and only 20–50% of triploid females were inseminated compared to 100% of sexual females, in populations with high triploid frequency (den Bieman 1988). Males would be expected to discriminate, if possible, as mating with a parthenogen wastes their sperm. Schlupp et al. (1994), however, have revealed an advantage to male sailfin mollies (*Poecilia latipinna*) of consorting with female gynogens, as this enhances their attractiveness to conspecific sexual females and therefore increases their chances of mating. Perhaps differentiation in micro-distribution and phenology rather than mate discrimination will result in an increased chance of insemination of the sexual female (Uzzell 1969; Booij and Guldemond 1984). Future work could investigate possible mate discrimination mechanisms in *L. lineatus*. Other suggested mechanisms of maintaining coexistence include reduced reproductive capacity of pseudogamous forms compared to host species (Uzzell 1964; Uzzell 1969) and competitive interactions between the two forms (Wilbur 1971). Some studies suggest that niche separation and differential food exploitation between sexual and clonal forms allows stable coexistence (Vrijenhoek 1978; Kirkendall and Stenseth 1990). Kirkendall and Stenseth (1990) suggest that mate selection is likely to be an important stabilising mechanism when niche overlap is high, i.e. in pseudogamous parthenogenetic systems. However, any stabilising mechanism must be frequency-dependent: either negatively, whereby more asexual females are inseminated when at a lower frequency (den Bieman 1988), making mate discrimination seem ineffective; or positively, whereby more asexual females are inseminated when at a higher frequency (Loyning and Kirkendall 1996). The latter

appears to be the case with *L. lineatus* in the present study and so the stabilising mechanism is as yet inconclusive.

7.5.1. Development Times

Initial trials to measure the development times of diploid and triploid *L. lineatus* found that the time from shedding of eggs (birth) to the appearance of young was shorter in triploids than in diploids. The rate of development of *L. lineatus* has been shown to be 15 days from the enclosure of the embryos in the cocoon (release of clitellum) to hatching and appearance of young (Giese and Pearse 1975). This is comparable to the results for diploids in the present study, but the development time of triploids was shorter. Previous studies of the relative growth of diploid and triploid forms of fish have revealed a variety of results depending on age and species (Ihssen et al. 1990). Some studies have shown juvenile triploid fish to have a growth advantage over juvenile diploids. Induced triploids of male and female mud loaches (*Misgurnus mizolepis*) were heavier than their diploid counterparts between 3 and 9 months old (Kim et al. 1994) and triploids had larger cells than diploids although without necessarily attaining a larger ultimate size (Benfey and Sutterlin 1984). In these studies, however, diploids and triploids were housed in separate tanks, making it hard to relate growth performances of diploids and triploids to competitive efforts. Other studies have shown that juvenile diploids have an improved growth performance over juvenile triploids (Mair 1993; Carter et al. 1994; Cherfas et al. 1994; Benfey 1999). This can be attributed to differences in competitive behaviour for shared resources and a subsequent difference in food intake favouring the diploids (Carter et al. 1994). As fish reach reproductive maturity it is generally found that triploids have a growth advantage: shown in plaice hybrids *Pleuronectes* spp. (Lincoln 1981), channel catfish *Ictalurus punctatus* (Wolters et al. 1982), rainbow trout *Oncorhynchus mykiss* (Guo et al. 1990) and Asian catfish *Clarius macrocephalus* (Fast et al. 1995). In the present study, the time from the appearance of young to the appearance of reproductively mature adults was very similar in diploids and triploids, supported by other studies showing equal growth performances of diploid and triploid adults (rainbow trout *Salmo gairdneri* Benfey et al. 1989, sea bass *Dicentrarchus labrax* Felip et al. 2001 and tilapia *Oreochromis niloticus* Hussain et al. 1995).

It would be interesting to specifically investigate possible mate discrimination mechanisms in *L. lineatus* as a stabiliser of the diploid:triploid system and to identify and investigate other possible stabilising mechanisms. It is also important to perform further studies to ascertain life-history parameters in order to theoretically model, empirically test and eventually fully understand the unique reproductive system of *L. lineatus*.

