THE REPRODUCTIVE PERFORMANCE OF ROE DEER IN RELATION TO ENVIRONMENTAL AND GENETIC FACTORS

by

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Submitted for the Degree of Doctor
of Philosophy at Southampton University

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April 1993

UNIVERSITY OF SOUTHAMPTON
ABSTRACT
FACULTY OF SCIENCE
BIOLOGY
Doctor of Philosophy

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This thesis investigates the influence of density-dependent and density independent factors on the reproductive performance of roe deer (*Capreolus capreolus*) in Britain. Studies were based on fifteen wild populations, in Forestry Commission commercial plantations and on Ministry of Defence training areas. Extensive data were available for each site from cull records covering between 5 and 21 years, and included the number of embryos present and eviscerated body weight for each female shot, totalling more than five thousand deer. For the nine Forestry Commission sites, which form the core of the study, the ovaries and the lower jaw bone were also collected from each animal. The age of each of these animals was assessed by tooth sectioning.

Reproductive performance was assessed in terms of age at first breeding, percentage ovulation (corpora lutea present in the ovaries), average number of successfully fertilised ovulations per ovulating doe (as number of corpora lutea) , percentage pregnancy (embryos present) and average number of embryos per pregnant doe. Precocious reproductive activity among kids rarely resulted in successful pregnancy, while the majority of yearlings did conceive, but generally produced smaller litter sizes than older does. A uniform level of reproductive performance was observed among all animals over two years old within a single population.

Generally, little significant variation in reproductive performance between years within a site was detected. However, at the two sites with the longest span of data available (21 and 12 years) some significant variation between years in potential litter size was observed; this variation was entirely explained by correlated variation in maternal body weight. A sequence of increasing reproductive cost was suggested as one male, one female, two females, two males, according to the average body weight of the mother. Between populations, reproductive performance was highly variable (although adult ovulation always approached 100%). Percentage pregnancy, average number of corpora lutea, and average number of foetuses were all highly correlated with maternal body weight, but this relationship was insufficient to explain the very highly significant differences between populations in reproductive performance. Poor reproductive performance was positively correlated with the tendency to produce a male biased sex ratio.

Over the period of this study, data were collected for each site on climate, population density, habitat quality and genetics. Of the indices of habitat quality, average length of the lower mandible was found to be the best indicator of food availability in relation to population density. There was significant correlation between reproductive performance in a particular year and the average jaw length as adults of animals born in that same year at the two sites with the longest span of data available, but little correlation of performance and climate between years within a site. Between populations, ovulation success was correlated with body weight and average jaw length, but climatic factors were best able to explain differences in subsequent success of implantation (% pregnancy and number of embryos per doe).

Using starch gel electrophoresis of liver and kidney to detect isozyme differences, the level of genetic variability among roe deer in Britain was estimated to be fairly low (13.3% polymorphic loci, 3.2% average heterozygosity). At the only polymorphic locus that could be screened reliably (Mannose phosphate isomerase), two alternative alleles were found in six populations. No relationship between such a low level of genetic variability and reproductive performance could be detected. The genetic relationships between populations were also investigated by comparing the cranial morphology, using skulls collected from culled females. Three distinct groups of genetically similar populations were identified using this technique. Variation in skull morphology was significantly correlated with differences in reproductive performance between populations, in particular, potential litter size, even when variation in environmental factors had been accounted for, suggesting some genetic influence on performance variation of roe deer in Britain.

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ACKNOWLEDGEMENTS

Firstly, I would like to thank Rory Putman for devising the project, supervising it and me and still remaining (relatively) sane. The depths of his enthusiasm and encouragement know no bounds and his ability to provide light relief was always welcome.

My thanks also go to the Wildlife Branch of the Forestry Commission, and in particular, my co-supervisor Phil Ratcliffe, who oiled the wheels of bureaucracy sufficiently to allow me access to the information on which this study is based. The Commission staff involved in the collection of the material are far too numerous to mention, but I am greatly indebted to them for their efforts over the past decade. Likewise, I should like to thank all the deer managers and coordinators of the Ministry of Defence who provided me with similar data specifically for this study, and also Colonel James Baker of Lands 3 for providing access to this information.

Jochen Langbein has provided advice, expertise and distractions, particularly on matters of fitness, and I look forward hopefully to working with him in the near future. I am also grateful to Andy Peace for his advice on all matters statistical, and Gary Carvahlo for devoting a substantial period of time to initiating me in the intricacies of gel electrophoresis. My thanks also to Mark Barber for providing the bench space and equipment to facilitate that part of the project for two months, three months, six months... Pat Lowe provided some data on skull morphometrics for comparison, and I am grateful to him for his generosity.

My thanks also to the boys in blue across the country, who saddled me with a paultry six endorsement points, allowing me to transport the delicate tissue samples to the freezer with the utmost haste. I would also like to thank Wayne, who has provided the most welcome of distractions over the last three years, and supported me throughout.

Finally, I must acknowledge the deer, who made the supreme sacrifice, laying down their lives in the name of science.

List of Abbreviations

Chemicals

PMS	Phenazine Methosulfate
PMSF	Phenylmethylsulfonyl Flouride
MTT	(3-[4,5-Dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide
ATP	Adenosine 5-Triphosphate
ADP	Adenosine 5-Diphosphate
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
Tris	Tris (hydroxymethyl) methylamine
$MgCl_2$	Magnesium Chloride
$MnCl_2$	Manganese Chloride
NaOH	Sodium Hydroxide
HCl	Hydrochloric Acid
NH_4Cl	Ammonium Chloride

Statistical Terms

ANOVA	Analysis of Variance
PCA	Principal Component Analysis
PC	Principal Component
RDA	Redundancy Analysis
R	Roger's Distance

Scientific Names of Deer Species Cited

Subfamily **Cervinae**

Fallow deer	*Dama dama*
Red deer	*Cervus elaphus*
Sika deer	*Cervus nippon*
Wapiti	*Cervus canadensis*

Subfamily **Odocoilinae**

Moose	*Alces alces*
Mule deer	*Odocoileus hemionus*
Reindeer or caribou	*Rangifer tarandus*
Roe deer	*Capreolus capreolus*
White-tailed deer	*Odocoileus virginianus*

Subfamily **Muntiacinae**

Muntjac	*Muntiacus reevesi*

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

The theoretical outcome of unrestricted population growth and regulation was encapsulated by Malthus (1798) when he observed that, if individuals are independent and identical, populations will either expand indefinitely with a constant doubling time or disappear. The capacity for such rapid increase is well illustrated by the proliferation of the human population, which has doubled in periods as short as twenty five years. Similarly, Darwin (1872) estimated that from a single pair of elephants (*Loxodonta africana*), one of the slowest known breeders, a population of nineteen million individuals would result from about seven hundred and fifty years of such unlimited increase. Although humans have developed technologies that have permitted something close to this unchecked growth to continue, the elephant population is sadly not so abundant.

The assumption underlying the above model is simplistic and, in fact, individuals within a population are not identical and do not act independently. As population density increases the rate of growth will slow because the reproductive rate of each individual decreases due to environmental resistance (Pearl and Reed 1920). Thus, if the population response to density is linear, the growth of the population will be logistic, described by a sigmoid growth curve (Fig. 1.1). Although this model still assumes individuals are identical and that there is no time lag between population growth and the consequent effects of that growth, it usefully illustrates the potential impact of increasing density on the dynamics of a population.

The first experiments that demonstrated such density-dependent effects used *Drosophila* to show that competition for food at high density caused first, a decrease in reproductive performance, followed by an increase in adult mortality (Pearl 1927). Reproductive success was impaired due to a higher incidence of neo-natal mortality among larvae, and was expressed at levels of food limitation substantially lower than the level at which adult mortality increased. In a similar set of experiments on house mice,

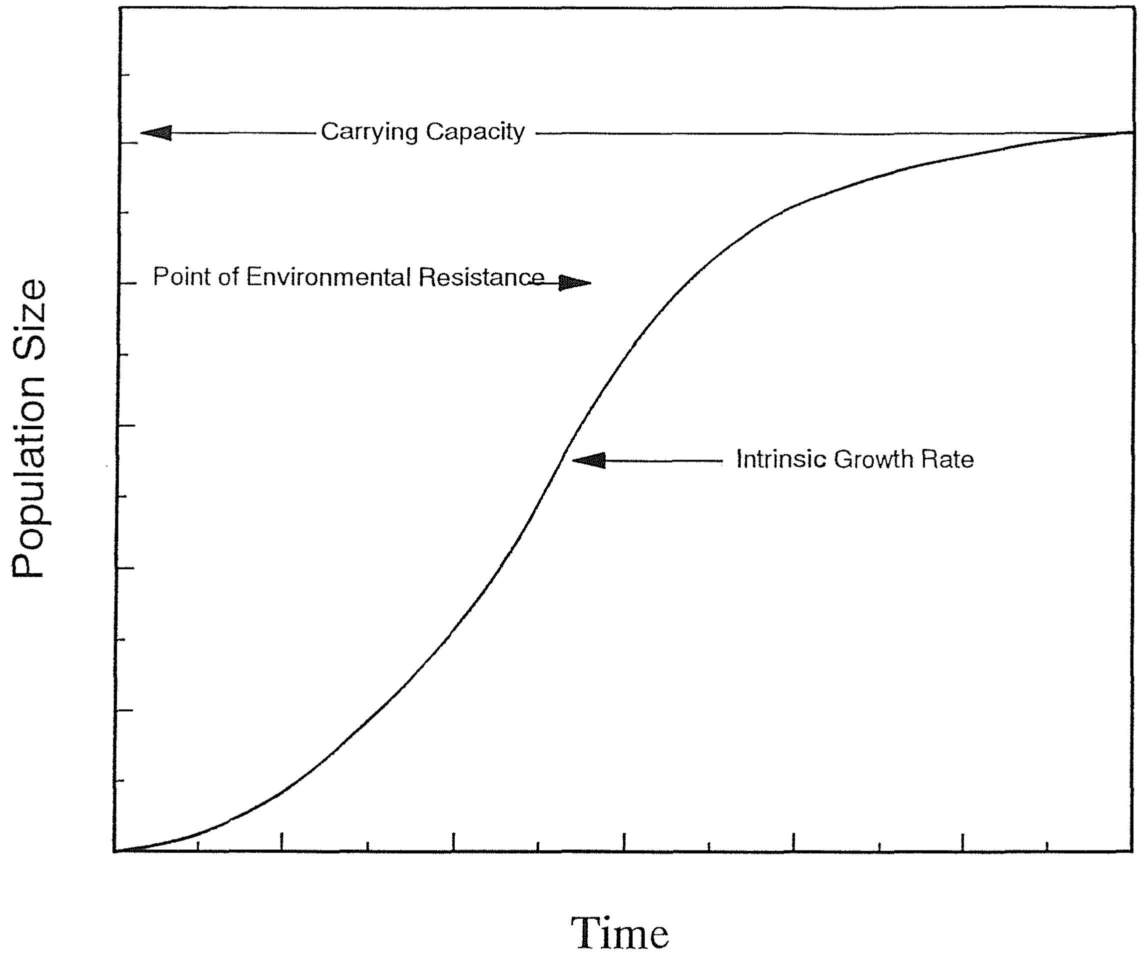


Figure 1.1: The theoretical logistic curve illustrating exponential growth and the density-dependent response.

(*Mus musculus*) reproduction in an enclosed population ceased abruptly when the population had expanded to the point where food was limiting (Strecker and Emlen 1953).

As Pearl's experiments demonstrate, juvenile survival is often the most sensitive parameter in its response to increasing density, generally falling significantly prior to any density-dependent loss in fecundity (Hanks 1981). However, reduced survivorship among adults is often only observed when the demographic vigour of a population has decreased even further. In their studies on the red deer of the Isle of Rhum, Clutton-Brock et al. (1987b) found a strong density-dependent effect on juvenile survival, but were also able to detect some effect of rapidly increasing density on adult survivorship (Clutton-Brock et al. 1985). Furthermore, the differential in the survival rates between the age classes was more pronounced at high population density (Clutton-Brock et al. 1987b).

The above studies were conducted in enclosed systems, but in natural populations there may be opportunity to disperse. Where the carrying capacity of the habitat is approached, individuals may be forced to emigrate to avoid intense intra-specific competition, either for food, territories or some other resource (Strecker 1954). Furthermore, in territorial species such social pressures may act to regulate population size at a level below that at which competition would cause the habitat resources to become depleted (see Ozoga and Verme 1982). Finally, extrinsic factors such as predation and disease can act in a density-dependent way, and may prevent the population exceeding the carrying capacity of the habitat. Population growth among species of large mammals is often restricted to some extent by predation pressure (e.g. Messier 1991; Seip 1992).

The intrinsic growth rate (the slope of the growth curve, Fig. 1.1), and the carrying capacity of the habitat will also be affected by 'catastrophic', commonly termed density-independent, factors such as climate (Howard and Fiske 1911). Indeed, Andrewartha and Birch (1954) viewed climatic variation as the most important influence on natural populations, maintaining that all the published work apparently demonstrating density-dependence was restricted to laboratory studies. However, as the authors themselves state, a heterogeneous habitat affords a variable degree of protection from adverse climatic conditions and individuals in less sheltered sites will be more likely to die. Thus, as a population decreases in response to density-independent factors, an increasing proportion of the population will occupy favourable sites and the mortality rate will decline. This then can be termed a density-dependent response. In reality, populations may respond to both density-dependent and density-independent mechanisms and more recent research has concentrated on quantifying the relative impact of a variety of environmental factors (e.g. Harcourt 1971)

The simple models of population growth described above assume that all the individuals of a population are identical. However, natural populations are characterised by a high degree of genetic variation (e.g. Lewontin 1974). This variation can be associated with fitness differences and so forms the raw material on which natural selection acts. The strength of selection is determined by the fitness differential between genotypes and the heritability of those differentials. Individual fitness can only be measured in relative terms (Dobzhansky 1968) and research in this area has focused on the fitness of populations.

Thoday (1953) noted that the continued existence of a population, its persistence through time, is a vital component of population fitness (also see Murray 1985). Thus, researchers have examined the ability of populations to persist and to increase as indicative of inferior or superior genetic fitness. Even minor changes in the genetic constitution of a population can greatly influence the fitness, and hence competitive ability, of its individual members (Park et al. 1964). In some of the first intra-specific selection experiments, Dobzhansky et al. (1964) were able successfully to demonstrate competition between chromosomally polymorphic and monomorphic populations of Drosophila. Polymorphic populations consistently out competed monomorphic populations, resulting in a change of genotypic frequencies within the experimental system.

Hanks (1981) suggested that as the rate of increase of a population declines, parameters which contribute to the biological fitness of the population change sequentially. Firstly, juvenile mortality increases, followed by an increase in the age at first breeding, subsequently adult fecundity declines, and lastly mortality among adults increases. This thesis examines variation in a crucial but sensitive measure of biological fitness, reproductive performance, in a species of large mammal, the roe deer. In particular, density-dependent (food availability per individual), density-independent (climate) and genetic factors are examined for their ability to explain the observed variation in reproductive performance within and between populations.

The action and interaction of the parameters that can affect fitness are presented diagrammatically in Fig. 1.2. Environmental factors, both density-dependent and density independent, and genetic factors determine fitness through their effect on characteristics of individuals. However, research in population biology can often only address one particular aspect of a system, for example, focusing on either the environmental or the genetic constraints which limit fitness. Few studies have attempted to quantify the relative importance of both these factors. As Smith et al. (1984) propose, 'The overall goal should be to understand the ways in which environmental and genetic factors interact to produce populations with different characteristics and functional levels'. This study aims to evaluate the importance of genetics and the environment in determining fitness of roe deer in Britain.

Of the many components represented in Fig. 1.2, which combine and interact to define the environmental conditions an animal experiences, three factors, density, food availability and climate are relatively easy to measure and are often cited as the most influential factors limiting reproductive performance of large mammals (see reviews Sadleir 1969a; Sinclair 1973; Fowler 1987).

As outlined above, it has been illustrated experimentally that intra-specific competition for food can cause a decrease in reproductive performance where resources are limiting. Such a response has been observed in several species of deer (white-tailed deer: Woolf and Harder 1979; red deer: Albon 1983; reindeer: Skogland 1985; fallow deer: Langbein and Putman 1992). However, the effects of resource limitation are a result of the interaction of resource availability with animal density, and therefore, resources must be assessed in relation to the number of individuals sharing those resources to define the carrying capacity of a habitat (see Fig. 1.1).

Fluctuations in population density *per se* are often associated with changes in reproductive performance of deer (white-tailed deer: Teer 1984; reindeer: Skogland 1985; red deer: Clutton-Brock et al. 1987; roe deer: Maizeret et al. 1989; fallow deer: Langbein

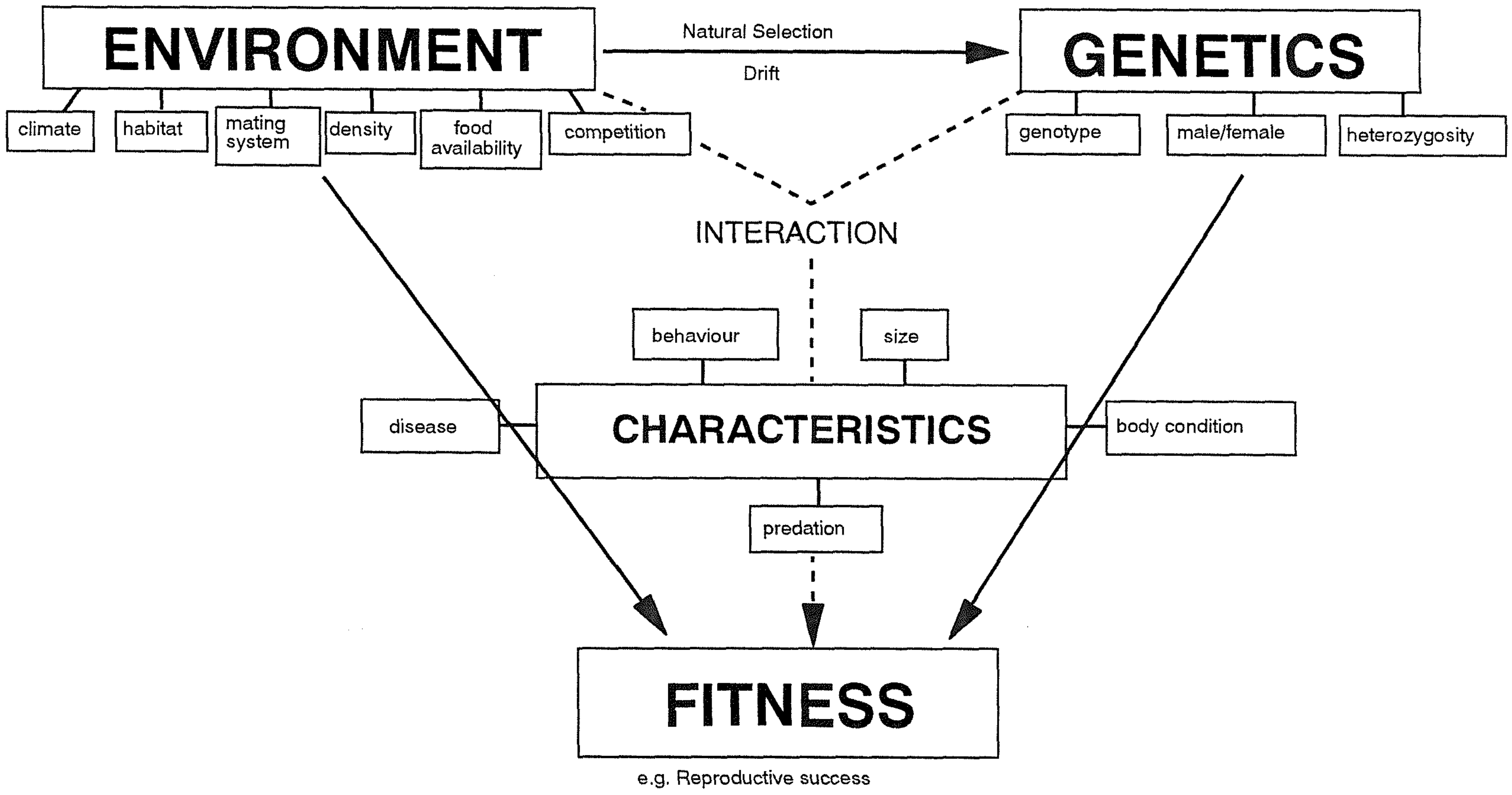


Figure 1.2: An illustration of the action and interaction of environmental and genetic factors on the fitness of individuals.

and Putman 1992). This relationship is usually assumed to signify resource limitation, however, other density mediated responses, such as disease or behavioral modification, could also be influential (Ozoga and Verme 1982; Vincent et al. *in press*). Chaetum and Severinghaus (1950) found that reproductive performance in populations of white-tailed deer was higher in areas of good range quality compared to areas of poorer quality. Subsequently, as density in one of the poor performance areas declined over several years due to hunting pressure, reproductive performance increased. Thus, both food availability and population density respectively were implicated in the population regulation of white-tailed deer. However, these comparisons, both between areas and between years within the poor performance area, considered only one or other of these factors, and it is therefore, not possible to conclude whether it was a single factor or the interaction of the two which suppressed reproductive performance.

Density-independent factors may also exert an influence on population growth among large mammals (Sinclair 1973). In deer this influence may be translated through increased levels of mortality, particularly among juveniles (Clutton-Brock and Albon 1983), but also may result in impaired reproductive performance (Albon et al. 1987). Adverse climatic conditions can be detrimental because of the associated increase in an animal's metabolic costs (Grace and Easterbee 1979; Ratcliffe 1987; Ratcliffe and Mayle 1992). However, the relationship between climate and reproductive performance is often mediated through habitat changes. Warm weather may advance the onset of the growing season and encourage the growth of vegetation throughout the summer, therefore increasing food availability (Albon 1983). In contrast, high rainfall during the winter may cause water logging and leaching of the soil's nutrients leading to poor growing conditions (Putman and Langbein 1992).

Thus, density-dependent and density-independent factors are often inter-dependent in their effect on fitness. The effects of these environmental factors are mediated through characteristics of individuals (Fig. 1.2), particularly body weight and condition (moose: Saether and Haagenrud 1983; reindeer: Skogland 1985; red deer: Mitchell et al. 1986; chamois (*Rupicapra rupicapra*): Bauer 1987). These relationships are often most strongly evident among juveniles (Hanks 1981), for example, puberty in deer may be dependent on juveniles achieving certain body weight thresholds (red deer: Hamilton and Blaxter 1980;

reindeer: Reimers 1983; fallow deer: Langbein and Putman 1992). Therefore, body weight and/or reproductive performance, in particular the age at first breeding, among sub-adult animals may be especially sensitive to fluctuations in environmental conditions.

The intrinsic rate of population growth of a species is limited by genetic constraints, but even small differences between populations at the molecular level may be associated with intra-specific variation in fitness. Furthermore, the fitness of a particular genotype varies according to the environmental conditions (Hedrick et al. 1976), causing an interaction effect (Fig. 1.2). This principle was outlined by Tansley (1917) who found that two closely related species of *Galium* grew best on soils they were generally found on in the wild but, in the absence of competition, were able to survive adequately on the alternative substrate. However, when the environment was altered by introducing the second species, competition favoured the species which was accustomed to that substrate.

In some species, in particular large mammals, at high population density individuals may alter their behaviour and disperse. This is probably a threshold response triggered by elevated competition for resources or mates and is particularly prevalent among juveniles in territorial species. Dispersal is often associated with high levels of mortality and/or reduced reproductive performance (Putman 1988). However, the level of this threshold response to density may be genetically mediated. Myers and Krebs (1971) found that certain individuals were genetically predisposed to disperse from the area of their birth in populations of field voles (*Microtus sp.*). This illustrates that there may be an interaction of environmental and genetic factors that is not purely additive.

Therefore, in order reliably to judge the influence of genetic variation on reproductive performance, it is necessary simultaneously to assess environmental and genetic influences. Ramsey et al. (1979) demonstrated that differences in juvenile mortality and reproductive performance between two adjacent herds of white-tailed deer were correlated with significant genetic differences at the molecular level. However, this correlation did not establish a cause and effect relationship as the authors stated that there may have been differences in food availability or quality between the two areas. Further work on the same population found that environmental factors and genetic characteristics interacted to determine body composition, and were therefore, implicated as potentially influencing reproductive success (Scribner et al. 1989). Pemberton et al. (1988) were able to determine the effects of genotype on the fitness of red deer, namely juvenile survival, in isolation from phenotypic variation. They showed that the probability of survival was significantly associated with molecular gene markers, even when the analysis controlled for variation in birth weight, birth date, locality, cohort and sex, all of which were known to affect survival of red deer calves.

Therefore, it is important to consider as many factors as possible, both environmental and genetic, to characterise the mechanisms behind variation in biological fitness of individuals or populations. Furthermore, samples should be drawn from populations to maximise the range of genetic and environmental variation, but also to provide replicates where one or other of the factors assessed is invariant.

1.2 THE ROE DEER

The way in which the environmental and genetic factors affect the reproductive performance of a population will be necessarily influenced by the biology and ecology of the particular species involved. Therefore, this section briefly summarises these characteristics for roe deer.

Taxonomy

The roe deer, *Capreolus capreolus*, with, among others, the white-tailed deer and the mule deer belongs to the subfamily Odocoilinae within the Cervidae family. Two subspecies are commonly recognised, the European roe *C. c. capreolus* and the Siberian roe *C. c. pygarus* (Lister 1984), and recent morphological (Sokolov et al. 1985a) and karyotype (Sokolov et al. 1986a) studies have provided firm evidence for this distinction. On the basis of similar morphological studies, further subdivision of both these subspecies groups has been proposed (Danilkin et al. 1985; Markov et al. 1985) but requires more detailed examination. Lonnberg (1910) described what he thought to be an indigenous British race in Scotland (*C. c. thotti*) on the basis of coat colour, however, this distinction is not widely accepted (Staines and Ratcliffe 1991).

Distribution and History

The European roe deer currently inhabits every country except Ireland within the continent. In Britain, roe deer have been present intermittently since the Middle Pleistocene era about four hundred thousand years ago, although they were restricted to southern Europe during the colder stages of this period (Lister 1984). In historical times, roe deer flourished in Britain until the fourteenth century, when the royal protection afforded by the forest laws was removed and they were subjected to hunting (Whitehead 1964). From this time numbers steadily declined until the eighteenth century when, with the exception of the Scottish highlands, they were thought to be extinct throughout Britain (Bewick 1790). In addition to the high hunting pressure, particularly during the civil war, the extensive destruction of woodland for timber and the difficulty of maintaining captive roe herds were major factors accelerating this decline in abundance (Whitehead 1964).

Over the last two centuries the area of woodland in Britain has increased substantially and, during this period, roe from various sources have been reintroduced to a number of areas where they were no longer present. From a point of near extinction two hundred years ago, the range and number of roe deer in Britain has increased rapidly. It has been estimated that roe deer populations can expand by 25 to 30% annually, if conditions are favourable (Loudon 1978). The current distribution of this species is presented in Fig. 1.3 (see Staines and Ratcliffe 1991). It is widespread throughout Scotland and northern England and is increasing its range. In southern England it is present from western Kent to Cornwall and is spreading north towards the midlands. There is also a thriving population in East Anglia which is expanding westward. Roe are now considered by many land owners as a pest species (Loudon 1982), and in the last decade over fifteen thousand animals a year have been culled in Britain.

The reintroductions and translocations of roe deer within Britain are poorly documented, and the origin and number of animals released often unknown. The dates of some introductions (taken from Higgins, not dated) are summarised in Table 1.1 and the corresponding points of release are superimposed on Fig. 1.3, however, this list is undoubtedly incomplete. The origin of the released animals is established in only a few of these cases (Table 1.1), and the wild populations which formed were often supplemented by further subsequent reintroductions of deer of unknown origin.

Habitat Use, Diet and Social Organisation

The roe deer is typically a woodland species, found in coniferous, deciduous or mixed forest where food and cover are sufficiently available. However, populations of roe are also established on the open moor of the Scottish highlands (Cumming 1966) and in the vast open agricultural landscapes of central Europe (Zejda and Homolka 1980), where groups of sixty or seventy animals have been observed (Kaluzinski 1982a). In the wooded habitat generally utilised by British roe, animals are usually solitary, but density varies widely between 8/km^2 in medium aged closed canopy areas and over 25/km^2 in young stands where food and cover are both abundant (Staines and Welch 1984; Loudon 1987). Similarly, range size within this habitat is highly variable, estimated to be as small as 4 hectares (Loudon 1979) or as large as 60 hectares (Johnson 1984), and is probably related to the availability of resources.

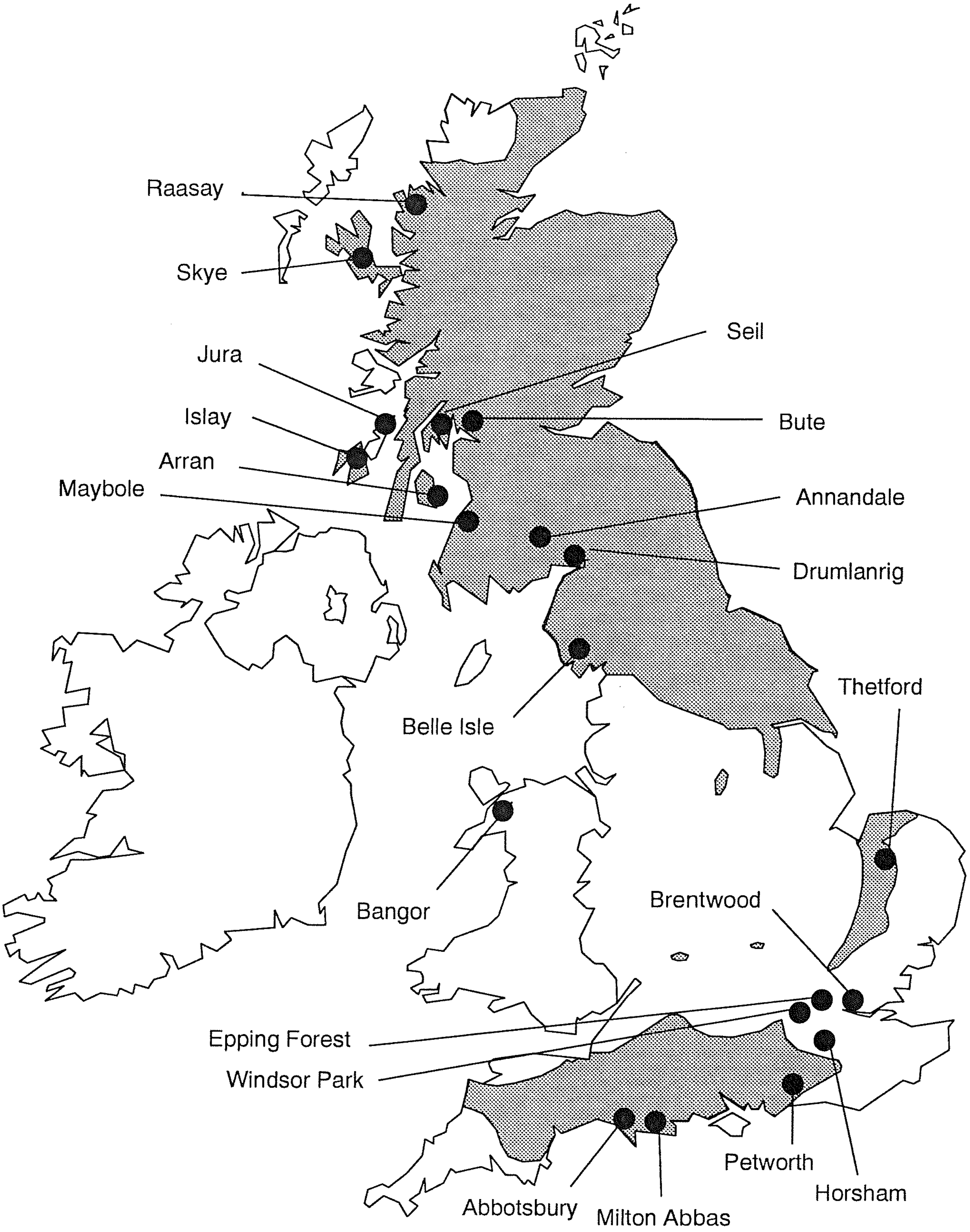


Figure 1.3: The current distribution of roe deer in Britain and some points of recent known re-introductions (see Table 1.1).

Table 1.1: Documented introductions of roe deer to Britain (taken from Higgins, not dated).

Site of Inroduction	Date	Origin of Animals	Number Released
Raasay, NW Scotland	1700's	Not known	
Isle of Skye, Scotland	1700's	Not known	
Isle of Jura, Scotland	1500's 1800's	Not known Not known	
Isle of Islay, Scotland	1700's	Not known	
Seil, Scotland	1957	Not known	
Bute, Scotland	1850's	Not known	
Isle of Arran, Scotland	1700's	Not known	
Maybole, Strathclyde, Scotland	1820's	Not known	
Annandale, Dumfries, Scotland	1854	Not known	
Drumlanrig, Scotland	1860	Not known	
Belle Isle, Windermere	1913	Austria	12
Bangor, Wales	1874	Germany and Dorset	
Thetford, Norfolk	1884	Wurtenburg, Germany	12
Brentwood, Essex	1892	Not kown	2
Epping Forest, Essex	1883 1884	Dorset Not known	6 8
Windsor Park, Berkshire	1825 1850	Dorset Petworth	4
Horsham, Sussex	1931	Not known	
Petworth, Sussex	1800's 1890	Not known Scotland	
Milton Abbas, Dorset	1800	Perth, Scotland (Ire)	
Abbotsbury, Dorset	1820's	Not known	

The roe is generally considered to be a relatively primitive deer species and as such, has a relatively small rumeno-reticular system which is not particularly well developed (Hofmann 1973). Consequently, it is unable efficiently to digest the cellulose in plant matter and must, therefore, select foods of low fibre but high nutrient content (Putman 1988). Thus, this species is termed a concentrate selector (Hofmann 1973), taking nutrient rich buds, shoots and non-graminaceous herbs preferentially (Jackson 1980). However, the feeding strategy of the roe deer must be more versatile in winter when ivy, conifers, ferns and even heather supplement their diet. In agricultural areas commercial crops may also be an important component of the diet (Putman 1986), but even in this environment the roe remains a selective feeder, taking the early crop shoots and the cereal itself when ripe, but in between, searching out the broadleaved weeds within the crop (Kaluzinski 1982b).

Roe deer throughout most of their range and in most habitats are strongly territorial, however, this behaviour is seasonal, essentially breaking down during the winter. As already noted, among the field roe of Europe territoriality is suppressed and large aggregations of animals can be observed year round. In Britain roe establish non overlapping territories with respect to the territories of other animals of the same sex (Bramley 1970), which are often delineated by natural features. Where overlap does occur within a single sex, it is generally believed that this is due to philopatric daughters remaining within their mother's range (Loudon 1979). Animals which are unable to acquire a territory will disperse from the area during their second or third year. Buck territories are generally larger than those of does and this is probably due to bucks maximising the number of female territories with which their own territory overlaps (Johnson 1984).

Reproduction

The rut takes place during late July and early August, however parturition does not occur until the following May or June. This constitutes the longest apparent gestation period among deer, however, the fertilised egg does not implant in the uterus wall until approximately half way through this period (Short and Hay 1966). This delayed implantation may be an adaptation enabling roe to expand their range as far north as the Swedish arctic circle, as reproductive effort of both sexes is concentrated into the climatically less harsh spring and summer months (Loudon 1982). Females normally breed for the first time at 14 months of age, but reproductively active kids have occasionally been recorded (Ratcliffe and Mayle 1992). Roe are thought to be monoestrus (Gaillard et al. 1992) but a 'false rut' in October may signify a resurgence of sexual activity in some does, or alternatively, may be a consequence of yearling animals only achieving reproductive condition later in the season (Ratcliffe and Mayle 1992). Roe are multiparous, producing up to three, but more usually one or two kids per litter.

1.3 SUMMARY OF OBJECTIVES

The objectives of this study can be summarised as follows:

1. Is there variation in reproductive performance of British roe deer between populations or between years within a single population?

2. Does reproductive performance vary with age, and is such variation more pronounced in some populations?

3. Can variation in reproductive performance within and between populations be explained entirely in terms of maternal body weight?

4. Is variation in reproductive performance related to density-dependent (food availability and quality) and/or density-independent (climate) factors?

5. What level of genetic variation is there within and between populations of British roe deer?

6. To what extent is variation in reproductive performance linked to genotypic properties?

CHAPTER 2

METHODS

2.1 THE STUDY AREAS

The data which form the basis of this thesis were derived from fifteen study sites, nine owned and managed by the Forestry Commission, and six by the Ministry of Defence, from which roe deer were shot in sufficient numbers to provide biologically meaningful sample sizes. The sites, the locations of which are presented in Fig. 2.1, were selected to ensure a wide distribution throughout Britain (falling between latitudes of 50^0 and 57^0 30'), and thus, to represent the full spectrum of climatic variation.

All nine of the Forestry Commission sites were commercial plantations, essentially composed entirely of conifers. The predominant species were Scot's pine *(Pinus sylvestris)*, Sitka spruce *(Picea sitchensis)* and Corsican pine *(Pinus maritima)*, but Japanese larch *(Larix raempferi)*, Douglas fir *(Pseudotsuga sp.)* and Norway spruce *(Picea abies)* were often also present. Each site was a mosaic of small, single species compartments, planted at different times over the last fifty years. Generally, in any one site approximately 25% of these compartments were mature stands (planted pre 1950), 40% medium aged, closed canopy thickets (planted between 1950 and 1970), 15% open canopy thickets (planted between 1970 and 1985) and 20% restock sites in the establishment phase (planted after 1985). In most sites, there was, in addition, a small proportion of ground left fallow after felling operations, and some sites also supported a very small area of broadleaved woodland. Only one Forestry Commission site, Spadeadam, differed significantly from this general pattern. At this site, mature stands were almost entirely absent, and the majority of compartments were recently planted, mostly during the 1970's. However, the surrounding land, owned by the Ministry of Defence, was also commercial coniferous plantation, and was largely comprised of older age stands.