CHAPTER 8

GENERAL DISCUSSION

8.1. INTRODUCTION

The theme of this thesis has been the problem of the maintenance of sexual reproduction with its costly requirement for males which do not directly produce offspring (Williams 1975; Maynard-Smith 1978). The principal object of investigation was a theoretical model demonstrating how the two-fold cost in capacity for growth for sexual populations, due to their comprising 50% males, can be compensated by relatively small advantages in competitive ability in crowded conditions (Doncaster et al. 2000; Pound et al. 2002; Doncaster et al. 2003). Specifically, I performed a series of experiments and field surveys investigating the role of genetic variation in conferring an advantage to sexual populations, using species of the freshwater crustacean genus *Daphnia* and obtaining knowledge on the biology and population dynamics of the pseudogamous parthenogenetic reproductive system of the littoral oligochaete *Lumbricillus lineatus*. This chapter discusses these results with respect to the problems of extrapolating to populations in the natural world and in relation to previous theoretical and empirical studies, and suggests key issues for future research.

8.2. LIMITATIONS OF THE STUDY

The experiments performed in the present study followed very precise and tightly focussed hypotheses that did not attempt to explain the whole paradox of sex, but only certain aspects of it in the context of specific models and reproductive systems. Experiments were designed specifically to test for a short-term competitive advantage of genetically diverse populations in the presence of genetically uniform populations, and to quantify any competitive release attributable to genetic variation (Chapters 3 and 4). Theory predicted that genetic variation would confer niche breadth, and hence competitive advantage. The choice of *Daphnia* as an empirical model for testing this theory had some potential

disadvantages to set against the advantages of measurable genetic variation and testable competitive impacts. Although wild populations have long-term stability (Gurney and Nisbet 1998; Murdoch et al. 1998), they undergo seasonal changes in abundance and can cycle at least under laboratory conditions (McCauley et al. 1999). In sexual forms, males tend to be produced as environmental conditions deteriorate and the products of sexual reproduction hatch from diapausing eggs. Sexual and obligate asexual forms may therefore compete out of phase with each other, reflected as differences on a temporal niche axis in nature. Our experiments were nevertheless informative about the likely outcomes of competition for the period during which sexual forms invest in the production of males. The parthenogenetic (asexual) phase of seasonally sexual forms used in the study allowed control over genetic variation and our method of competing populations comprising several clones against single-clone populations allowed us to measure directly any competitive release attributable to genetic variation alone (Chapters 3 and 4). The *Daphnia* system suits Lotka-Volterra dynamics at this experimental timescale, involving continuous reproduction from the mixing of individuals and resources in a 3-dimensional habitat. Furthermore, the aquatic habit and fast growth of *Daphnia* facilitate measuring competitive impacts.

In the case of experiments and surveys on *Lumbricillus lineatus* (Chapters 6 and 7) the greatest limitation is inherent to the reproductive system. Results gained and conclusions drawn can only be applicable to *L. lineatus*, as systems of sperm-dependent parthenogenesis seem to have their own complex and specific ecological, genetical and historical aspects, preventing direct extrapolation to other pseudogamous species. Despite this limitation, however, the empirical examinations of a particular reproductive system have enabled us to explore some of the ecological problems of coexistence for sperm-dependent parthenogens in general.

Despite the numerous theoretical explanations and mathematical models of the maintenance of sexual reproduction, there has been a distinct lack of empirical studies in designed to quantify theoretical predictions (Wuethrich 1998). Empirical testing is paramount, however, to the acceptance or dismissal of any theory. This study is the first to test a model based on fundamental principles of population

dynamics (Doncaster et al. 2000), through carefully designed and executed laboratory experiments and field surveys.

8.3. THE ECOLOGICAL MODEL

The many previous tests of ecological costs of sex were based on the Tangled Bank model of Sib Competition (Bell 1982). This is an incomplete model of population dynamics in the sense that it considers only differences in intra-specific competition and does not use them to callibrate the consequences for inter-specific competition at carrying capacity. Sib Competition models consider influences of genetic variation on the intensity of competition amongst sexual siblings compared to competition amongst identical asexual siblings, and advantages of sex are inferred from observing a higher growth rate for sexual populations with lower competition amongst siblings. Empirical experiments tended to find insufficient reductions in competitive intensity to cancel the two-fold cost of males, which led to the general rejection of Bell's Tangled Bank model in the late 1980s (Schmitt and Ehrardt 1987; Kelley 1989). The rejection of this theory, however, does not detract from the potential validity of ecological explanations in general, since Sib Competition alone fails to take account of the relative strengths of inter- and intra-specific competition.

The Lotka-Volterra models developed by Doncaster et al. (2000), Kerszberg (2000) and Pound et al. (2002) describe the complete dynamic of the ecological costs of males. This is because they account for the ecological expression of genetic variation in terms of reduced intra-specific competition (sib model) and, in addition, its consequence for inter-specific competitive release (Frozen Niche Variation). Lotka-Volterra models make the robust prediction that a small competitive release can suffice to cancel a two-fold disadvantage in intrinsic capacity for population increase. This type of trade-off between competition and growth is a well-known principle of ecology, which explains the persistence of fast-growing fugitive species amongst dominant competitors (Nee and May 1992). Its novel application to the cost-of-sex problem simply focuses on the other side of this dynamic: the persistence of a slow-growing competitor in the presence of a fast-growing invader. Although sexual and asexual sibling species tend not to coexist in

the natural world (Peck et al. 1998), the model predictions for coexistence solve the immediate problem of sex, of its slow intrinsic capacity for growth. They show how the sexual mode can persist over ecological timescales, providing the opportunity for it to express its many other advantages over evolutionary timescales.

Lotka-Volterra models of ecological dynamics are closer in concept to Vrijenhoek's (1979) Frozen Niche Variation hypothesis: the verbal assertion that asexual lineages arise in a sexual population with genotypes frozen to those of the progenitor parent. Several empirical studies present evidence of limited genetic variation in asexual clones relative to sexual populations, constraining the clonal phenotype to a narrower ecological niche (Vrijenhoek 1979; Hebert et al. 1988; Honeycutt and Wilkinson 1989; Jokela et al. 1997; Semlitsch et al. 1997). Evidence exists that such niche differentiation may result in coexistence of sexual and asexual types at various scales of resolution, for example: lizards (Case 1990); enchytraeid worms (Christensen et al. 1992); crustaceans (Barata et al. 1996); molluscs (Fox et al. 1996); fish (Vrijenhoek and Pfeiler 1997); insects (Becerra et al. 1999) and frogs (Negovetic et al. 2001).

Tests of the new quantitative model in this thesis have focused on the ecological expression of genetic variation. This focus has required devising novel experiments to consider how genetic variation can influence the impact of asexual on sexual competitors, relative to the impact that the sexual individuals have upon themselves. The experiments on *Daphnia* were designed to separate genetic variation from the males that cause it, in order to separate estimates of competitive release from intrinsic costs of male presence. This separation was not possible for experiments on the hermaphroditic *Lumbricillus lineatus*, which could nevertheless explore relative net reproductive success resulting from costs of investing in male gametes and associated benefits of genetic variation.

8.4. PRINCIPAL FINDINGS AND SUGGESTIONS FOR FUTURE WORK

The two distinct parts of the present thesis, concerning respectively *Daphnia* and *L. lineatus* as model organisms, contrast with each other in terms of the information they have provided on ecological costs of sex. The study of genetically diverse and genetically uniform populations of *Daphnia* gave ecological answers to the question of the persistence of sexual reproduction, providing information on the ecology of the cost of sex. It suggested that the ecological advantage conferred by the genetic variation inherent to sexual reproduction may suffice to permit immediate coexistence with asexual invaders. In contrast, the study of population ratios of sexual diploid to asexual triploid *L. lineatus* only increased our difficulty in understanding the ecology of pseudogamy, providing information mainly on the evolution of sex. Here I discuss the main findings from each model organism and then attempt to bring them together in a discussion of the implications and wider perspectives of the work.

The role of genetic variation in providing a competitive advantage over genetically uniform populations was studied using *Daphnia* species (Chapters 2–4). The testing of the ecological model for the coexistence of sexual and asexual forms of a species (Doncaster et al. 2000), sought to estimate the competitive impacts within and between sexual and asexual populations (Chapter 3). Intrinsic birth rates and growth rates in isolation and in competition were measured between genetically diverse and genetically uniform populations of *Daphnia*, and competition coefficients were calculated. It was demonstrated that genetic variation contributes to competitive release in the presence of genetically uniform populations, as the latter exert more of a negative impact on each other than they do on competing genetically diverse individuals ($\alpha_{12} < 1$). The competitive release was small in magnitude but predicted to be sufficient for immediate coexistence of sexual and asexual populations of *Daphnia pulex*, even if the sexual type were to invest equally in production of males and females, thus giving it half the population growth capacity of an otherwise identical asexual lineage. It would be interesting to elucidate the specific mechanisms of success of the genetically diverse groups and populations of *Daphnia* Further experiments suggested that competitive advantage