There was, thus, a fairly high degree of uniformity between Forestry Commission study sites in the vegetation structure within compartments of similar age. Generally,

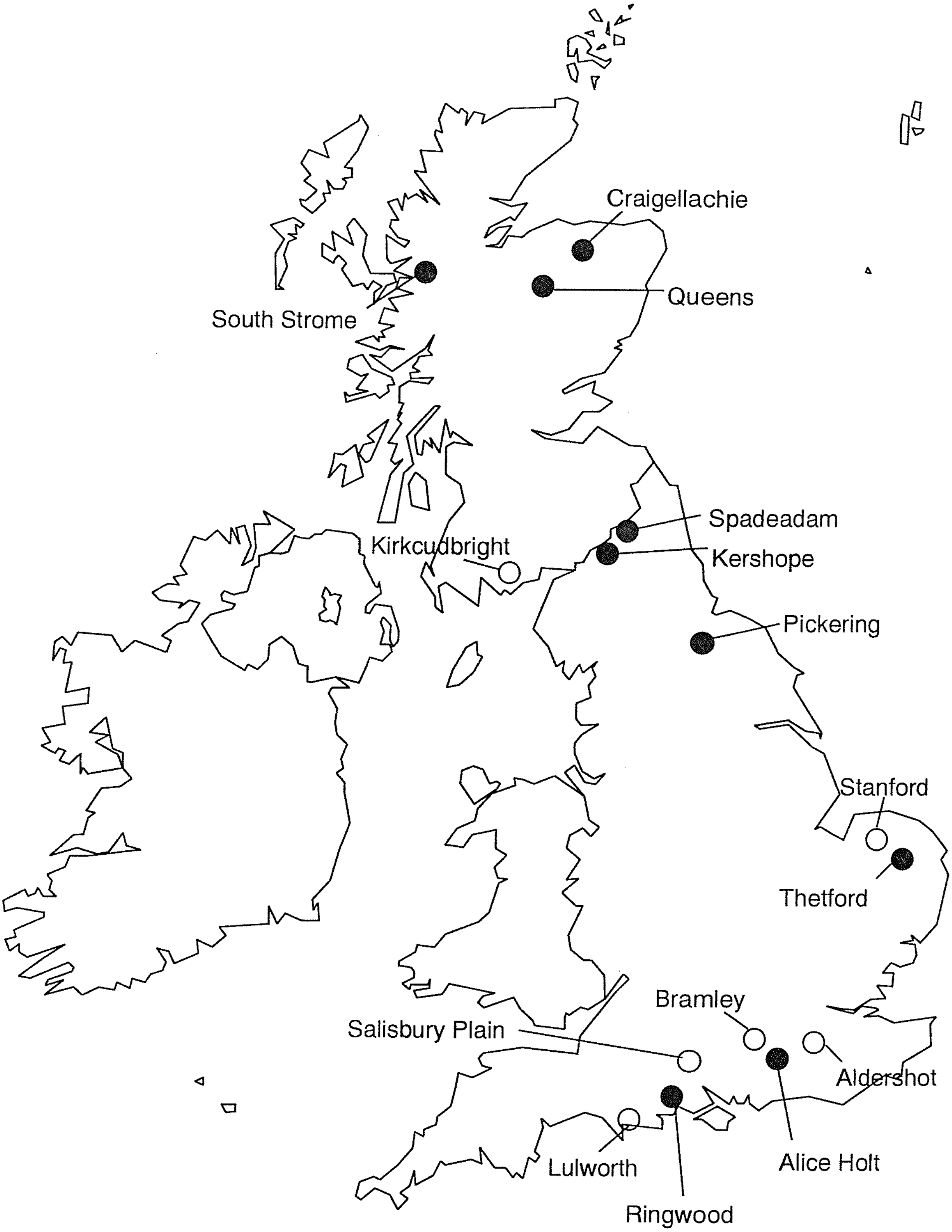


Figure 2.1: The location of the study sites in Britain, filled circles are Forestry Commission plantations, open circles are Ministry of Defence training areas.

mature stands contained a good ground flora of bramble, holly, herbs, grasses, mosses, bracken and some fungi. There was often an understorey of hawthorn and birch. In contrast, the closed canopy stands (thicket and pole stage) had very poor ground vegetation, and were, in fact, usually entirely barren. In the younger age compartments (pre-thicket and re-stock stage), heather was often the main component of the ground flora, but bracken, moss and herbs were often also present.

The Forestry Commission sites were widely distributed over the geographical range of British roe deer, nonetheless, these areas were generally similar in habitat structure, essentially comprising of commercial coniferous forest. To provide a greater diversity of environmental types considered in the study, and to extend the range of variation in habitat quality, six Ministry of Defence sites were also included. These study sites comprised a more variable and fragmented habitat structure. These areas are used for military training and therefore, contain a fairly large proportion of open ground, either grassland, scrub, or agricultural land. However, almost all sites included some small pockets of woodland of oak, birch and occasionally some conifers. Areas of scrub containing hawthorn, blackthorn, birch, heather and gorse were also common. Kirkcudbright was the only site entirely devoid of tree cover, however, in this site the hawthorn, gorse and grass sward scrub was particularly deep, offering a considerable degree of shelter and concealment.

2.2 DATA COLLECTION

2.2.1 Cull Data

Cull Records

The Ministry of Defence deer managers and the Forestry Commission rangers routinely collected detailed information from each animal that was shot or involved in a fatal accident. Detailed records of this type have been maintained for a period of about 8 years for all Forestry Commission sites, and 5 years for Ministry of Defence sites. The data for culled females only (shot between October 23rd and March 1st) were transcribed from these records. Information recorded included a code number, date killed, location at death, 'clean' body weight (total weight less viscera, but with head and feet on), estimated age from tooth eruption and tooth wear (see below) and reproductive status (see below). The availability of this, and other information for each study site is summarised in Table 2.1.

Age

The age in years of each animal was estimated by the stalker immediately after death, by examining the dentition for eruption and tooth wear. In addition, for the Forestry Commission study sites only, lower jaw bones from each animal shot were retained. For these animals, but not for deer shot on Ministry of Defence land, a more accurate assessment could subsequently be made, by sectioning the teeth to count incremental growth rings in the cementum layer (Aitken 1975; Ratcliffe and Mayle 1992).

In roe deer, eruption of all permanent teeth is complete by the age of 10 to 15 months of age. Therefore, any animals shot in the doe season which had only two molars and a tricuspid third premolar (Fig. 2.2a) were necessarily kids (in their first winter). Animals which had a complete set of permanent teeth (Fig. 2.2b), but where little wear was evident on the third molar were likely to be yearlings (in their second winter) (Ratcliffe and Mayle 1992).

Table 2.1: A summary of the data availability for each study site (+ indicates a data sample was available).

Study Site	Cull Record Information							Climate Data	Dung Counts	Rumen Liquor	Body Tissues	Skulls (n)
	Study Period		Number of Corpora Lutea	Number of Embryos	Sample Size	Jaws						
	Period 19-	No. Yrs					Sample Size					
Alice Holt	70-90	21	+	+	360	+	268	+	+	+	+	12
Thetford	79-90	7	+	+	705	+	584	+	+	+	+	8
Ringwood	83-90	8	+	+	235	+	183	+	+	+	+	5
Spadeadam	83-90	7	+	+	279	+	224	+	+	+	+	9
Kershope	83-90	8	+	+	382	+	279	+	+	+	+	14
Pickering	82-90	9	+	+	681	+	464	+	+	+	+	10
Craigellachie	81-90	9	+	+	432	+	347	+	+	+	+	5
Queens	82-90	7	+	+	115	+	106	+	+	+	+	11
South Strome	83-90	7	+	+	113	+	128	+	+	+	+	12
Salisbury Plain	86-90	5	-	+	231	-	\	+	-	-	+	6
Lulworth	85-90	6	-	+	144	-	\	+	-	+	+	6
Aldershot	85-90	6	-	+	65	-	\	+	-	-	+	-
Bramley	85-90	6	-	+	88	-	\	+	-	-	+	-
Stanford	85-90	6	-	+	129	-	\	+	-	+	+	7
Kirkcudbright	85-90	6	-	+	112	-	\	+	-	+	+	10

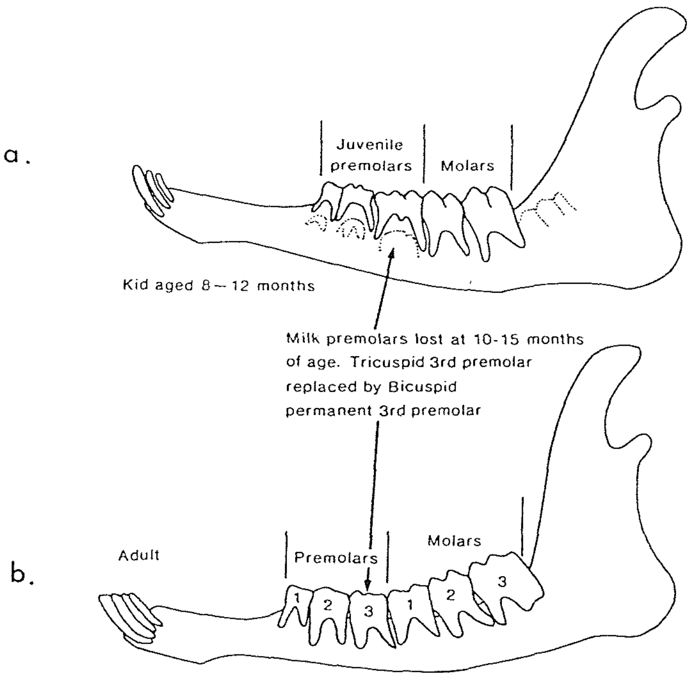


Figure 2.2: a. Juvenile and b. Adult dentition.

Tooth sectioning allowed precise ageing of yearlings and older animals. Distinct layers of dental cement are laid down annually between the roots of a tooth as it grows. When the tooth is sectioned, these layers can clearly be seen with the aid of a microscope as alternate light and dark bands (Aitken 1975). For each available jaw, the second molar was cut in two, slightly behind the mid-line, using a hacksaw (Fig. 2.3a), and then removed from the jaw. The surface of the anterior portion of the tooth was polished on fine carborundum paper and then wiped clean to reveal cement pad (Fig 2.3b). The tooth section was mounted in plasticine and viewed with a direct light source at between x10 and x40 magnification. The number of light coloured layers was assessed (Fig. 2.3c). (Each represents one summer's growth; the thin, darker line represents growth during a single winter when resources are relatively restricted.) The tooth was polished, washed and examined repeatedly to confirm the number of layers present. Where the banding pattern was indistinct, a second tooth from the other half of the jaw was extracted, and the process repeated. The age of the animal (in years) was estimated as equal to the number of wide, light coloured bands observed (see Ratcliffe and Mayle 1992).

Reproductive Condition

1. Embryos.

The number of embryos was assessed at the time of death at both Forestry Commission and Ministry of Defence study sites. The uterine horns of each animal were cut longitudinally and embryos counted (Strandgaard 1972a). Roe deer exhibit the phenomenon of delayed implantation (Short and Hay 1966), the fertilised blastocysts only becoming implanted in the uterus wall during late December or early January (Aitken 1974). Therefore, for the first five months after the rut it is not possible to assess pregnancy in this way, and only does shot after the first of January were considered for this parameter (see Ratcliffe and Rowe 1985).

2. Corpora lutea.

From Forestry Commission sites only, ovaries were available from each shot female (see Table 2.1). Ovaries were collected by stalkers, and stored in dilute formalin solution, labelled with the animal code. In the laboratory, the ovaries were examined for

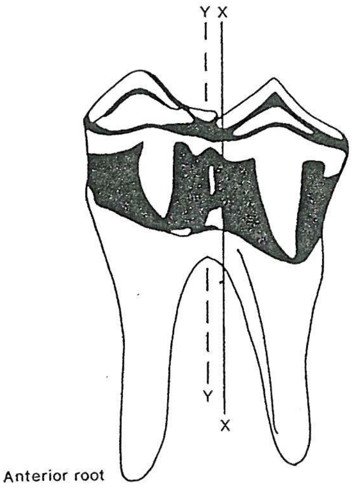


Figure 2.3a: Line of cut (X-X) of the molar to the posterior side of the mid-line (Y-Y).

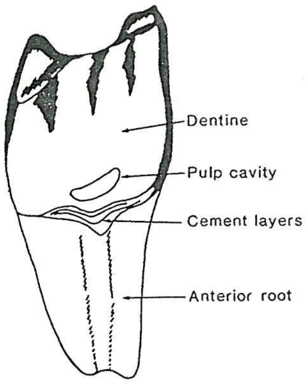


Figure 2.3b: Transverse section through the molar to reveal the cementum pad.

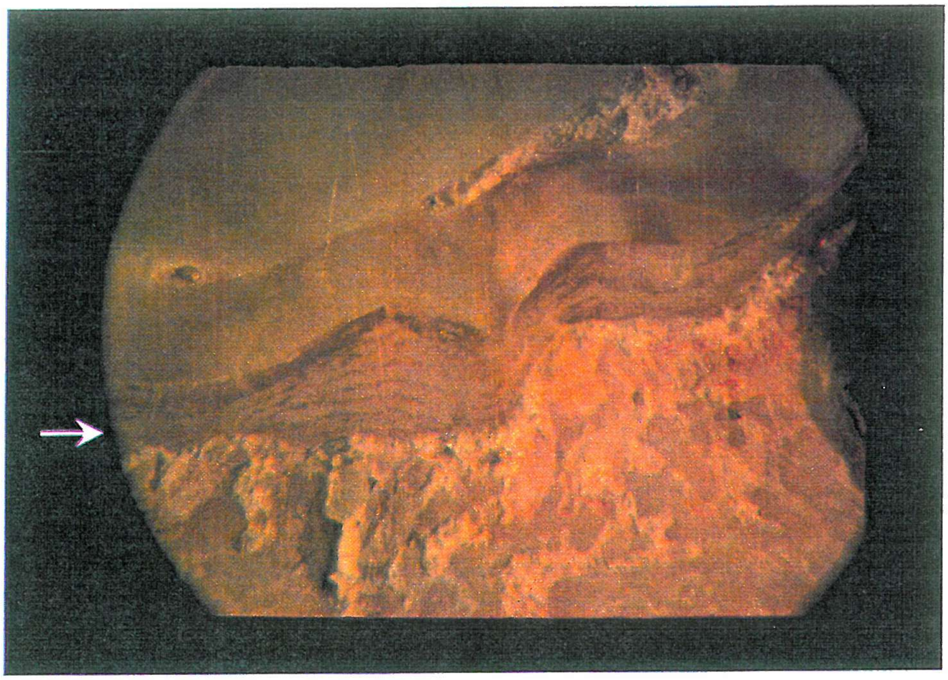


Figure 2.3c: A sectioned tooth showing the cementum layers (indicated by the arrow).

corpora lutea, yellow bodies within the ovary which only persist following successful fertilisation (Strandgaard 1972a). The ovary was sliced two or three times longitudinally, and the corpora lutea counted with the aid of a hand held magnifying glass (Ratcliffe and Mayle 1992). A single corpus luteum is formed from the fertilisation of each egg released from the ovary, and therefore, the number of such bodies represents the potential number of offspring. Although embryo development is not immediate in roe deer (see above), because of the phenomenon of delayed implantation (Short and Hay 1966; Aitken 1974), corpora lutea are apparent immediately after fertilisation, and can, therefore, be assessed throughout the duration of pregnancy.

2.2.2 Resource Availability and Population Density

Skeletal development is dependent on the level of nutrition an animal receives, particularly just prior to, and in the first few months after birth (Ellenberg 1974; Skogland 1983). Therefore, two skeletal measures were used as indices of the relative availability of resources in the year of an individual's birth, (as resource availability in relation to the number of animals sharing that resource). In addition, the level of free ammonia in the rumen liquor reflects the nutritional state of an animal at the time it was sampled (Klein 1962), and therefore, is a useful independent index of resource availability in relation to animal density. Absolute densities can be estimated from standing crop faecal counts (see Putman 1984). The rationale underlying these indices is addressed more fully in Chapter 4. The methodology is given below.

Skeletal Measurements

Skeletal size was assessed on the basis of two measurements on the lower mandible (these were retained from all animals shot on Forestry Commission land, see above). The length of the lower mandible was measured from the point where the first incisor enters the gum at the distal end to tip of the processus angularis at the proximal end (Ratcliffe 1987). The thickness of the mandible was assessed by measuring the height of the diastema at its thinnest point. These measurements were taken using vernier callipers and were recorded in millimetres to one and two decimal points respectively. The lower jaws of all shot animals are routinely retained by the Forestry Commission rangers, and were therefore available for measurement for a number of years from all Commission study sites (see Table 2.1). A small number of jaws were available from one or two recent culls at some of the Ministry of Defence sites.

Rumen Liquor Quality.

Rumen samples were collected from shot animals during the 1990/91 doe season. After evisceration, the rumen-reticulum was opened and the contents thoroughly mixed. A handful of the contents was placed in a muslin bag and the bag was squeezed gently, allowing the expelled liquor to drain into a polythene sample bottle containing 4ml of 40% formalin. The bottle was filled to a marked level which denoted a total volume of 40ml, thus providing an accurate 10% dilution. The bottle was marked with the individual deer code and stored in a cool building, ready for analysis.

The rumen samples were subjected to colorimetric analysis to determine the level of free ammonia using the indophenol-blue reaction (Searle 1984). In the reaction, ammonium-nitrogen is oxidised by sodium hypochlorite and then coupled with a phenolic compound (sodium salicylate) to produce the indophenol-blue colour. The formalin preservative was found to interfere with the assay, as it reacted with some of the reagents. In order to overcome this problem, the free ammonia had first to be released from the rumen liquor, and then reabsorbed in a solvent (boric acid), prior to determination (Ratcliffe 1987). The reagents and procedure essentially follow those described in Allen (1989).

Reagents

a. Nitrogen standards.

Stock solution: 0.191g of dry NH_4CL was dissolved in water and diluted to 500ml to provide a solution of 0.1mg per ml ammonium-nitrogen (actually 0.129mg/ml ammonium).

Working solution: The working solution of 0.01mg per ml ammonium-nitrogen was prepared daily by a ten fold dilution of the stock solution.

b. Combined reagent.

The combined reagent contained 0.125M sodium potassium tartrate (Rochelle salt), 0.11M sodium salicylate, 0.002M sodium nitroprusside and 0.5M sodium hydroxide. The solution was stirred until all constituents were dissolved and stored at +2°C.

c. Sodium hypochlorite solution.

The supplied stock solution (10% available Cl) was diluted to give a solution with 0.15% available Cl.

Procedure

a. Sample pre-treatment.

The rumen liquor was filtered through Whatman 41 paper with the aid of a tap-mounted vacuum pump. 5ml of the filtrate was placed in a 3cm petri-dish. This dish was placed inside a 6ml petri-dish containing 5ml of 2% (w/v) boric acid. Then 2.9g of sodium hydroxide was added and a 5cm petri-dish placed over the reaction as a lid. The addition of the alkali, sodium hydroxide, displaces the equilibrium below to the right, releasing all the ammonia, which is then reabsorbed in the boric acid.

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

The system was left for 16 hours to allow the reaction to go to completion before the level of ammonia absorbed in the acid was estimated.

b. Estimation of ammonium ions absorbed in the boric acid.

Although absolute values of ammonia concentration were not required, a standard curve was constructed daily to ensure the reproducibility of the reaction procedure.

A series of eleven test-tubes was set up containing a pipetted volume between 0 and 1ml (0, 0.1, 0.2, ...0.9, 1.0) of working solution.

A further number of tubes were labelled and set aside for samples.

To each of the tubes containing the standard solution, 0.5ml of blank boric acid was added.

To each sample tube, 0.5ml of boric acid containing the absorbed ammonia from the appropriate reaction dish was added (see above).

From this point standards and samples were treated in an identical way.

3ml of combined reagent was added and each tube was agitated with a bench vortex.

0.4ml of sodium hypochlorite was added.

Water was added to make each tube up to a total volume of 5ml.

The solutions were mixed thoroughly with the vortex and left in a water bath at 40°C for ten minutes.
The absorbence of each tube was measured at 660nm on a spectrometer, against the calibration curve tube containing no standard solution.

For each sample, the determination of free ammonia was carried out twice. Generally, with regard to safety, it was sufficient to follow standard laboratory procedure. However, some of the constituents of the combined reagent are particularly harmful (corrosive and toxic), so this solution was handled with extreme care, wearing protective clothing, gloves and respiration apparatus.

Pellet group counts

Population density can be estimated using the faecal standing crop in a given area. Although it is not possible to calculate accurate densities of animals without detailed information on the defecation rate and the faecal decay rate, this technique has often been used to provide an index of population density for comparisons between different areas (Putman 1984). Standing crop dung group counts were carried out at all Forestry Commission study sites in March/April 1990, October/November 1990 and again in March/April 1991, during the collection of culled material. It was not possible to carry out counts in the Ministry of Defence sites, as the ground vegetation was generally extremely dense due to the open nature of these areas, and therefore, it was often completely impossible to find deposited pellet groups. Furthermore, the habitat in these sites was fragmented and, unlike the Commission sites, not of uniform type. Thus, the decay rate and the proportion of missed pellet groups was likely to be highly variable between sites, and so sites were not directly comparable.

For each Commission site, the proportion of each habitat type was calculated under the following categories:

Restock (up to 7 years old)
Pre-thicket (8 to 14 years old)
Thicket (15 to 27 years old)
Pole (28 to 40 years old)
Mature (over 40 years old)

In each site, a number of points were randomly placed on Commission stock maps, such that the number of these points in each habitat category was directly proportionate to the percentage area of that habitat type. The points were placed to ensure as much as possible of the study site was sampled.

In the field, each point was located and a distance of 100 metres measured on a compass bearing towards the centre of that habitat compartment. This belt transect of 100x1 metre was searched thoroughly for pellet groups, and the number found was recorded. A pellet group was defined as a cluster of six or more pellets (Ratcliffe and Mayle 1992). If dung groups were found on the edge of the transect, they were included in the count only if more than half the pellets fell inside the plot. Where two or more groups had been deposited on the same spot, it was usually possible to separate them on the basis of size and colour. A time allotment of 25 minutes for a single transect was followed to ensure consistency in searching intensity between transects and study sites.

Defecation and pellet decay rates were assumed to be relatively constant across the study sites in the very similar habitat types of the Commission's coniferous plantations. Therefore, the standing crop of pellet groups was expressed as the average number of groups per 100 metre transect, and sites were ranked according to this figure. This provided an index of population density for comparisons between study sites (Staines and Ratcliffe 1987).

2.2.3 Climate

Meteorological data, examined in relation to reproductive performance of different roe populations around Britain, were based on records from the weather station nearest to each study site, and were extracted from monthly reports published by the Meteorological Office (HMSO). From these data, the following six parameters were extracted to describe climatic conditions at each site:

Average monthly winter temperature, °C (November to March)
Average monthly mid-winter temperature, °C (January to February)
Average monthly number of days snow lie (November to March)
Average monthly winter rainfall, mm (November to March)
Average monthly summer temperature, °C (May to June)
Average monthly summer rainfall, mm (May to June)

2.2.4 Genetics

Two methods, starch gel electrophoresis and cranial morphometrics, were employed to determine the genetic relationships of British roe deer.

Cranial Morphometrics

The heads of up to twenty culled females were retained by the rangers at each study site except Bramley (where storage facilities were not available), and were collected during routine visits. The sample sizes available for this analysis are presented in Table 2.1. The heads were cleaned by 'cooking' in a conventional autoclave for approximately one and a half hours. This enabled the flesh to be removed relatively easily, while preserving all the delicate bones of the skull intact. A series of eighteen skull parameters, illustrated in Fig. 2.4, were measured to the nearest millimetre using callipers. Only heads of animals which were at least eighteen months old were used. These measurements essentially followed those used by Lowe and Gardiner (1974), selected to represent all the skull's dimensions, and to detect as many variations in skull morphology as possible. The statistical approach adopted in the analysis of these data is detailed in Chapter 5.

Starch gel electrophoresis

Samples of liver, kidney and muscle tissue were taken from culled female animals at each site during 1990 and 1991. The minimum sample size was set as twenty, but in practice it was not always possible to obtain this number. The samples were placed in labelled polythene sachets and frozen in a domestic freezer at -10°C. The time elapsed from kill to freezing was recorded. If this period exceeded 6 hours the sample was discarded, but, in practice, the time elapsed was usually approximately two hours and always less than four. The tissues were collected from each site within three months and were transported packed in ice. On arrival, each sample was snap frozen in liquid nitrogen and then stored at -70°C until required for electrophoresis (see Richardson et al. 1986).

Theoretical aspect

Gel electrophoresis is a system designed to separate proteins on the basis of their size, shape and charge. Different alleles of the same gene usually produce proteins that have an altered amino acid sequence, and are, therefore, often different in three dimensional shape or electrical charge. Thus electrophoresis of proteins can detect some

A. Condylo-basal Length: The posterior surfaces of the occipital condyles to the most anterior point of the premaxillae (the prosthion).

B. Basilar Length: The median position of the ventral margin of the foramen magnum to the prosthion.

C. Rostrum Length: The orbital margin of the lachrymal to the prosthion of the longer side.

D. Palatal Length: The posterior junction of the palatines (staphylion) to the prosthion.

E. Frontal Length: The prosthion to the alveoli of the second pre-molar.

F. Premaxillary Length: The anterior point to the posterior point of the longer nasal.

G. Nasal Length: The anterior point to the posterior point of the longer nasal.

H. Nasal Breadth: The maximum breadth of the nasals.

I. Maxillae Width: The minimum width of the maxillae.

J. Orbital Breadth: The maximum breadth of the frontals across the orbital processes.

K. Inter-orbital Width: The minimum inter-orbital width.

L. Brain Case Breadth: The maximum breadth of the brain case.

M. Condylar Breadth: The maximum condylar breadth.

N. Nasal Depth I: The minimum depth between the nasal suture at the proximal end of the maxillae and the inter-maxillary suture.

O. Nasal Depth II: The minimum depth between the inter-nasal/frontal suture and the inter-maxillary/palatine suture.

P. Palatal Depth: The minimum depth between the staphylion and the inter-frontal suture.

Q. Rear Length I: The distance between the inter-nasal/frontal suture and the posterior of the skull.

R. Rear Length II: The distance between the inter-maxillary/palatine suture and the median position of the ventral margin of the foramen magnum.

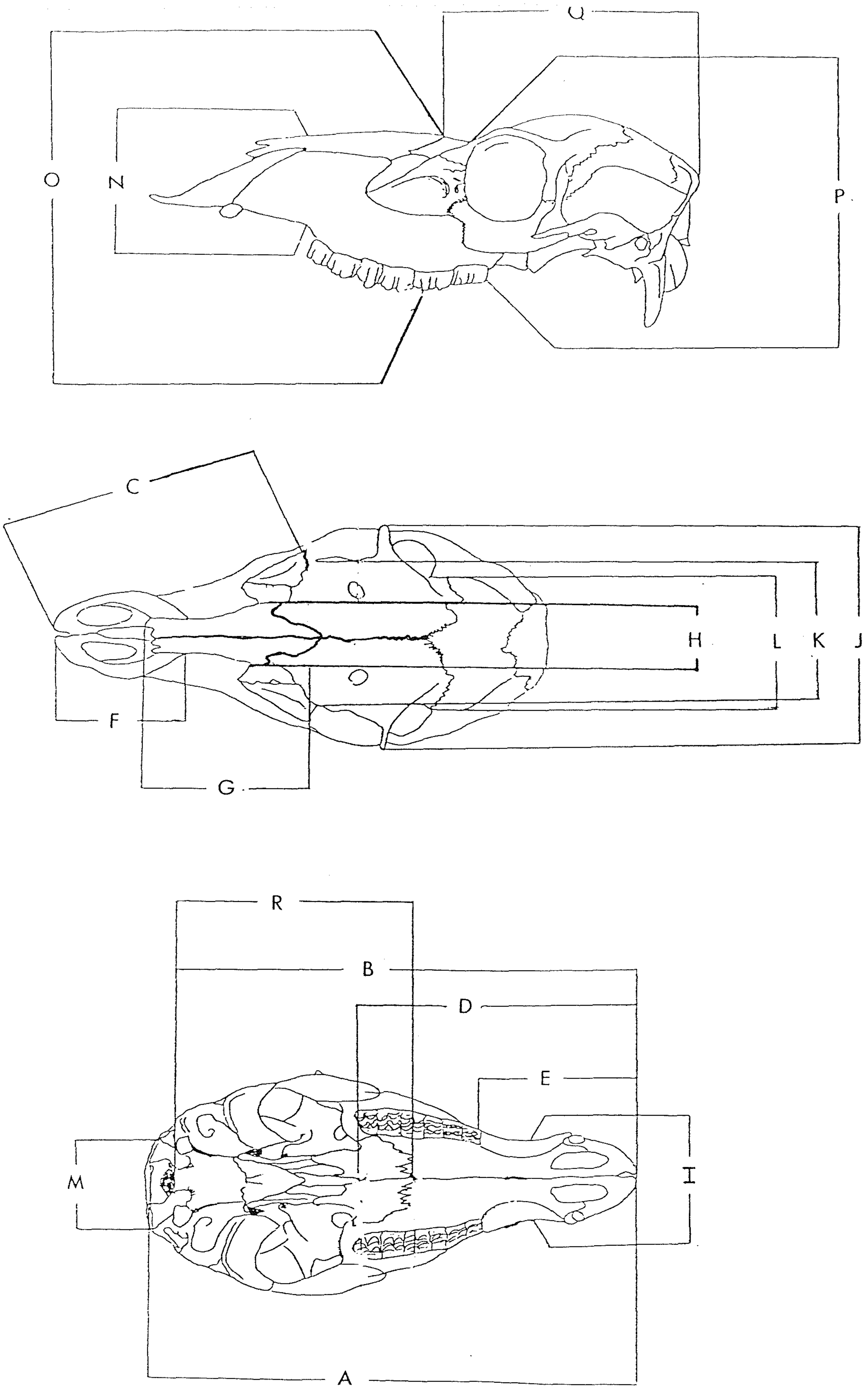


Figure 2.4: Eighteen skull measurements for cranial morphometrics (key opposite).

of the differences in the primary genetic code between alleles. The gel acts as a molecular sieve, allowing proteins to migrate through, but providing some resistance to this movement. The molecules migrate in response to an electrical gradient applied across the gel. Most proteins are negatively charged at the pH levels employed in electrophoresis, and so migrate towards the anode. The speed of this migration depends on the size, shape and charge, and therefore, protein products of different alleles at the same locus will often migrate different distances. The relative mobilities of these proteins (usually enzymes) can be visualised by a number of techniques. The most common of these is to use the enzymic reaction for which the particular protein is a catalyst to produce a coloured product identifying the location of the protein on the gel. However, not all allelic differences at the DNA level will be detected by this method. The redundancy of the genetic code means that approximately one third of base substitutions do not result in a change in the amino acid coded for. Furthermore, even where base substitution does cause an alteration in the amino acid sequence of the protein product, a proportion of these changes do not confer an altered shape or net charge on the protein molecule. Therefore, the level of variability detected by this method is likely to be an underestimate. (see Smith 1976).

Starch was selected as the electrophoretic medium for a number of reasons. Firstly, it was the cheapest method, and therefore, allowed the number of samples screened at a number of enzyme loci to be maximised, given set financial limitations. Starch gels can be sliced and stained for more than one enzyme, making them more cost efficient, but also more efficient in the use of time and samples. Finally, starch is the most widely used medium, and hence, the composition of reagents is well established. In particular, it is the most common medium employed in genetic studies on ungulates, especially roe deer, and therefore, was used to make the results of this study directly comparable to those obtained by others.

Preparation

a. Gels: The gels were prepared the evening prior to an electrophoretic run to allow adequate time for them to set, essentially following the procedure of Smith (1976, Chapter 7), but were always used the following morning. In routine screening, four gels were run simultaneously so the preparation procedure below was repeated twice.

For two gels of 12% concentration, 66g of high grade hydrolysed starch (Connaught Laboratories, London) was placed in a large conical flask with a side arm and 400ml of the appropriate gel buffer added (see below). The mixture was swirled by hand for approximately one minute to suspend the starch, and then heated over a bunsen burner, maintaining constant agitation (using an asbestos glove). The mixture became progressively more viscous until, at approximately 90°C, the mixture suddenly became clear and fluid again. At this point, the flask was removed from the heat, and the side arm of the flask was attached to a water powered vacuum pump via a suitable trap. A stopper was inserted and the mixture was evacuated for two minutes until all air bubbles were removed, while stirring constantly. The mixture was then poured into two gel moulds, each comprising of a perspex frame (internal dimensions 180x140x6mm) on top of a glass plate (200x160mm). Immediately, a second glass plate of the same dimensions was positioned along one end of the frame, and gently lowered onto the gel, taking care not to trap any air bubbles. The plate was pushed down to within 1mm of the gel frame to allow for contraction as the gel cooled. The gels were left at room temperature over night, but transferred to the cold room (4°C) at least an hour before use.

b. Samples: The samples were kept on ice at all times during preparation to prevent enzyme degradation. A small piece of the appropriate tissue (see below), about 2x2x2mm, was taken from the frozen block using a sharp knife and placed in a labelled eppendorf tube, and the sample block immediately returned to the freezer. Generally, between ten and twelve samples were run on each gel so the procedure was repeated until there were a total of about fifty tubes, each containing an individual sample. To each tube, 20 microlitres of homogenising buffer (see below) was added, and the tissue was homogenised using a micropestle (the pestle was washed and dried after each sample). The tubes were then centrifuged for a short pulse (approximately 5 seconds) at 13000g.

Gel loading

Samples were loaded on to the gel following the procedure outlined by Manlove et al. (1975). The top plate was carefully removed from the gel and, while still inside the mould, the gel was sliced vertically along its shorter dimension to form an origin, at a point 5cm from one end of the gel. A piece of filter paper (Whatman No. 1), no bigger than 0.5x0.5cm, was dipped into the supernatant of each sample tube, soaked for five seconds and then blotted on tissue to remove excess liquid. The paper was then loaded by prising the gel apart at the origin, and placing the paper between the cut surfaces (the forceps were washed and dried between the loading of each sample). Sample papers were arranged along the gel spaced at 1cm intervals, leaving at least 1cm clear at each side of the gel. A paper containing ferritin was also loaded, to provide a marker with constant relative mobility. When all papers were loaded, a few specks of bromophenol blue were dotted on the surface of the gel, just in front of the origin. This dye allows the progress of the buffer through the gel to be visualised.

Electrophoresis

Approximately 250ml of the appropriate tank buffer (see below) was poured into each well of a standard electrophoretic tank. The gel was placed in the tank with the origin nearest the cathodal well. A cloth wick was soaked in the running buffer, the folded edge drawn out of the well and applied, one to either end of the gel. Care was taken to ensure good contact between the gel and the wicks, and that the wicks dipped evenly into the running buffer of each well. The gel was then covered with a plastic sheet to prevent desiccation. Gels were run at a constant current of 20 or 35mA (see below), for between three and a half and four hours at 4°C.

Gel staining

Staining procedure followed the system set out by Richardson et al. 1986). The sample papers were removed from the gel and the gel frame taken away. The gel was then cut horizontally into three slices using fine grade steel wire mounted on a cutting board, and perspex formers of the appropriate height (Smith 1976, Chapter 7). Each slice was transferred to a separate glass plate, cut surface uppermost, for staining. This procedure allowed a single gel to be stained for three different enzyme systems. An appropriate staining solution (see below) was mixed with an equal volume of 2% agar solution (maintained at 65°C), taking care to add the agar slowly while stirring to prevent enzyme denaturation, and the mixture was poured over one slice of the gel. Once the agar overlay had set, the plates were incubated in the dark at 37°C. The gels were viewed at least every fifteen minutes to monitor the development of the coloured bands. When development was optimal, the banding pattern was recorded on diagram sheets and, where appropriate, the gel was photographed.

Screening strategy

Initially, a series of twenty enzyme systems were investigated. By testing a variety of buffers, tissue types and electrophoretic conditions (pH, strength of current, running time), ten of these systems, constituting fifteen putative enzyme loci, were found to produce banding patterns that were clear and readily interpretable. The running conditions and tissues used for each system are summarised in Table 2.2. Screening proceeded, running samples from a variety of populations on a single gel. When an electrophoretic variant was identified, repeat gels were carried out to provide confirmation. Putative variant samples were only considered actually different, where there was significant mobility differences when they were run adjacent to a sample of known mobility on the same gel. Thereafter, examples of each of the variants was included on every subsequent gel to provide controls for direct comparisons (see Richardson et al. 1986 for detailed strategical approaches).

Reagents

a. Buffer Systems.

i. Citrate morpholene.

Tank buffer: 0.04 M citric acid
adjust to pH 7.4 with N(3 aminopropyl) morpholene
Gel buffer: dilute tank buffer 1:9

ii. Poulik (see Smith 1976).

Tank buffer: 0.3M boric acid
0.05M NaOH (adjust to pH 8.2)
Gel buffer: 0.076M Tris
0.005 Citric acid (adjust to pH 8.7)

Table 2.2: Electrophoretic running conditions for each enzyme system.

Enzyme System	Buffer	pH	Current (mA)	Run Time	Tissue Used
Adenylate kinase	Poulik	8.7/ 8.2	20	3.5 hrs	Liver/ Kidney
Glucose-6-phosphate dehydrogenase	Citrate morpholene	7.4	35	4 hrs	Liver
Glycerol-3-phosphate dehdrogenase	Citrate morpholene	6.1	25-30	4 hrs	Liver
Isocitrate dehydrogenase	Citrate morpholene	7.4	35	4 hrs	Liver
Lactate dehydrogenase	Citrate morpholene	6.1	25-30	4 hrs	Liver
Malate dehydrogenase	Citrate morpholene	6.1	25-30	4 hrs	Liver
Malic enzyme	Poulik	8.7/ 8.2	20	3.5 hrs	Liver/ Kidney
Mannose phosphate isomerase	Citrate morpolene	7.4	35	4 hrs	Liver
Phosphoglucose isomerase	Poulik	8.7/ 8.2	20	3.5 hrs	Liver/ Kidney
Superoxide dismutase	Poulik	8.7/ 8.2	20	3.5 hrs	Liver/ Kidney

b. Homogenisation Buffer.

Stock solution:	Beta mercaptoethanol	3.12 microlitres
	Triton	25.0 microlitres
	PMSF	50mg
	0.1% Sodium azide	25.0 microlitres
	in 5ml of distilled water	
Working buffer:	0.1M Tris-HCl pH 8.0	200 microlitres
	Stock solution	306 microlitres
	Distilled water	494 microlitres
	NAD or NADP	10mg

c. Stains.

All stain recipes below were adapted from standards given in Brewer and Sing (1970), Shaw and Prasd (1970), Selander et al. (1971) and Siciliano and Shaw (1976), (the numbers in brackets refer to the E.C. number, see Richardson et al. 1986).

i. Adenylate kinase (2.7.4.3)

Glucose	50mg
ADP	25mg
NADP	15mg
$MgCl_2$	20mg
0.1M Tris HCl pH8	25ml
PMS	10mg
MTT	10mg
Hexokinase	20 units
Glucose-6-phosphate dehydrogenase	30 units

ii. Glucose-6-phosphate dehydrogenase (1.1.1.49)

Glucose-6-phosphate	150mg
NADP	16mg
$MgCl_2$	10mg
0.1M Tris HCl pH8	20ml
MTT	5mg
PMS	8mg

iii. Glycerol-3-phosphate dehydrogenase (1.1.1.8)

Glycerol-3-phosphate	400mg
Sodium pyruvate	300mg
NAD	30mg
0.1M Tris HCl pH8	25ml
MTT	8mg
PMS	8mg

iv. Isocitrate dehydrogenase (1.1.1.42)

Isocitric acid	30mg
NADP	8mg
$MgCl_2$	25mg
0.1M Tris HCl pH8	25ml
MTT	5mg
PMS	8mg

v. Lactate dehydrogenase (1.1.1.27)

D-L Lactic acid	400mg
NAD	60mg
$MgCl_2$	20mg
0.1M Tris HCl pH8	20ml
MTT	5mg
PMS	8mg

vi. Malate dehydrogenase (1.1.1.37)

L Malic acid (sodium salt)	200mg
NAD	60mg
$MgCl_2$	25mg
0.1M Tris HCl pH8	25ml
MTT	10mg
PMS	10mg

vii. Malic enzyme (1.1.1.40)

L Malic acid (sodium salt)	250mg
NADP	8mg
$MnCl_2$	25mg
0.1M Tris HCl pH8	25ml
MTT	5mg
PMS	8mg

viii. Mannose phosphate isomerase (5.3.1.8)

Mannose-6-phosphate	80mg
NADP	8mg
$MgCl_2$	40mg
0.1M Tris HCl pH8	25ml
MTT	8mg
PMS	8mg
Phosphoglucose isomerase	14 units
Glucose-6-phosphate dehydrogenase	20 units

ix. Phosphoglucose isomerase (5.3.1.9)

Fructose-6-phosphate	40mg
NADP	8mg
$MgCl_2$	25mg
0.1M Tris HCl pH8	25ml
MTT	5mg
PMS	8mg
Glucose-6-phosphate dehydrogenase	15 units

x. Superoxide dismutase (1.15.1.1)

NADP	16mg
$MgCl_2$	10mg
0.1M Tris HCl pH8	25ml
MTT	10mg
PMS	10mg

Safety precautions generally followed standard laboratory procedure, but when handling particularly hazardous reagents, such as PMSF, sodium azide, PMS, and MTT which are potential carcinogens and mutagens, protective clothing and breathing apparatus were worn.