increases in proportion to genetic variation (Chapter 4). Large groups of genetically diverse individuals of *D. obtusa* invading into genetically uniform recipient populations increased their representation by slightly more than small invading groups, in contrast to large groups of genetically uniform individuals invading into genetically diverse recipient populations which diminished on average by considerably more than small invading groups. This interaction between group size and invader type supported the hypothesis for genetically diverse invaders that larger groups, with inherently greater genetic variation, had greater competitive advantage when invading into genetically uniform populations than did smaller groups with less genetic variation. These studies contribute to the understanding of the role of genetic variation in the mechanism of how sex may persist in the face of invasion by asex. This competitive release of genetically diverse populations of *Daphnia* competing against genetically uniform populations with a two-fold advantage in growth capacity ($\alpha_{12} < 1$; Chapter 3), may also be sufficient to enable an invading genetically diverse group to experience competitive release and to increase in number within the population more than a genetically uniform invading group could (Chapter 4). It has been shown theoretically how a sexual population could withstand invasion of multiple asexual clones, provided the sexual population retains a resource refuge (Pound et al. 2002). It was beyond the scope of the experiment of Chapter 4, however, to determine whether the proportionate changes in genetically diverse and genetically uniform invading groups were due to higher fecundity of the better competitor or greater mortality of the worse competitor. It would be interesting to further investigate the specific mechanisms of success of the genetically diverse groups and populations. The suggestion that larger genetically diverse groups invade more successfully than smaller ones cannot be directly extrapolated to sexual and asexual populations, because sex would later on have to pay the price of producing males to sustain the genetic variation. The competitive release experienced by the sexual groups and populations may be enough, however, to provide the time required for sex to express its long-term advantages of genetic variation in meeting the demands of environmental change. An extended invasion experiment (of genetically diverse and genetically uniform groups and populations of *Daphnia*) would reveal the eventual outcome of displacement or stabilisation of recipient populations and invading groups. In

addition, the genotype of *Daphnia* appeared to have an effect on the success of invasion of genetically diverse and genetically uniform groups and populations (Chapter 4), with both genetically diverse and uniform groups invading more successfully when exhibiting the homozygous slow (SS) allele for the allozyme glucose-6-phosphate isomerase (GPI). Further work could build on the preliminary observations made on the genetic variation present in temporary pond *Daphnia* populations in the U.K. (Chapter 2) and the effect of genotype on competitive ability (Chapter 4) by quantitatively investigating the potential relationships between life-history traits and allozyme variation.

The pseudogamous reproductive system, natural distribution and habitat requirements of the littoral oligochaete *Lumbricillus lineatus* were investigated (Chapters 6 and 7). A series of field surveys of *L. lineatus* performed at sites in the U.K. (Chapter 6) sought to obtain knowledge of the distribution and habitat requirements of the worm in order to further model this unique system of sperm-dependent parthenogenesis. It was found that populations of *L. lineatus* are more restricted today, both geographically and in terms of local habitat requirements, compared to 40 years ago (Tynen 1972). The species was found only in areas with good drainage of coarse or pebbly sand, on sheltered beaches experiencing little physical disturbance. The obligatory coexistence of the asexual parthenogenetic form with the sexual hermaphroditic form prevented direct measurement of intrinsic birth rates. It was therefore impossible to calculate competition coefficients directly to attempt to predict and explain coexistence of the two forms, as achieved with *Daphnia* (Chapter 3). Instead, a series of trials was devised to test for advantages of genetic variation by measuring the somatic fitness and reproductive effort of out-crossing hermaphrodites and genetically identical clones of *L. lineatus* over time and under varying conditions of temperature and food quality (Chapter 7). Competitive ability was estimated indirectly from comparisons of the rate of somatic growth and from egg production, measured by the number of developed free-floating eggs in the coelom of an adult worm. At lower temperatures and higher food quality, reproductive outputs of diploids and triploids were each inversely proportional to the abundance of the other. This dynamic appeared to favour eventual reversion to diploid-only populations as a result either of diploids out-performing triploids, or of triploids out-performing diploids and the

triploid-only population crashing in the absence of diploid sperm. Samples of natural populations of *L. lineatus* taken on Anglesey showed diploid:triploid ratios of 4:1, suggesting that pseudogamy may arise frequently in diploid-only populations. It would be interesting for future research to investigate this possibility. The pseudogamous reproductive system of *L. lineatus* is relatively new to science, with the investigations of the present study being among few studies performed to date. Much more work is required in order to ascertain life-history parameters of the worm, such as generation times, and more specific habitat and geographic requirements, to enable the successful modelling and the eventual understanding of this unique system and how it persists in nature. It would be interesting specifically to seek mechanisms of mate discrimination in *L. lineatus* as a possible stabiliser of the diploid:triploid system and to identify and investigate other possible stabilising mechanisms. Furthermore, it would be useful to investigate the frequency at which populations of diploids and triploids crash in nature, and the frequency at which new pseudogamous forms arise by mutation. Experiments could be performed either *in situ* or in laboratory conditions, with diploid populations physically isolated within the surrounding natural environment receiving *ad libitum* resources and renewed seawater. An extensive study of this nature, involving the manipulation of biotic and abiotic conditions (for example, temperature or density, respectively) may reveal much required information on the natural occurrences of ancestral diploid *L. lineatus* and the natural invasion of its mutant polyploids.

8.4.1. Implications for Natural Populations

Several assumptions must be made when extrapolating the results of the present study to populations in the natural world. For the purposes of precise hypothesis testing, we did not include *Daphnia* males in our experiments of Chapters 3 and 4. Any advantage of genetic variation in natural populations of *Daphnia,* as measured here, will be counterbalanced to some degree by the cost of producing males to sustain the genetic variation. Our theoretical calculations estimated this balance, on the assumption that males constitute half the population and are identical to females in all respects other than a zero birth rate. Copepod males are known to express aggressive mating behaviour, however, which may extend to harassment of asexual

females (Brewer 1998). This would further increase the competitive advantage of the sexual mode in natural populations. It would be interesting to investigate male harassment in mixed sexual and asexual populations and any adaptations by females to avoid it, in order to measure its effects on inter-specific competition.

The competitive release of sexual populations of *Daphnia* (Chapters 3 and 4) was likely to be actuated by a combination of responses to the impure mix of edible and inedible algae and bacteria that was provided as food. Natural food resources would undoubtedly be impure mixes, although further work is required to elucidate the precise mechanisms responsible for linking genetic variation to breadth of diet and consequently to niche breadth. The response to competitive release was expressed in laboratory populations through the fertility of females and the survival of their newborn offspring. The different mean growth rates of the genotypes suggested that the heterogeneous food supply provided sufficient habitat variation against which different genotypes could express variation in ecological advantages. Extrapolation to the natural world requires assuming that free-living *Daphnia* experience a similar if not greater level of heterogeneity and variation in resources, presenting a Tangled Bank of microhabitats to such populations, and possibly providing an even greater release from competition than observed in laboratory populations. In such heterogeneous habitats as the temporary ponds where *Daphnia* live, adapting to new environments is essential to long-term survival. The short-term advantages to parthenogenesis are cancelled by small competitive advantages to a genetically diverse sexual population, maintaining cyclically parthenogenetic populations in nature.

Natural populations may frequently experience invasions of genetically diverse groups into genetically uniform populations, and genetically uniform groups into genetically diverse populations (Chapter 4). Obligately and cyclically parthenogenetic forms of *D. pulex* exist sympatrically in North American and Canadian ponds (Hebert et al. 1988) and to a lesser extent further south (Lynch et al. 1989). The overflowing and mixing of neighbouring ponds in the rainy season may cause large numbers of one reproductive form to invade into an established population of the other. The consumption of diapausing eggs by mammals or migratory waterfowl may result in invasions of smaller groups, following their

ejection in faeces (Crease et al. 1997). The design of the present study realistically reflects these natural occurrences, since the genetically diverse and genetically uniform individuals were not physically separated, and the limited but impure mix of edible and inedible algae as well as bacteria in the food allowed competitors to express different feeding niches. The impure food mix is likely also to have dampened any tendency for populations to cycle in nutrient-rich conditions, as inedible algae utilise the nutrients required by the edible algae (Murdoch et al. 1998; McCauley et al. 1999).

The invasion events of genetically diverse individuals into genetically uniform populations reflect the evolutionary origin of sexual reproduction, where sex (meiosis) evolved in primitive eukaryotes of the Kingdom Archezoa (Cavalier-Smith 1995). The reciprocal invasion events, in which genetically uniform groups invaded into genetically diverse populations, reflect the ongoing ecological pressures on sexual populations in nature that are continuously susceptible to spontaneous production of more rapidly reproducing asexual mutants. There is indeed strong support for the arrival of obligately parthenogenetic lines by the paternal transmission of a dominant gene for sex-limited meiosis suppression (Innes and Hebert 1988). Such evidence suggests that the gene could spread through a cyclically parthenogenetic population, resulting in a genotypically diverse group of obligate parthenogens. It is reasonable to assume that this process is continuing and therefore that some obligate parthenogen clones have originated relatively recently (Innes and Hebert 1988). The experimental result that larger genetically diverse groups invade more successfully than smaller ones (Chapter 4) suggested a direct relationship of competitive advantage to genetic variation. Although it did not include the price of producing males to sustain the genetic variation, this cost can be absorbed by relatively small competitive release (Chapter 3), affording the sexual population time to express its long-term potential to adapt to environmental change. This long-term advantage may result in the eventual displacement of asexual invaders or competitors in regions where sexual and asexual populations of *D. pulex* coincide (Hebert et al. 1988), and could be investigated with an extended invasion experiment.