CHAPTER 3

VARIATION IN REPRODUCTIVE SUCCESS

3.1 INTRODUCTION

The equilibrium size of an animal population can be seen as the net balance of birth, death, immigration and emigration. While migration is essentially a behavioural response of individuals to the conditions they experience, the birth rate and the mortality rate of a population reflect the reproductive success and survivorship of the individuals that constitute that population. An individuals life time reproductive success is determined by its ability to survive, the age at which it first breeds, the number of times it gives birth and the number of offspring delivered per pregnancy. From this point of view performance of a population can be measured by investigating the average of these parameters within the population.

This chapter describes the degree of variation in female reproductive success, and hence potential recruitment, found within and between roe deer populations across Britain. At the population level, this variation can manifest itself in three ways: firstly, through variation in the age at which the individuals first breed, secondly, through variation in the percentage of fecund adults in the population, and lastly, in multiparous species such as roe deer, through variation in average litter size.

Each of these three parameters has been shown to vary considerably between populations of roe deer. Although roe are generally thought to breed for the first time in their second year (e.g. Strandgaard 1972b; Pole 1973; Kaluzinski 1982a), some instances of precocious reproduction of kids have been recorded (Borg 1970; Ratcliffe and Mayle 1992). However, in some populations, most does may not achieve breeding condition until they are over two years old (Borg 1970). The percentage of adult does breeding is generally high, often estimated as approximately 90% or higher (e.g. Chaplin et al. 1966; Prusaite et al. 1977; Kaluzinski 1982a). However, values 20 or 30% lower were reported by Bathurst (1988) for several populations of roe deer in Britain. Roe are multiparous and, although does will generally only produce twins (Strandgaard 1972b), exceptional cases of individual animals carrying five (Myrberget and Milton 1971), or even six (Cherkasov 1962) embryos have been recorded. The average potential litter size of different populations has been estimated to vary considerably, between a recorded minimum of 1.6 (Ellenberg 1978) and maximum of 2.2 (Borg 1970) kids per reproductively active female.

A further, qualitative variation in reproductive output involves modification of the sex ratio of progeny. Although this does not directly affect the number of offspring produced, it may have an important impact on effective population recruitment, for example, through altering rates of dispersal. In addition, if either of the sexes experiences a greater degree of variation in reproductive success, the sex of any offspring produced may also affect the inclusive fitness of an individual (Trivers and Willard 1973). Investment of reproductive effort in this sex may, therefore, be associated with a greater potential gain in fitness for the parent, but may also be a riskier strategy. There is little recorded information on the sex ratio at birth in roe deer (but see Prior 1968; Borg 1970; Strandgaard 1972b), however, Ellenberg (1978) found widely differing ratios between some populations of central Europe.

While roe deer generally conceive in a brief, synchronised rutting period in late July or early August, does do not give birth until the following May (Prior 1968). Amongst mammals there is a good general relationship between gestation period and body size (Clutton-Brock and Harvey 1978), however, judged on this scale, the apparent ten month gestation of roe deer seems overly protracted. This is accounted for by a delay of approximately five months in the implantation of the fertilised egg (Aitken 1974), commonly termed embryonic diapause (Short and Hay 1966), and is purportedly an adaptation to northern climates, ensuring parturition will occur in the relatively hospitable spring or summer months (Loudon 1982). Following fertilisation, the blastocyst enters a state of suspended development or diapause, and does not implant in the uterus wall until late December or early January. However, during this interim period, pregnancy can still be detected by the presence of corpora lutea, yellowish glands which develop inside the ovary and persist only if fertilisation occurs. Throughout the text of this thesis the presence of corpora lutea is taken to be indicative of successful ovulation with fertilisation, but not necessarily implantation, of the egg.

Therefore, because of the reproductive cycle of roe deer, each of the three measures of reproductive success, outlined above, can be estimated for a population by examination of both the number of corpora lutea in the ovaries (indicating the number of fertilised ovulations) and the number of embryos in the uterus (the proportion of fertilised ova successfully implanted). Thus, this chapter characterises variation in reproductive success of populations of roe deer across Britain using seven parameters, six quantitative and one qualitative:

a. Age at first breeding
    i) Ovulation (corpora luteum(a) present)
    ii) Pregnancy (embryo(s) present)

b. Percentage of post-pubertal animals reproductively active
    i) Ovulation (corpora luteum(a) present)
    ii) Pregnancy (embryo(s) present)

c. Average litter size
    i) number of corpora lutea present
    ii) number of embryos present

d. Sex ratio of embryos

Some preliminary analysis of reproductive performance of British roe deer populations has suggested a high degree of variability in some of these parameters, both between sites, and between sampling years within a single site. Ratcliffe and Mayle (1992) described large differences across Britain in potential recruitment to the population, as measured by average number of corpora lutea and average number of foetuses per doe, in seven Forestry Commission sites. They also identified actual differences in juvenile recruitment to the population, calculated from winter counts of the average number of kids at foot. Bathurst (1988), also using data from Forestry Commission cull records, found significant differences between sites in age at first breeding, percentage of adults pregnant and potential litter size. In addition, significant variation between years in percentage of animals pregnant, but not in litter size, was identified at some sites.

The variation in reproductive performance of roe deer in Britain documented in this chapter is derived from data on fifteen study sites from across Britain (nine Forestry Commission, six Ministry of Defence). Thus, this study includes most of the habitat types that roe deer occupy in Britain, from commercial coniferous plantations to coastal dunes, and encompasses a very wide geographic range with highly variable climatic features. The data set covers a period of between six and twenty one years for each study site, and comprises of a total of over five thousand individual animals. However, it should be noted that only embryo data, and not corpora lutea data were available for Ministry of Defence sites.

3.2 RESULTS

3.2.1 The Effects of Age on Reproductive Performance

Age at First Breeding

Maturation of roe deer is a rapid process, particularly for females, and does generally complete growth within the first eighteen months of life (Prior 1968). Sexual maturity is also reached equally early, with females often conceiving at fourteen months. However, precocious reproduction of kids has been documented only at a few locations in Britain (Ratcliffe and Mayle 1992).

Table 3.1 shows the reproductive performance of kids and yearlings, in each study site, as measured by percentage of animals with corpora lutea and/or foetuses. Due to small sample sizes the data were pooled for all years.

i) Percentage Ovulation

From the table, it is immediately obvious that percentage ovulation rates (animals with recognisable corpora lutea) approach 100% for yearlings in all sites, the sole exception being Queens. Ovulation rates among kids were low and generally less than 10%, except at Pickering, where almost half of all kids sampled had ovulated.

An analysis of variance without replication (here after ANOVA) on arc-sine transformed percentages failed to reveal any significant differences between sites for either age group, and yearling ovulation rates were not significantly lower than those of adults at the same site (data for adults is presented in Table 3.2).

ii) Percentage Pregnancy

Table 3.1 also illustrates the degree of variability between sites in percentage of yearlings and kids with implanted foetuses. Clearly, kid pregnancies were rare and only occurred to any significant extent at Pickering. Yearling pregnancy, however, was a relatively common occurrence, and was recorded at over 50% in more than half the study sites. However, the difference between sites in yearling pregnancy rate was not significant.

Table 3.1: Percentage of does ovulating and pregnant among a. kids and b. yearlings (# indicates a percentage based on less than six samples).

a. Kids

Study Site	Percentage Ovulation	Sample Size	Percentage Pregnancy	Sample Size
Alice Holt	11	47	0	24
Thetford	5	110	1	96
Ringwood	4	51	0	35
Spadeadam	1	81	0	51
Kershope	9	103	0	49
Pickering	48	232	8	167
Craigellachie	0	75	0	24
Queens	0	9	0#	4
South Strome	17	12	0#	1
Salisbury Plain	-		0	43
Lulworth	-		0	7
Aldershot	-		0	7
Bramley	-		0	23
Stanford	-		0	25
Kirkcudbright	-		0	16

b. Yearlings

Study Site	Percentage Ovulation	Sample Size	Percentage Pregnancy	Sample Size
Alice Holt	100	51	96	27
Thetford	98	181	81	118
Ringwood	100	12	71	7
Spadeadam	92	25	56	16
Kershope	95	38	35	23
Pickering	99	67	74	39
Craigellachie	97	107	62	34
Queens	74	19	55	11
South Strome	100	11	100#	1
Salisbury Plain	-		36	14
Lulworth	-		75	12
Aldershot	-		75#	4
Bramley	-		83	6
Stanford	-		44	9
Kirkcudbright	-		45	11

Table 3.2: Percentage of does ovulating and pregnant a. adults (2-5 yrs) and b. mature (6+ yrs), # indicates percentages based on less than six samples.

a. Adults

Study Site	Percentage Ovulation	Sample Size	Percentage Pregnancy	Sample Size
Alice Holt	99	156	92	101
Thetford	99	271	91	170
Ringwood	99	127	86	80
Spadeadam	98	138	73	82
Kershope	100	161	68	92
Pickering	99	298	78	190
Craigellachie	100	179	70	67
Queens	100	52	64	25
South Strome	97	69	77	26
Salisbury Plain	-		84	58
Lulworth	-		79	34
Aldershot	-		81	27
Bramley	-		86	37
Stanford	-		96	28
Kirkcudbright	-		85	26

b. Mature

Study Site	Percentage Ovulation	Sample Size	Percentage Pregnancy	Sample Size
Alice Holt	100	30	88	16
Thetford	97	73	85	46
Ringwood	98	44	80	30
Spadeadam	100	50	79	33
Kershope	98	45	63	24
Pickering	97	70	82	49
Craigellachie	100	40	71	14
Queens	96	24	75	12
South Strome	100	23	91	11
Salisbury Plain	-		78	9
Lulworth	-		77	26
Aldershot	-		100#	1
Bramley	-		50#	2
Stanford	-		80#	5
Kirkcudbright	-		100#	2

In many cases, notably Alice Holt, Thetford, Pickering, Queens, South Strome, Lulworth, Aldershot and Bramley pregnancy rates approached those of the adult age classes. However, overall, the difference between yearling and adult pregnancy rates within each site was very highly significant (ANOVA: $p<0.001$), (data for adults are presented in Table 3.2). This discrepancy in pregnancy rate between yearling and adults (Fig.3.1) was most apparent at Spadeadam, Kershope, Salisbury Plain, Stanford and Kirkcudbright.

Age Related Variation in Reproductive Success of Post-Pubertal Animals

From these analyses, it is clear that roe does can not be considered as adults with respect to reproduction until they have passed their second birthday. In order to compare reproductive success between populations, or between years within a single population, we must first establish to what degree, if any, subsequent performance may vary with increasing age of an adult.

Therefore, reproductive performance of adults in individual age classes of two years old, three years old, four years old, five years old, six years old and animals older than six was examined within each study site. Yearlings were not included as it was already established that reproductive rates among this age class were lower. A oneway ANOVA for each forest showed that, among adults, there was little identifiable pattern of change in fecundity with age, whether assessed by average number of corpora lutea or average number of foetuses per doe. In only one case was the variation across these classes found to be significant (Spadeadam $p<0.05$). This irregularity seemed to be due to a low fecundity rate among two and three year olds at this site, although the shortfall in these groups was not significant when compared against each of the other age classes in a multiple range test.

Thus, it seems that variation in fecundity with age, of animals of two years and older, is minimal. However, inspection of the data suggested there may be a slight decline in fecundity among older animals at some sites. Therefore, the analysis was repeated in a series of two way ANOVAs of reproductive performance against study site and animal age, but, in this case, with adults split into just two sub-classes, adults (2-5 years) and mature (6 years plus). Yearlings were also included in the analysis as a separate group.

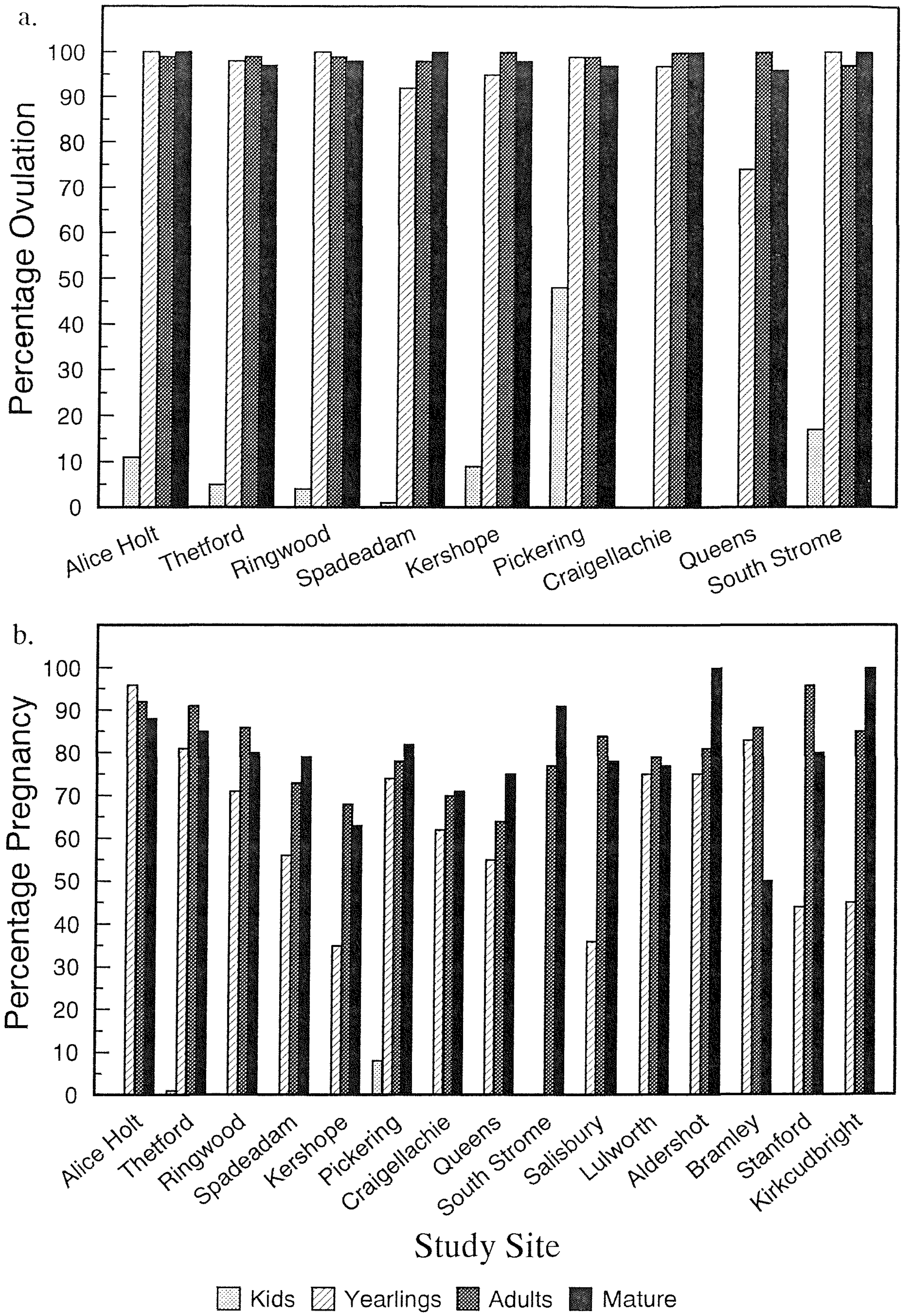


Figure 3.1: The percentage of does a. ovulating and b. pregnant among kids, yearlings, adults (2-5 years) and mature animals (6+ years) in each study site.

i) Percentage Ovulation

As has already been described above for yearlings, percentage ovulation rates for animals over one year old approached 100%. Table 3.2 gives the values separately for adults and mature animals and shows the lowest rate to be 96% (Queens: mature). It is, therefore, not surprising that a two way ANOVA could detect no differences between age classes within each study site.

ii) Average Number of Corpora Lutea Per Ovulating Doe

The number of corpora lutea per ovulating doe was highly variable ($p<0.001$) between age classes within each study site (Table 3.3). A one way ANOVA for each study site in turn found that in four out of nine cases there was significant variation between age categories (Thetford, Kershope, Pickering, Craigellachie).

However, it seems that the vast majority of this variation is due to a reduction of corpora lutea production among yearlings, as at each of these four sites yearling values were significantly lower ($p<0.05$) than either or both of the adult age classes. In contrast, the difference in corpora lutea production between the two adult age classes was never significant. If any pattern did exist, it was that mature animals tended to produce slightly more corpora lutea than the adult age class, as was the case in four of the study sites.

iii) Percentage Pregnancy

The wide variation in pregnancy rates between sites, evident for yearlings, is also apparent in Table 3.2 for both adult and mature age classes. In addition, a two way ANOVA without replication showed there to be very highly significant differences ($p<0.001$) between age classes within each study site.

However, from Table 3.2, it is apparent that again there is no consistent pattern across the study sites in age related pregnancy for animals over two years old, and the significant result from the two way ANOVA can be attributed purely to the shortfall in yearling pregnancy. In fact, in seven out of the fifteen cases mature pregnancy rates marginally exceeded those of the corresponding adult class, but the situation was reversed at eight sites.

Table 3.3: Average number of corpora lutea and foetuses per doe a. adults (2-5 yrs) and b. mature (6+ yrs), standard error in brackets; # denotes values based on less than six samples.

a. Adults

Study Site	Average No. Corp. Lutea	Sample Size	Average No. Foetuses	Sample Size
Alice Holt	2.09 (.03)	154	1.90 (.05)	91
Thetford	1.98 (.03)	269	1.80 (.04)	135
Ringwood	1.78 (.04)	126	1.69 (.06)	67
Spadeadam	1.64 (.05)	129	1.43 (.06)	60
Kershope	1.86 (.03)	161	1.60 (.06)	62
Pickering	2.20 (.06)	294	1.97 (.04)	148
Craigellachie	1.71 (.04)	179	1.72 (.08)	46
Queens	1.31 (.07)	52	1.00 (.00)#	5
South Strome	1.78 (.06)	67	1.42 (.12)	19
Salisbury Plain	-		1.94 (.05)	49
Lulworth	-		1.70 (.09)	27
Aldershot	-		1.91 (.11)	22
Bramley	-		2.00 (.08)	32
Stanford	-		1.93 (.05)	27
Kirkcudbright	-		1.82 (.08)	22

b. Mature

Study Site	Average No. Corp. Lutea	Sample Size	Average No. Foetuses	Sample Size
Alice Holt	2.10 (.12)	30	1.85 (.10)	13
Thetford	2.08 (.09)	71	1.57 (.09)	35
Ringwood	1.93 (.08)	43	1.63 (.10)	24
Spadeadam	1.78 (.07)	50	1.73 (.09)	26
Kershope	1.95 (.06)	44	1.80 (.11)	15
Pickering	2.22 (.07)	68	2.00 (.07)	40
Craigellachie	1.60 (.08)	40	1.20 (.13)	10
Queens	1.22 (.09)	23	1.33 (.33)#	3
South Strome	1.74 (.09)	23	1.50 (.17)	10
Salisbury Plain	-		1.71 (.18)	7
Lulworth	-		1.75 (.12)	20
Aldershot	-		2.00 (-)#	1
Bramley	-		1.00 (-)#	1
Stanford	-		1.50 (.29)#	4
Kirkcudbright	-		2.00 (.24)#	2

iv) Average Number of Foetuses Per Pregnant Doe

Table 3.3 also presents the average number of foetuses per pregnant doe for each study site. This parameter varied very significantly ($p<0.001$) between age categories within each study site.

A one way ANOVA found significant variation between age categories at five out of fifteen study sites. Once again, the majority of variation was due to the discrepancy in production between yearlings and the two adult age classes (yearling foetus production was significantly lower than one or both of the adult age classes at three sites). However, a degree of inconsistency was once again detected: the yearling age class was more productive than one (or both) of the age classes in five out of fifteen cases, although this was never significant statistically.

In pairwise comparisons, adults had more foetuses on average than mature animals at seven of the sites (significantly so in two cases, $p<0.05$), but the situation was reversed at eight sites, although only significantly once ($p<0.05$), in the anomalous case of Spadeadam previously referred to. This lack of a consistent pattern was born out in the two way ANOVA, where the interaction term of age and study site was very highly significant ($p<0.001$).

3.2.2 Variation in Reproductive Success Within and Between Study Sites

Within Study Sites

In addition to variation in reproductive success between study sites and between age categories, Bathurst (1988) found that percentage pregnancy rates, but not average number of corpora lutea or foetuses, varied significantly between years within a single site. Therefore, similar analyses were carried out here to assess the degree of annual variation in reproductive success within sites. Since the analysis in the above subsection revealed very few differences in fecundity between adults of different ages, the data were considered with all animals over two years old pooled into a single adult age class. The analyses were then repeated for yearlings separately.

i) Percentage Ovulation

A two way ANOVA without replication (factors study site and year) revealed no differences in percentage ovulation between years within any site. Values for all forest-year groups approached 100%.

ii) Average Number of Corpora Lutea Per Ovulating Doe

Table 3.4 illustrates the degree to which the three variable measures of adult reproductive success vary between years for three study sites, including Alice Holt and Thetford. These two sites, with the longest span of available data, exhibited a relatively high degree of year to year variation in performance.

A high degree of variation within a site between years in corpora lutea production was identified ($p<0.001$). However, in almost all study sites, with the exceptions of Alice Holt and Thetford, data are only available from 1983 onwards. When the analysis was restricted to 1983 to 1990, the variation between years was much reduced, although still remaining significant ($p<0.05$).

The effect of year was further investigated by means of a one way ANOVA on each study site in turn. In five out of nine cases the year to year variation was found to be significant at the five percent level, and at a further two sites, Pickering and Ringwood, the result was borderline ($p=0.053$ and $p=0.059$ respectively). However, no consistency was found in the effect of a particular calendar year across the study sites; a highly productive year in one site did not necessarily correspond to productive years at other locations. Thus, the pattern of year to year variation within a site was particular to that site. Pairwise comparisons of individual years within a site in a multiple range test identified only a very few significant differences.

This is illustrated in Table 3.4, where no consistent pattern can be distinguished and was emphasised by the very highly significant interaction term ($p<0.001$) in the two way ANOVA.

When the same analysis was repeated for yearlings, it revealed no significant variation in corpora lutea production within a site between years.

Table 3.4: The percentage of does pregnant, and the average number of corpora lutea and foetuses per doe in each year of the study, at Alice Holt, Thetford and Pickering (includes all animals over 2 years old).

Year	**Average Number of Corpora Lutea**			**Percentage Pregnancy**			**Average Number of Foetuses**		
	Alice Holt	Thetford	Pickering	Alice Holt	Thetford	Pickering	Alice Holt	Thetford	Pickering
1970	2.00	-	-	100	-	-	2.00	-	-
1971	2.33	-	-	100	-	-	2.33	-	-
1972	1.75	-	-	100	-	-	1.67	-	-
1973	2.09	-	-	100	-	-	1.80	-	-
1974	2.12	-	-	100	-	-	1.60	-	-
1975	2.00	-	-	100	-	-	1.33	-	-
1976	2.33	-	-	100	-	-	2.20	-	-
1977	2.30	-	-	100	-	-	2.40	-	-
1978	2.11	-	-	100	-	-	1.92	-	-
1979	2.00	1.96	-	100	88	-	1.82	1.81	-
1980	2.27	1.97	-	100	97	-	1.63	1.82	-
1981	2.50	2.15	-	100	94	-	3.00	1.75	-
1982	2.14	2.04	2.42	100	94	91	2.33	1.81	2.30
1983	1.50	2.06	2.17	67	86	60	2.00	1.79	1.83
1984	2.00	-	2.24	100	-	60	1.86	-	2.06
1985	2.13	-	2.20	83	-	90	2.00	-	1.97
1986	1.88	-	2.23	60	-	86	2.00	-	1.96
1987	2.50	-	2.36	50	-	91	2.00	-	2.00
1988	2.00	-	2.10	80	-	60	1.88	-	2.10
1989	2.00	1.86	2.14	-	81	91	-	1.42	1.90
1990	2.07	1.83	2.07	75	81	84	1.78	1.75	1.95

iii) Percentage Pregnancy

A two way ANOVA without replication (study site/year) found significant variation in percentage adult pregnancy within sites between years ($p<0.05$), when all available data were included. However, when, as above, the analysis was restricted to the period 1983 to 1990 only, the variation between years within a site in percentage pregnancy rates was no longer significant.

Data for the period prior to 1983 concerned only two study sites, Alice Holt and Thetford, and it was at these sites where year to year variation in the pregnancy rate was most apparent. In both these cases, the rate of pregnancy has tended to decrease with time (Table 3.4). This decline was particularly apparent at Thetford where the drop constituted a potentially significant fall (circa 10%) over the six year span where there were no data available for this site.

A similar analysis on the percentage of pregnant yearlings found no significant variation between years within a study site, although this analysis was confounded by small sample sizes.

iv) Average Number of Foetuses Per Pregnant Doe

No significant variation in foetus production was found within a site between years for either adults or yearlings. Neither was the site-year interaction term significant, and no pattern in the effect of year on foetus production across forests was discernable. Identical results were obtained whether using the full data set, or restricting the years included in the analysis to the period 1983 to 1990.

A one way ANOVA confirmed this lack of inter-year variation: of the fifteen study sites in the analysis, the variation between years in foetus production was significant only at Alice Holt ($p<0.05$). Table 3.4 shows the yearly average number of foetuses per doe for three sites, including Alice Holt.

Between Study Sites

The above section has illustrated that fecundity varies little from year to year within a study site. However, the fecundity of yearlings has been demonstrated to be considerably lower than that of older animals in the same population. Among animals over two years old there may be some age related differences in reproductive success, but no consistent pattern was evident. Therefore, to compare reproductive performance between study sites, the data were pooled for all years, but the three age categories, yearling, adult (2-5 years) and mature (6 years plus), were retained.

i) Percentage Ovulation

There were no significant differences in the ovulation rate of adults or yearlings between study sites. Values approach 100% in all cases (Tables 3.1 and 3.2).

ii) Average Number of Corpora Lutea Per Ovulating Doe

The number of corpora lutea produced per doe was highly variable between study sites within each age class (ANOVA: $p<0.001$).

Pickering, Alice Holt and Thetford had higher than average number of corpora lutea per doe in both the adult age sub-classes (Table 3.3), as well as in yearlings. In contrast, Queens, and to a lesser extent, Craigellachie and Spadeadam exhibited considerably lower values. A Scheffes multiple range test found that the pairwise differences between the study sites in these high productivity and low productivity groups was always significant for adults (Table 3.5a) and usually so for the mature class (Table 3.5b).

The same test for yearlings revealed a similar pattern of differences (Table 3.5c), although in this case, corpora lutea production among yearlings at Ringwood was also high.

iii) Percentage Pregnancy

A two way ANOVA without replication (study site/age) revealed highly significant differences in percentage pregnancy between study sites within each age class ($p<0.001$).

Because the data were in percentage form, it was not possible to statistically test these differences for particular pairs of sites by means of a multiple range test (such tests require standard deviations, which cannot be calculated from a single percentage value).

Table 3.5a: Multiple range test on the average number of corpora lutea per ovulating doe for adults (2-5 yrs), (* denotes significance at $p<0.05$).

Study Site	Qu'ns	Spadeadam	Crg'ach	Sth. Strm	Ringwood	Kershope	Thetford	Alice Holt	Pickering
Queens									
Spadeadam									
Craigellachie	*								
Sth Strome	*								
Ringwood	*								
Kershope	*								
Thetford	*	*	*						
Alice Holt	*	*	*	*	*	*			
Pickering	*	*	*	*	*	*	*		

Table 3.5b: Multiple range test on the average number of corpora lutea per ovulating doe for mature animals (6+ yrs), (* denotes significance at $p<0.05$).

Study Site	Qu'ns	Crg'ach	Sth. Strm	Spadeadam	Ringwood	Kershope	Thetford	Alice Holt	Pickering
Queens									
Craigellachie									
Sth Strome									
Spadeadam									
Ringwood	*								
Kershope	*								
Thetford	*	*							
Alice Holt	*								
Pickering	*	*							

Table 3.5c: Multiple range test on the average number of corpora lutea per ovulating doe for yearlings (* denotes significance at p<0.05).

Study Site	Qu'ns	Spade-adam	Crg-'ach	Sth. Strm	Kersh-ope	Thet-ford	Ring-wood	Pick-ering	Alice Holt
Queens									
Spadeadam									
Craigellachie									
Sth Strome									
Kershope	*								
Thetford	*		*						
Ringwood	*								
Pickering	*	*	*						
Alice Holt	*	*	*						

However, by examining Table 3.2 it is clear that Alice Holt, Thetford, Ringwood, Pickering, South Strome, Salisbury Plain, Aldershot, Stanford and Kirkcudbright all had relatively high pregnancy rates (above 80%), whereas Queens, and perhaps Kershope to a lesser degree, had rather lower rates.

iv) Average Number of Foetuses Produced Per Pregnant Doe

The number of foetuses produced per doe was also highly variable between study sites within each age class (ANOVA: $p<0.001$).

Once again, Alice Holt and Pickering had the two highest values, in this case for foetus production, while Queens, Spadeadam and Craigellachie showed reduced performance, although in addition, South Strome and Kershope also had low average values in two of the three age categories. However, the pattern here was not as consistent as that identified for corpora lutea production (Table 3.3). Multiple range tests revealed relatively few significant differences in pairwise comparisons of study sites. These were in the adult age category where Pickering and Alice Holt does, on average, produced significantly more foetuses than Spadeadam does ($p<0.05$).

Among yearlings, foetus production was high at the same sites, Alice Holt, Ringwood and Pickering but low at Spadeadam, Kershope, Craigellachie, Lulworth and Bramley. The samples from Queens and South Strome consisted of only one individual. There were no significant differences between sites in pairwise comparisons.

3.2.3 Variation in the Sex Ratio of Embryos

The sex ratio of kids at birth, as estimated from the ratio of male to female embryos, varied widely between study sites, shown in Table 3.6. Furthermore, the evidence presented here complied with the four principal canons identified by Clutton-Brock and Iason (1986) for studies of sex ratio variation. That is, sample sizes were generally in excess of fifty individuals, the overall difference in the data was significant at the five percent level, there was a significant departure from parity in at least one instance (in fact two) and the independent variable (in this case the population) was equally subdivided between cases.

Table 3.6: Sex ratio for each study site with the associated partitioned G-values (* denotes significant deviation from parity at $p<0.05$; # denotes value based on less than six samples).

Study Site	**Number of Foetuses**			**Sex Ratio**	**G-Value**
	Male	Female	Total (n)		
Alice Holt	79	83	162	0.95	0.080
Thetford	211	190	401	1.11	1.058
Ringwood	78	67	145	1.16	0.889
Spadeadam	53	39	92	1.36	2.148
Kershope	52	33	85	1.58	4.335*
Pickering	147	147	294	1.00	0.000
Craigellachie	26	40	66	0.65	3.028
Queens	2	3	5	0.67	$0.297^{\#}$
South Strome	20	8	28	2.50	5.317*
Salisbury Plain	47	44	91	1.07	0.075
Lulworth	33	28	61	1.18	0.337
Aldershot	19	27	46	0.70	1.433
Bramley	30	23	53	1.30	0.978
Stanford	11	22	33	0.50	3.707
Kirkcudbright	15	23	38	0.65	1.753

The sex ratio values ranged from 0.50 (i.e one male for every two females) at Stanford to 2.50 (five males for every two females) at South Strome. The significance of the variation between sites and the departures from the expected one to one ratio were calculated by a replicated goodness of fit test, which indicated that the heterogeneity observed in Table 3.6 was significant ($p<0.05$). The partitioned G values for each individual study site are also given in the table. These were compared with the chi-squared value of significance at the five percent level, which with one degree of freedom is 3.841.

It is, therefore, apparent that, although there was significant variation in sex ratios between study sites, in only two instances, Kershope and South Strome, did this variation indicate a significant departure from a one to one ratio. Despite this, however, it was possible to identify sites where the does tend to produce more males, (Spadeadam, Kershope, South Strome, Bramley), and sites where the does tend to produce more females, (Craigellachie, Aldershot, Stanford, Kirkcudbright). At the remainder of the study sites, does tend to produce kids in an approximate one to one ratio.

3.3 DISCUSSION

Reproductive success defines the potential for variation in the level of fecundity, and is, therefore, a major determining factor governing the dynamics of a population. Together with mortality and migration, it defines the recruitment potential to the population, and is, therefore, of prime importance when assessing a population's capacity for expansion.

The data presented here show that there is extensive variation in reproductive performance of roe deer populations in Britain. Whether measured in terms of age at first breeding, or ovulation and pregnancy rates of reproductive adults, significant variation was detected in comparisons between study sites. Only the percentage of animals ovulating (in all age categories) showed a high degree of consistency between the study sites (c.f. Bathurst 1988). In addition, there was also significant qualitative variation in reproductive output of populations, demonstrated by an inconsistent sex ratio of embryos in the cull data.

3.3.1 Effects of Age on Reproductive Success

From an extensive review of the literature, Hanks (1981) summarised the sequence of events indicative of declining demographic vigour in a population of large mammals to be:

1. Increase in juvenile mortality.
2. Increase in age at first breeding.
3. Decrease in adult fecundity.
4. Increase in adult mortality.

This sequence highlights the importance of age at first breeding in such populations, suggesting that this will be a more sensitive indicator of the populations reproductive potential than adult fecundity. Precocious reproduction can significantly increase the effective population size (proportion of individuals that are reproductively active) and hence vastly enlarge the potential rate of expansion of the population.

Mitchell (1973) and Clutton-Brock et al. (1982) have shown that red deer hinds will not conceive until they are two years old in the resource limited moorland populations of Scotland, whereas Ratcliffe (1984, 1987) commonly found pregnant yearlings in populations occupying the higher quality habitat of the Scottish commercial coniferous forests. Langbein (1991) found that the vast majority of variation in fecundity between the fallow deer populations of British parks was translated through fluctuations in the level of yearling pregnancy. We may, therefore, focus on the reproductive performance of the youngest age classes as likely to be the most sensitive indicator of variation in reproductive success between populations.

From the data for roe deer presented here, there is little indication for any consistent relationship of reproductive performance with age among animals of two years or older. In many mammalian species, reproductive performance reaches a pinnacle at a certain age, and thereafter declines (e.g. for red deer, Clutton-Brock and Albon 1984). Such a response has been suggested for roe deer in a few studies (Borg 1970; Kaluzinski 1982a). In this study, however, comparisons of reproductive performance between single year adult age classes revealed very few significant differences, and these were equally likely to involve an increase or a decrease in performance with age. Only at Spadeadam was a significant pattern identified. In this case, there was a significant shortfall in productivity among two and three year olds, which may indicate that puberty is substantially delayed at this site.

However, there is considerable evidence in support of Hanks' theory, suggesting reproductive decline of roe deer populations is initially most apparent among the youngest age categories. Clearly, among some populations of roe deer in Britain, kids may exceptionally become sexually mature (such precocious reproduction has previously been reported by Ratcliffe and Mayle 1992). However, while data presented here indicate significant ovulation among kids at Pickering (48%) and, to a lesser extent, South Strome (17%), in reality very few, if any, of these animals actually implant a foetus and carry it through to full term (pregnancy rates recorded were 8% and 0% respectively). Successful reproduction among yearlings is certainly a more common phenomenon: ovulation rates approach 100% in all sites, and a proportion of these animals in each population successfully implant a foetus. Differences between populations in percentage of yearlings pregnant strongly suggests significant variation in age at first breeding across the country.

The data from this parameter alone suggests a tentative classification of populations into performance categories as follows:

High performance:	Alice Holt, Thetford, Ringwood, Pickering, Lulworth, Aldershot, Bramley.
Low performance:	Spadeadam, Kershope, Craigellachie, Queens, Salisbury Plain, Stanford, Kirkcudbright.

(The sample at South Strome consisted of only a single individual.)

Among the high performance populations, yearling pregnancy rates are often in excess of 70%, and are thus approximately equivalent to those of adults (Alice Holt, Thetford, Pickering, Lulworth, Aldershot, Bramley). Several authors have identified a significant shortfall in pregnancy rate among this age class (Borg 1970; Bathurst 1988; Gaillard et al. 1992). Therefore, yearling pregnancy rates as high as these indicate a potential for a substantial contribution of this age class to population recruitment at these sites, and furthermore, suggest that these populations may be approaching their maximal reproductive capacity, at least in terms of percentage of animals pregnant.

In contrast, at Queens, the yearling and adult pregnancy rates are, once again, approximately equivalent, but, in this case, at a much lower level. The yearling pregnancy rate at this study site is low at only 55%, but the equivalent parameter for adults is only 5% higher. This might suggest that reproductive performance has been suppressed among both yearlings and adults, stages two and three in the sequence proposed by Hanks (1981).

It is worth noting at this point that among non-professionals (the deer managers at the Forestry Commission, but not at the Ministry of Defence, are professional wildlife managers), the term yearling can sometimes become confused and misappropriated to kids. Lower jaws for accurate ageing were only available for Forestry Commission sites and, in the analysis of the Ministry of Defence data, ageing generally relied on the field estimates of the deer managers. Therefore, this is one possible explanation for the low rates of yearling pregnancy in the otherwise high performance Ministry of Defence populations of Salisbury Plain and Kirkcudbright (see next section).

3.3.2 Variation in Reproductive Success Within and Between Sites

Little variation was apparent in any of the measures of reproductive performance used, when comparing different years within each study site. Only the average number of corpora lutea per ovulating doe varied significantly between years and then, only in some sites. There was no pattern in the effect of a particular calendar year. In contrast, Bathurst (1988) identified significant variation between years in percentage pregnancy within some populations of roe deer in Britain, but found no year to year variation in average number of corpora lutea or embryos per pregnant doe.

A few studies on deer have been able to identify significant variation in reproductive success between years at a particular location. Verme (1989) documented a drop in percentage pregnancy rate of white-tailed deer in southern Michigan from 60% to 40% between 1952 and 1982, and studies on the same species in Texas estimated a drop in reproductive performance of between 33 and 60% over a four period, depending on the age class (Teer 1984). Average corpora lutea production among roe deer at Kalo, Denmark fluctuated between 1.9 and 2.4 per doe over a nineteen year period (Strandgaard 1972b), but sample sizes in this case were low. Vincent and Bideau (1991) found that the number of kids at foot per doe fell from 1.45 to 1.04 over a nine year period in a roe deer population near Paris. In each of these studies (the exception is Teer 1984), data were generally available for a long period (between nine and thirty years), and may have been associated with drastic environmental change.

Therefore, it is perhaps not surprising that little inter year variation in reproductive success could be detected in this study. With the exception of Alice Holt and Thetford, data were only available for a fairly short time span, generally covering between six and eight years. It is doubtful whether any environmental change over such a time period (e.g. change in density or food availability) would be pronounced enough to be translated into a modified rate of reproductive productivity. It is pertinent that Alice Holt and Thetford, with data covering twenty one and twelve years respectively, account for three of the six significant inter year variations in reproductive success mentioned above. Thus, it seems that to identify significant variation in reproductive success of a deer population over time, may require an extremely lengthy study period, as the population regulation response may be very slow.