The suggestion that triploid *L. lineatus* perform relatively poorly from a starting point of numerical disadvantage, and relatively successfully from a starting point of numerical advantage (Chapter 7), implies that diploid-triploid coexistence is finely balanced in this pseudogamous species. Poorly performing triploids at relatively low triploid frequency will clearly lead to a triploid extinction, whereas triploids performing well at a relatively high triploid frequency leads towards a diploid extinction, from which triploid extinction must necessarily follow. Numbers of pseudogamous females would increase in a population until the number of sexual individuals is diminished (Clanton 1934), eventually leading to the insemination of only pseudogamous forms and the subsequent demise of the sexual diploid population. This suggests that pseudogamous lineages may experience frequent extinctions in nature. The observed abundance of triploids in my field surveys (Chapter 6; average of 25% of all *L. lineatus*) implies that new triploid lineages may also arise frequently from natural diploid populations. Hybridisation between sympatric sexual and parthenogenetic forms has been demonstrated to lead to the appearance of new genetically diverse parthenogenetic lineages in natural populations of freshwater planarians (Pongratz et al. 1998).

Stabilising mechanisms may be in action in natural populations to prevent the crash of finely balanced *L. lineatus* populations. A likely mechanism proposed and discussed is mate discrimination (Chapter 7), whereby males avoid wasting their sperm discriminating against parthenogens. Mate discrimination is known for many species (Uzzell and Goldblatt 1967; McKay 1971; den Bieman 1988; Loyning and Kirkendall 1996), but requires confirmation in *L. lineatus* specifically. Other possible mechanisms of maintaining coexistence in nature include differentiation in micro-distribution and phenology between sexual and asexual individuals that increases the chance of insemination of the sexual females (Uzzell 1969; Booij and Guldemond 1984). It would be interesting to further investigate the degree of clonal variation present in *L. lineatus* parthenogens, as demonstrated for other pseudogamous parthenogens (Pongratz et al. 1998). Coexistence may be stabilised by any reductions to the reproductive capacity of pseudogamous forms compared to host species (Uzzell 1964; Uzzell 1969), also by interference competition between the two forms (Wilbur 1971), and by niche separation and differential food exploitation between sexual and clonal forms (Vrijenhoek 1978; Kirkendall and

Stenseth 1990). Stabilising mechanisms in natural *L. lineatus* populations appear to be positively density-dependent, whereby more asexual females are inseminated when at a higher frequency (Loyning and Kirkendall 1996).

8.4.2. Implications for the Cost-of-Sex Problem

The age-old debate about the cost of sexual reproduction continues to stimulate much theoretical and empirical research. With many of the key aspects of the problem of the cost of sex having been proposed originally in the context of evolutionary biology, insufficient attention has been paid to the population dynamic context. The results of the present study, in providing some support to the recent theoretical predictions of Doncaster et al. (2000), emphasise the relevance to the cost-of-sex problem of fundamental principles of ecology. A review paper is now timely, comprising a rethink on the cost of sex to include properly worked out effects of density-dependence, and expanding on the information provided in Chapter 1. Such a review would need to acknowledge that other ecological factors influence species coexistence in addition to density-dependent competition. For example, harshness (e.g. mortality and stress) and temporal and environmental fluctuations (e.g. disturbance, weather variation and seasonal changes) can affect coexistence by their interaction with—or generation of—other mechanisms, including niche differentiation and classical resource partitioning. Coexistence will be favoured if fluctuations open up temporal or spatial niche opportunities. In allowing coexistence, harshness creates the potential for co-evolutionary adjustments such as character displacement among the coexisting species. Other density-dependent limitations also affect coexistence, for example, responses to predation and to the physical environment, and coexistence may be facilitated by interactions between these factors under environmental fluctuations (Chesson and Huntly 1997).