It is apparent that the vast majority of variation in reproductive success can be attributed to differences between populations. This inter population variation is very highly significant for three of the four parameters ($p<0.001$), although is perhaps less pronounced for percentage pregnancy rates. From the available literature, Bobek (1977) defined a measure of variability in fecundity between roe deer populations. Using the average number of corpora lutea per doe, he took the ratio of minimum to maximum performance rates of roe deer populations, from studies across Europe. His calculation of 1.3 was well below equivalent figures for mule deer (Nellis 1968) or for white-tailed deer (Chaetum and Severinghaus 1950), of 1.9 and 3.1 respectively. However, the figure for roe deer based on data from this study alone is 1.7 and this rises to 2.0 when data from other roe deer studies are included.

The values calculated for each of parameters of adult reproductive performance of populations in this study are generally within the range reported for roe deer, although the poor performance sites exhibit particularly low reproductive rates. Roe does older than two years will, almost without exception, ovulate and be fertilised (e.g. Chapman and Chapman 1971; Ratcliffe and Mayle 1992). The proportion of animals that then go on to full pregnancy is also generally high (e.g. Chaplin et al. 1966; Prusaite et al. 1977; Kaluzinski 1982a), however, the values for some of the populations in this study, particularly sites in Scotland and northern England, were considerably lower than any previously reported.

The average potential litter size of roe deer varies more significantly, illustrated by the wide range in reported rates of corpora lutea and/or embryos per pregnant doe, between 1.7 (Chapman and Chapman 1971) and 2.5 (Borg 1970), and between 1.6 (Ellenberg 1978) and 2.3 (Borg 1970), respectively. From this study, the potential litter size estimated for British roe deer was generally within these ranges, however, the values for the poorest performance sites, once again, fell below the lower limit of this scale. Sadleir (1969b) suggested that there was evidence that, in many mammalian species, populations at higher latitudes within the range of a particular species exhibited larger litter sizes than those at more southerly latitudes. Borg (1970) cited the fecund Swedish and, to a lesser degree, Danish populations of roe deer as evidence that roe complied with this general trend. However, the results presented here directly contradict this hypothesis, as, within Britain, the opposite trend of decreasing litter size with increasing latitude is evident. Furthermore, the British populations with the lowest potential litter size are at an approximately equivalent latitude to those in Denmark and southern Sweden.

Taking all the parameters of adult reproductive success in combination (with the exception of percentage ovulation rate which was shown to be uniformly high), it is possible to divide populations into high, medium and low performance categories, as was done earlier for the yearling data. The groupings for the nine Forestry Commission sites only are as follows are as follows:

High performance: Alice Holt, Thetford, Pickering.
Medium performance: Ringwood, Kershope, South Strome.
Low performance: Spadeadam, Craigellachie, Queens.

Data for the Ministry of Defence sites only relates to pregnancy, therefore, based on only two parameters (percentage pregnant and average number of foetuses per pregnant doe), all the six Ministry of Defence sites, Salisbury Plain, Lulworth, Aldershot, Bramley, Stanford and Kirkcudbright, were assigned to the high performance category.

These classes seem well defined and are in good agreement with those derived from the yearling data. The main irregularity between the two classifications concerns three Ministry of Defence sites, Salisbury Plain, Stanford and Kirkcudbright, where there was an apparent shortfall in yearling pregnancies. However, as was highlighted earlier, there may be some confusion among the deer managers at these sites over the term yearling, which may, on occasion, be misapplied to kids.

Otherwise, there is generally a high degree of consistency between the three reproductive performance parameters at each site, and well delimited boundaries to each category. South Strome is the one significant exception, as, although it falls into the medium performance category, foetus production was rather low at this site. It may, therefore, be more appropriate to view this case as a low-medium performer. The difference between members of the high and low performance groups in the various reproductive parameters was often significant in pairwise comparisons. Thus, it seems that there are large differences between study sites in potential recruitment to the population across the country, and sites can be conveniently assigned to high, medium and low performance categories.

It is worth noting that variation in percentage pregnancy was not as pronounced as variation in corpora lutea or foetus production. Generally, at most sites pregnancy rates were in excess of 75%, and only at Queens was the rate for adults substantially lower. However, it should be remembered that whether an individual produces one, two or three offspring in a particular breeding season may be of less consequence to the lifetime reproductive success of that individual, than whether the individual becomes pregnant or fails to conceive at all.

3.3.3: Variation in the Sex Ratio of Embryos

Three contrasting theories have been proposed to explain observed deviations from a one to one sex ratio of young at birth. Each of these theories assumes that breeding adults will manipulate the sex ratio of their offspring to maximise their own fitness (Trivers and Willard 1973), as has been shown for invertebrates (Charnov 1982). The mechanism underlying this adjustment may be active resorption of the foetus (Gosling 1986) or simply neo-natal mortality, possibly induced by maternal neglect (Trivers and Willard 1973). However, as Maynard Smith (1980) states, manipulation of the sex ratio prior to birth would be less wasteful of reproductive effort. Each of the hypotheses was developed to explain the adaptive significance of sex ratio manipulation for species with different life history parameters, and are, as yet, largely unconfirmed (for a review see Clutton-Brock and Iason 1986).

Trivers and Willard (1973) first proposed the theory that females in better condition should invest more in the production of offspring of that sex with the greatest variation in expected reproductive fitness. In many polygynous species, males actively compete for matings, and certain successful individual males will gain a large number of matings, while others will entirely fail to copulate (e.g. Clutton-Brock et al 1988; Langbein and Thirgood 1989), causing a pronounced skew in reproductive success among the males. In this situation, the success of an individual male will be determined, to some extent, by its body size and, therefore, will be strongly influenced by the degree of parental investment from its mother. Clutton-Brock et al. (1981) have shown that the reproductive fitness and survivorship of male offspring may be much more dependent on the amount of milk received from the mother than is the fitness of females. Therefore, to maximise her own inclusive fitness, a female should only produce a male if she herself is in superior condition, and so can devote enough reproductive effort to ensure the offspring will be relatively successful. By contrast, the optimal strategy for mothers in poor condition should be to produce females, which will be relatively assured of some reproductive success (unlike subordinate males), albeit at a lower level than dominant males.

Clutton-Brock et al. (1984) invoked this hypothesis to explain the excess production of males by dominant red deer hinds that were in superior body condition. The authors showed that, for this species, the sons of dominant hinds (in better than average condition and with high reproductive performance) were more successful than daughters, but daughters of subordinate hinds were more successful than sons. Rutberg (1986) also found excess production of male calves among yeld hinds, presumably in better than average condition (red deer: Mitchell et al. 1986), in American bison.

The second of these theories takes account of the fact that, in sexually dimorphic species, the costs of gestation and lactation are dependent on the sex of the progeny. If the costs of successfully producing a male are higher than those of producing a female, mothers may only attempt this when they are in better than average body condition (Clutton-Brock and Iason 1986). If a female attempts to produce males when in poor condition herself, she may not be able to recover condition, and may, therefore, suffer impaired reproductive success in the future. This concept, applied to multiparous species, forms the basis of the fractional offspring hypothesis (Williams 1979). This theory postulates that mothers will maximise their fitness by expressing their reproductive potential to maximum effect. If sons are more costly than daughters, mothers in poor condition will tend to produce more female singletons, and those in slightly better condition will produce more male singletons. However, mothers in better condition still will maximise their fitness by using their higher reproductive potential to produce two females or even one male and one female. Thus, Williams suggested that there is a sequence of increasing reproductive cost under this hypothesis of one female, one male, two females, one male and one female, two males, three females etc.

Finally, differential investment in male and female offspring might result from a difference in the potential for future competition posed by those offspring. The local resource competition hypothesis, proposed by Clark (1978), suggests that, where resources are limiting, the costs of rearing whichever sex may exhibit philopatry will exceed the costs of producing offspring of the sex which tend to disperse from their mother's home range. In this context, resources may include food, space, mates, or a combination of these (Clutton-Brock et al 1982).

In the multiparous white-tailed deer, which are both polygynous and sexually dimorphic, McGinley (1984) suggested that, if males experience greater variation in reproductive success, females with only just sufficient reproductive effort to produce two poor quality females should, instead, concentrate their effort into producing one high quality male. In contrast, Caley and Nudds (1987) argued that the data on the North American white-tailed deer, reviewed by Verme (1983), do not in fact fit the fractional offspring hypothesis. Instead, they invoked local resource competition as an adaptive explanation for the skewed sex ratios in the Odocoileus. Male white-tailed deer tend to disperse as yearlings, where-as female offspring commonly remain within their mother's home range for two and a half years (Verme 1983). Thus, production of female progeny may result in a greater loss of reproductive potential to a mother over her lifetime, because the daughter will compete with her for resources and matings (Clutton-Brock and Iason 1986).

Roe deer are a territorial species and substantial polygamy is unlikely (Staines and Ratcliffe 1991). Furthermore, there is only a minimal degree of sexual dimorphism in this species; females are generally only one or two kilograms lighter, on average, than males (Staines and Ratcliffe 1991). Thus, it seems unlikely that there is any *a priori* differential between the sexes in expected reproductive success, and production of either sex will constitute a similar immediate metabolic cost to the mother. Twinning is common in roe deer and so, according to William's fractional offspring hypothesis, we would expect to see an excess of male offspring among does only having a single foetus. From our data, this is not the case, as there is a tendency among does having singletons to produce more females.

However, as is the case for white-tailed deer, several authors suggest that dispersal of young is far greater, and occurs earlier in male roe deer than is the case for females (Strandgaard 1972b; Klein and Strandgaard 1972; Bideau et al *in press*), particularly at high densities where conditions may be limiting. However, at low densities, it seems there may be no differences in dispersal patterns between the sexes (Cederlund 1983; Bideau et al. 1987). This suggests that local resource competition is likely to be the theory most applicable to roe deer, and that it will manifest itself most strongly in populations where these 'resources' are most limiting.

From the data presented in section 3.2.3, it is indeed apparent that there is an association between low performance populations, which are presumably resource limited, and populations producing an excess of male foetuses. In fact, there is a significant inverse correlation (Spearman's Rank Correlation, $p<0.01$) of the sex ratio with the average number of foetuses produced per doe in the population. An excess of female foetuses are produced in the high performance populations of Aldershot, Stanford and Kirkcudbright, and a deficit of females are produced in the low or medium-low performance populations of Spadeadam, Kershope and South Strome. This deficit at Kershope and South Strome is a significant departure from a one to one sex ratio ($p<0.05$). Verme (1969) found a similar inverse relationship between the number of fawns per doe and the proportion of males produced in a population of North American white-tailed deer. Furthermore, he was also able to show that this response was associated with diet quality, as animals on limiting resources tended to produce an excess of males. In roe deer, Borg (1970) found from post-mortem examinations that does generally produced male and female kids in equal proportions. However, following one particularly harsh winter, when resources were certainly limiting (mortality due to starvation was very high) the sex ratio was 1.4:1 in favour of males. Ellenberg (1978) found that the sex ratio varied widely in different roe populations, between 0.33 and 3.0, and related this to variation in resource availability. Thus, does experiencing good nutritional conditions tended to produce more female offspring, where-as undernourished does tended to produce more males.

There are, however, two exceptions to this pattern in this study. Firstly, Craigellachie, a low performance population, shows a female biased foetal sex ratio, and secondly, Bramley, a high performance population, exhibits a male biased sex ratio in foetuses. In addition, the sex ratio in the high performance populations of Alice Holt, Thetford and Pickering is approximately one to one. The latter results may not be so surprising, as there is nothing in the local resource competition hypothesis that implies animals under non-restrictive conditions will produce an excess of females. However, Clutton-Brock et al. (1982) suggested that, in some species, females in rich environments will tend to produce more daughters over whom they have greater control of reproductive success, for example, through assisting them in gaining access to favourable habitat patches, (DeGayner and Jordan 1986).

The results presented here suggest that British roe deer may adjust the sex ratio of their progeny according to the conditions they experience. Where reproductive performance is low and resources may be limiting, there is a tendency for does to produce an excess of male offspring, the theoretical expectation defined by the local resource competition hypothesis, seemingly the most applicable theory for this species.

This chapter has illustrated the high degree of variability, both quantitative and qualitative, in the reproductive potential of roe deer populations across Britain. The subsequent two chapters attempt to explain the possible causes of this level of variation in terms of environmental and genetic factors respectively.

CHAPTER 4

REGULATING FACTORS AFFECTING REPRODUCTIVE SUCCESS

4.1 INTRODUCTION

The previous chapter presented evidence of a highly significant level of variation in reproductive success in roe deer populations across Britain. This chapter will investigate the relationship between this observed variation and a number of environmental factors, which might be expected to influence reproductive performance: in particular, climate, population density and forage quality. First, the reproductive data will be examined in relation to variations in maternal body weight, to ascertain to what extent the weight of a female determines her reproductive potential. Subsequently, variation in reproductive success will be related to climate and range conditions, as outlined above, to assess the influence of these variables on the reproductive performance of roe deer.

Body Weight

In many animal species the fecundity of an individual female is often linked to her body weight. In mammals, a clear relationship has been demonstrated between sexual maturity and body weight, with puberty dependent on the attainment of some critical threshold body mass (cattle: Sorensen et al. 1959; rats: Widdowson et al. 1964; humans: Frisch 1984). Red deer hinds on the Scottish moorland are generally of relatively low body weight, and commonly do not conceive until their third year (Mitchell 1973). However, among the heavier populations of the coniferous woodlands, where habitat quality is higher, hinds regularly breed successfully as yearlings (Ratcliffe 1987).

Even post-puberty, maternal body weight may continue to have an effect on fecundity, with the probability of becoming pregnant in a given year dependent on body weight (red deer: Mitchell 1973; Clutton-Brock et al. 1983; Albon et al. 1986; reindeer: Reimers 1983; roe deer: Gaillard et al. 1992). Furthermore, many studies have demonstrated that there is a threshold body weight for successful reproduction, such that animals which fail to reach that critical threshold, fail to ovulate and will not conceive in that season (red deer: Mitchell and Brown 1974; Hamilton and Blaxter 1980; reindeer: Reimers 1983; fallow deer: Langbein and Putman 1992). The costs of gestation and lactation are considerable in mammals. In populations where females are in generally poor condition, these costs may be substantial enough to prevent some females, which give birth in one season, achieving the critical body weight threshold by the following rut. In consequence, adult females in such populations may breed only in alternate years (Clutton-Brock et al. 1983; Reimers 1983; Skogland 1985; Mitchell et al. 1986).

In roe, one of the few multiparous species of deer, maternal body weight may also influence litter size. Both potential litter size (number of corpora lutea) (Blant 1987), and actual number of kids successfully reared (Ellenberg 1978) have been shown to increase with increasing maternal body weight in this species. A similar relationship has also been shown in white-tailed deer, where litter size varies with maternal body weight among both juveniles and adults (Ozoga and Verme 1982).

However, although it is certainly true that body weight is a prime determinant of reproductive output in mammals, it is not the only factor influencing reproductive success. Population density, resource availability, climate and even genetic factors may all contribute to determine reproductive performance, either through an effect on body weight, or directly and independently of such a relationship. Therefore, this observed relationship between body weight and fecundity does not necessarily resolve, but merely rephrases the question of causality, requiring the factors that cause variation in body weight itself to be explored. Thus, while this chapter will investigate the relationship between reproductive performance and body weight of roe deer, the relationship of fecundity with density, habitat quality and climatic variables will also be explored.

Climate

Climatic factors may have a significant impact on the reproductive performance and the survivorship of large mammal populations (Sinclair 1973). The level of recruitment to the population in three species of ungulates, mule deer, wapiti and bighorn sheep (*Ovis sp.*), was shown by Picton (1984) to be subject to climatic variation. These effects may be direct, or may operate through climatic influence on other environmental factors such as habitat quality, and individual climatic variables can have both positive or negative effects.

In harsh environments, the energetic costs of maintenance activities vary according to the temperature, windspeed, rainfall and snow cover: under adverse conditions, heat loss of red deer may be doubled (Simpson 1976; Grace and Easterbee 1979). Deep snow may restrict the mobility of deer and increase the energetic demands of movement (Borg 1970), deny them access to food (Mitchell and Staines 1976), and also increase susceptibility to predation (Cederlund and Lindstrom 1983). In addition, cold temperatures may affect the normal functioning of rumen microbes, reducing fermentation efficiency (Grace and Easterbee 1979), and a lack of free water can also interfere with the digestive system (Borg 1970). Such a direct impact of climate is often translated through increased levels of juvenile mortality (Clutton-Brock and Albon 1983; Cederlund and Lindstrom 1983; Ratcliffe and Mayle 1992), but can also affect mortality among adults (Albon 1983). Adverse climatic conditions, such as high levels of rainfall during the autumn, may also result in impaired reproductive performance (Albon et al. 1987). Mech et al. (1987) found that snow accumulation had a significant effect on the reproductive success of both white-tailed does and moose cows, by restricting movement and, therefore, access to food.

However, the relationship between climate and performance is often mediated through habitat changes. For example, climatic variables such as high summer temperature and/or low summer rainfall promote the growth of heather (Miller 1979), an important component of the roe deer's diet in upland habitats (Staines and Welch 1984). This effect of climate on forage production was cited by Albon (1983) as an important factor determining the nutritional state of red deer hinds. However, Teer (1984) found that rainfall had a significant, but opposite, impact on the quality of the range vegetation, and noted a consequent positive correlation of precipitation rate with fecundity in a white-tailed deer population in Texas. Putman and Langbein (1992) found that prolonged winters were associated with lower body weights and elevated levels of mortality in British park fallow deer, due to the delay in onset of the growing season, while high rainfall had a negative impact on pasture production, probably due to water logging and leaching of the soil's nutrients.

Thus, average monthly temperature and average monthly rainfall for both summer and winter, as well as average monthly temperature for mid-winter (January-February) and average monthly snow lie, were examined to determine the influence of these climatic variables on the reproductive performance of roe deer in Britain.

Density in Relation to Habitat Quality

A further potentially significant environmental factor we should consider for its possible influences on both body weight and reproductive performance of roe deer, is population density. However, measures of absolute density of animals, although often of interest, do not take into account the potential capacity of the habitat to support the population. Even at high animal densities, if food availability and quality are also high, the carrying capacity of the habitat may not be reached, and the population will, therefore, be characterised by heavy and fertile individuals. Indeed, in a number of studies on enclosed deer populations supplied with high levels of artificial feed, fecundity remained high despite extremely high animal density (roe deer: Ellenberg 1978; white-tailed deer: Ozoga and Verme 1982; fallow deer: Langbein: 1991). Despite a seven fold increase in density, reproductive performance actually also increased in an enclosed white-tailed deer herd when supplementary feed was given ab libitum (Ozoga and Verme 1982). Furthermore, in areas of poor range quality, even if the absolute density of animals is low, reproductive performance may be impaired due to the effects of resource limitation. Thus, the effective density experienced by the animals themselves is a complex function of absolute numbers in relation to the food resources available.

The best evidence for an effect of density on reproductive performance comes from studies of year to year density changes in single populations experiencing similar range conditions over the period of study. Teer (1984) noted a decline in fecundity over a five year period in the white-tailed deer population of the Llano Basin in Texas, and suggested this was due to an inverse relationship with deer density, which had increased to a point where the food supply was no longer adequate to meet the nutritional demands of the population. In an unexploited population of roe deer, population growth ceased and recruitment levels fell to 0.54 from 0.76 kids per doe, when density reached about 20 animals /100 ha., despite a constant level of range quality (Prusaite et al. 1973). However, Klein and Strandgaard (1972) found that populations of roe deer living in habitats of inferior quality were still able to meet their growth requirements, but only provided deer densities were also low.

Because of these complex interactions of habitat quality and absolute population density, an index which combines consideration of both food availability and animal density within a population is of greater value than information on either of these elements in isolation. Such an index will, therefore, reflect quantity and quality of resources available to each individual. In this chapter, average adult jaw size of animals born in a particular year (length of lower jaw and minimum height of diastema), and average rumen liquor quality of animals shot in a given year (amount of available ammonium nitrogen) are used as measures of habitat quality in relation to animal numbers. These indices will be related to reproductive success to determine to what degree habitat quality may delimit reproductive potential. Samples were taken from females only, and indeed, Huot (1988) notes that best results for such indices will be obtained when females only are considered.

1. Cohort Jaw Size

Jaw size has often been used as an index of skeletal development in deer (e.g. Mitchell et al. 1976; Staines 1978; Skogland 1983; Suttie and Mitchell 1983; Ratcliffe 1987; De Crombrugghe 1989). Of four skeletal indices (body length, foot length, jaw length and tooth row length), Challies (1978) found jaw length of red deer to be the most appropriate, because of its ease of measurement and sensitivity to fluctuations in level of nutrition. In fact, it has been suggested that one of these measurements, foot length, is determined to a greater extent by genetic factors (Klein 1964; Hartl et al. 1991b). In contrast, although jaw bone length in red deer calves was found to reflect the body condition of the mother, heritability was presumed to be negligible, as there was no direct correlation between jaw length of mother and calf (Mitchell et al. 1986). Ellenberg (1974) for roe deer, and Skogland (1983) for reindeer, found that lower jaw length was an extremely good predictor of total body size, and both authors noted that resource availability around the time of birth determined the jaw length of an individual. Skogland found that when the level of nutrition a calf received was inadequate, either during gestation or shortly after birth, this resulted in slower skeletal growth rates, and this retardation was not compensated for in later life. The mandible has a high growth priority shortly after birth but does not increase in size significantly after the first year of growth

(Langvatn 1977; Huot 1988) and, hence, the development of the jaw of newly borns will be particularly sensitive to resource limitation. In addition, birth may be delayed when food is scarce and, therefore, the active growth season for newly borns in their first year may be curtailed, and this will be reflected in smaller skeletal size of the mature animal (Skogland 1983). Several authors have noted that compensatory growth of the jaw as an adult is minimal (Ellenberg 1974; Suttie 1981; Skogland 1983). Thus, average jaw length and average diastema height of a cohort are used in this chapter as indices of resource availability at the time of that cohort's birth.

2. Rumen Liquor Quality

The level of nitrogen in washed rumen contents has been shown to be a good indicator of diet quality (Staines and Crisp 1978; Reimers 1983). Levels of free ammonia in the rumen liquor most accurately reflect the nitrogenous content of the food an animal has ingested (e.g. Klein 1962; Bailey 1969). Colorimetric analysis can determine this level, which has been used as a measure of nutritional status in red deer (Ratcliffe 1987). In this chapter, the average level of free ammonia in rumen samples taken from animals shot in a given year is used as a reflection of the resource availability in that same year.

3. Pellet Group Counts

Roe deer, as a territorial species, may also be subject to direct effects of density as a result of crowding (Klein and Strandgaard 1972). Social factors in high density populations may result in roe does becoming stressed, and cause a decline in condition and reproductive success (Vincent et al. *in press*). In a population of white-tailed deer at high density, but with unlimited nutritional resources, Ozoga and Verme (1982) observed that doe fecundity approached maximal levels. However, at even higher density, reproductive performance among yearlings decreased, and the authors suggest that this was due to increased levels of buck harassment. No such effect was noted by Teer (1984) in a wild population of the same species, but territoriality in this species is generally less pronounced than in roe. Therefore, reproductive performance in different populations will be considered in relation to absolute population density, assessed by pellet group counts.

4.2 RESULTS

4.2.1 Variation in Body Weight

Variation in doe body weight, both between study sites, and between kids, yearlings and adults within a site, is considerable and is presented in Table 4.1. All weights presented are 'clean weights' (eviscerated but with head and feet on) and are given in kilograms. The lowest values were found at Queens, where mature animals were, on average, only 12kg clean weight. These are among the lowest values recorded for the species. Generally, however, average body weights of animals of two years of age and over fell between 14 and 19kg, with the heaviest populations at Alice Holt, Salisbury Plain and Bramley.

Kids were, on average, approximately 35% lighter than mature animals in the same population, whereas yearlings, at the Forestry Commission sites, were often as heavy, and were never more than 1kg lighter than adults. At the Ministry of Defence study sites yearlings were, on average, always 10 to 15% lighter than adults. It is perhaps worth reiterating at this point that there is occasionally a certain amount of confusion regarding the correct terminology used to describe animals less than one year of age, which are sometimes erroneously referred to as yearlings. This possible source of error may explain the shortfalls of both the body weight and reproductive performance (see Chapter 3) of yearlings, observed at some of the Ministry of Defence sites, where ages were assessed only by stalkers, and jaws were not available for confirmation.

4.2.2 The Effects of Body Weight on Age Related Reproductive Performance

Analysis of variance of reproductive performance, using age at first breeding, proportion of fecund adults and average potential litter size, explored in Chapter 3 was repeated including maternal body weight as a covariate. For analysis of percentage ovulation and percentage pregnancy the covariate entered was the average body weight of a particular class of animals, but when considering actual number of corpora lutea or foetuses produced by individual does, the actual weight of each individual was entered as covariate.

Table 4.1: Average female body weight (eviscerated, head and feet on, in kilograms) for kids, yearlings and adults (2+ yrs) in each study site, standard errors are given in brackets.

Study Site	**Kids**		**Yearlings**		**Adults**	
	Body Weight	Sample Size	Body Weight	Sample Size	Body Weight	Sample Size
Alice Holt	12.9 (.16)	94	18.1 (.19)	59	18.4 (.12)	207
Thetford	9.1 (.21)	130	13.6 (.13)	197	14.1 (.11)	378
Ringwood	10.5 (.23)	53	16.1 (.33)	12	16.4 (.14)	170
Spadeadam	9.8 (.24)	73	14.8 (.50)	24	15.0 (.15)	182
Kershope	11.4 (.16)	107	15.4 (.39)	42	16.0 (.15)	233
Pickering	12.8 (.12)	247	16.5 (.26)	66	17.2 (.11)	368
Craigellachie	9.7 (.21)	86	14.3 (.17)	114	14.7 (.13)	232
Queens	7.6 (.37)	9	11.2 (.44)	20	12.1 (.18)	86
South Strome	10.7 (.43)	12	14.1 (.57)	11	14.8 (.19)	90
Salisbury Plain	13.9 (.20)	90	16.0 (.50)	27	18.7 (.15)	114
Lulworth	10.8 (.29)	35	14.8 (.43)	22	16.3 (.18)	87
Aldershot	13.8 (.39)	11	17.1 (.38)	12	18.7 (.32)	42
Bramley	11.8 (.33)	30	15.5 (.32)	8	17.8 (.23)	50
Stanford	10.8 (.35)	42	14.7 (.47)	23	16.4 (.18)	64
Kirkcudbright	11.7 (.22)	37	14.5 (.64)	17	17.1 (.23)	58

Hoggarth (unpubl. data) found that there was little significant loss of doe body weight over the winter in several British roe populations. This conclusion was tested by a series of regressions of body weight against the date shot for each age class within each site. Although these tests identified a slight trend of weight loss through the winter in about half the sites, this trend was very rarely significant, and constituted only a minor weight differential. Therefore, the actual body weight of culled animals was used in the following analyses.

Age at First Breeding

An examination of the proportion of individuals within a population ovulating or pregnant in relation to body weight, among juveniles, revealed that much of the age related variation within a site in these two parameters, detailed in the previous chapter, is explained by a corresponding variation in body weight.

Variation in body weight accounted for the very highly significant difference ($p<0.001$) between kid and yearling ovulation rate observed within study sites. Likewise, the highly significant difference between kids and yearlings in percentage pregnancy is accounted for almost entirely by body weight. In both cases body weight is an extremely important factor in establishing these age related differences in fecundity ($p<0.001$) of juveniles.

Body weight was also a very highly significant factor ($p<0.001$) in explaining the difference between yearling and adult pregnancy rates within a site. When body weight was included as a covariate, the difference between yearling and adult pregnancy rates falls from a p-value of <0.001 to <0.05, but is still significant.

In comparisons between sites, differences in yearling pregnancy were not correlated with differences in body weights of the populations. Nor was it possible to identify any threshold body weights relating to percentage pregnancy rates applicable across all study sites. Indeed, inspection of the data (Table 4.1) emphasises that if any such thresholds exist, they are specific to a particular population. For example, the yearling pregnancy rate of 35% at Kershope is one of the lowest among the populations studied. However, the average body weight of these animals is substantially higher than that of the adult age classes at several of the other study sites with superior adult pregnancy rates. It should be noted, however, that these comparisons involve cull weights. Although the difference between cull weight and weight at the rut may be minimal among adults (see above), it is likely that yearlings will still be growing during the post-rut period, and may actually be heavier when shot.

The Effects of Body Weight on Age Related Variation in Reproductive Success of Post-Pubertal Animals

Post-pubertal ovulation rates, within or between sites approach 100% in all cases (see Chapter 3). Therefore, no effect of the average body weight of the age class on adult ovulation rate could be detected. However, the very highly significant differences in pregnancy rate between age classes within a study site, previously identified, ($p<0.001$) were found to be entirely explained by variation in average body weight (two way ANOVA without replication).

Inclusion of individual maternal body weight as a covariate, explained some of the variation between age classes in average number of corpora lutea and average number of foetuses produced, and was highly significant in the analysis ($p<0.001$). However, although the sum of squares in both cases was notably reduced, age remained a very highly significant determinant of both corpora lutea and foetus production ($p<0.001$).

4.2.3 The Effects of Body Weight on Variation in Reproductive Success Within and Between Study Sites

The variation in reproductive success between years within a study site was also examined in relation to body weight.

There was no variation in ovulation rates between years within a site. The significant variation in pregnancy rate between years within a site was entirely explained by differences in average maternal body weight (the covariate, body weight, was significantly correlated with percentage pregnancy between years, $p<0.05$).

There was significant variation between years, within a site in corpora lutea production, but this was also largely due to variation in maternal body weight. Body weight was significantly correlated with the average number of corpora lutea per doe in a given year ($p<0.001$), and when body weight was included as a covariate, between year variation only remained significant if the analysis included the period 1979 to 1983 ($p<0.05$). The data for this period were drawn solely from Alice Holt and Thetford. However, the site-year interaction factor remained very highly significant ($p<0.001$), indicating that the year to year patterns of variation in this parameter were specific to each site.

There was no significant variation between years within a site in the average number of foetuses per doe. However, in contrast to the general trend, there was significant correlation between foetus production and maternal body weight between years at Alice Holt and Thetford ($p<0.05$). It is notable that it is at these two sites where the data spans the widest number of years.

For yearlings, the variation between years within a site was not significant for any of the parameters of reproductive success. Therefore, inclusion of body weight as a covariate in each of the four analyses had little effect.

The variation in reproductive success between study sites was also reexamined with the inclusion of body weight as a covariate. There were no differences between sites in percentage ovulation rate within any age class. Variation in body weight could not explain the large differences between sites in each of the other three reproductive parameters. Despite the fact that body weight was significantly correlated with reproductive success in each case ($p<0.001$), the variation between sites in reproductive performance remained very highly significant ($p<0.001$) when body weight was included as a covariate in the analysis. The relationship of body weight with each of these three measures of reproductive success are illustrated in Figs. 4.1, 4.2 and 4.3. It is apparent that, in each case, Thetford has a higher than predicted reproductive success estimated from body weight data as, on all three graphs, it falls below the best fit line. Queens also has a higher than predicted percentage pregnancy (Fig. 4.2). In contrast, Alice Holt, despite an actually high value in absolute terms, has a somewhat lower than predicted rate of corpora lutea production (Fig. 4.1), and both Salisbury Plain and Aldershot have a lower than predicted rate of percentage pregnancy (Fig 4.2).

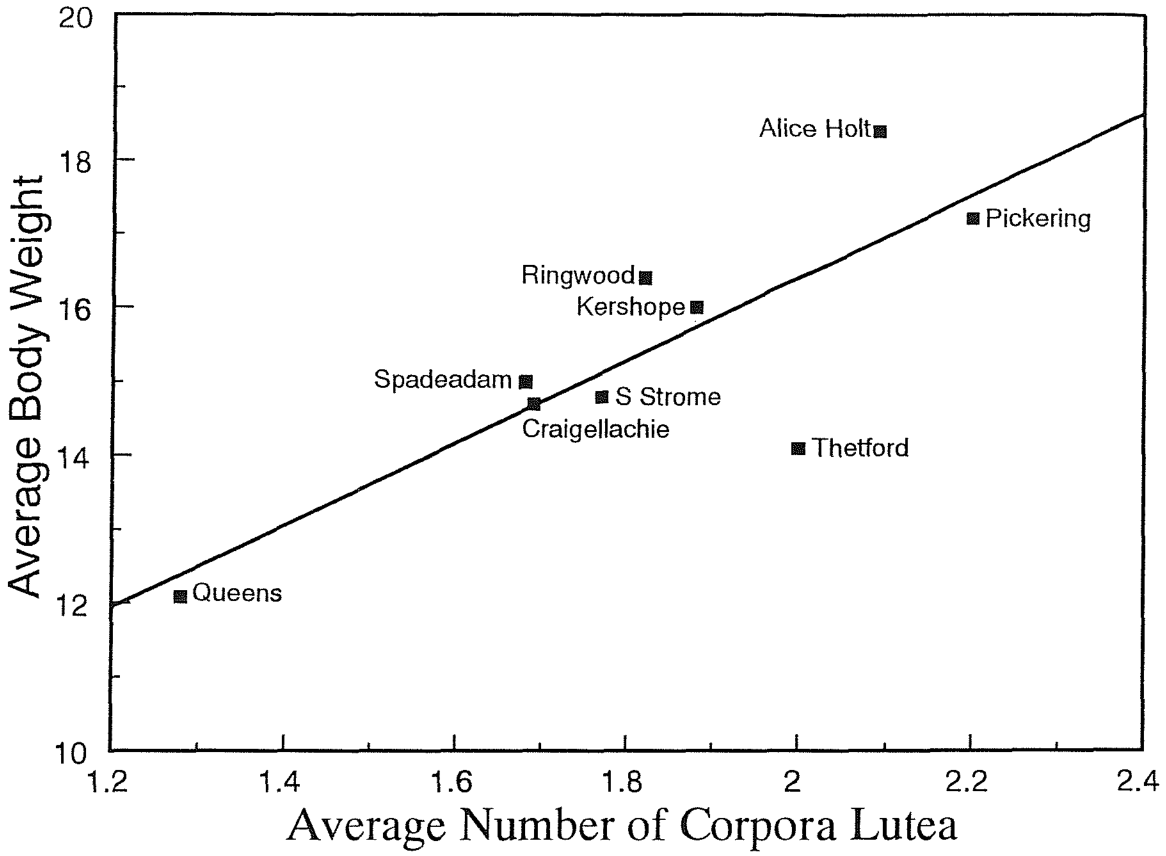


Figure 4.1: The relationship between the average number of corpora lutea per ovulating adult (2+ years) and average maternal body weight at each study site.

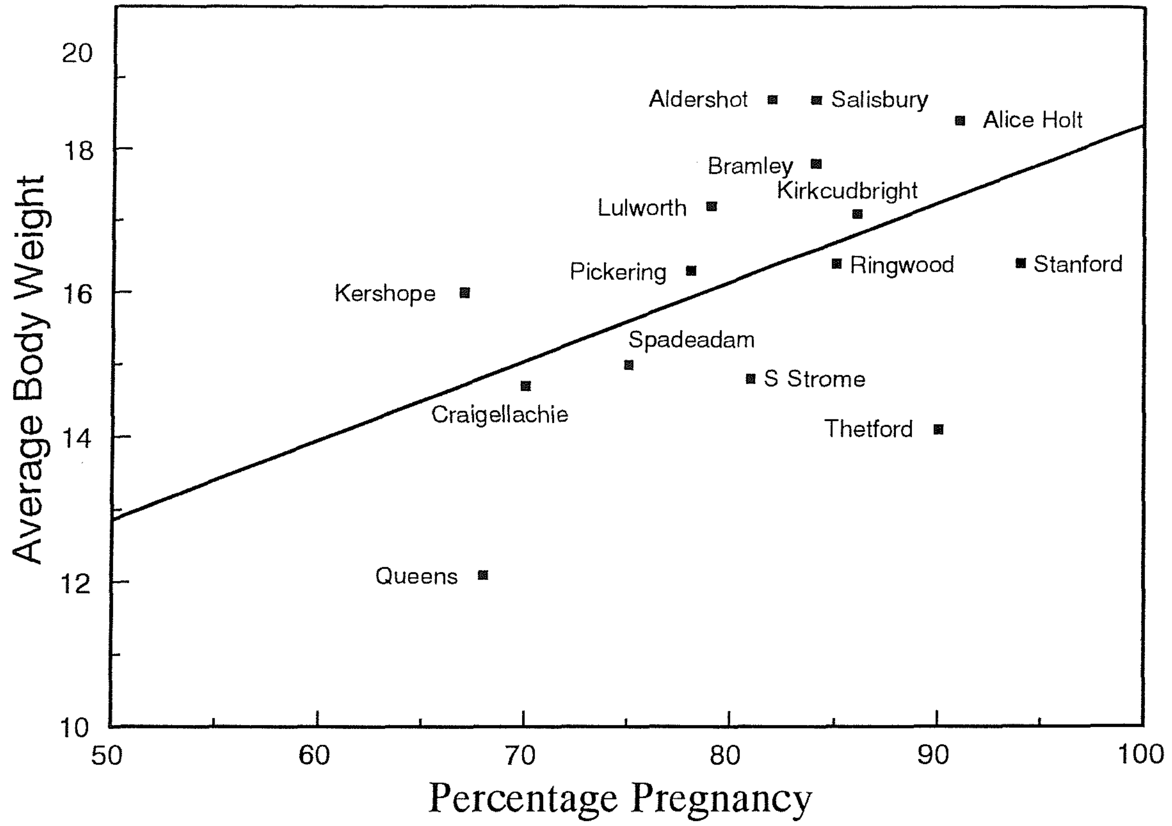


Figure 4.2: The relationship between percentage pregnancy among adults (2+ years) and average maternal body weight at each study site.

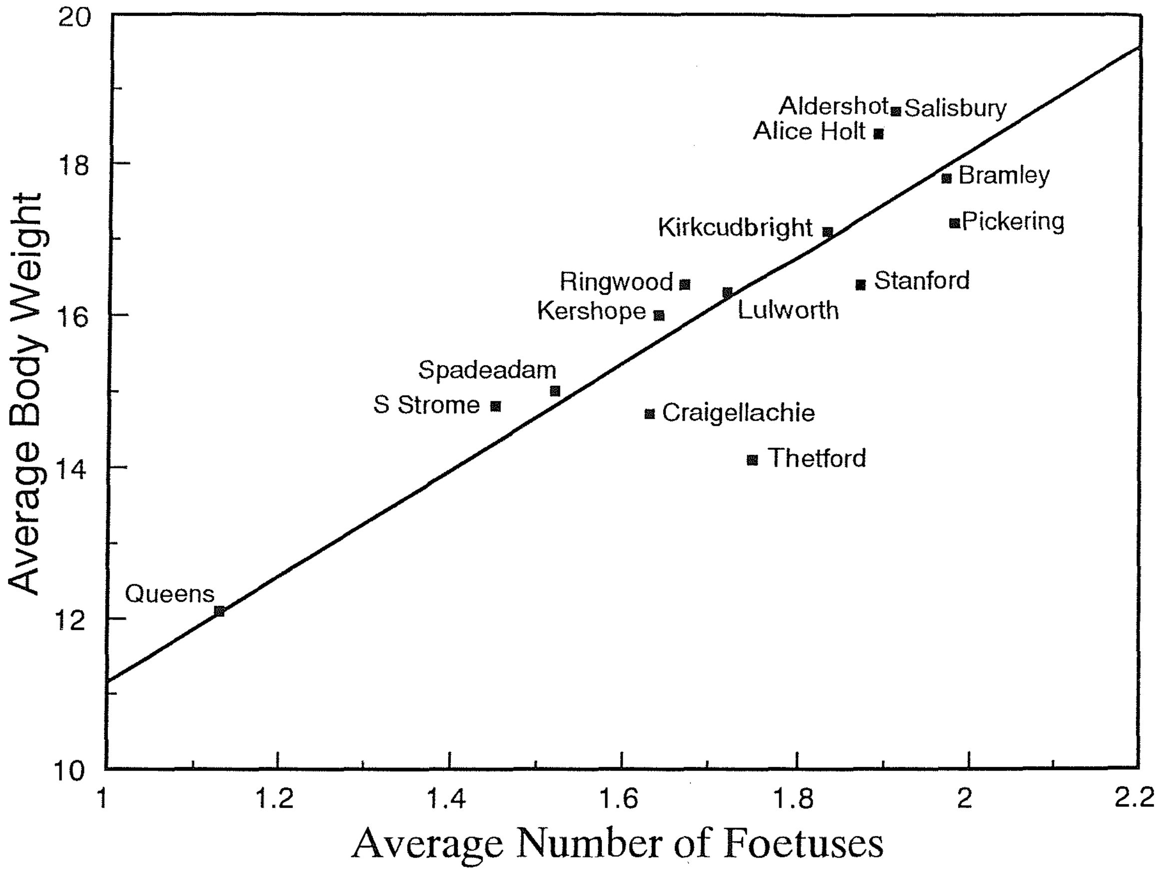


Figure 4.3: The relationship between the average number of foetuses per pregnant adult (2+ years) and average maternal body weight at each study site.

A similar analysis which considered yearlings only, found no correlation between body weight and corpora lutea production, and the variation of this parameter between sites remained very highly significant ($p<0.001$) when weight was entered as covariate. In contrast, the highly significant variation between sites in average number of foetuses per pregnant yearling was entirely explained by the animal's body weight.

4.2.4 The Effects of Body Weight on the Sex and Number of Embryos Produced

As a final element in the initial analysis of the effects of body weight on reproductive performance, the highly variable sex ratios detailed in the previous chapter were also examined in relation to maternal body weight.

A strong inverse relationship of embryo sex ratio with average body weight of the population was detected in across site comparisons. A population with a relatively low average body weight was more likely to produce an excess of males, although, perhaps due to small sample sizes, the correlation was marginally non-significant ($p=0.053$). A similar, but significantly stronger, relationship of body weight with the number and sex of foetuses produced by individual does within each site was also evident.

Number of Embryos

A one way ANOVA for each study site, using the number of foetuses as classes, showed that the variation in maternal body weight between classes of animals carrying different number of foetuses was significant at only three out of fifteen sites, Ringwood ($p<0.01$), Spadeadam ($p<0.01$) and Kirkcudbright ($p<0.05$). However, despite this, a fairly consistent pattern of body weight in relation to the number of foetuses carried is evident across all the study sites (Table 4.2). Does carrying two foetuses were, on average, heavier in all but two cases, than those with one or no foetuses; this difference was significant for these same three sites (all $p<0.05$). However, does carrying one foetus were sometimes heavier (at eight sites) and sometimes lighter (at six sites) than barren does. Among yearlings, the same pattern is apparent, but without any exceptions. At the six sites where sample sizes were large enough, twinning does are, on average, always the heaviest but barren does are, on average, always heavier than those carrying a single foetus.

Table 4.2: Average body weight (eviscerated, head and feet on, in kilograms) of adult females (2+ yrs) carrying nought, one or two foetuses, in each study site, standard errors are given in brackets. # denotes a value based on a sample size of less than six.

Study Site	Number of Foetuses Present					
	0		1		2	
	Body Wt.	n	Body Wt.	n	Body Wt.	n
Alice Holt	18.0 (.55)	10	18.0 (.25)	17	18.1 (.16)	81
Thetford	13.4 (.63)	22	13.6 (.34)	47	14.3 (.17)	117
Ringwood	16.1 (.52)	17	15.5 (.29)	30	16.9 (.20)	61
Spadeadam	15.0 (.34)	29	14.0 (.26)	41	15.2 (.28)	45
Kershope	15.1 (.41)	37	15.2 (.49)	28	15.9 (.23)	48
Pickering	16.9 (.39)	47	17.8 (.45)	20	17.1 (.18)	146
Craigellachie	13.7 (.37)	24	13.6 (.36)	22	14.4 (.35)	31
Queens	11.9 (.25)	12	11.9 (.46)	7	12.7# (-)	1
South Strome	14.5 (.65)	7	14.4 (.37)	15	14.8 (.53)	13
Salisbury Plain	18.6 (.77)	11	18.8 (.35)	7	18.5 (.24)	47
Lulworth	15.4 (.63)	13	15.9 (.42)	14	16.5 (.27)	32
Aldershot	16.5 (1.01)	6	16.9 (.69)	8	18.1 (.33)	17
Bramley	18.7 (.61)	6	16.8# (.26)	3	17.6 (.32)	29
Stanford	14.1# (1.40)	2	17.0# (.51)	4	16.5 (.28)	27
Kirkcudbright	16.4# (.72)	4	14.4# (.88)	4	17.3 (.35)	18

Sex of Embryos

This relationship was analyzed further by looking at the differences between average body weights of animals according to the sex of the foetuses carried. Table 4.3 gives the average clean weights for each category at all sites except Queens, where these data were not available. A series of Wilcoxon matched pairs tests identified a high degree of ordering among these classes (p-values are given in Table 4.4 for two tailed tests).

From the table we can see that does carrying one male foetus were, on average, lighter than all other pregnant animals. This difference was statistically significant for comparisons with all categories of twinning does ($p<0.01$), but only marginally so for the comparison with does carrying a single female foetus ($p=0.056$), if the test is a two tailed one. The next lightest category, does carrying a single female foetus, were lighter on average than does carrying female twins or mixed sex twins (both $p<0.05$) but not significantly lighter than does carrying male twins ($p=0.28$). Among twinning does, those carrying two males were lighter on average than does carrying female twins but the difference was not significant, at least when the test is considered to be two tailed ($p=0.11$). In addition, there was a tendency for does with male twins to be lighter on average than does with mixed sex twins but this result was not statistically significant. Does with twin females were, on average, equally likely to be heavier or lighter than those carrying mixed sex twins.

Thus, there is a sequence of number and sex of foetuses carried by a doe with increasing average body weight, as follows: one male, one female, two males, two females and one of each sex.

Table 4.3: Average body weight (eviscerated, head and feet on, in kilograms) of adult females (2+ yrs) according to the number and sex of the foetuses present, in each study site (#= sample <6).

Study Site	**Number and Sex of Foetuses Present**									
	1 Male		1 Female		2 Males		2 Females		1 Male/ 1 Female	
	B.Wt.	n	B.Wt.	n	B.Wt.	n	B.Wt.	n	B.Wt.	n
Alice Holt	18.2#	4	18.0	12	18.1	17	18.3	18	18.7	29
Thetford	13.1	37	13.3	32	14.2	41	14.3	36	14.3	78
Ringwood	15.6	13	15.4	12	16.8	15	15.8	10	17	35
Spadeadam	13.4	21	14.5	13	15.0	11	15.1	8	14.9	10
Kershope	14.2	13	14.7	10	16.2	10	16.8#	2	15.7	19
Pickering	15.0	6	17.1	13	17.1	35	16.9	34	16.8	54
Craigellachie	13.1	9	13.3	12	13.6#	2	14.2	7	14.4	12
South Strome	14.2	6	14.6#	5	13.4#	5	-	-	13.9#	3
Salisbury Plain	14.5#	3	19.6#	2	17.7	12	18.5	10	19.2	19
Lulworth	16.4	12	13.6#	5	15.6	6	15.7	7	15.8	8
Aldershot	16.4#	1	17.7#	3	18.1#	4	18.3	7	18.2	7
Bramley	16.7#	3	16.4#	2	18.3#	3	-	-	17.1	21
Stanford	12.3#	1	17.3#	2	15.0#	1	16.8	6	16.1	8
Kirkcudbright	12.7#	1	15.0#	3	-	-	18.7#	3	17.3	14

Table 4.4: P-values for pairwise comparisons of differences between average body weight of adult females (2+ yrs) according to the number and sex of the foetuses present (Table 4.3), in all study sites, using Wilcoxon Matched Pairs Tests.

No. and Sex of Foetuses	1 Male	1 Female	2 Males	2 Females	1 Male/ 1 Female
1 Male					
1 Female	0.056				
2 Males	0.009**	0.279			
2 Females	0.005**	0.049*	0.110		
1 Male/1 Female	0.003**	0.048*	0.249	0.814	

4.2.5 Indices of Habitat Quality and Population Density

The three separate indices of habitat quality in relation to population density and one index of absolute population density were explored to assess the extent of variation between populations and, where appropriate, within populations between years. These independent indices were then related to the four parameters of adult reproductive success to investigate the influence of density and resource availability on population fecundity.

Jaw Length and Diastema Height

There is considerable variation in jaw size between populations (Table 4.5), an index which reflects habitat quality and the number of animals sharing the resources. From the table it is apparent that the average jaw length at adulthood of animals born in any year is highly variable between populations (2 way ANOVA site/year: $p<0.001$), ranging from 147.43mm at Thetford to 160.18mm at Ringwood. The average minimum diastema height shows a similar degree of variability ($p<0.001$), with a minimum average value of 9.53mm at Spadeadam and a maximum of 11.49mm, also at Ringwood. Although the variation in these two indices across the study sites seems generally to correspond, and there is a significant correlation of the two in all cases ($p<0.001$), the two measures are not totally dependent, as correlation between these two jaw indices accounts for less than 20% of the variation in either measure.

A one way ANOVA identified significant variation between years within a study site in the average jaw length of adults of different cohorts (groups of animals born in the same year) in four out of the nine sites considered, Alice Holt ($p<0.01$), Thetford ($p<0.001$), Spadeadam and Kershope (both $p<0.05$), (variation at Pickering just misses significance, $p=0.078$). In almost all sites considered, (seven out of nine), there is a pattern of falling average jaw length with calendar year (Table 4.6). The example of Thetford is used in Fig 4.4 to illustrate this trend. The exceptions are Alice Holt, where the average jaw length fell from an originally high value to a lower level in the late seventies, but has risen again over the last decade, and Ringwood, where there is no detectable pattern. There is little equivalent variation in average diastema height of a cohort within a study site over time. Only at Alice Holt ($p<0.001$) (and possibly Thetford, $p=0.069$) is this variation significant and no consistent trends were apparent at this site or, indeed, any of the others considered.

Table 4.5: Average jaw length and average minimum diastema height of adult does (2+ years) in each study site, standard errors are in brackets. All measurements given in millimetres.

Study Site	Average Jaw Length	Average Diastema Height	Sample Size
Alice Holt	159.1 (.30)	10.8 (.05)	268
Thetford	147.4 (.24)	10.6 (.03)	584
Ringwood	160.2 (.38)	11.5 (.06)	183
Spadeadam	149.6 (.33)	9.5 (.04)	224
Kershope	151.5 (.28)	9.6 (.04)	279
Pickering	155.9 (.22)	10.0 (.03)	464
Craigellachie	151.8 (.29)	10.1 (.04)	347
Queens	148.3 (.57)	9.8 (.07)	106
South Strome	152.2 (.39)	9.8 (.06)	128

Table 4.6: Average jaw length of adults (2+ years) from each year’s cohort, at each study site (minimum sample size of 5)

Year	**Study Site**								
	Alice Holt	Thetford	Ringwood	Spadeadam	Kershope	Pickering	Craig ’chie	Qu’ns	South St’me
1966	163.1	-	-	-	-	-	-	-	-
1967	-	-	-	-	-	-	-	-	-
1968	160.8	-	-	-	-	-	-	-	-
1969	157.4	-	-	-	-	-	-	-	-
1970	157.8	-	-	-	-	-	-	-	-
1971	159.7	-	-	-	-	-	-	-	-
1972	156.6	-	-	-	-	-	-	-	-
1973	159.5	151.3	-	-	152.1	-	-	-	-
1974	155.1	151.8	-	-	-	-	-	-	-
1975	157.7	149.6	-	153.9	155.1	157.4	150.4	-	-
1976	155.0	150.7	-	-	151.4	159.4	154.2	-	-
1977	157.7	148.4	160.8	-	156.1	157.0	153.9	-	-
1978	157.2	148.3	161.2	150.7	155.0	156.6	152.4	147.7	153.8
1979	159.7	147.5	162.8	152.0	151.3	156.5	153.9	152.1	154.5
1980	161.2	147.0	160.0	151.0	150.6	156.2	153.0	148.9	151.9
1981	-	145.8	161.7	150.4	150.6	156.2	151.8	147.6	152.3
1982	161.2	147.6	160.6	148.4	151.5	156.4	151.2	148.6	151.8
1983	-	146.7	159.6	149.0	150.7	157.0	149.9	145.1	151.7
1984	160.5	147.8	157.6	147.9	148.8	155.6	152.0	149.3	151.0
1985	162.5	147.9	159.9	149.1	150.7	155.6	152.1	-	149.7
1986	159.6	146.5	159.7	149.4	151.8	155.8	152.3	149.4	151.0
1987	160.1	145.9	159.5	149.2	154.9	154.3	152.1	143.9	-
1988	157.8	144.1	-	147.4	152.9	153.0	150.9	-	-
1989	-	140.4	-	145.0	150.8	154.8	-	-	-
1990	-	-	-	-	-	-	-	-	-

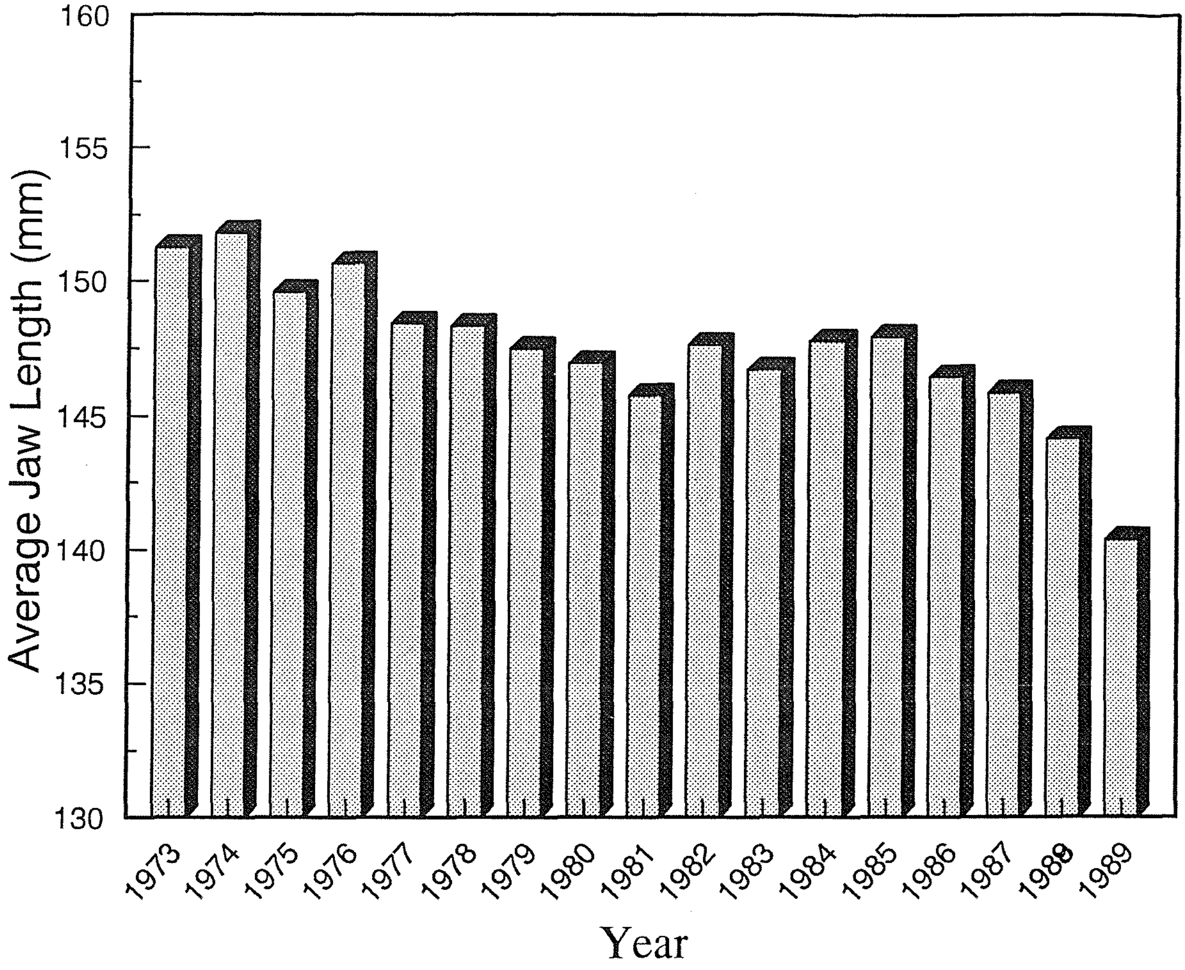


Figure 4.4: The average jaw length as adults of each year's cohort at Thetford.

Rumen Liquor Quality

The average amount of free ammonium nitrogen in the rumen liquor of samples collected from 1990 only, from twelve study sites, is shown in Table 4.7. The figures are of comparative use only as it was not possible to convert them into absolute concentrations. Analysis involved between site comparisons only, as samples were only available from a single year. Values ranged from 0.10 at Spadeadam to 0.797 at South Strome (absorbence at 660nm), and this variation was significant ($p<0.05$).

There was a significant correlation across study sites of this index of habitat quality with average jaw length ($p<0.05$), but not with average diastema height, of the population.

Pellet Group Counts

Pellet counts were conducted at all Forestry Commission sites during 1990 and 1991. Such counts can be used to calculate the absolute number of deer using a given area. This type of calculation requires specific knowledge of deer defecation rates, plus information on decay rates of dung pellets in different locations and habitats. In this thesis, however, this measure of population density was used purely for comparative purposes, and, furthermore, information on differential rates of decay was not available. Pellet counts were only carried out at Forestry Commission sites, where the structure and composition of the habitats are similar (see Chapter 2), therefore, variation in decay rate between sites was assumed to be negligible. Although this assumption was almost certainly not fully satisfied, the introduced error is unlikely to distort the results sufficiently to invalidate a rank ordering of study sites, based on this index. Because the dung count data were to be used in this way, it was deemed inappropriate to attempt to convert the counts obtained into actual deer densities, and pellet group densities were used as a relative index of deer abundance only.

For each site examined, the proportion of each habitat type was calculated under the following categories, according to the age of the stand: restock site (up to 7 years), pre-thicket (8 to 14 years), thicket (15 to 27 years), pole (28 to 40 years) and mature (40 years plus). In each site, a number of 100 by 1 metre transects were walked, so that the proportion of each habitat type sampled was approximately equal to the proportion of that

Table 4.7: Average absorbence value (at 660nm) derived from the Indophenol Blue reaction to determine the level of free ammonium-nitogen in the rumen liquor, standard errors are given in brackets. # denotes a value based on less than six samples.

Study Site	Average Absorbence	Sample Size
Alice Holt	0.552 (.159)	10
Thetford	0.192 (.106)	20
Ringwood	0.480 (.120)	18
Spadeadam	0.100 (.081)	20
Kershope	0.169 (.096)	22
Pickering	0.123 (.062)	20
Craigellachie	0.619 (.188)	21
Queens	0.219 (.109)	10
South Strome	0.797 (.131)	14
Lulworth	0.366 (.299)	10
Stanford	0.102[#] (.118)	3
Kirkcudbright	0.235 (.114)	10

same habitat in the whole of the study site. Thus, the intensity of sampling was weighted to take account of variation in forest structure between sites. The figures presented in Table 4.8 are given as the number of pellet groups per hundred metre transect of one metre width.

There was a wide variation in this index of population density between the study sites ($p<0.01$). Thetford appeared to hold the highest density of animals with a count of almost thirteen pellet groups per transect. Likewise, the figures in the northern forests of Spadeadam, Craigellachie and Queens were also relatively high. In contrast, the figure of only 1.67 at South Strome indicated a density less than one seventh of that found at Thetford.

This index of absolute density was examined for correlations with the indices of habitat quality in relation to population density, derived earlier. There was a significant correlation between pellet group counts (data available from 1990/91 only) and average jaw length ($p<0.05$) of a population (all years combined), but there was no correlation with either average diastema height or average rumen liquor quality (1990/1) of the population.

4.2.6 Variation in Reproductive Success in Relation to Indices of Habitat Quality and Population Density

Jaw Size

For each population, the reproductive performance of adult does in a particular year was examined in relation to the average adult jaw length and average adult diastema height of the cohort which was born in that same year.

No correlation was detected between percentage ovulation of adult females in a population in a given year with either the average adult jaw length or the average adult diastema height of does born in that same year. Neither was there any correlation of percentage pregnancy in the year of a cohort's birth with average adult jaw length of that cohort.

Table 4.8: Average number of pellet groups per plot (1x100 metre transects) and number of plots sampled at each study site, standard errors are given in brackets.

Study Site	Pellet Groups Per Plot	Number of Plots
Alice Holt	7.58 (2.53)	26
Thetford	12.98 (3.13)	42
Ringwood	3.47 (1.50)	17
Spadeadam	10.57 (2.14)	28
Kershope	6.23 (1.07)	44
Pickering	2.74 (0.56)	42
Craigellachie	10.16 (2.02)	32
Queens	9.96 (1.48)	24
South Strome	1.67 (0.41)	24

There was, however, a significant correlation between both corpora lutea production ($p<0.05$) and foetus production ($p<0.01$) with average adult jaw length of a cohort at South Strome. The latter relationship was also significant at Alice Holt ($p<0.05$) and Thetford ($p<0.001$).

The three measures of adult reproductive success in a given year, percentage pregnancy, corpora lutea production and foetus production, were significantly correlated with the average diastema height of a cohort born in that same year, at both Thetford and Spadeadam (all correlations $p<0.05$). (However, in the case of Spadeadam, these correlations indicated a negative relationship.) In addition, there was a significant relationship of foetus production with the average diastema height of a cohort at Alice Holt ($p<0.05$).

Reproductive success may be affected, not only by the conditions that deer experience immediately prior to conception, but also by the conditions experienced in the previous winter. The above analyses limit investigation to the relationship between reproductive success and habitat quality indices (jaw size of a cohort born in the same year) that only reflect the conditions of the preceding summer (see Chapter 2). To account for this, the analyses were repeated using average jaw length and diastema height calculated from overlapping biannual periods within each population. For each of the four reproductive parameters, correlations using biannual periods produced exactly the same results. The only differences concerned the associated levels of significance.

To investigate the influence of habitat quality in relation to population density on variation in reproductive success across populations, the data for each study site were combined to include all years. This increased the sample sizes available, and took account of the fact that the effect of a particular calendar year may not be consistent at different locations. Spearman's rank correlations were used to test the association of these population averages, because of the genetic element that may be exist in differences of gross average jaw size.

With the exception of the percentage ovulation rate, each of the parameters of adult reproductive success showed a significant correlation with one or other of the jaw size indices across populations, and this correlation was always at the five percent level (Table 4.9). Where the relationship of a reproductive success parameter and the jaw size index was not significant, it was only marginally not so, with p-values between 0.06 and 0.10.

The relationship between percentage pregnancy and average diastema height is plotted in Fig 4.5. From this graph, it seems that Spadeadam and South Strome had higher pregnancy rates than predicted by this index (they fall below the line), whereas Ringwood had a lower than predicted rate. Similarly, Fig. 4.6 plots the relationship of average jaw length with corpora lutea production. It is evident from this graph that Ringwood again, but also Alice Holt, had lower average corpora lutea production than predicted by jaw length. In contrast, it seems that Thetford had higher than predicted corpora lutea production. A fairly strong relationship of average diastema height with average number of foetuses produced is illustrated in Fig. 4.7. However, at Ringwood foetus production was considerably lower than that predicted by this index.

These graphs were re-plotted with each year from each site as a single data point. These plots revealed the degree of clustering and/or overlap between study sites for these relationships, however, it was not possible to make any statistical inferences from such analysis, because each data point was not independent of each of the other points on the graph. Despite this, the plots could be used for descriptive analysis; when this was done the same general pattern emerged. However, there was a distinct division of Alice Holt into two clusters consisting of data from the 1970's and 1980's in separate groups. Generally, where reproductive performance at Alice Holt was lower than that predicted by jaw size, it was during the 1980's when this shortfall was most acute.

The relationships of reproductive success with habitat quality (jaw size) between study sites were investigated further, by comparing data from each population derived from only a single calendar year, and repeating for each year where most sites were represented (1983, 1986 and 1988). Few significant relationships were detected, but this analysis may have been confounded by inconsistent effects of a particular calendar year at different study sites across Britain, as well as small sample sizes.

Table 4.9: P-values for correlations between reproductive performance of adults in a population and average jaw size at that study site (jaw length and minimum diastema height), all years' data combined. (* denotes a significant correlation at the five percent level.)

Jaw Index	**Reproductive Performance**			
	Percentage Ovulation	Average No. of Corp. Lutea	Percentage Pregnancy	Average No. of Foetuses
Av. Jaw Length	0.500 ns	0.046 *	0.082 ns	0.102 ns
Av. Diastema Height	0.383 ns	0.060 ns	0.012 *	0.034 *

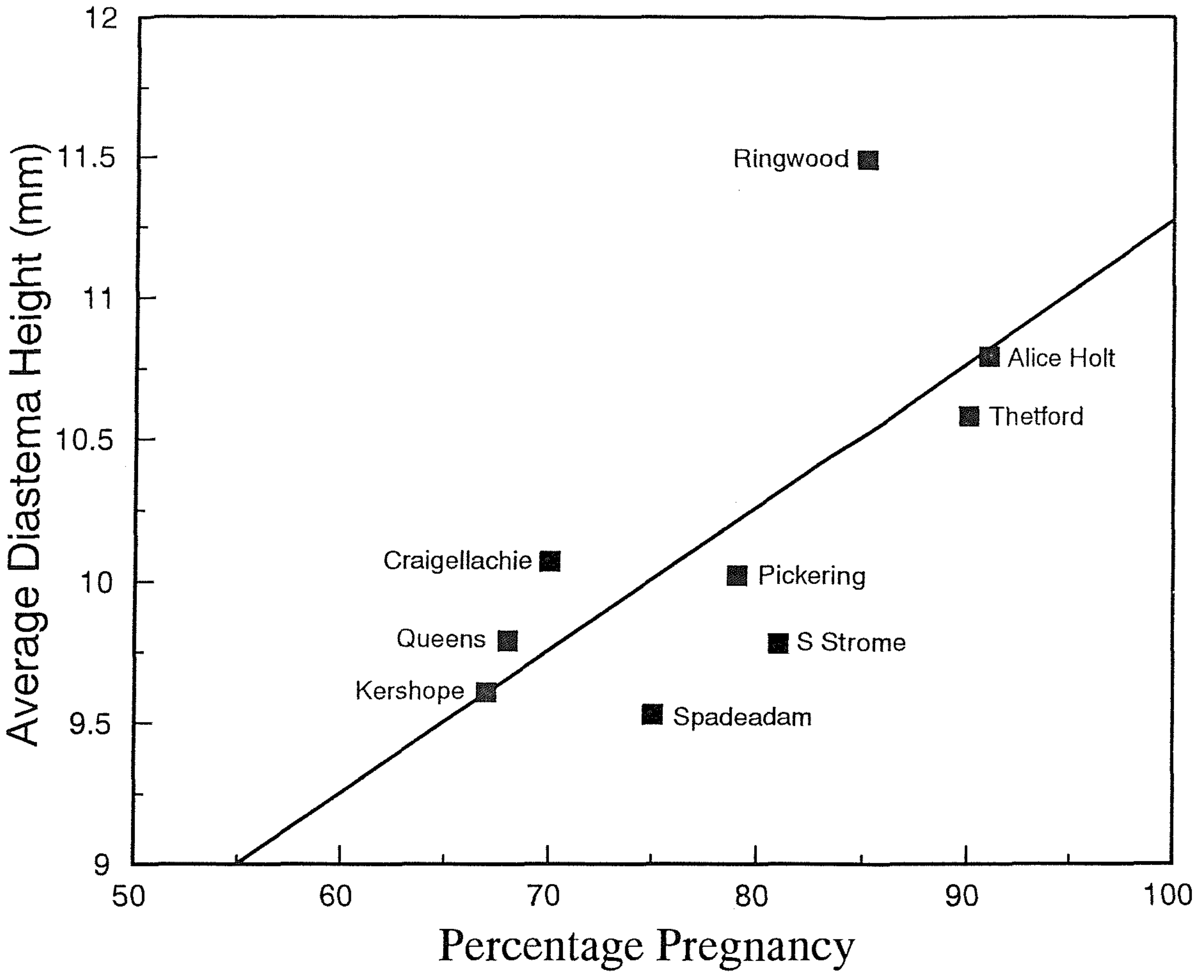


Figure 4.5: The relationship between percentage pregnancy among adults (2+ years) and average minimum diastema height of adults at each study site.

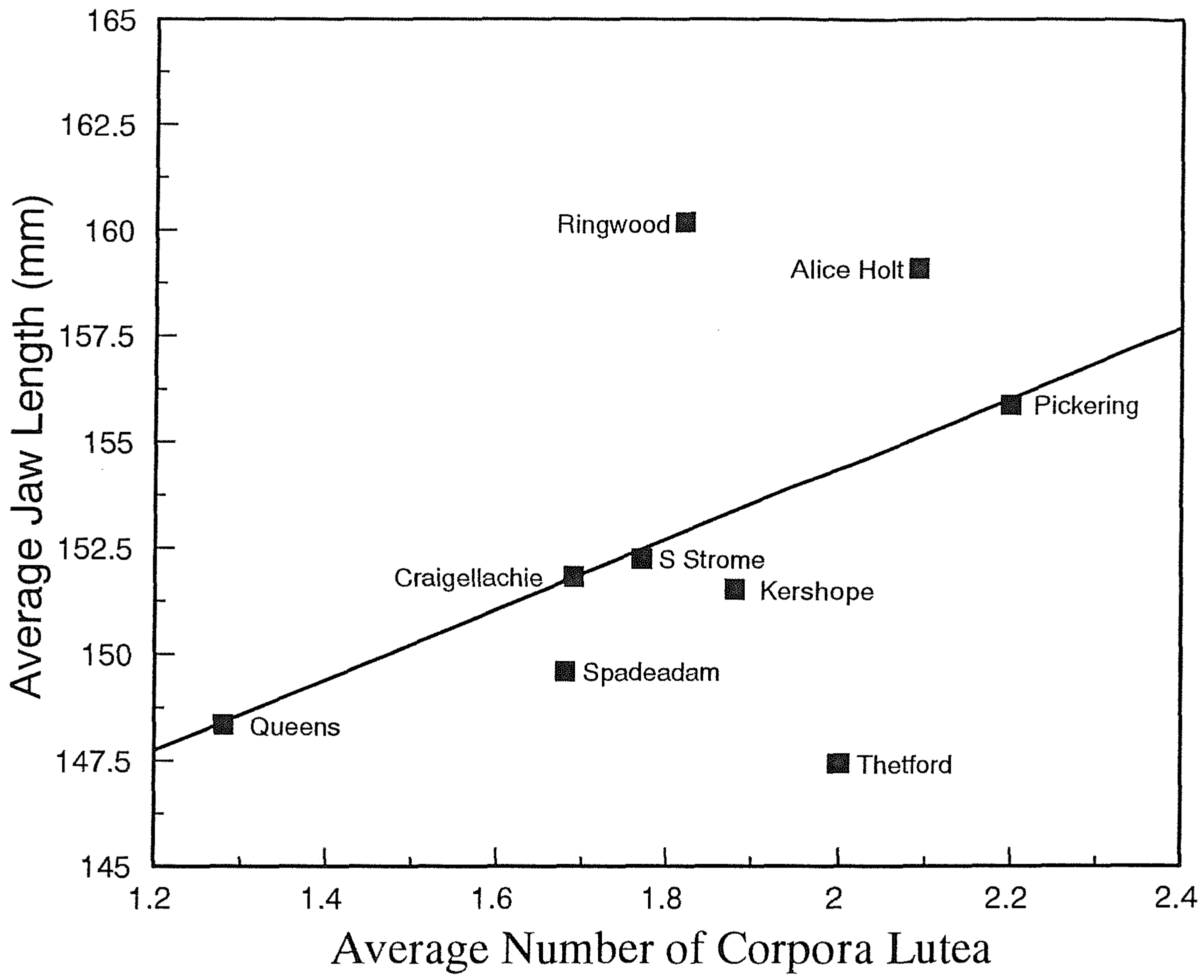


Figure 4.6: The relationship between average number of corpora lutea per ovulating adult (2+ years) and average jaw length of adults at each study site.

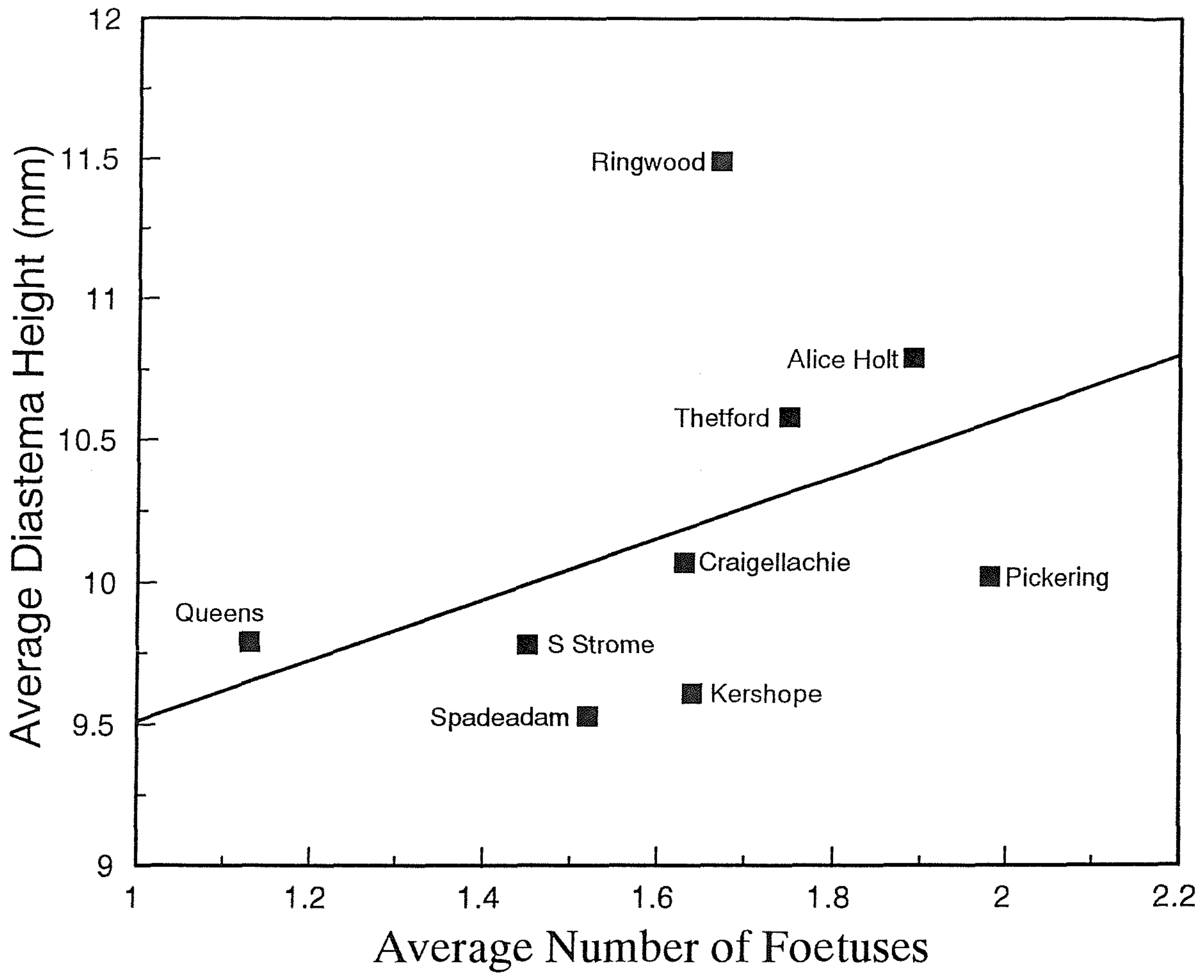


Figure 4.7: The relationship between average number of foetuses per pregnant adult (2+ years) and average minimum diastema height of adults at each study site.

Rumen Liquor Quality

The four parameters of adult reproductive success from 1990 data were examined for relationships with forage availability per individual, as estimated by rumen liquor quality (samples were available from 1990 only). There was a significant correlation between adult percentage ovulation of a population and average rumen liquor quality ($p<0.001$), but this was the only significant relationship. This result is, in any case, not particularly helpful, as values for this reproductive parameter fall between 97 and 100% at all sites.

Pellet Group Counts

There were no significant relationships between any of the four measures of adult reproductive success (calculated as an average over all years from all available data) and pellet group counts from each of the study sites. In pooling data for all years, however, it is assumed that animal density, as assessed by pellet counts, was unlikely to have changed substantially over the short period from which the reproductive success parameters were calculated. When analyses was repeated using data from 1990 only (the year in which the dung surveys took place), there was a significant negative relationship across study sites between percentage of does pregnant in 1990 and pellet group counts.

4.2.7 Effects of Climate

Six measures of climatic conditions were chosen for their ability to reflect the year round meteorological conditions experienced by the roe deer populations at the different study sites. These were average monthly winter temperature (November to March), average monthly winter rainfall (November to March), average monthly mid-winter temperature (January to February), average monthly winter snow lie (November to March), average monthly summer temperature (May to June) and average monthly summer rainfall (May to June).

The variation in these six climatic variables between study sites (all years combined) is summarised in Table 4.10. It is apparent from this table that there is a high degree of inter-correlation of the six variables selected. Indeed, in almost every case, each climatic variable was very highly significantly correlated with each of the other five (Pearson's univariate correlations on population averages of pairs of climatic variables,

Table 4.10: Average climatic conditions at each study site (all years combined) - average monthly winter (Nov-March) temperature (°C); average monthly winter (Nov-March) rainfall rainfall (mm); average monthly mid-winter (Jan-Feb) temperature (°C); average monthly winter (Nov-March) snow lie (no. days snow lie at 0900 hrs); average monthly summer (May-June) temperature (°C); average monthly summer (May-June) rainfall (mm).

Study Site	Winter Temp.	Winter Rainfall	Mid-Win Temp.	Winter Snow	Summer Temp.	Summer Rainfall
Alice Holt	4.84	73.9	3.61	1.59	12.5	53.9
Thetford	4.77	54.0	3.64	1.69	12.5	56.7
Ringwood	5.51	81.9	4.09	0.68	13.1	45.3
Spadeadam	2.93	150.2	1.84	6.80	10.1	76.1
Kershope	2.93	150.2	1.84	6.80	10.1	76.1
Pickering	4.01	67.6	2.55	4.70	10.8	53.6
Craigellachie	3.74	69.5	2.67	4.79	10.8	68.3
Queens	2.69	96.9	1.25	8.94	10.3	60.6
South Strome	5.15	168.2	3.99	1.78	11.4	69.1
Salisbury Plain	6.10	80.0	4.95	0.60	13.0	38.6
Lulworth	6.95	83.0	5.71	0.47	13.2	32.5
Aldershot	5.14	63.8	3.93	2.60	13.3	49.1
Bramley	4.96	88.6	3.57	1.53	13.0	53.3
Stanford	4.77	54.0	3.64	1.69	12.5	56.7
Kirkcudbright	5.27	89.1	4.31	0.17	11.0	62.3

$p<0.001$). The exception was average monthly winter rainfall, which was generally correlated with each of the other variables at the five percent level, but was not significantly correlated with average monthly mid-winter temperature ($p=0.26$).