The difficulty in understanding how diploid *L. lineatus* persists in nature in competition with the pseudogamous parthenogenetic triploid forms is only increased by the results of the present study. The model of Schley et al. (submitted) demonstrates how coexistence requires a more than two-fold competitive advantage to the sexual form to compensate its two-fold disadvantage in growth capacity. The

precise genetic and ecological aspects of any sperm-dependent parthenogenetic system appear to be unique to that system, limiting the scope of extrapolation to other species. The complication of the apparently finely balanced system of diploid and triploid *L. lineatus* in nature for understanding the coexistence of sex and asex, along with the suggestion that biparental inheritance can be possible under pseudogamous parthenogenesis (Beukeboom et al. 1996a), only serves to highlight more unanswered questions. Perhaps pseudogamous parthenogenesis is not strictly a clonal mode of reproduction. Although the potential transmission of parasitic B chromosomes is likely to be detrimental, for example by reducing cocoon production and slowing adult growth (Beukeboom et al. 1998), there may be other aspects of this biparental inheritance that offer a previously unrecognised benefit upon the pseudogamous population. For example, there is a potential exchange of chromosome sets within pure parthenogenetic populations of *Schmidtea polychroa* (Beukeboom in press). Screening of chromosome frequencies in *L. lineatus* would serve to ascertain whether such inheritance occurs in this particular system and whether it could contribute to the dynamics of diploid:triploid ratios in natural populations. The system of reproductive parasitism of the triploid pseudogamous form on the sexual diploid form of *L. lineatus* can be likened to the hypothesis on the maintenance of sexual reproduction in the short term that relies on the theoretical predictions of the Red Queen (see Chapter 1). Such a suggestion must assume that common host diploid genotypes are more likely to be parasitised by the pseudogamous triploid, and that there would be an advantage to rare host genotypes, which are less likely to be parasitised. There is as yet no empirical support for this suggestion. Indeed, host–parasite dynamics are particularly difficult to measure in natural populations, as they require long-term detailed studies (Michiels et al. 2001), where sexual and asexual forms should coexist in the field and differ only in their reproductive mode. Such studies have been successfully performed on insects (Weinzierl et al. 1999), snails (Lively 1987), fish (Lively et al. 1990) and geckos (Moritz et al. 1991). More extensive theoretical models and field studies are required in order to fully understand the unique reproductive system and potential host–parasite dynamics of natural populations of *L. lineatus* highlighted in this study.

8.5. CONCLUDING REMARKS

Having first appeared amongst primitive eukaryotes in the Cambrian era (Cavalier-Smith 1995), sex has evolved during 500 million years to become the most diverse and widespread mode of reproduction in the animal world. That it should prevail over the many other intrinsically faster modes of asexual reproduction has been a core problem for evolutionary biologists since Darwin, and remains so today (Maynard-Smith 1978). The work described in this thesis provides evidence for a competitive release due specifically to the genetic variation inherent to sexual reproduction. This advantage has been quantified in the context of fundamental principles of population dynamics, which distinguish realised from intrinsic costs of sex. Even the small release observed under experimental conditions is predicted to compensate sex for its inherent slowness due to investment in males. On the other hand, similar trade-offs between competitive advantage and growth capacity appear to destabilise the dynamics of sperm-dependent parthenogenesis. Together these results pave the way for an eventual re-evaluation of the role of ecology in answering the evolutionary question of why sex exists.

APPENDICES

APPENDIX 1: RECIPE FOR ZOOPLANKTON MEDIA

Daphnia were housed in zooplankton media when in culture in the laboratory or undergoing experimental treatments. All compounds and solutions were made using chemicals obtained from Fischer Scientific or Sigma. Stock solutions were made up using the recipe shown in Table A1.1. The vitamin solution was created using ingredients listed in Table A1.2 and stored in 20 ml eppendorf tubes in the freezer until required.

Table A1.1—(A) Zooplankton medium stock solutions and (B) vitamin solution recipes, taken from Lynch et al (1986).

Zooplankton medium compound	g l^{-1}
$CaCl_2 \cdot 2H_2O$	2.65
$FeCl_3 \cdot 6H_2O$	0.11
KCl	5.00
KH_2PO_4	0.60
K_2HPO_4	0.60
$MgSO_4 \cdot 7H_2O$	4.00
$NaNO_3$	5.00
$NaSiO_3 \cdot 9H_2O$	2.00
Biotin	0.01
Vitamin solution	0.03

Vitamin solution compound	g l^{-1}
Calcium pantothenate	7.0
Vitamin B12	0.0003
Thiamin	0.6
Riboflavin	0.4
Nicotinamide	1.3
Folic acid	3.3
Putrescine	0.3
Choline	5.0
Inositol	11.0

50 ml of each of the stock solutions of Table A1.1 was poured into a 5-l jerry can, along with an appropriate portion of the defrosted vitamin solution, and the medium was made up to 5 l using distilled water. The medium was thoroughly mixed and ready to supply to *Daphnia* cultures. Zooplankton media was suitable for approximately 2 weeks.