In two cases, (Thetford and Stanford; Spadeadam and Kershope), the values presented are identical as the nearest weather station was the same for each site due to their proximity. There is clearly a wide degree of variation in climate between the other study sites however, reflecting their geographical location. There was more than a three fold difference in average monthly winter rainfall between the wettest study site, South Strome (168.2mm), and the driest, Thetford (54.0mm). Generally, at most sites, winter rainfall averaged between 70 and 100mm per month, but at South Strome, Spadeadam and Kershope the monthly average exceeded 150mm. There was less variation in average monthly summer rainfall, the maximum of 76.1 found at Spadeadam and Kershope was only just double that of 32.5mm at Lulworth. There was a similar pattern in average monthly January-February temperature. Among the study sites in England, this parameter averaged over 4°C whereas in Scotland it generally fell below 3°C. The exceptions were the west coast populations of South Strome and Kirkcudbright where the average monthly January-February temperature was equivalent to those found in England. These were the three variables used in the analysis of the effects of climate on reproductive performance (see below).

To examine the influence of climatic variation on reproductive success; both between years within a site, and between sites, these climatic variables were introduced into stepwise multiple regressions (Snedecor and Cochran 1967) in relation to each of the four parameters of adult reproductive success. To overcome the problem of inter-correlation among the climatic variables, only three of the six variables were included in the multivariate analysis below. Average monthly mid-winter temperature (January-February) and average monthly winter rainfall (November-March) were not inter-correlated, but were strongly correlated with each of the other variables, therefore, they were selected. Average monthly summer rainfall (May-June) was also included as it was significantly correlated with both pregnancy rate and foetus production in the preliminary univariate correlations (see below), and provided representation of summer climatic factors in the analysis.

Variation in Reproductive Success Between Years Within a Site in Relation to Climate

In multiple regressions, there were no correlations between adult ovulation rate or corpora lutea production between years and any of the climatic variables, at any of the study sites. However, in multiple regressions on adult percentage pregnancy, average monthly mid-winter temperature was inversely correlated with between year variation in this parameter at Spadeadam ($r^2=0.65$, $p<0.05$). In multiple regressions of foetus production within a site between years, climatic variables were selected at a few sites: at Thetford, foetus production (number of foetuses implanted per adult female) was correlated with both average monthly mid-winter temperature (negative) and average summer rainfall (cumulative $r^2=0.97$, both $p<0.01$). Conversely, at South Strome and Stanford, summer rainfall was negatively correlated to foetus production ($r^2=0.89$, $p<0.05$; $r^2=0.99$, $p<0.001$ respectively) and at Spadeadam, average monthly mid-winter temperature was positively correlated to foetus production ($r^2=0.63$, $p<0.05$).

Multiple regressions were also used to investigate the relationship of the reproductive performance of yearlings in a particular year with climatic conditions in that same year, within each site. There were significant correlations between corpora lutea production and average summer rainfall in a particular year at Thetford ($r^2=0.72$, $p<0.05$) and Spadeadam ($r^2=0.99$, $p<0.01$), the latter an inverse relationship. There were also significant correlations of yearling percentage pregnancy with the average mid-winter temperature that year at two sites, Kershope and Craigellachie ($r^2=0.92$ and 0.78 respectively, both $p<0.05$), but, in the latter case, this was again an inverse relationship. There were no significant correlations of climate in a particular year with yearling ovulation rate or foetus production.

Variation in Reproductive Success Between Sites in Relation to Climate

The relationship of climate and reproductive success between study sites was examined within single calendar years, and with data pooled for all years.

1. Calendar Years

Four calendar years were chosen, 1983, 1986, 1988 and 1989, as most sites were represented in these years. The multiple regressions for this section used only adult data for comparisons across sites, as the yearling sample sizes derived from single years were too small.

No climatic variables were selected for inclusion in the regression equations of percentage ovulation or corpora lutea production with climate in any of the four years. However, in the multiple regression of percentage pregnancy with the climatic variables, there was a significant association of summer rainfall in 1983 ($r^2=0.63$, $p<0.05$), and a significant inverse correlation with winter rainfall in 1988 ($r^2=0.45$, $p<0.05$). In addition, in 1986 there was a significant inverse association of winter rainfall ($r^2=0.70$, $p<0.001$) with foetus production across sites.

In the above analysis, the influences of climate are considered to be immediate, but equally the effects of a severe winter may carry over into the following breeding season. Therefore, still using data from single calendar years, the analysis was repeated, but correlating the reproductive success from a particular year with climatic data from the previous winter. No differences between this and the above analyses were found.

2. Population Averages (all years combined)

The above analyses only consider data from four years. Therefore, to investigate the cumulative influence of climatic conditions over a number of years on the variation in reproductive performance between study sites, gross population averages were calculated for the four measures of adult reproductive success and the six climatic variables. The relationship between each of these climatic variables and each of the parameters of reproductive success was then examined in a series of univariate correlations. This analysis revealed no significant relationships between the average climatic conditions at a particular study site and the population average for percent ovulation or corpora lutea production among adults at the same site. In contrast, the population average for adult percentage pregnancy was significantly correlated with four of the six climatic variables (winter temperature, mid-winter temperature, snow lie and summer rainfall; all $p<0.05$), and adult foetus production was significantly correlated with all six (all $p<0.05$).

These relationships were explored further, by subjecting each of these population averages for measures of reproductive success to multiple regressions against the average climatic conditions at each study site. These analyses were repeated for yearlings and adults.

In these analyses, no climatic variables were selected for inclusion in the regression equations of percentage ovulation or corpora lutea production in adults. However, among yearlings, there was significant association of average monthly mid-winter temperature with ovulation rate ($r^2=0.59$, $p<0.05$), and with corpora lutea production ($r^2=0.68$, $p<0.05$).

Average monthly mid-winter temperature was also selected for inclusion in the regression equations of adult pregnancy rate ($r^2=0.38$, $p<0.05$), and of foetus production for both adults ($r^2=0.30$, $p<0.05$) and yearlings ($r^2=0.80$, $p<0.01$). In addition, there was a significant inverse association of winter rainfall with yearling pregnancy rates ($r^2=0.58$, $p<0.05$).

4.2.8 The Relative Effects of Density Dependent and Density Independent Factors

Environmental variables such as population density, food availability and climate may be strongly inter-related (see Section 4.1). In particular, the effects of climate may operate through the restriction of food resources, for example, by limiting the length of the growing season. In this case, habitat quality indices such as jaw size or body weight may reflect a substantial amount of the climatic influence. However, climatic effects may also act independently, for example, through the costs of thermoregulation. In order to assess the relative, independent influence of i). Climate, ii). Population density in relation to available resources, and iii). Body weight, on reproductive performance, a further set of multiple regressions were carried out, including not only climatic variables, but also body weight, jaw size and pellet group counts (the last for between site analysis only).

Between Years Within Each Site

The few significant relationships between climatic variables and adult reproductive success between years within each study site, were, in four out of the five cases, replaced by stronger relationships with jaw size and body weight. In the same way, the climatic variables selected in the equations of yearling reproductive success were supplanted by jaw size and/or body weight, when these variables were included in the regressions.

Between Study Sites

When body weight, jaw size and pellet counts were also considered, to determine the relative influence of these variables and climatic factors on variation in reproductive performance between study sites (using data pooled for all years), a fairly consistent pattern emerged in the analysis of both adult and yearling data sets. Generally, climatic variables still seemed to account for variations between sites in rates of pregnancy and foetus production but, in contrast, body weight was the major determinant of ovulation rate and corpora lutea production. Table 4.11 summarises these results, giving the correlation coefficients and associated significance values.

Table 4.11: Multiple regressions of reproductive performance (four measures) of a population against climatic variables (average monthly mid-winter temperature, average monthly winter rainfall and average monthly summer rainfall), habitat quality indices (body weight, average jaw length and average minimum diastema height) and the density index, dung counts for a. adults (2+ years) and b. yearlings. (Significance levels: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

Reproductive Performance	**Adults**			**Yearlings**		
	Variable Selected	Cumm. R^2	Sig.	**Variable Selected**	Cumm. R^2	Sig.
Percentage Ovulation	-			Body Weight	0.62	*
Av. No. of Corp. Lutea	Body Weight	0.67	**	Body Weight Mid-Win Temp	0.80 0.96	** **
Percentage Pregnancy	Mid-Win Temp	0.35	*	Winter Rain	0.58	*
Av. No. of Foetuses	Body Weight Winter Rain	0.73 0.84	*** *	Mid-Win Temp Dung Counts	0.80 0.94	** ***

4.3 DISCUSSION

It is widely accepted that body weight is a crucial determinant of reproductive success in mammals. Several authors have found that age specific differences in fecundity among mature red deer hinds could be explained entirely by differences in body weight (Hamilton and Blaxter 1980; Albon et al. 1986). Similarly, a disparity in fecundity of milk and yeld red deer (Clutton-Brock et al. 1983; Mitchell et al. 1986) and reindeer (Skogland 1985) has also been attributed to body weight variation. Body weight has also been cited as a highly important factor affecting age at puberty (reindeer: Reimers 1983; red deer: Ratcliffe 1987; fallow deer: Langbein and Putman 1992), and therefore, influential in defining the recruitment potential of a population.

For roe deer specifically, several authors have noted previously that body weight may affect doe fecundity. Ratcliffe and Mayle (1992) suggested a link between maternal weight and the probability of ovulation and conception, particularly among juveniles. This was also observed by Bathurst (1988), who found that kids which had ovulated were significantly heavier than those that were still sexually immature in several populations. In addition, in populations where average fecundity was high, yearling body weight was correspondingly high, and *vice versa*. In a single population in western France, Gaillard et al. (1992) found that pregnant yearlings were significantly heavier than non-breeding animals of the same age. However, no clear relationship between body weight and fecundity among adult does was detected by either of these authors.

Evidence that variation in litter size in roe deer is linked to maternal body weight has been provided by several authors. Loudon (1987) claimed that variation in body weight could explain the differences observed in the average number of corpora lutea per doe between yearlings and adults, as well as differences between roe deer populations, in this fecundity measure. Some studies of roe deer populations in Europe have also identified a strong positive correlation between body weight and average litter size among adult does. Blant (1987) found that does with two and three corpora lutea were 12% and 31% heavier respectively, than does carrying only a single corpus luteum. In one population in central France, the number of kids seen per female fell from 1.62 to 1.3 over an eight year period, and was matched by a decrease in average body weight of approximately 1.5kg (Maizeret et al. 1989). A further study, in Tuscany, Italy, compared two roe deer populations and found an average weight differential of 1.25 kg was positively associated with a significant disparity (over 30%) between the populations in the number of embryos found per culled female (Lovari et al. 1991).

Results from the present study confirm that body weight is indeed of considerable importance in determining reproductive success in British roe deer. This is particularly obvious within single study sites, where the high degree of variation in reproductive success between kids, yearlings and adults, the variation between individual animals in the sex of their offspring, and the rather smaller degree of variation in reproductive performance between years within a single population is largely explicable in terms of body weight.

4.3.1 Age at First Breeding in Relation to Body Weight Thresholds

It has been proposed that decreasing demographic vigour of a population will be first evident among the juvenile age classes (for a review see Hanks 1981). Thus, the relationship of body weight and fecundity is often strongest in these animals. Indeed, the proportion of young adults breeding has been linked to body weight in many ungulate species (red deer: Mitchell 1973; moose: Saether and Haagenrud 1983; reindeer: Skogland 1985; chamois: Bauer 1987; roe deer: Gaillard et al. 1992). Moreover, several authors have demonstrated that there are specific weight thresholds which must be achieved in order to breed successfully (Reimers 1983; Ratcliffe 1987). Langbein and Putman (1992) calculated such a weight threshold of 32kg for British park fallow deer, at which 50% of yearling does were likely to produce fawns.

In this study, the differences between kids and yearlings, and yearlings and adults, in percent ovulation and pregnancy within a site appear to be entirely weight related. This implies that there are threshold body weights for successful reproduction in roe deer. Loudon (1987) defined a threshold of 18kg (eviscerated weight when culled), above which there was a 90% chance of roe does, from several populations in northern Britain, having two or more corpora lutea. However, from the evidence of this study, it is clear that such a threshold will be specific to a single population. In some highly fertile populations, such as Thetford, very few does exceeded this threshold of 18kg, yet the average number of corpora lutea produced per doe at this site was exactly two. Indeed, between site differences in number of corpora lutea produced per doe (but not number of foetuses implanted) among yearlings persisted, even when variation in body weight was accounted for in the analysis.

At almost all sites in this study, yearling ovulation rates approached 100%, and were generally equivalent to rates among adults. The obvious exception was Queens, where only 74% of yearlings ovulated. Average body weight was much the lowest at this site, for all age classes, suggesting the possibility of a weight threshold for successful ovulation, generally applicable across Britain. However, at three sites where the animals were particularly heavy, Alice Holt, Pickering and, to a lesser extent, Kershope, the average weight of kids comfortably exceeded the yearling average at Queens of 11.16kg. Despite this, only at Pickering was kid ovulation more than a rarity. It should be noted that this analysis was necessarily based on cull weights, and it is likely that the weight differential between cull weight and weight at the rut will be greatest among kids, so comparisons of cull weights between different age classes of animals may be inappropriate.

It seems more likely, therefore, that critical thresholds for sexual maturity are not universal, but are defined independently in different populations. Clutton-Brock and Albon (1989) showed that body weight thresholds for successful reproduction among red deer hinds were highly variable between five different Scottish populations. In one population in this study, Queens, the difference between the average weight of yearlings and adults, generally less than 0.5kg at all other sites, was almost 1kg. This suggests that a higher proportion of yearlings have failed to achieve this particular critical threshold weight, and may explain the shortfall of yearling ovulation at this site.

4.3.2 Number and Sex of Embryos in Relation to Body Weight

It has been proposed that the concept of threshold body weights within populations also applies to the sex, as well as the number, of progeny a female deer is able to produce. The main theories that address the question of why animals may adjust the sex ratio of their offspring were discussed in the previous chapter (for a review see Clutton-Brock and Iason 1986).

These hypotheses variously predict that higher weight females in better condition will produce an excess of either male or female young. Williams (1979), for white-tailed deer, proposed the fractional offspring hypothesis, which suggests there is a sequence of increasing costs in reproductive effort of one female, one male, two females, two males, due to the differential metabolic demands of rearing male or female offspring. This model predicts that heavier females will tend to produce, on average, an excess of male progeny. In contrast, the local resource competition hypothesis (Clark 1978) predicts that the greatest cost to the mother of successful reproduction may accrue through increased competition for limited resources, if her offspring are philopatric. Thus, where the degree of philopatry is differential between the sexes, lighter animals in poorer condition will tend to produce a deficit of the philopatric sex.

In Chapter 3, local resource competition was suggested as the theory most suitable to explain the adaptive significance of variation in the offspring sex ratio, according to the biological characteristics of roe deer. Thus, we would expect to find that heavier animals produce more females on average. This is indeed the case, and the results indicate a sequence of increasing reproductive cost of one male, one female, two males, two females. It appears that the costs of rearing females are so much higher, that does in average condition may, with almost equal cost, produce male twins or a female singleton. Furthermore, the negative correlation of the embryo sex ratio with average body weight across study sites suggests that does in populations where resources are limiting will tend to produce an excess of male offspring.

This is in direct contrast to the predictions of Williams (1979) for white-tailed deer, but in agreement with Verme (1985), who proposed, from his observations on the same species, that the sequence suggested by Williams should be reversed. The fractional offspring hypothesis (Williams 1979) may be more applicable to white-tailed deer, where there is a degree of sexual dimorphism, a central tenet of this theory. In contrast, the size differential between the sexes in roe deer seems small and there are no obvious differential energetic costs associated with gestation and lactation for rearing either sex.

Gestation and lactation certainly seem to be a considerable drain on energetic reserves of a roe doe. Although does with any number of corpora lutea are, on average, always heavier than does that have not ovulated, non-pregnant does are equally likely to be heavier, as they are to be lighter, than females carrying a single foetus. Mitchell et al. (1976) have shown that, in red deer, the costs of reproduction are such that hinds will generally not conceive in the year following successful reproduction, and this has been related to the significantly higher body weights of yeld hinds (Mitchell et al. 1986). However, it is unlikely that such reproductive costs will result in alternate year breeding in roe deer (Gaillard et al. 1992), although these costs could be expressed through variation in litter size. This suggests that does fail to ovulate because they have not reached the critical threshold body weight. But their energetic costs are reduced relative to pregnant animals, so that pregnant does with singletons will, on average, eventually fall below the weight of barren animals.

4.3.3 Habitat Quality Indices

Body weight itself can be considered as a reflection of the environmental conditions that an animal has experienced. Klein and Strandgaard (1972) state that body weight fluctuations are often in response to other factors, such as density, food availability and climate, and these factors will therefore, indirectly affect fecundity. Factors such as population density (white-tailed deer: Morton and Chaetum 1946; roe deer: Klein and Strandgaard 1972; reindeer: Skogland 1985; red deer: Mitchell et al. 1986), food quality (white-tailed deer: Verme and Ozoga 1980; reindeer: Skogland 1983; red deer: Ratcliffe 1987; fallow deer: Langbein and Putman 1992) and climate (red deer: Albon et al. 1987; fallow deer: Putman and Langbein 1992) have all been shown to affect body weight and are, therefore, implicated as key influences on the reproductive success of a population. In addition, such factors, particularly population density and climate, may have an independent impact on reproductive success which is not translated through body weight variation.

The environmental conditions at a particular location are reflected in the growth and development of animals experiencing those conditions. Skogland (1983) found that resource limitation of reindeer calves during the late stages of gestation caused a delay in birth, and hence, curtailed the period available for growth, resulting in skeletally small animals with low body weights. Furthermore, such growth deficits can generally not be compensated for in later life (Hamilton and Blaxter 1980; Suttie 1981; Albon et al. 1986).

compensated for in later life (Hamilton and Blaxter 1980; Suttie 1981; Albon et al. 1986). Thus, skeletal size may be used as an indicator of conditions an individual experienced around the time of its birth.

Of the indices of habitat quality investigated in this study, the length of the lower jaw appears to be the most useful. This index is correlated with each of the others (positively with body weight, diastema height and rumen liquor quality, and negatively with pellet group counts) across the study sites, and shows the highest degree of variation between years within sites. Challies (1974) found an inverse relationship of jaw length and population density in red deer and concluded that this skeletal measure was sensitive to differences in nutrition an individual received. This index has also been used by several other authors, particularly in studies on red deer: differences between populations in average jaw length have been detected (Suttie and Mitchell 1983), but not between milk and yeld hinds (Mitchell et al. 1986). Albon et al. (1986) found an inverse relationship of jaw length and fecundity when comparing animals of the same body weight. Thus, more fecund animals, although skeletally smaller, were presumably in better condition, as a greater proportion of their body weight must have been due to fat reserves. This illustrates the fact that jaw length will co-vary with body weight to a certain degree, as both these parameters are influenced by the environment.

Despite the lack of variation between years at most sites in this study, there is a general trend of decreasing average jaw length of a cohort towards the present day in seven out of the nine study sites. This pattern is statistically significant at Thetford where, over the fifteen year period when cohort sample sizes are big enough, average jaw length of animals born in a given year has fallen by 11.4mm (Table 4.6). It is generally accepted that roe deer are currently expanding their range (Staines and Ratcliffe 1991), and it is possible that absolute density is increasing. However, there are no empirical data on population densities over the last decade with which to judge this hypothesis.

Roe deer prefer the early stages of growth of coniferous plantations, in particular the restock and pre-thicket stages (Staines and Welch 1984). However, once the trees are fifteen to twenty years old and the canopy begins to close, the potential carrying capacity drops due to the decrease in available food and cover. Much of the commercial coniferous

1940 and 1965. Consequently, the habitat at these sites was best suited to the requirements of roe deer during the sixties and seventies. As the canopy began to close over a greater percentage of these plantations during the eighties, and less restocking took place, it is likely that the carrying capacity of these forests fell significantly. Forest age structure was cited by Prior (1968), as having potentially large effects on the body weight and reproductive performance of roe deer.

4.3.4 Adult Reproductive Success in Relation to Body Weight and Habitat Quality

Generally, the small degree of variation in reproductive success between years within a site, identified in Chapter 3, is explained by a concomitant variation in body weight. Although there is some residual variation between years within a site in corpora lutea production, not explained in terms of body weight, this is not significant when the analysis is restricted to the period 1983 to 1990. This must be attributed to the fact that only two sites, Alice Holt and Thetford are represented in the data prior to 1983, and therefore, analysis of the unrestricted data set is unbalanced. This point is further highlighted by the fact that the few significant correlations of reproductive success with body weight, across years within a site, occur at these same two sites.

The average jaw length of a cohort may reflect resource availability at the time of that cohort's birth (Ellenberg 1974; Skogland 1983). In the present study, few significant relationships of the jaw length of a cohort with reproductive success of the animals breeding in the year of birth of that cohort could be detected. This was true whether reproductive success was related to the average jaw length of kids born in that year, or average jaw length of kids born in that year and the preceding year (to account for the possible impact of resource restriction during gestation). Only eleven out of seventy eight relationships were significant, and these, again, mainly concerned Alice Holt and Thetford. All the parameters of reproductive success except percent ovulation were correlated with average jaw size of the cohort born in that year at Thetford, while at Alice Holt, only foetus production was significantly correlated with jaw size.

In a further analysis, not presented in detail in this thesis, there was, likewise, no correlation of reproductive success in a given year and the average adult body weight of a cohort born in that same year. Indeed, inspection of the coefficients of variation within cohort born in that same year. Indeed, inspection of the coefficients of variation within each site for average body weight of animals shot in a single year and for body weight of a cohort indicated that animals shot in a particular year are more likely to have similar body weights than those from a cohort. This suggests that, for roe deer, the main source of year to year variation of body weight is variation in body fat due to the immediate conditions, rather than skeletal size, determined in the early stages of growth. This is contrast to the results of Gaillard et al. (1992), who found that the body weight of yearling roe does could be linked to environmental conditions at birth.

The data records for Alice Holt and Thetford span twenty one and twelve years respectively, far longer than for any other site. In an eight year study of a roe deer population in central France (Maizeret et al. 1989), the number of kids at foot fell 20% and was matched by a significant drop in average body weight. Therefore, it seems likely that the lack of variation in reproductive success, body weight and jaw length, between years within a study site, may simply reflect the short time period and stable conditions when data were collected at the majority of sites.

Ratcliffe and Rowe (1985) suggested that fecundity in roe deer may not always necessarily be negatively related to population density. Furthermore, Loudon (1987) found a positive correlation of density with fecundity, and concluded that roe deer did not comply with the general trend of density dependence among cervids. However, these authors fail to take account of the fact that density must be considered in relation to the available resources. In the latter study, a high density of animals was found in young plantations, where food availability and quality will generally also be high, whereas low densities were found in areas where the canopy was closed, and the availability of suitable forage would therefore, be much lower. This is supported by the fact that animals in the high density plantations were significantly heavier than those in the closed canopy areas, indicating that the density of animals was more closely approaching the carrying capacity of the habitat in the latter case. These different stages of forest growth will have different carrying capacities, and therefore, direct comparisons of absolute density are not appropriate.

Some authors have postulated that population regulation in roe deer will be stress (Ratcliffe and Rowe 1985; Vincent et al. *in press*). Bobek (1977) states that, as a consequence of territoriality, the reproductive rate of roe deer will be density independent. Klein and Strandgaard (1972) recognise the potential importance of this mechanism in population regulation of roe deer, but also emphasise that such a phenomenon would not act independently of the resource base. In fact, Bobek (1977) states that the availability of summer forage will set the minimum territory size, and defines the resulting maximum density of animals as the social carrying capacity. However, it should still be possible for a population to exceed such a capacity and, as a consequence, experience density dependent effects. If this were not the case, roe populations would presumably exhibit a ubiquitously uniform level of body condition and, hence, reproductive success. However, several authors have shown density dependent effects on body weight and fecundity in this species (Prusaite et al. 1973; Maizeret et al. 1989; Ballon et al. 1990; Lovari et al. 1991; Gaillard et al. 1992), and both measures vary widely between populations in this study. Furthermore, in an enclosed roe population at extremely high density, Ellenberg (1978) observed no loss in doe fecundity, provided adequate nutrition was available. The author concluded that the food availability per individual influenced reproductive success in roe deer, rather than density *per se.*

In this study, maternal body weight was found be highly influential in explaining variation in adult reproductive performance between study sites (the p-value of the covariate, body weight, was always less than 0.001). Indeed, the differences between sites in percentage pregnancy, corpora lutea production and foetus production were each correlated with average body weight of the population. However, even when variation in body weight was accounted for, the differences between sites, for all three parameters, were still very highly significant ($p<0.001$). Furthermore, these differences between study sites in percentage pregnancy, corpora lutea production and foetus production were all significantly correlated with the average jaw size of the population. This suggests that the habitat quality and/or population density is having a significant impact on both body weight and reproductive performance of roe deer populations, but there may also be other factors which contribute to this high degree of variability across Britain.

4.3.5 Climatic Effects on Reproductive Success

As noted earlier, the effects of climate on reproductive success may be direct

(Albon et al. 1987; Ratcliffe 1987) or indirect, for example, by influencing the growth patterns of vegetation and thus, affecting the quantity and quality of available forage (Albon 1983; Teer 1984; Putman and Langbein 1992). These indirect effects of climate may, therefore, be translated through changes in body weight. Indeed, almost all the significant correlations of climatic variables and adult reproductive success, between years within a site, in this study were supplanted by body weight and/or jaw size, when these factors were included in multiple regressions. This indicates that year to year variation of climate will affect the reproductive performance of a roe deer population indirectly, by influencing the primary productivity of the vegetation, and hence, the condition of the animals living on that range.

Similarly, the significant correlations, between sites, of yearling ovulation rate and corpora lutea production with climate were supplanted by body weight in multiple regressions, although winter climate explained a significant proportion of the residual variation in the latter case. In addition, although climatic variables were not able to explain the variation in ovulation rate or corpora lutea production among adults, body weight was selected in the multiple regression on corpora lutea production. (The ovulation rate was invariable between sites, see Chapter 3.) This suggests that any climatic influences on variation in ovulation success between populations tend to be expressed through variation in body weight.

However, climate was found to have a strong independent effect on variation between populations in both adult and yearling pregnancy. The average winter climatic conditions (temperature and/or rainfall) remained in all four regression equations, even when body weight and jaw length were also considered. Indeed, although univariate correlations revealed no significant relationship between climate at a study site and ovulation among adults (percentage ovulation or corpora lutea production) at that site, strong associations were identified between all climatic variables and percentage pregnancy and foetus production. Body weight, in addition to climate, explained a significant proportion of the total variation in average number of foetuses produced, but not in percentage pregnancy, between populations. This was probably a consequence of the strong relationship between body weight and corpora lutea production, as the number of foetuses a doe implants is necessarily limited by the number of ova that were successfully fertilised at the rut.

This suggests that, in general, ovulation, which occurs during the relatively mild summer months in roe, is not influenced directly by climatic factors, but is subject to control through body weight thresholds, according to the density of animals in relation to the food availability. Any effects of climate on ovulation are translated indirectly, through maternal body weight. Summer climate has been shown to affect the body weight of red deer through its influence on heather production (Albon 1983), and such effects may play an important role in determining ovulation in roe deer, particularly among yearlings. In addition, climatic influences on variation in fecundity among adults, between years within a site, may also be translated through altered rates of growth and development. However, it is worth noting in this context that Britain experienced a relatively mild decade during the eighties, and extreme weather conditions were rare. In circumstances where climatic conditions at a single site were more variable, it may be possible to detect a direct influence of climate on year to year variation in pregnancy rates, similar to that noted between sites.

In contrast, the percentage of animals subsequently becoming pregnant and the number of foetuses successfully implanted in a particular population is, to a larger degree, influenced by winter climatic factors. This presumably relates to the fact that implantation and active gestation eventually occur during the most hostile months of winter. This influence is, probably due to the costs of thermoregulation and maintenance activities, which may vary considerably according to temperature, windspeed and rainfall (Clutton-Brock and Albon, 1983), particularly during the winter. In addition, there is an independent impact of climate on the average number of corpora lutea produced in yearlings, over and above the effects of body weight. It is likely that a yearling doe, breeding for the first time, will be only marginally above the necessary weight threshold, and will, therefore, be particularly sensitive to the direct influences of inclement weather (see Table 4.11 in Section 4.2 for a summary of these results).

From the data presented in this chapter, it is evident that body weight, habitat quality in relation to population density (jaw size), absolute deer density (pellet counts) and climate are all influential, to a greater or lesser degree, in determining the reproductive success of roe deer in Britain. The differences in reproductive potential within populations are small, and often seem to be mediated through variation in body weight which, itself, may reflect slight variations of habitat quality, density or climate.

In contrast, the variation in reproductive performance between populations is pronounced, and cannot be explained purely in terms of habitat related factors or climate. However, each of these are important: variation in ovulation success is strongly related to maternal body condition which, itself, responds to variation in range quality, and the proportion of animals subsequently becoming pregnant is largely dependent on climatic conditions. The data on average body condition of the study populations suggest that the animals at Ringwood and, to a lesser extent, at Alice Holt, Salisbury and Aldershot, are performing at a level lower than that predicted by their condition, while those at Thetford, and to a lesser extent, Queens and Spadeadam are performing at a higher than predicted level. This implies that there may be reasons, other than environmental factors alone, which can explain this variation of reproductive performance between populations of roe deer in Britain.

CHAPTER 5

GENETIC VARIATION IN RELATION TO REPRODUCTIVE SUCCESS

5.1 INTRODUCTION

This thesis has considered variation in the reproductive success of roe deer in relation to environmental parameters; in particular, the way in which factors such as climate, density or range quality may limit the reproductive potential of animals which experience these conditions. However, the reproductive success of an individual may also be constrained by its genetic constitution. Certain genotypes may be positively or negatively associated with phenotypic traits that confer an advantage on an individual in terms of its reproductive success. Furthermore, the response of an individual to the environmental conditions it experiences may also be modified, according to its genetic type. Selection may act in different ways in a heterogeneous environment (Hedrick et al. 1976), and, therefore, genotypes will often have different fitness under differing environmental conditions. (However, in practice, it is unlikely that these dual influences of genetic constitution on reproductive success, both direct and interactive, will be independent, and the observed effects will be evident in combination.)

A population may be defined as 'a reproductive community of individuals who share a common gene pool' (Dobzhansky 1950). Therefore, by definition, two individuals from the same population will, on average, be genetically more related to each other than they are to a third individual from a different population, assuming populations to be discrete, but individually panmictic. Thus, populations will be composed of genetically similar individuals, and therefore, if particular genotypes are associated with minor differentials in fecundity rate, when these differentials are averaged over populations, a significant disparity in reproductive success between populations may be evident.

In purely molecular terms, heterozygosity *per se* (the occurrence of dissimilar alleles at any one locus) may also be associated with differential levels of fitness, through the phenomenon of heterozygous advantage. At the level of the individual, the average fitness of a genotype tends to increase with the number of heterozygous loci it contains (Turelli and Ginzburg 1983). When this concept is translated to the population level, a relationship emerges between the level of heterozygosity within a population and average fitness of that population, as measured by various criteria (Soule and Wilcox 1980).

The first genetic studies of mammals focused on an assessment of the degree of dissimilarity of morphological characters, particularly for taxonomic purposes. Subsequently, such techniques have been applied to the analysis of intra-specific skeletal variation in relation to geographical distance, and, therefore, reproductive isolation, of sub-populations (e.g. humans: Howells 1966; mouse: Berry and Jakobson 1975; fox (*Urocyon littoralis*): Wayne et al. 1991). This approach is often the most practical, but must be employed with care as it is not always possible to discern to what degree the chosen characters are under genetic, rather than environmental, control (Corbet 1978).

More recently, the techniques of molecular biology have been adopted by ecological geneticists to examine genetic variation at the individual, population and species level. The most widely used and applicable for such studies is protein electrophoresis (Lewontin and Hubby 1966; Harris and Hopkinson 1976). This method enables a set of enzyme loci to be screened for genetic polymorphisms. Although the variation revealed by this technique may be selectively neutral (Kimura 1983), the information derived can provide an indicator of the degree of genetic similarity between individuals or groups of individuals. Such similarities, or differences, can be expressed quantitatively in three ways: allele frequency describes the occurrence of a particular allele at one locus over the group of individuals studied; the average heterozygosity of a group is estimated by calculating the proportion of heterozygotes for each locus, and averaging over all loci; the proportion of polymorphic loci simply denotes the percentage of loci examined where the most common allele has a frequency of less than 0.95 (e.g. see Hartl and Clark 1989).

Polymorphism of isozymes is extensive and has been identified in almost all natural populations (humans: Harris 1966; *Drosophila*: Lewontin and Hubby 1966; mouse:

Selander and Yang 1969; plants: Cleg and Allard 1972). Nevo (1978) summarised the results of electrophoresis studies from 243 species (Fig. 5.1). From the graph, which plots average heterozygosity against the proportion of polymorphic loci for major taxonomic groups, it is apparent that there is a negative correlation between genetic variability within a group, estimated from allozyme studies, and the degree of evolutionary complexity of that group. This lead Nevo to propose that the more primitive, or generalist, species will exhibit higher levels of genetic variation, which permits a greater degree of flexibility for further evolutionary adaptation (Nevo 1983; Nevo et al. 1984).

The graph illustrates that, in accordance with their high degree of evolutionary complexity, mammals exhibit relatively low levels of genetic variation. Smith et al. (1991) evaluated the extent of variation among ten species of deer and found that it did not differ significantly from that calculated for mammals in general (Nevo et al. 1984). However, reported levels of genetic variation do differ between cervid species, but also between different studies of the same species (reviewed in Dratch and Pemberton 1992). It is not clear whether these inconsistencies are population specific or merely due to differences in methodological approach.

Selander and Kaufman (1973) proposed the 'Environmental Grain Hypothesis' which predicted that large, mobile, warm blooded animals, such as deer, would exhibit a low degree of genetic variability because their larger body size provided a greater potential for maintaining internal homeostasis, irrespective of external conditions. Therefore, these animals required less genetic flexibility for adaptation in the face of a fluctuating environment. However, values reported in the literature for the degree of genetic variability among cervids, although not always consistent, are often as high as those reported for mammals in general (Baccus et al. 1983; Smith et al. 1991). Several studies on white-tailed deer have revealed polymorphism at a total of 27 out of 35 loci (77%) when these data are pooled (Smith et al. 1984). Equivalent figures for some less extensively studied species are somewhat lower, for example, red deer 24% (Hartl et al. 1990), roe deer 30% (Hartl et al. 1991a) mule deer 18% (Smith et al. 1990), reindeer 15% (Roed 1985), moose 16% and elk 11% (Baccus et al. 1983). Furthermore, among cervids there is no obvious correlation between body size and genetic variability (Baccus et al. 1983; Wooten and Smith 1985). Table 5.1 gives the values of genetic variability among some species of ungulates.

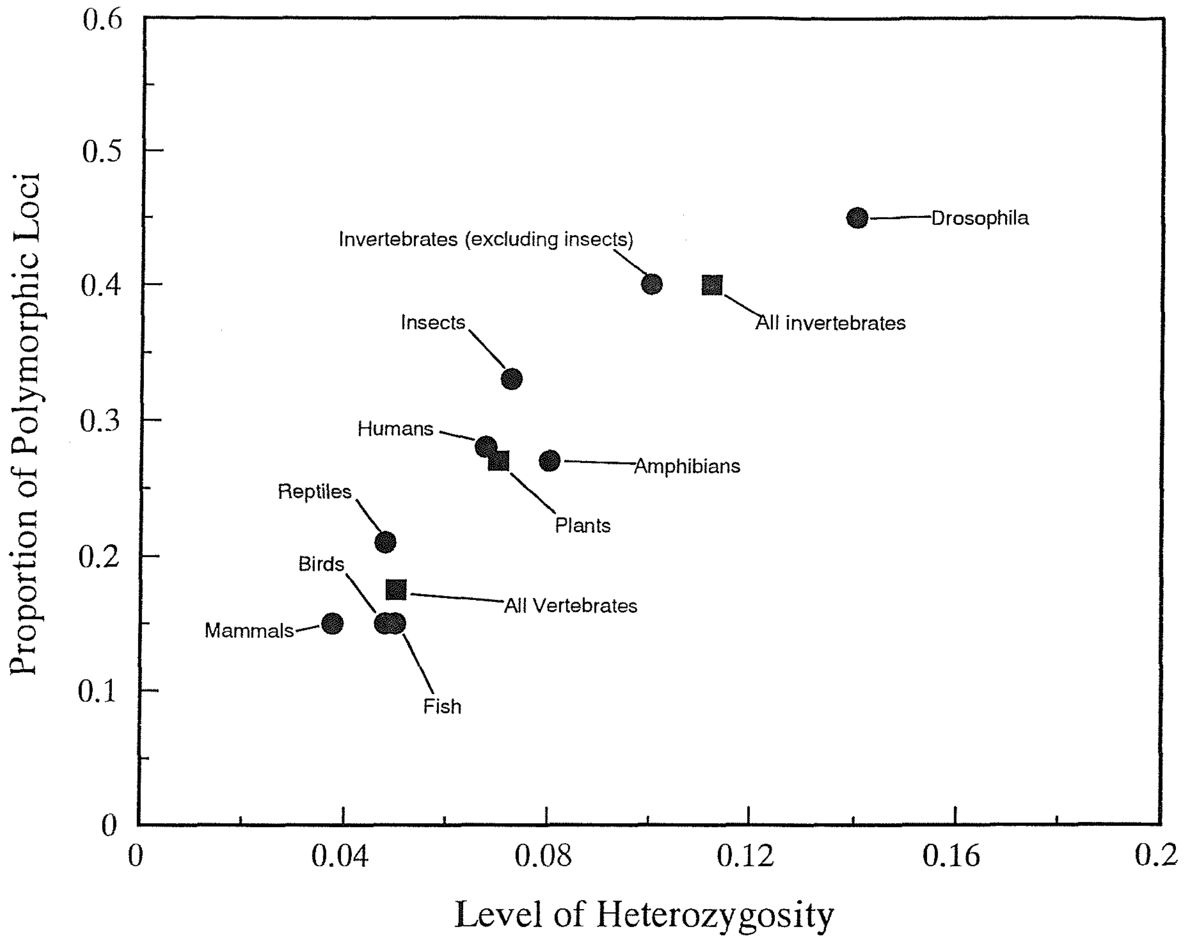


Figure 5.1: The level of genetic variation (proportion of polymorphic loci and average heterozygosity) estimated by electrophoresis of isozymes for some major taxonomic groups.

Table 5.1: The extent of genetic variation (percent of loci examined that were polymorphic) estimated by electrophoresis of isozymes in some species of ungulates.

Species	No. Loci	Percent Polymorphic	n	Reference
European bison (*Bos bonasus*)	15	6.7	4	Hartl et al 1988
Alpine chamois (*Rupicapra rupicapra*)	55	18.2	53	Pemberton et al 1989
Ibex (*Capra ibex*)	15	20.0	149	Stuwe et al 1991
Fallow deer (*Dama dama*)	30	0.0	794	Pemberton et al 1985
Moose (*Alces alces*)	23 19	4.3 15.8	180 165	Ryman et al 1977 Baccus et al 1983
Reindeer (*Rangifer tarandus*)	19 34	5.3 23.5	20 198	Baccus et al 1983 Roed 1985
Red deer (*Cervus elaphus*)	43 19 34	23.5 15.8 20.6	365 27 594	Hartl et al 1990 Baccus et al 1983 Gyllensten et al 1983
Roe deer (*Capreolus capreolus*)	40 19 22	30.0 10.5 13.5	239 24 24	Hartl et al 1991a Baccus et al 1983 Sokolov et al 1986b

Estimates of intra-specific genetic variability for a particular species are frequently conflicting. A low level of variation was initially postulated for several species (moose: Ryman et al. 1977; elk: Cameron and Vyse 1978; fallow: Pemberton and Smith 1985), while other authors have found the same species to be polymorphic for some loci (moose: Ryman et al. 1980; elk: Baccus et al. 1983; fallow: Hartl et al. 1986). This suggests that the extent of intra-specific genetic variation may vary considerably according to the provenance and breeding history of the population under consideration, and, perhaps, also depends on the experimental approach adopted by the investigators.

Electrophoretic studies of roe deer have, likewise, revealed both high (Hartl and Reimoser 1988; Hartl et al. 1991a) and low (Baccus et al. 1983; Gyllensten et al 1983) levels of genetic variability. In fact, Hartl et al. (1991a) found the total proportion of polymorphic loci among populations of roe deer in central Europe to be approximately 30%, and concluded that roe were one of the genetically most variable species of deer yet studied. This relatively high level of genetic variability in roe deer has been cited as evidence (Hartl and Reimoser 1988) in support of Harrington (1985), who hypothesised that this species, as an r-selected, primitive species and a habitat generalist, would exhibit high levels of polymorphism (see Nevo 1984). In contrast, Baccus et al. (1983) found significant polymorphism at only two out of nineteen loci, and Sokolov et al. (1986b) identified only three polymorphisms among twenty-two screened loci. In two further studies, genes for both haemoglobin (Maughan and Williams 1967) and transferrin (Gyllensten et al. 1983) were found to be monomorphic.

Where these electrophoretic studies of deer have examined more than one population, significant allele frequency differences between populations have sometimes been identified (white-tailed deer: Manlove et al. 1976; moose: Chesser et al. 1982a; red deer: Gyllensten et al. 1983; elk: Dratch and Gyllensten 1985; reindeer: Roed 1986). The only comparative study of roe deer to date also found significant inter-population differentiation, accounting for approximately 10% of the total genetic variability identified in this study (Hartl et al 1991a). In particular, the degree of genetic dissimilarity was especially high in comparisons between populations in Hungary and those in Austria. This was attributed to the completely fenced border between these two countries which effectively prevented gene flow. However, in a study including both Siberian and

European roe, only one between population allele frequency difference was identified, and the estimated genetic differentiation between these two subspecies was more typical of poorly isolated populations of the same subspecies (Sokolov et al. 1986b).

A particularly contentious issue surrounding the interpretation of isozyme variation concerns the degree to which such variation is subject to natural selection. It is now generally accepted that the majority of allozyme substitution is selectively neutral (Kimura 1983). However, despite the associated difficulties, particularly when studying deer, there have been some successful demonstrations of selection acting on a single locus, or at least, on genotypes with which a particular allozyme marker is linked (Baccus et al. 1977; Chesser et al. 1982b). A significant association between antler size, a parameter often taken as indicative of animal quality, and the frequency of a particular allozyme has been demonstrated in moose (Ryman et al. 1977), white-tailed deer (Smith et al. 1983) and red deer (Hartl et al. 1991b). More specifically related to productivity, and in particular, reproductive success, Pemberton et al. (1988) showed a strong correlation of allelic frequencies at three enzyme loci and the rate of juvenile survival in red deer, even when the analyses were controlled for phenotypic and environmental factors which may also affect reproductive success. Further work by these authors has revealed that the same genotypes that confer a greater probability of juvenile survival, in contrast, are positively associated with delayed puberty, and reduced reproductive success and survival as an adult (Pemberton et al. 1991). This suggests a possible mechanism, counterveiling selection in different fitness components, which can maintain polymorphism within populations.

In addition, the association of multi-locus heterozygosity and various parameters of fitness, particularly in white-tailed deer, has been taken as indicative of natural selection acting at the molecular level. The ovulation rate (Maffei and Woolf 1987), the number of foetuses per doe (Johns et al. 1977; Chesser and Smith 1987; Rhodes et al. 1992), the rate of foetal development (Cothran et al. 1983; Maffei and Woolf 1987) and female adult body size (Cothran et al. 1983; Chesser and Smith 1987) have all been shown to be positively correlated with the level of heterozygosity in this species. However, several authors have failed to show such a relationship. Furthermore, Chesser and Smith (1987), in their work on the white-tailed herd of the Savannah River Plant, South Carolina, suggested that although highly heterozygous deer produced more offspring, developed faster in utero, attained larger adult body size and were, thus, in better condition at conception, highly homozygous deer had faster post-natal growth rates, reached puberty earlier and could confer greater investment in their progeny because they tended to give birth to singletons only. Thus, in certain circumstances, an individual that successfully produced one offspring in its first breeding season would make a more significant contribution to the population gene pool than an individual that waited until its second season to produce two (e.g. Cole 1954). This again suggests that selection acts on single loci in a complex way and contributes to the maintenance of genetic polymorphism.

Rees (1969, 1970) established that metric and non-metric (epigenetic) variation in the cranial morphology of white-tailed deer was largely determined by genetic differences. However, the morphology of an individual skull is not related to a single gene locus, rather, cranial variation will reflect the action of many gene complexes. Rees (1969) estimated that each of the twenty skull parameters measured was under the control of approximately ten different loci. Thus, this technique is unable to distinguish between two closely related individuals, but provides a powerful tool to discriminate between species, sub-species and even sub-populations.

Lawrence and Bossert (1975) successfully used this technique to distinguish between species of wolf and coyote, as well as between locality races of each species. Among ungulates, cranial morphometrics has provided valuable information concerning the degree of hybridisation between red and sika deer in northern Britain (Lowe and Gardiner 1975; Ratcliffe et al. 1991), and its consequences for the genetic integrity of native red deer. The taxonomic status of British feral muntjac was established by comparing their skull characteristics with those of known origin (Chapman and Chapman 1982). Furthermore, this technique has also been employed to distinguish between genetically and geographically isolated sub-species groups of red deer from mainland Britain, Ireland and the rest of Europe (Lowe and Gardiner 1974).

The most emphatic evidence concerning the ability of skull shape to reflect genetic divergence within a species was found by Rees (1970). Using cranial morphometrics, two populations of white-tailed deer which had been reproductively isolated from each other by the Strait of Mackinac, Michigan, for four thousand years were successfully distinguished. Of several environmental factors considered (geology, climate, forage availability etc.), only latitude, and hence photoperiod, was associated with the shape differences of the cranium. However, this association was not sufficient to explain the morphological differences observed, and Rees concluded that reproductive isolation had lead to genetic divergence of these two populations.

Comprehensive studies of roe deer morphology, based on quantitative craniometric analyses, have provided a genetic basis for classifying the European (*Capreolus capreolus*) and the Siberian roe (*C. pygargus*) as distinct species (Sokolov et al. 1985a). Indeed, the differences in skull morphology between these groups were found to be due to differential growth tempos among pre-pubescents for all the skull characters measured, although the differential varied according to the character (Sokolov et al. 1985b). At a finer level of discrimination, a further division of the Siberian roe into three sub-species (*C.p. pygargus, C.p. tianschanicus and C.p. melanotis*) has been proposed (Danilkin et al. 1985). Similar analyses of skulls of European roe from France, Bulgaria, Belorussia, the Ukraine and the Baltic area suggested that there is considerable morphological heterogeneity over the range of this species which may warrant sub-species status for certain groups (Markov et al. 1985).

Genetic divergence between roe deer populations in Poland was found by Markowski and Markowska (1988) using analysis of non-metric cranial traits. In contrast, Zima et al. (1989) found that roe deer from four geographical regions of Czechoslovakia could not be distinguished on the basis of metric or non-metric skull variation, and concluded that this was due to a relatively high level of genetic homogeneity of roe deer in this area. A further study in the same country also failed to identify between population differences in skull shape, despite significant variation in absolute skull size (Zejda and Koubek 1988). The authors identified the environmental parameters of geography, climate and forage availability as significant influences on absolute size of the skull, but the two populations studied were presumed to be genetically similar on the basis of cranial shape alone. The only such analysis to date of British roe deer compared three populations, one from the south, one from the north and one from a park population (Lowe and Gardiner

1983). Each population was found to be genetically discrete on the basis of cranial morphology, however, this study was conducted on rather small sample sizes.

This study has used both starch gel electrophoresis of isoenzymes and cranial morphometrics to determine the degree of genetic variation within and between roe deer populations in Britain. Few studies have employed this dual approach (but see Wayne et al. 1991). This genetic variation was then examined for its ability to account for the variation in reproductive success between populations previously described. Although cranial morphometrics is a widely used technique, none of these studies have attempted to relate genetic identity, as determined by skull shape, to fitness components of the population.

5.2 RESULTS

5.2.1 Electrophoresis

Up to twenty individuals from each population were screened for a total of ten enzyme systems, representing fifteen presumptive isoenzyme loci (in some cases fewer than twenty samples were available). The preparation of samples and electrophoretic methods for each enzyme system are fully described in Chapter 2. Significant polymorphism was judged according to the 95% criteria (the most common allele has a frequency of less than 0.95). In practice, a rare allele was identified in only one enzyme, and in this case, in only one individual, so this criterion was not bridged. In all cases, the observed banding patterns conformed to those expected from the quaternary structure of the protein, and followed the descriptions given in the literature (Darnall and Klotz 1975; Harris and Hopkinson 1976; Selander et al. 1971). Where a locus was polymorphic, alleles were given a superscript which denoted their mobility relative to that of the most common allele, which was designated by the superscript 100.

Adenylate Kinase

Although two loci are generally apparent in this system, only one was identified (*Ak-1*). The enzyme is monomeric and, therefore, double banded heterozygotes and single banded homozygotes were both identifiable. All bands migrated anodally. The relative mobilities of the homozygotes suggested the presence of up to three alternative alleles in each population, however, separation was not sufficiently clear and repeat gels sometimes revealed inconsistency in the scoring of the homozygotes. This locus was designated as polymorphic in all populations, but the frequencies of each allozyme could not be calculated.

Glucose-6-Phosphate Dehydrogenase

One locus with dimeric products (*G6pdh-1*) was identified in this system and was monomorphic in all populations. Each individual had a single anodal band (see Hartl and Hoger 1986).

Glycerol-3-Phosphate Dehydrogenase

Two loci, both dimeric, were identified and found to be monomorphic in all populations in this enzyme system. Three cathodal bands were observed in close proximity. Two of these represented the two loci (*G3pd-1* and *G3pd-2*), while the middle band was a heterodimer, formed from the inter-locus hybridisation of the polypeptide products of the two loci.

Isocitrate Dehydrogenase

A single anodally migrating band was observed, representing the product of one dimeric locus (*Idh-1*) which was monomorphic in all populations. There are generally two loci, one cytoplasmic one mitochondrial, associated with this system (see Harris and Hopkinson 1976) but only one of these was successfully identified, presumed to represent the cytoplasmic form.

Lactate Dehydrogenase

This system was characterised by a widely separated five banded pattern, three anodal bands and two cathodal (Fig. 5.2). The enzyme is tetrameric, and this pattern was the result of hybridisation between the primary products of two loci (*Ldh-1* and *Ldh-2*) to form hybrid inter-locus heterotetramers (e.g. see Manlove et al. 1975). The staining intensity of the enzyme products of the cathodal locus (*Ldh-2*) was poor, resulting in a gradient of band intensity up the gel (the lower two bands are difficult to distinguish in Fig. 5.2). The pattern was invariable within and between all populations.

Malate Dehydrogenase

Two sets of bands, one anodal and one cathodal, were observed and presumed to represent two loci, both dimeric. The cathodal pattern consisted of a single band and was invariant in all samples (*Mdh-2*). The anodal pattern was more complex and was comprised of three closely positioned bands whose relative staining intensity seemed to vary (*Mdh-1*). This pattern has been observed in other studies and interpreted as a polymorphism (see Linnell 1989). However, in this study, the variability was not reproducible with any consistency, and therefore the pattern was taken to represent a single, invariant allele with associated sub-bands (a common phenomenon in this system). Thus, the two loci of this system were found to be monomorphic in all populations.

Malic Enzyme

There are two tetrameric malic enzyme loci, one cytoplasmic and one mitochondrial, but as is generally the case in this system, only one was demonstrable (*Me-1*). A single fast anodally migrating band was observed and was invariant in all populations.

Mannose Phosphate Isomerase

This isozyme was polymorphic in six of the fifteen populations, Alice Holt, Thetford, Ringwood, Salisbury, Aldershot and Stanford. One locus with two alternative alleles with anodally migrating primary products was identified (*Mpi-1*). The double banded heterozygote, typical of a monomeric enzyme, and both single banded homozygotes were observed in all populations (Fig. 5.3) where this isozyme was polymorphic (see Hartl and Hoger 1986). The most common allele identified was designated 100, and the second allele was designated 115 on the basis of its relative mobility. In the nine populations where this system was monomorphic, all individuals were 100/100 homozygotes.

Phosphoglucose Isomerase

The pattern observed in this system was complex but conformed to that previously described (see Gyllensten et al. 1983). Two loci with dimeric products generally produced a three banded pattern anodal to the origin. The central band represented one locus (*Pgi-2*) and was invariant in all populations. The outer two bands were both products of a second locus (*Pgi-1*) which was also ubiquitously monomorphic, except for a rare variant in one animal from Kershope which was a heterozygote of the common allele (designated 100) and a fast migrating allele (250). In this case, a series of seven bands was observed due to the formation heterodimers between products of the two alleles and products of the two loci.

Superoxide Dismutase

This system consists of two dimeric loci, both of which were demonstrable as single, anodally migrating bands (*Sod-1* and *Sod-2*). Both loci were monomorphic in all populations.

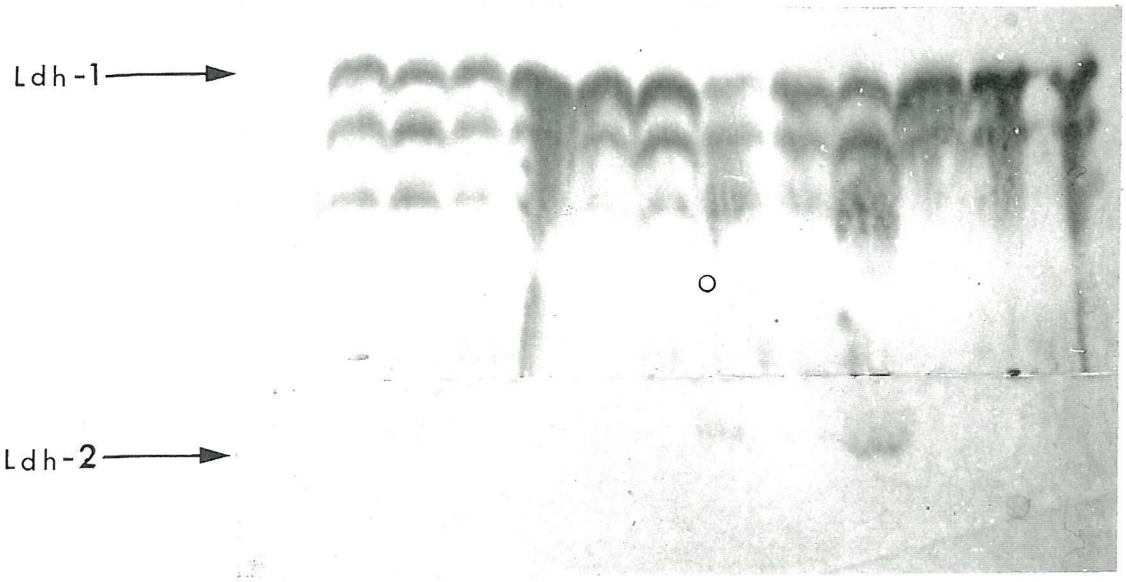


Figure 5.2: The five banded pattern of the tetrameric Lactate dehydrogenase system. The top and the bottom bands are the primary products of *Ldh-1* and *Ldh-2* and the three intermediate bands are heterotetrmers formed from the hybridisation of the products of the two loci. The lower bands are less distinct due to the poor activity of *Ldh-2.*

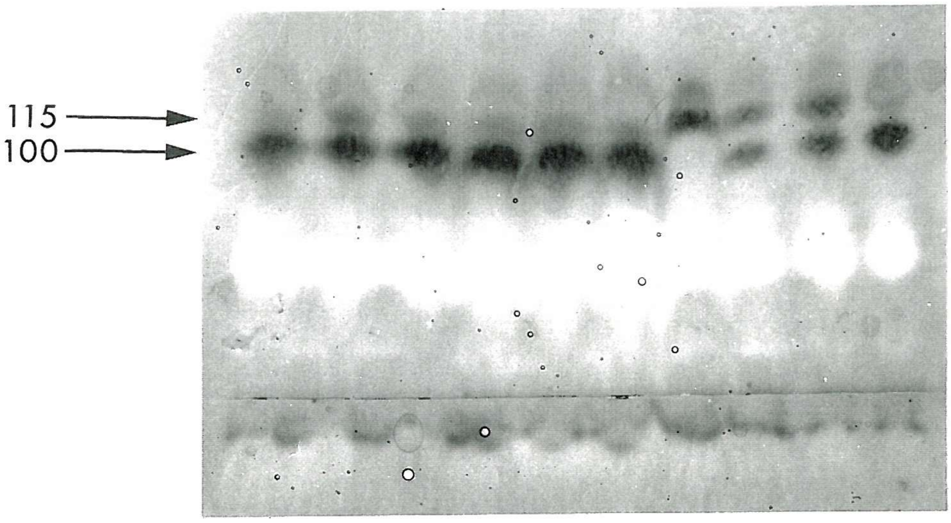


Figure 5.3: The two banded pattern of the monomeric Mannose phosphate isomerase system. Both homozygotes and the double banded heterozygote are shown.

Of the fifteen presumptive loci, significant polymorphism was demonstrated in two, Adenylate Kinase (*Ak-1*) and Mannose Phosphate Isomerase (*Mpi-1*). A rare Phosphoglucose Isomerase (*Pgi-1*) allelic variant was detected in one individual, but because the frequency of this allozyme was less than 0.0025, the system was not considered polymorphic.

The total proportion of polymorphic loci for roe deer in Britain was estimated as 13.3%, and the average heterozygosity (proportion of heterozygotes averaged over all loci) was calculated as 3.2% This compares with estimates for central European roe of 30% and 4.9% respectively (Hartl et al. 1991a). Thus, these estimates of the level of variation across loci are rather lower than those obtained in the only other comprehensive electrophoretic study of roe deer, but are typical for mammals in general (averages of 12.8% and 3.3% respectively, Baccus et al. 1983).

The average level of genetic variation within single populations was estimated by the average proportion of polymorphic loci and the average heterozygosity (averaged from the figures for each population) as 9.34% (standard deviation 3.35%) and 3.1% (sd 1.8%) respectively. The equivalent figures obtained by Hartl et al. (1991a) were 15.8% (sd 2%) and 5.1% (sd 1.5%). These figures must be interpreted with care as, in some cases, sample sizes were less than twenty (Nei 1975), but it is unlikely that the bias due to small sample sizes will distort the results significantly (Gorman and Renzi 1979). Furthermore, the average heterozygosity calculated from the population averages was almost exactly equal to the gross average of the study.

Table 5.2 lists the population allele frequencies for *Mpi-1*, the only polymorphic locus for which these values could be calculated. The values for each population were tested for their compliance with Hardy-Weinberg equilibrium. Chi-squared tests revealed no significant deviations from this equilibrium ($p>0.05$), but the more sensitive Smith's H statistic (Smith 1970) identified a deficiency of heterozygotes at Thetford ($p<0.05$). This may be due to population sub-structuring, as Thetford forest covers a wide area, but is more probably the result of small sample sizes (see Richardson et al. 1986).

Table 5.2: Allele frequencies at the *Mpi-1* locus and the average heterozygosity (calculated over all loci) for each population. # denotes a value based on less than ten samples.

Study Site	**Allele Frequency**		**Sample Size**	**Average Het. (%)**
	Mpi-1^{100}	*Mpi-1*^{110}		
Alice Holt	0.533	0.467	20	5.77
Thetford	0.525	0.475	18	5.00
Ringwood	0.973	0.027	19	3.00
Spadeadam	1.000	0.000	20	2.40
Kershope	1.000	0.000	20	2.10
Pickering	1.000	0.000	20	1.90
Craigellachie	1.000	0.000	20	2.50
Queens	1.000	0.000	10	4.00
South Strome	1.000	0.000	13	3.60
Salisbury Plain	0.893	0.107	14	0.50
Lulworth	1.000	0.000	12	1.70
Aldershot	0.750	0.250	2	$3.30^{\#}$
Bramley	1.000	0.000	18	3.30
Stanford	0.526	0.474	19	4.30
Kirkcudbright	1.000	0.000	20	0.00

Table 5.3: Roger's Genetic Distance (R) between pairs of populations (monomorphic populations not included).

Study Site	Thetford	Stanford	Alice Holt	Salisbury Plain	Ringwood
Thetford					
Stanford	0.001				
Alice Holt	0.008	0.007			
Salisbury Plain	0.368	0.367	0.360		
Ringwood	0.448	0.447	0.440	0.080	

There was significant variation in *Mpi-1* allele frequencies between populations for this locus, whether all sites were included (chi-square = 176.8, 14 d.f., $p<0.001$), or only those six sites where the locus was polymorphic considered (chi-square = 31.8, 5 d.f., $p<0.001$). In situations where allelic frequencies are only available for two alleles at a single locus, it is not possible to give particularly meaningful measures of genetic identity between populations. When Rogers' distance (R) was calculated (Rogers 1972), the values generated were equal to the differences between pairs of populations in the raw allele frequencies (of either allele) because the calculation relied on data from a single locus. Such measures are of more empirical value when data from several independent loci are incorporated, however, Table 5.3 gives the value of R for comparisons between the polymorphic populations for exploratory use only. (A value of zero implies the populations are identical.) Aldershot is not included because the sample size was too small.

It was notable that two of the polymorphic populations which abut one another geographically, Thetford and Stanford, had almost identical allele frequencies and, therefore, an R value which was only marginally above zero. These two populations were also closely related to Alice Holt based on the R values from this one locus. The frequency of the Mpi-1^{115} allele was rather lower at both the remaining polymorphic study sites, Ringwoood and Salisbury, and the R value between these sites was consequentially low. The genetic distance between the south-eastern populations (Alice Holt, Thetford, Stanford) and the central southern populations (Ringwood, Salisbury) was estimated by this method to be relatively high. All populations in northern Britain plus Lulworth and Bramley were monomorphic, consisting of Mpi-$1^{100/100}$ homozygotes only, and by implication, were more closely related to Ringwood and Salisbury than to the three populations in the south-east.

5.2.2 Cranial Morphometrics

The skull is the most complex bone structure in the body and is highly variable, reflecting variation in genetic stock. Cranial morphometric studies are based on the assessment of skull shape from the measurement of numerous distances. The measurements taken are generally well established. In this study, the eighteen distances taken essentially followed those used by Lowe and Gardiner (1974), in their work on red deer. These variables were illustrated in Fig. 2.4. Where sampling was sufficiently intensive, between ten and twenty adult doe skulls from each population were measured, although for some sites, less than ten were available (see Table 2.1). Many morphometric studies have shown important results based on small sample sizes (see Blackith and Reyment 1971). Furthermore, the ability to discriminate between groups of skulls increases as the square root of the sample sizes (Lawrence and Bossert 1975) and therefore, to increase the precision of this discrimination by a significant degree would require vastly greater sample sizes. In a cranial analysis of red and sika deer, Hunt (1987) found that sample sizes of more than six were sufficient to provide a high level of discrimination in both inter- and intra-specific comparisons, and the addition of supplementary data did not result in significant improvement in the resolution provided by this technique.

Cranial studies of deer have either concentrated on a single sex or analyzed male and female data separately. In addition, juveniles are generally excluded from the analysis. In this study, only doe skulls were available from all populations, so the problem of variation in skull characteristics between the sexes did not arise. All skulls measured were from does of at least eighteen months, and generally older than two years. For roe deer cranial studies, the minimum age of animals included in the analysis has been set at two years for does (Sokolov et al. 1985a) and three years for bucks (Zejda and Koubek 1988).

Some authors have employed methods of standardising morphometric data to account for heterogeneity in absolute size of skulls, and to control the weighting given to each individual measurement. Lawrence and Bossert (1975) divided each parameter by the overall length of the skull and expressed the measurements as a proportion of absolute size. Other authors have subjected the data to logarithmic transformation in order to generate parameters with variances which are independent of each other (Schonewald-Cox et al. 1985). However, in this study the untransformed raw data were used, as it was felt that such a transformation lead to a loss of information about real differences in skull size and shape. Furthermore, the statistical approach that was adopted distinguished between variation in absolute size and shape.

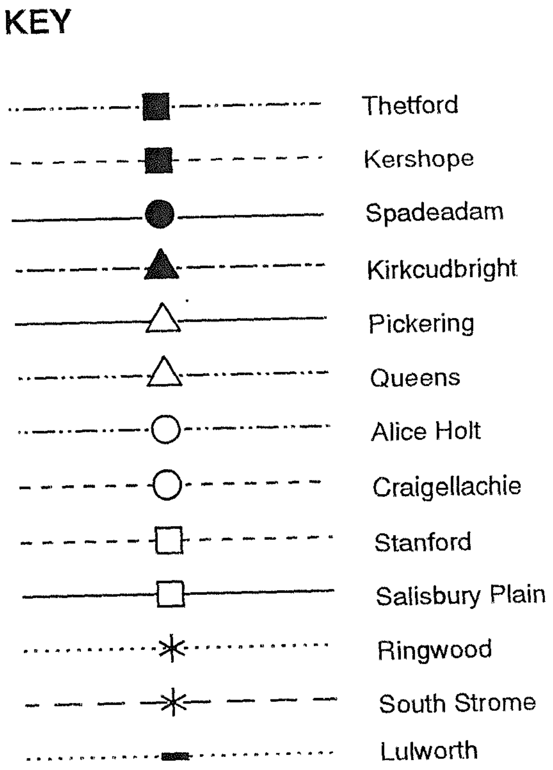
Principal Components Analysis (PCA) is a multivariate ordination technique that arranges points (samples) in a two dimensional space according to their dissimilarity across a number of variables (e.g. see Jongman et al. 1987). PCA constructs theoretical variables that best explain the underlying structure of a data matrix by minimising the total residual sum of squares when fitting these straight line theoretical variables. These generated variables are called principal components, and can be used as axes on which the data are plotted to visualise the heterogeneity. A number of PCA axes can be derived but each explains a decreasing proportion of the residual variance. In practice, the first two, or possibly three, components will explain the significant proportion of the overall variation. Unlike discriminant analysis (Jongman et al. 1987), PCA makes no *a priori* assumption of the inter-relationship of individuals, based on a knowledge of their group identity, and so is a more stringent test of the degree of similarity between individuals.

The results of a PCA, only including skulls for which it was possible to measure all eighteen parameters, showed that the first three PCA axes explained 45%, 22% and 15% respectively, a total of 82%, of the heterogeneity in skull morphology of 118 samples. The principal components for each of the first three PCA axes are given in Table 5.4. From this table it can be seen that the factor loadings for PC 1 are all of the same sign, indicating that this axis essentially describes a size vector. The high values associated with the rostrum length, the premaxillary length and, to a lesser extent, the condylo-basal length and the basilar length (see Fig. 2.4), show that this axis is defined by the overall length of the skull. Thus, in the plot of PC 1 against PC 2 (Fig. 5.4), those individuals towards the left of the axis have longer skulls and those towards the right, relatively shorter skulls.

In contrast, the factor loadings of the eighteen skull variables for PC 2 have both positive and negative values, indicating this axis describes a shape vector. The same variables are again the most influential, but in this case, in a relative manner. Towards one end of the axis skulls have a relatively longer premaxillary length and a relatively shorter rostrum length, but towards the other end the opposite is true. The fairly high, negative factor loadings for the condylo-basal length and the basilar length on this axis suggest that individuals towards the top of the graph are shorter overall than those at the bottom (Fig. 5.4). This seems to contradict the findings based on PC 1, however, it must be remembered that the second axis is derived with the constraint that it is uncorrelated

Table 5.4: Factor loadings for the first three principal components in a PCA of eighteen skull measurements (see Fig. 2.4) from 118 skulls, originating from 13 populations.

Skull Measurement	**PC 1**	**PC 2**	**PC 3**
Rostrum Length	-755	-430	637
Premaxillary Length	-558	911	-38
Basilar Length	-261	-189	-409
Condylo-basal Length	-244	-275	-528
Palatal Length	-194	-168	-304
Rear Length I	-166	-77	-134
Rear Length II	-153	-92	-171
Frontal Length	-137	-17	-205
Orbital Breadth	-132	-13	-93
Nasal Length	-118	36	-261
Nasal Depth II	-97	21	-115
Palatal Depth	-91	-73	-135
Nasal Depth I	-31	19	-36
Inter-orbital Width	-62	-16	-137
Brain Case Breadth	-55	-18	-60
Maxillae Width	-49	-37	-59
Condylar Breadth	-31	19	-36
Nasal Breadth	-20	-43	-47


KEY
Thetford
Kershope
Spadeadam
Kirkcudbright
Pickering
Queens
Alice Holt
Craigellachie
Stanford
Salisbury Plain
Ringwood
South Strome
Lulworth

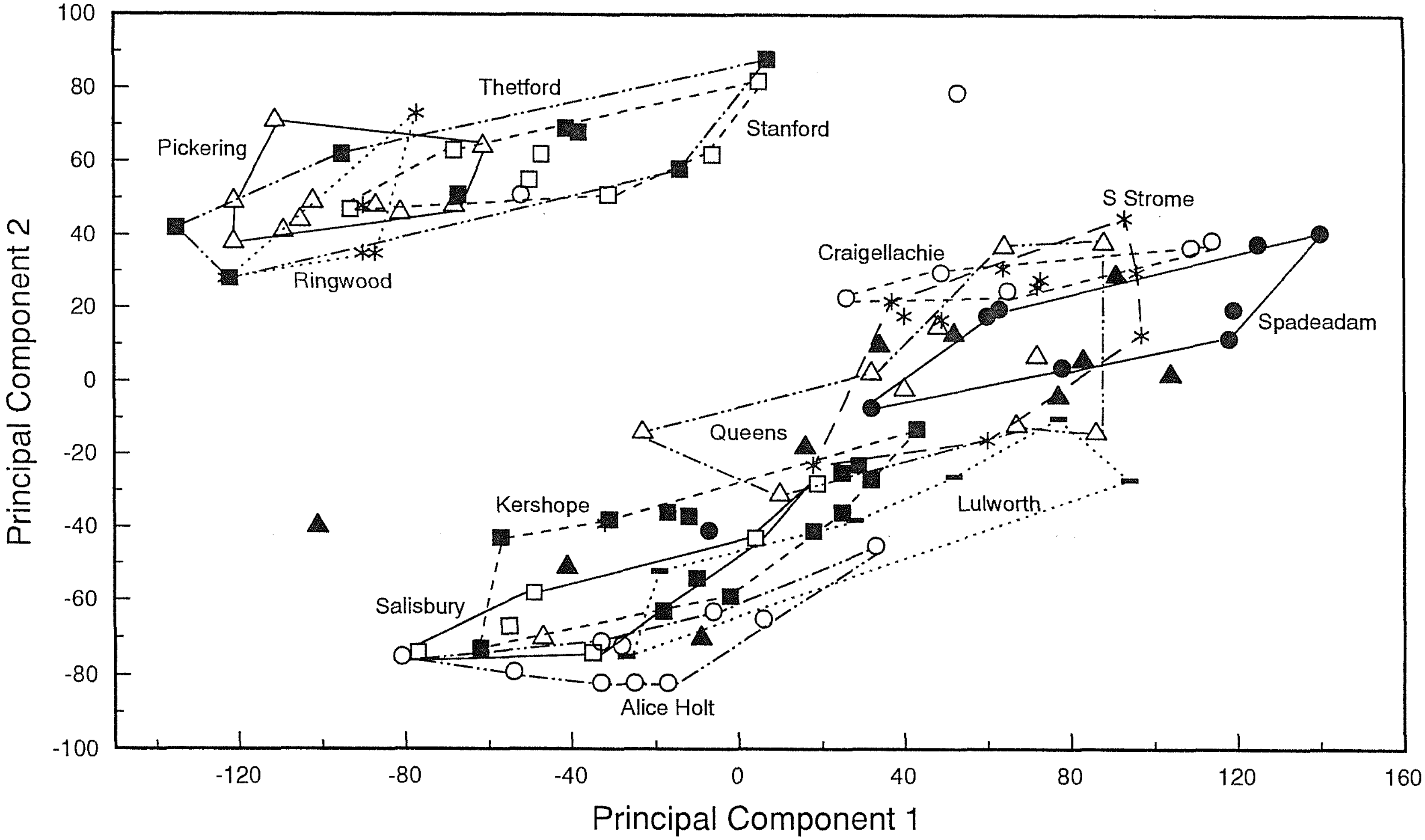


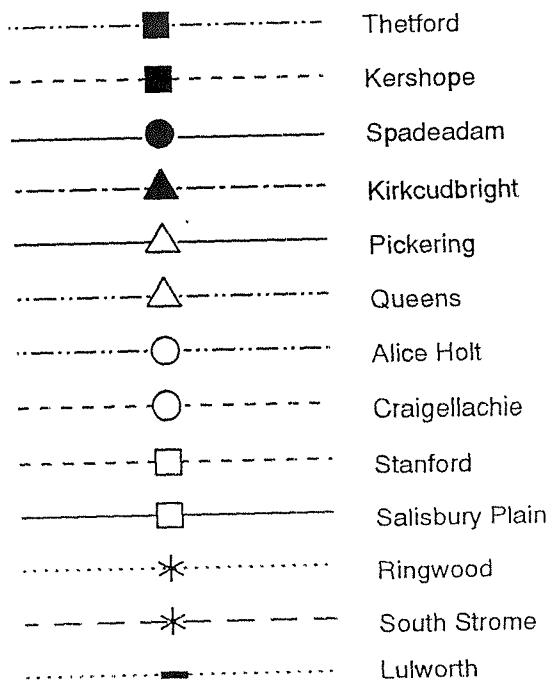
Figure 5.4: Plot of Principal Component 1 vs Principal Component 2 derived from a PCA on 118 skulls from 13 populations (see opposite for site codes).

with the first, so this result must be considered in relation to the other variables. Thus, at the top of the figure skulls have long premaxillae relative to their overall length, and at the bottom relatively short premaxillae.

The between population variation in skull morphology described by the first three PC's (i.e. the separation of the predefined geographical groups along each axis) was significant in each case (one way ANOVA on PC scores: $p<0.001$ for all three axes). By plotting PC 1 against PC 2 (to visualise the heterogeneity in skull morphology described by the first two principal components, which explained the majority of the skull variation), it was possible to clearly distinguish two groups of skulls (Fig. 5.4). The smaller cluster contains the skulls from Thetford, Ringwood, Pickering and Stanford. Skulls from the remaining nine populations make up the larger, more dispersed cluster. These groupings are well delineated and there is no cross over between the two. This suggests that there is a distinct division of roe deer populations into two types, based on the morphological characteristics of the skull. Those on the top left of Fig. 5.4 (Thetford, Ringwood, Pickering, Stanford) have long skulls, and in particular, long premaxillae. Those in the large cluster to the bottom right of the figure are characterised by relatively shorter skulls, with especially short premaxillae.

The larger of the two clusters in Fig. 5.4 was rather dispersed, and suggested there may be a further level of discrimination within this group. To test this hypothesis, PC 3, which explained a significant proportion of the residual variance, was considered. The factor loadings again indicate that this PC is a shape vector determined by the relationship of overall length of the skull relative to the length of the front portion. Towards one end of this axis skulls tend to have a long front portion relative to their overall length and at the other the situation is reversed. Thus, in the plot of PC 2 against PC 3 (Fig. 5.5), which displays the heterogeneity in skull morphology described by the second and third principal components, skulls placed towards the top of the figure have disproportionately long frontal portions. Two distinct clusters are again evident in Fig. 5.5, but in this case the composition of these clusters has changed. The group to the left, comprised of Alice Holt, Kershope, Salisbury and Lulworth, is characterised by skulls with short frontal portions, mainly because of their particularly short premaxillae. Within the larger group there is some degree of discrimination. The populations of Thetford, Ringwood, Pickering

KEY


Thetford
Kershope
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Pickering
Queens
Alice Holt
Craigellachie
Stanford
Salisbury Plain
Ringwood
South Strome
Lulworth

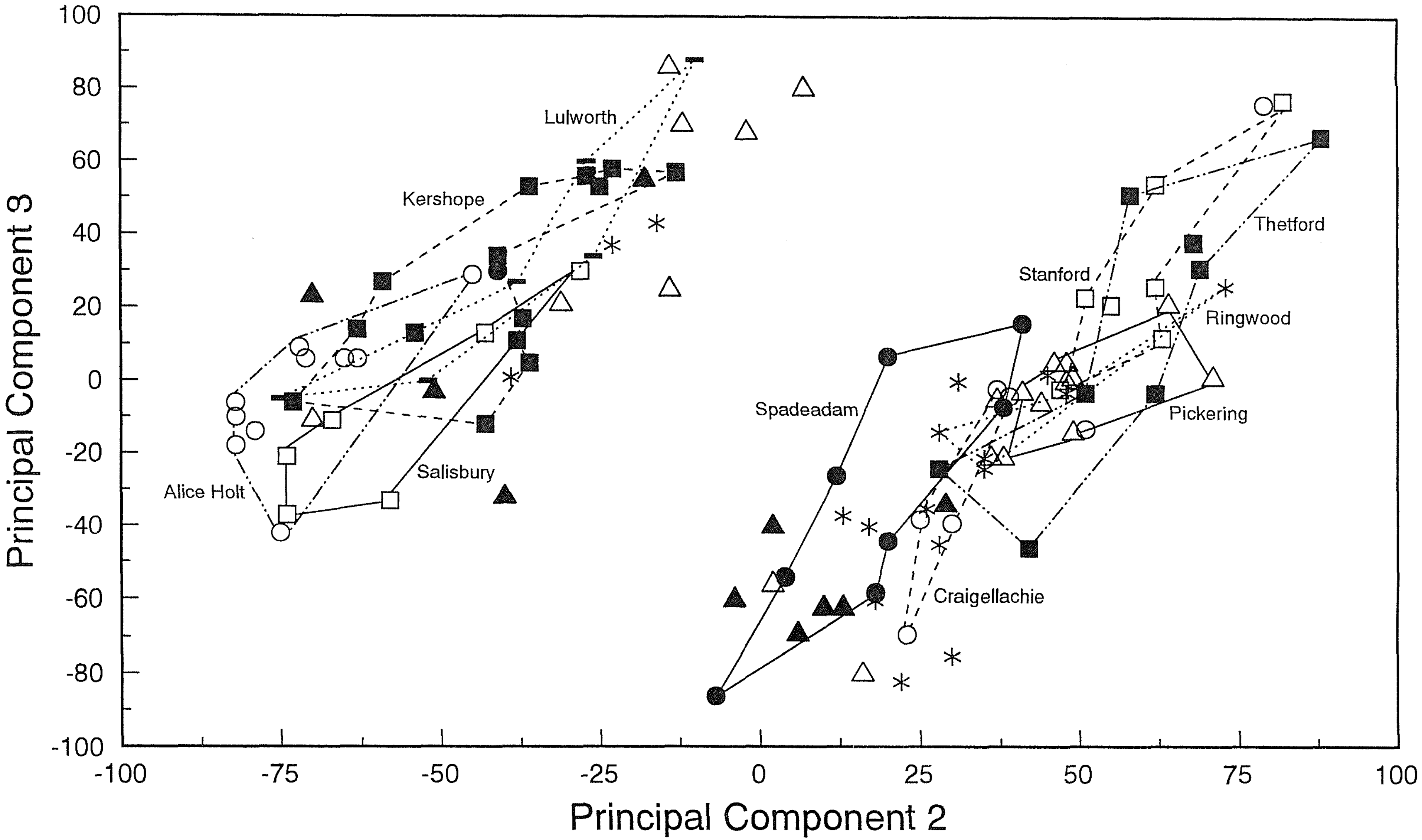

Figure 5.5: Plot of Principal Component 2 vs Principal Component 3 derived from a PCA on 118 skulls from 13 populations (see opposite for site codes).

and Stanford are again tightly clustered. These skulls have relatively long frontal portions, but most of this length is attributable to their especially long premaxillae. Skulls from Spadeadam and Craigellachie also have relatively long premaxillae, but this does not result in a correspondingly long frontal portion.