APPENDIX 2: CELLULOSE ACETATE ELECTROPHORESIS METHODS

Cellulose acetate electrophoresis was used to investigate *Daphnia* genotypes in Chapters 2 and 4 using common allozymes. The chemicals and solutions (Table A2.1) and the stain recipes for the allozymes used in this thesis (Table A2.2) are shown below along with any significant alterations of, or additions to, the original methods. For full methodology and details of the equipment used refer to the handbook of Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis by Hebert & Beaton (1989).

Additional Notes on Methodology:

1. Large individual *Daphnia* were ground vigorously for at least 10 seconds and the resilient carapace was removed to prevent its undesired application to the cellulose plate and interference of protein movement. Fresh individuals were used for each run to ensure minimal degradation of enzymes.
2. 10 µl distilled water added to the *Daphnia* in each well, in order for enough to be taken up and applied to the plate.
3. Stain mixtures were prone to degrade even when frozen, and so were discarded once over 2 weeks old.
4. Stain mixtures were warmed to room temperature before the addition of the unstable ingredients and agar to prevent the premature setting of the agar.
5. 1.6% agar was used.
6. Stain mixture with agar was poured steadily from left from to right across the centre of the plate for a smooth distribution and to ensure staining of all protein previously applied to plate.

7. GPI was found to stain clearest and sharpest when run at 200 V (2 mA) for 30 mins.

Table A2.1A—Chemical list with concentrations and manufacturer details. All solutions should be made up to 1 l using distilled water

Stain ingredient	Sigma catalogue #	Concentration
Sodium azide	S-2002	250 mg^{-1}
Nicotinamide adenosine diphosphate (NAD)*	N-7381	2 mg^{-1}
Fructose-6-phosphate (F6P)*	F-3627	20 mg^{-1}
MTT	M-9138	10 mg^{-1}
PMS	P-9625	2 mg^{-1}
DL-lactic acid	L-1375	-
Fumaric acid*	F-1506	100 mg^{-1}
Benzaldehyde	B-6259	-
L-Malic acid	M-9138	-
Glucose-6-phosphate dehydrogenase (G6PDH)**	G-5885	300 mg^{-1}
MDH**	M-7383	200 mg^{-1}

*Solutions should be fixed with 1 ml sodium azide stock ml^{-1} for storage.

**Enzymes only available as concentrated solutions or solids.

Table A2.1B—solutions list with ingredients and instructions

Solution	Ingredients
Malic substrate	3.68 L-Malic acid; 20 ml Tris HCl, pH = 9 180 ml distilled water; adjusted to pH = 8
Starch solution	33 ml 1M K_2HPO_4; 66 ml 1M KH_2PO_4 600 mg NaCl; 10 g soluble starch 900 ml distilled water
Iodine solution	16.6 g KI; 25.4 g I_2 2 l distilled water; diluted 1:2 for use

Table A2.2—stain recipes for the allozymes commonly investigated in Chapters 2 and 4 of this thesis

Ingredients for stain	Amount to add	Notes
Glucose-6-Phosphate Isomerase (GPI)		
Tris HCl, pH = 8	1 ml	
NAD	1.5 ml	
F6P	5 drops	
MTT	5 drops	
PMS	5 drops	Add immediately before use
G6PDH	10 µl	
Agar	2 ml	
Lactate Dehydrogenase (LDH)		
Tris HCl, pH = 7	1 ml	
NAD	1.5 ml	
F-6-P	5 drops	
DL-lactic acid	4 drops	
MTT	5 drops	Add immediately before use
PMS	5 drops	
Agar	2 ml	
Malate Dehydrogenase (MDH)		
Tris HCl, pH = 8	1 ml	
NAD	1.5 ml	
Malic substrate	13 drops	
MTT	5 drops	Add immediately before use
PMS	5 drops	
Agar	2 ml	
Fumarate Hydratase (FUM)		
Tris HCl, pH = 7	1 ml	
NAD	1.5 ml	
Fumaric acid, pH = 8	5 drops	
MTT	5 drops	Add immediately before use
PMS	5 drops	
MDH	50 µl	
Agar	2 ml	
α-Amylase (AMY)		
Starch solution	2 ml	
Agar	2 ml	Add immediately before use
Iodine solution	1 l	Remove agar once set and

		place plate in tray of iodine solution
Aldehyde Oxidase (AO)		
Tris HCl, pH = 8	0.6 ml	
Benzaldehyde	1 drop	
MTT	5 drops	Add immediately before use
PMS	5 drops	
Agar	2 ml	

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