Thus, on the basis of the results from these two plots combined, three distinct morphological types are evident among roe deer skulls. Thetford, Ringwood, Pickering and Stanford have long skulls with disproportionately long frontal portions, mainly due to very long premaxillae. Alice Holt, Kershope, Salisbury and Lulworth skulls are shorter because of a smaller premaxillae. Spadeadam and Craigellachie have the shortest skulls, but the premaxillae is not particularly short. The three remaining populations, Queens, South Strome and Kirkcudbright are broadly affiliated with the latter two groups, but cannot be discriminated further.

A series of multiple range tests on the PCA scores of axis 1 and 2 confirmed that the observed clustering in the PCA represented significant variation between populations in skull morphology. The skulls from the populations of Thetford, Ringwood, Pickering and Stanford were significantly different from those of all other populations ($p<0.05$) along PC 1 (Spadeadam, Kershope, Craigellachie, Queens, South Strome, Lulworth and Kirkcudbright) and/or PC 2 (Alice Holt, Kershope, Queens, Salisbury, Lulworth, and Kirkcudbright). The skulls of Spadeadam and Craigellachie, in addition to the comparisons already covered, were significantly different from those of Alice Holt, Kershope and Salisbury along both PC 1 and 2 (all $p<0.05$).

To examine this proposed sub-clustering further, Thetford, Ringwood, Pickering and Stanford skulls were omitted and the reduced data set submitted to PCA. This analysis explained a similarly high proportion (80%) of the overall heterogeneity in skull morphology, however, this was mainly attributable to the first two axes only, which explained 51% and 22% of the variance respectively. PC 1 again described the overall length of the skull and PC 2 was a shape vector, determined by the relative lengths of the frontal and rear portions of the skull. When these vectors were plotted against each other (Fig. 5.6), two discrete clusters were evident which confirmed the group composition suggested above. Discrimination was mainly on the basis of absolute size (PC 1): skulls

KEY

■	Thetford
■	Kershope
●	Spadeadam
▲	Kirkcudbright
△	Pickering
△	Queens
○	Alice Holt
○	Craigellachie
□	Stanford
□	Salisbury Plain
✳	Ringwood
✳	South Strome
▬	Lulworth

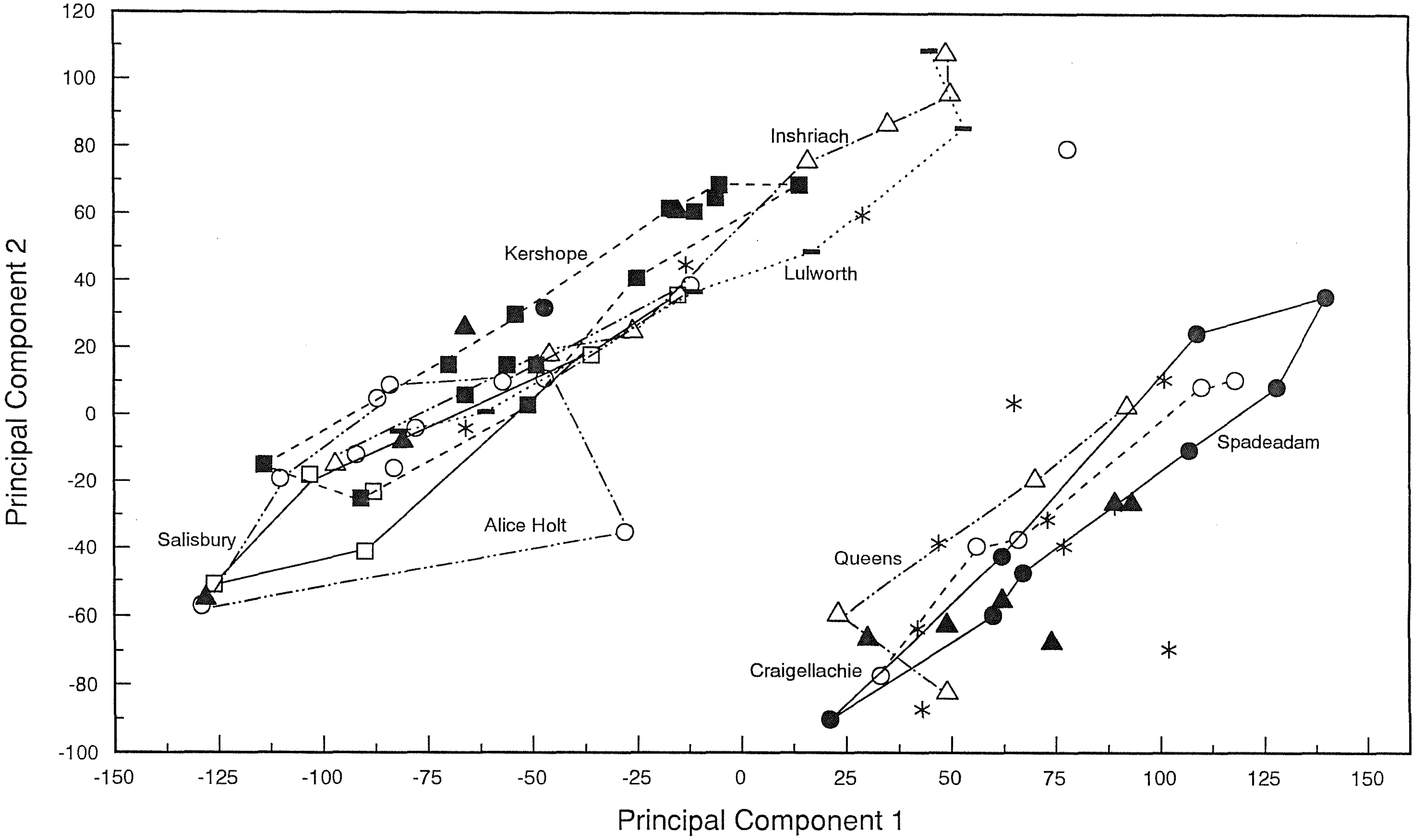

Figure 5.6: Plot of Principal Component 1 vs Principal Component 2 derived from a PCA on 91 skulls from 9 populations (see opposite for site codes).

from Spadeadam and Craigellachie were shorter, but in addition, had particularly short frontal portions (although not premaxillae).

This analysis also confirmed that skulls from Queens, South Strome and Kirkcudbright overlapped with both the short skull groups. However, when the data were re-plotted with skulls from two different forests within the Queens population (Inshriach Forest and Queens Forest) distinguished by separate codes, the situation was resolved in part. Skulls from the two forests were discriminated completely and each was associated with one of the two separate short skull groups (see Fig. 5.6). No such subdivision could be imposed for the data from South Strome and Kirkcudbright.

In these analyses the length of the premaxillae was the variable with the highest discriminatory power. To investigate whether this was the sole difference between skull clusters, a further PCA was run, but with this variable excluded. The results are represented in Fig. 5.7. The omission of the premaxillae variable vastly reduced the discrimination between two of the three clusters, which essentially coalesced. However, the Spadeadam-Craigellachie-Queens Forest cluster remained discrete on the basis of both absolute skull length (PC 1), and the relative length of the front portion (PC 2). This illustrates that the discrimination between the Alice Holt-Kershope-Salisbury-Lulworth-Inshriach Forest cluster and the Thetford-Ringwood-Pickering-Stanford cluster is based on their especially short and especially long premaxillae respectively. In contrast, the Spadeadam-Craigellachie-Queens Forest cluster is discriminated on the basis of a relatively short front portion of the skull.

In the course of this analysis, some supplementary cranial data were obtained for roe deer from various locations in Britain (Dr. V.P.W. Lowe, Institute of Terrestrial Ecology, Merlewood). In some cases, these additional skulls had originated from some of the same populations examined in this study, or were drawn from a similar geographical area. Therefore, these data was incorporated in a further PCA to examine the coincidence of the two data sets. In general, there was good agreement between the original and the corresponding supplementary skulls data which were derived from the same populations along the skull length axis, but less coincidence of purportedly similar groups along the shape axis. However, much of these supplementary data were identified by very general

KEY

■	Thetford
■	Kershope
●	Spadeadam
▲	Kirkcudbright
△	Pickering
△	Queens
○	Alice Holt
○	Craigellachie
□	Stanford
□	Salisbury Plain
✳	Ringwood
✳	South Strome
▬	Lulworth

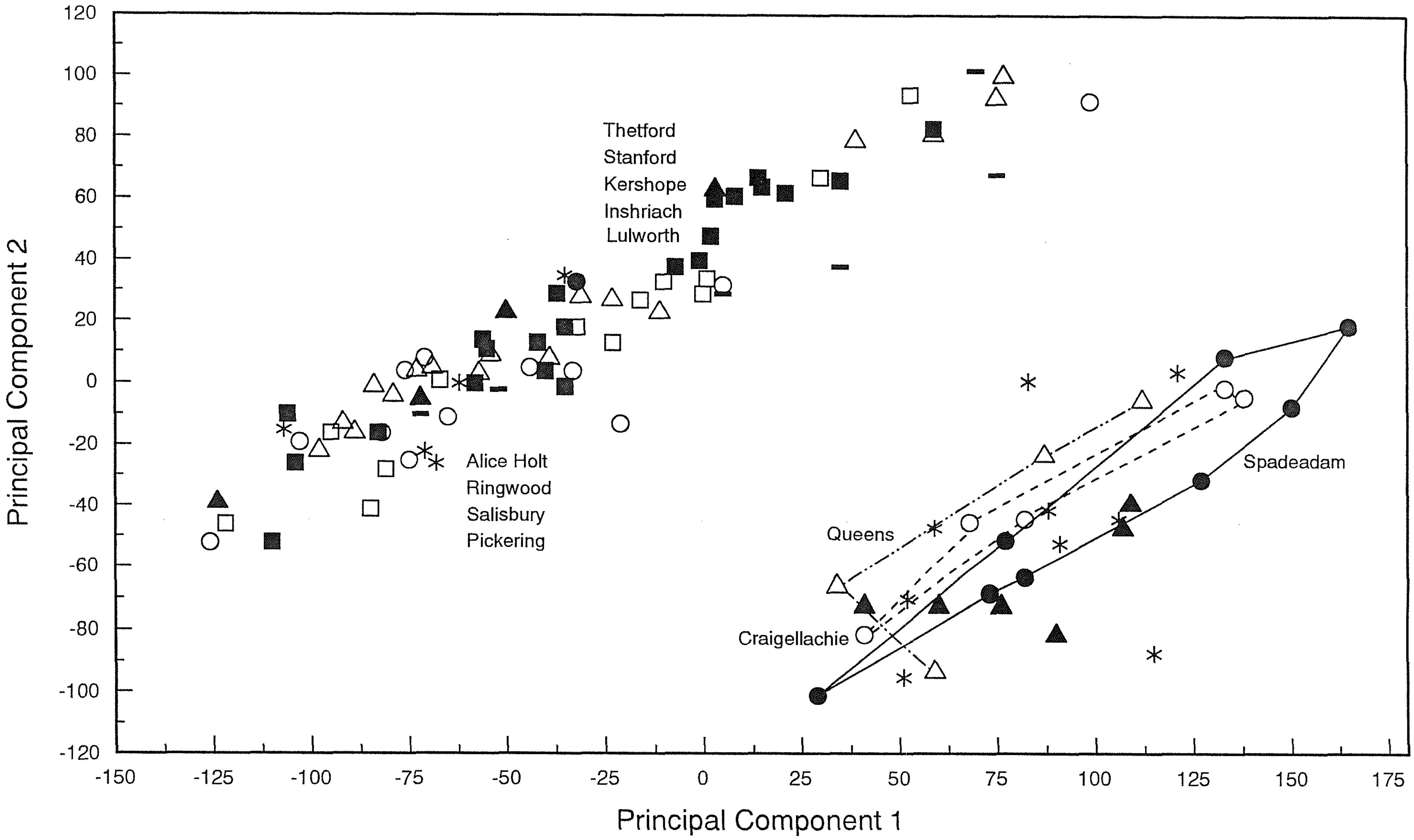

Figure 5.7: Plot of Principal Component 1 vs Principal Component 2 derived from a PCA on 118 skulls from 13 populations (see opposite for site codes), excluding the premaxillary length skull measurement (see text).

designations (e.g. North Yorkshire), and may not have been drawn from stock within close proximity to the populations of this study. In the two cases where the origin of the supplementary skulls was more clearly specified, Alice Holt and Inshriach Forest, there was good agreement between the two groups of data along both axes. This suggests that differences in cranial morphology may be localised and, therefore, these supplementary data were omitted in further analyses.

5.2.3 Genetic Variation in Relation to Reproductive Success

The level of electrophoretic variation observed was insufficient to characterise the degree of genetic similarity between the study sites in any detail. Allele frequencies were only available for a single locus, and it was, therefore, impossible to relate differences in genotype frequencies with variation in reproductive success between populations. Neither was it possible to determine the relationship of the genotype of a particular individual with its reproductive success as sample sizes were too small within populations, and between population comparisons were confounded by the combination of potential environmental influences and highly variable allelic frequencies. However, average heterozygosities over two loci could be calculated for each population and provided one way of relating electrophoretic variation to reproductive success.

The level of heterozygosity revealed by electrophoresis is often taken to represent the degree of heterozygosity over the whole genome. It is sometimes suggested that more heterozygous individuals or populations may have a selective advantage due to phenomenon of heterozygote advantage. A series of univariate correlations between populations of the average heterzygosity within a population, calculated from the two polymorphic loci (*Mpi-1* and *Ak-1*), and the gross average adult reproductive success of the population failed to reveal any significant relationships: percentage ovulation $r^2=0.39$, percentage pregnancy $r^2=0.30$, average number of corpora lutea per doe $r^2=0.11$, average number of foetuses per doe $r^2=0.20$, (all tests $p>0.05$). Neither was average heterozygosity within a population significantly related to body size of adults in the population: average body weight $r^2=-0.32$, average jaw length $r^2=0.07$, (both tests $p>0.05$).

The lack of significant correlation between average heterozygosity and population reproductive performance is probably due to the small number of polymorphic loci available and the consequent uniformity of heterozygosity levels between populations. In fact, the whole spectrum of population values for average heterozygosity fall within the ninety five percent confidence limits of the gross survey mean (mean=3.1%, sd=1.8%).

To determine the degree of influence of genetic differences, revealed by cranial morphometrics, on reproductive success, the skull data were reexamined in relation to the average reproductive performance of each population. In the previous subsection, PCA was used to allocate skulls to a position in a two dimensional space which best represented their relationship with each of the other skulls. To do this, the PCA constructed theoretical variables (PC's) which best explained the variability in the skull data matrix. In order to examine how skull morphology was linked with reproductive success, a related statistical technique, Redundancy Analysis (RDA), was employed. This technique is a canonical form of PCA which detects the pattern in the data matrix which is best explained by supplied 'environmental' variables (Jongman et al. 1987). Thus, the axes generated, which in PCA were theoretical variables, in RDA are linear combinations of these supplied 'environmental' variables.

An RDA was carried out on the skull data with the four parameters of average reproductive success of the population, percentage ovulation, percentage pregnancy, average number of corpora lutea and average number of foetuses, supplied as 'environmental' variables. This analysis attempted to relate variation in skull morphology to reproductive success of each population, and, hence, determine to what extent reproductive performance is limited by genetic control. The axes extracted by this analysis explained a total of 25.3% of the overall variation in skull morphology, and the first two axes explained 18.6% and 5.5% respectively. This was approximately one third of the percentage of the variation explained by the theoretical variables constructed in the PCA, but was still substantial.

The correlation between the skull data and the 'environmental' data is summarised by the species-environment correlation which was R=0.62 and R=0.52 for the first and second axes respectively. Table 5.5 gives the canonical coefficients and the associated t-values. These coefficients are equivalent to regression coefficients between each 'environmental' variable and the RDA axes. A t-value greater than 2.1 indicates that a

Table 5.5: Canonical (regression) coefficients for the relationship between measures of adult reproductive performance and the first three axes of the RDA on skull shape. The t-values for each correlation are also displayed (a value of greater than 2.1 indicates a statistically significant relationship, denoted by a *).

Reproductive Performance Variable	**PC 1**		**PC 2**		**PC 3**	
	Canon. Coeff.	t-value	Canon. Coeff.	t-value	Canon. Coeff.	t-value
Percentage Ovulation	0.112	1.48	0.260	4.87*	-0.050	-0.19
Av. No. of Corp. Lutea Per Doe	0.144	0.52	0.552	2.81*	0.060	0.65
Percentage Pregnancy	-0.022	-0.27	0.014	0.23	-0.130	-4.67*
Av. Number of Foetuses Per Doe	-0.534	-1.94	-0.465	-2.38*	0.015	0.16

Table 5.6: Canonical (regression) coefficients for the relationship between environmental variables (habitat quality indices, body weight, climatic variables) and the first three axes of the RDA on skull shape. The t-values for each correlation are also displayed (a value of greater than 2.1 indicates a statistically significant relationship, denoted by a *).

Explanatory Variable	**PC 1**		**PC 2**		**PC 3**	
	Canon. Coeff.	t-value	Canon. Coeff.	t-value	Canon. Coeff.	t-value
Jaw Length	2.29	3.60*	-3.20	-5.16*	0.22	0.36
Diastema Height	0.76	5.79*	0.04	0.33	-0.10	-0.73
Mid-Winter Temp	-1.32	-4.24*	0.99	3.28*	0.06	0.20
Summer Rainfall	0.98	2.65*	-1.65	-4.57*	0.08	0.22
Body Weight	-3.44	-4.05*	4.65	5.62*	-0.09	-0.11

particular variable was significantly influential in defining that axis. From the table it is apparent that the average number of foetuses is the supplied variable which is most important for defining axis 1, the skull length axis, but the correlation is not quite statistically significant. In contrast, all the reproductive success parameters except percentage pregnancy are highly influential in defining axis 2, the skull shape axis, and this influence is statistically significant. Thus, the differences in reproductive success between populations are, in part, explained by genetic factors, revealed by skull morphology.

Fig. 5.8 is a biplot of axis 1 and axis 2 which summarises the RDA. The term biplot refers to the dual representation of the species data (skulls) with the 'environmental' (reproductive success) data. The clustering of the skulls is the same as Fig. 5.4, but the relative orientation appears to be different because the figure is rotated relative to the axes, which, in this figure, are linear combinations of the reproductive success variables. Each individual reproductive success parameter is represented by an arrow. The length of this arrow is proportionate to the importance of that variable in explaining the ordination diagram and the arrow points in the direction of its maximum change. Arrows which point in the same direction are positively correlated and those that point in opposite directions are negatively correlated. An angle of 90^0 indicates that the two variables represented by those arrows are totally uncorrelated.

A similar analytical approach was employed to investigate the relative effects of genetic factors on reproductive success, once the possible influences of environmental factors on skull morphology had been accounted for. RDA was carried out on the skull data with reproductive success parameters, body weight, relative density indices (jaw size) and climatic parameters all supplied as the 'environmental' variables. These variables explained a total of 57% of the variation in skull morphology, accounting for approximately three quarters of the overall cranial variation which was explained by theoretical PC's. Table 5.6 gives the canonical coefficients and t-values for this test (for the environmental variables only). RDA requires the number of 'environmental', variables to be low and when this is not the case the canonical coefficients become unstable. Therefore, these values are of exploratory use only. However, it is apparent that, of the supplied variables, average body weight, average jaw length and average monthly summer

KEY

··–··–■··–··–	Thetford
- - - ■ - - -	Kershope
——●——	Spadeadam
–·–▲–·–	Kirkcudbright
——△——	Pickering
··–··–△··–··–	Queens
··–··–○··–··–	Alice Holt
- - - ○ - - -	Craigellachie
- - - □ - - -	Stanford
——□——	Salisbury Plain
·······✳·······	Ringwood
— — ✳ — —	South Strome
·······▬·······	Lulworth

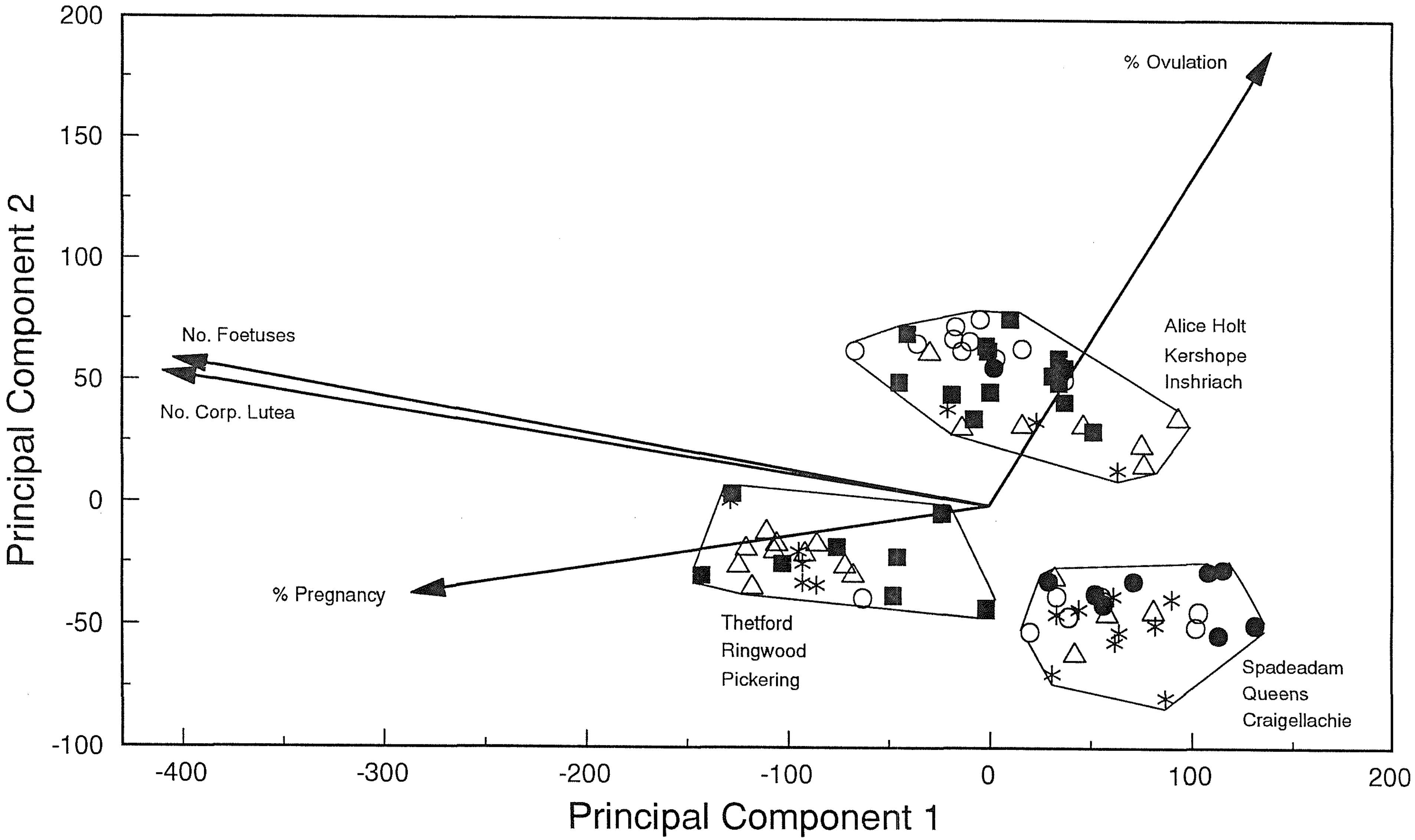

Figure 5.8: Plot of Principal Component 1 vs Principal Component 2 derived from a RDA on 118 skulls from 13 populations (see opposite for site codes), with adult reproductive success parameters supplied as explanatory variables (see text).

rainfall were the most influential environmental factors defining the two axes which best explain the overall pattern in the skull morphology data. Furthermore, this combination of variables represent the three areas previously implicated as important in determining reproductive success, body weight, climate and relative density.

These three environmental factors were then used as covariables in a partial canonical ordination. Partial RDA is identical to the standard form of RDA, but the variation in the data set which can be explained by specified covariables is first 'partialled out' and the target variables related to the residual variation (Jongman et al. 1987). Thus, specifying these three environmental factors as covariables permitted the relationship between genetic factors (represented by skull shape) and reproductive success to be examined in isolation because the environmental influence on cranial morphology was already accounted for.

Using this approach, the parameters of reproductive success accounted for a smaller, but still significant proportion of the variation in skull morphology (21%). The first axis accounted for 11% of the residual variation, just under one third of that explained by environmental factors and reproductive success parameters in combination. All four parameters of reproductive success were significantly influential in determining this axis. (Canonical coefficients and the associated t-values are given in Table 5.7). However, the second axis explained 9% of the residual variation, more than two thirds of that explained when all variables were included. Percentage pregnancy and the average number of corpora lutea were significant in determining this axis. The species-environment correlation (the correlation between the skull data and the reproductive success data) was R=0.74 for the first axis and R=0.72 for the second axis and was statistically significant ($p<0.01$) according to the Monte Carlo permutation test (Ter Braak 1988).

Table 5.7: Canonical (regression) coefficients for the relationship between measures of adult reproductive performance and the first three axes of the Partial RDA on skull shape (body weight, jaw length and summer rainfall as covariables). The t-values for each correlation are also displayed (a value of greater than 2.1 indicates a statistically significant relationship, denoted by a *).

Reproductive Performance Variable	**PC 1**		**PC 2**	
	Canon. Coeff.	t-value	Canon. Coeff.	t-value
Percentage Ovulation	-0.26	-5.38*	-0.03	-0.72
Av. No. of Corp. Lutea Per Doe	-0.94	-4.70*	-0.95	-4.99*
Percentage Pregnancy	0.29	4.98*	0.28	4.99*
Av. Number of Foetuses Per Doe	1.54	7.42*	0.05	0.23

5.3 DISCUSSION

5.3.1 Genetic Variation

A number of hypotheses have been proposed in an attempt to explain patterns of variation in biochemical genetic data among populations, species or taxa. The 'environmental grain' hypothesis predicts that large mammals will be genetically less variable than small mammals (Selander and Kaufman 1973). Under this hypothesis, roe deer are expected to be genetically invariable relative to other smaller mammals. The degree of biochemical genetic variability revealed in this study (percentage of polymorphic loci=13.3%; average heterozygosity=3.2%), although considerably lower than the values calculated by Hartl et al. (1991a) for this species, are in close agreement with those found for mammals in general (12.8% and 3.3% respectively, Baccus et al. 1983). This is in concordance with the generally accepted lack of such a negative correlation between body size and genetic variability among mammals (Baccus et al. 1983; Wooten and Smith 1985).

In contrast, the theories of Nevo (Nevo 1983; Nevo et al. 1984) predict that roe deer, as a primitive generalist species found across broad geographic, climatic and habitat spectra, will be relatively genetically variable. Because the degree of genetic variation among British roe deer was estimated as typical for mammals in general, this study does not provide evidence to support or refute this hypothesis. In fact, the value obtained for average heterozygosity was more in agreement with that cited by Nevo for specialist species (3.2%) than that for the generalists (5.4%).

It seems likely therefore, that such hypotheses are over simplifications, and the amount of genetic variability within species will depend on the breeding history of the studied population(s). The average heterozygosity for different populations of white-tailed deer, the most genetically variable deer species yet studied, range widely, between 5.6% and 9.2% (Smith et al. 1984). The white-tailed deer is often considered to be the New World equivalent of roe deer, as they are both r-selected (most other deer species give birth to a single offspring) habitat generalists, and have similar feeding strategies (Putman 1988). Hartl et al. (1991a), based on findings in central Europe, postulated that roe deer were also one of the most genetically variable deer species yet studied. However, other authors have, in agreement with the results of this study, found a rather lower level of electrophoretic variation in this species (Baccus et al. 1983; Gyllensten et al. 1983; Sokolov et al. 1986b).

Population bottlenecks may drastically affect the level of electrophoretic variation within a species. When the size of the breeding population is small, rare alleles will be readily lost due to the effects of genetic drift. Estimates of the proportion of polymorphic loci and the average heterozygosity in the cheetah, which is known to have experienced two such constrictions in population size, were 3% and less than 1% respectively (O'Brien et al. 1983). Pemberton and Smith (1985) found no electrophoretically detectable variation at 30 enzyme loci in fallow deer and postulated that this was due to a genetic bottleneck during the domestication of this species in Britain.

The extent and range of the roe deer in Britain was drastically reduced in recent times, possibly to the point of extinction in England during the eighteenth century (see Chapter 1). The remnant population was probably restricted to the Scottish highlands until deer were reintroduced to a number of areas in Britain from a variety of sources during the nineteenth century. This relatively recent population restriction may have caused a severe genetic bottleneck and it is likely that rare alleles were lost at this time due to genetic drift, resulting in a low level of electrophoretic variability in the native roe stock. In addition, it seems the reintroduced founder populations were often small and therefore prone to inbreeding and consequent loss of genetic variability. Gyllensten et al. (1983) found a very low level of electrophoretic variation in a Swedish red deer population which was founded from three individuals. Such genetic bottlenecks may have caused the rather low level of electrophoretic variability in British roe deer detected in this study.

However, although the origin of the subsequently reintroduced animals is not well documented, it seems certain that both native and continental deer were translocated to various parts of Britain (see Chapter 1 and Prior 1968). If these stocks of diverse origin were sympatric, this might result in increased genetic variability within populations due to the introduction of novel genes to the native stock. Alternatively, if such reintroductions occurred in isolation, the result might be an increased level of differentiation between populations as the founder effect caused different rare alleles to be lost in different, reproductively isolated populations. However, if many of the reintroductions in England involved Scottish stock, which were already relatively genetically invariable due to the bottleneck in the eighteenth century, then such population differentiation would not be expected.

It is difficult to establish how the complicated and poorly documented history of roe deer in Britain has affected levels of genetic variability and population differentiation on the basis of the electrophoretic results of this study. The average heterozygosity, calculated over only two loci, did not vary significantly between populations. Allele frequencies were only available from one polymorphic locus (with two alleles) which was fixed for one of the alleles in nine out of fifteen study sites. However, the allelic frequencies at this locus did vary significantly between populations.

Three simple models generally form the basis for interpretation of electrophoretic data (e.g. see Richardson et al. 1986). The single panmictic unit model is implicated where there is no significant allele frequency variation over the entire geographic range of the studied unit. This is clearly not the case for British roe deer as the frequency of the *Mpi-1*^{115} allele ranged between 0.475 and 0 in southern England, but was consistently absent in northern England and Scotland. The monomorphism of the *Mpi-1* locus in all study sites in northern Britain suggests that the single panmictic unit model might apply in this geographic area. However, it is probable that the roe deer of these populations are all descended from native stock and were subject to the genetic bottleneck two hundred years ago. Thus, it is not surprising that both the level of genetic variation within, and the degree of differentiation between these populations is low. Furthermore, it seems unlikely that animals ranging from the North York Moors to the west coast of Scotland constitute a single panmictic population.

The situation among the populations of southern England is more complex and must invoke one of the two other alternative population models. The discrete population model allows drift (or selection) to lead to significant genetic differentiation between populations which are reproductively isolated from one another. The isolation by distance model also incorporates genetic differentiation, but the change is gradual over a particular range. The two models can be distinguished because in the first, the change in allele frequencies will be at the same geographical point for several loci, but in the second, the changes at each locus will occur independently, as there is no underlying population structure limiting gene flow at particular geographical locations.

Because frequency data are only available for a single polymorphic locus, it is difficult to discern which of these models is most applicable to the roe deer of the south. The frequency of the *Mpi-1*^{115} allele ranges from 0.475 in East Anglia to 0.027 in Ringwood, Hampshire (and zero in Lulworth, Dorset), suggesting an east-west cline in allele frequency and therefore, implicating the isolation by distance model as the most appropriate. The East Anglian populations are purportedly descended from introduced German stock (see Chapter 1), and it is possible that these animals brought the polymorphism at the *Mpi-1* locus to eastern Britain, from where it began to spread as these animals and their descendants came into reproductive contact with native roe in the south. However, based on this single locus information, the population at Alice Holt, on the Hampshire-Surrey border, seems to be panmictic with the two East Anglian populations (Thetford and Stanford), but relatively reproductively isolated from the more geographically proximate populations (Ringwood, Hampshire and Salisbury Plain, Wiltshire). Furthermore, the population at Bramley in northern Hampshire was fixed for the slow allele at this locus indicating total reproductive isolation from the Alice Holt animals. Thus, it is equally possible that this polymorphism is present at various frequencies in the roe deer populations of Britain due to the action of drift on reproductively isolated units.

This complex situation can be illustrated graphically. Fig. 5.9 plots the degree of genetic relatedness between pairs of populations, as determined by Rogers' distance, against the geographical distance separating the same two populations. The two lines superimposed on the plot represent the expected relationship of genetic divergence and geographical distance under the two alternative models. The unbroken line represents the expectation under the isolation by distance model. In this situation, the value of R is small at low geographical distance but rises sharply as the distance increases. The curve gradually flattens out, and, at the point where it becomes parallel to the x-axis, there is no longer any genetic association implied. The broken line represents the expected relationship under the discrete population model. The value of R is again low at low

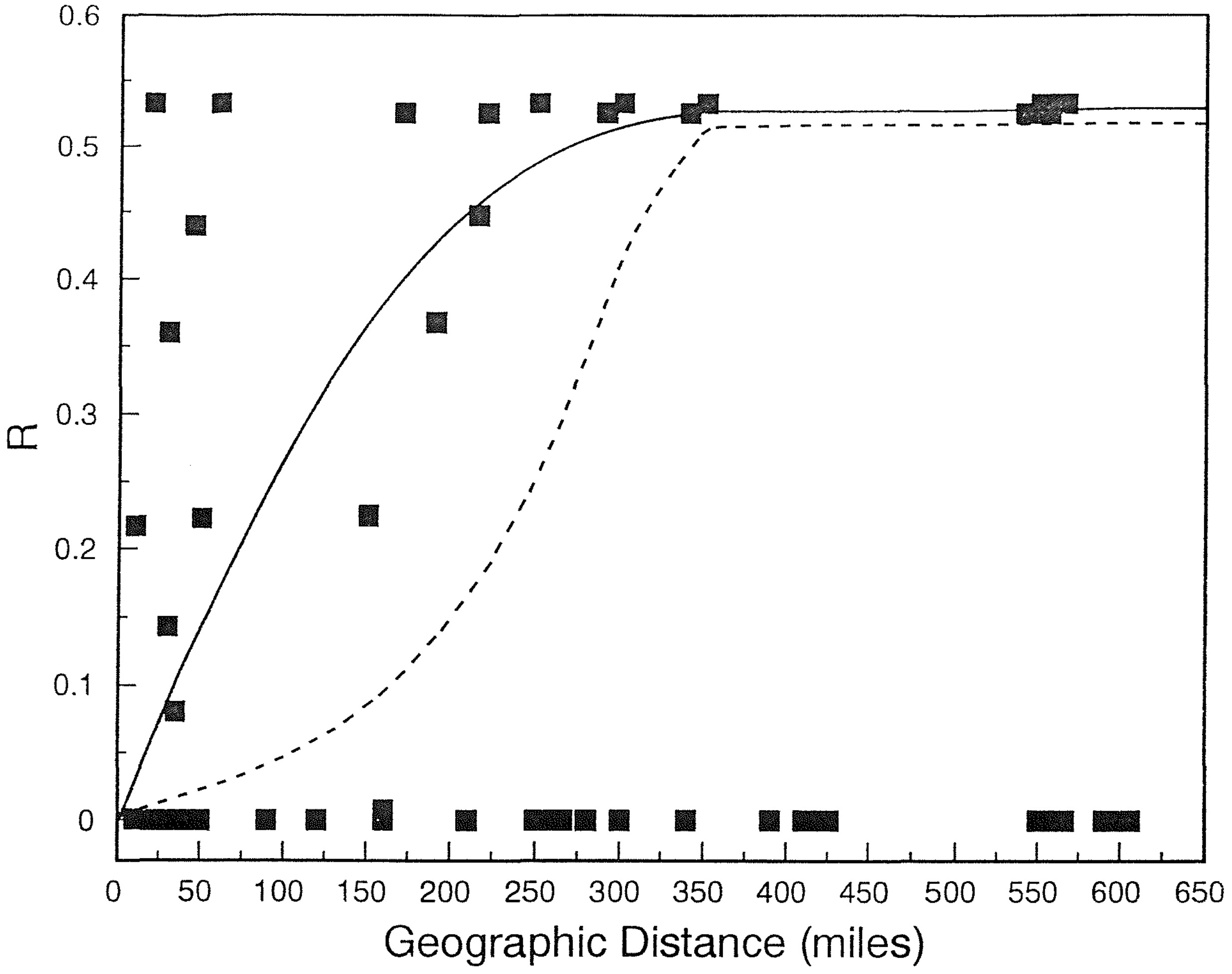

Figure 5.9: A plot of Roger's genetic distance (R) determined by electrophoresis of isozymes against geographical distance for pairs of populations. The lines represent alternative models of patterns of genetic variation. The unbroken line describes the expected relationship under the isolation by distance model and the broken line represents the expected relationship under the discrete population model.

geographical distance, but rises more slowly as distance increases. This is because of the close genetic relationship of populations which are near enough to be effectively panmictic with each other. The sharp rise of the curve, which follows, indicates the geographical distance which is large enough to create discrete populations through reproductive isolation.

Clearly, the R values obtained from this study cannot distinguish which of these models best describes the situation among British roe deer populations, as the data can be interpreted to fit either line. For example, the deer at Alice Holt appear to be relatively reproductively isolated from other populations within fifty miles, but panmictic with East Anglian animals more than two hundred miles away. Further data on *Mpi-1* allele frequencies at more geographically intermediate sites might help to resolve this question, but more especially, the identification and screening of additional polymorphic loci is necessary before any definitive conclusions can be made. Ideally, to distinguish between the above models, data from six polymorphic loci should be considered (Richardson et al. 1986).

In contrast, the discrimination between groups of populations with different skull morphology was relatively pronounced. The first three axes of the PCA explained a high proportion of the overall heterogeneity in cranial shape (82%), and skulls from ten of the thirteen populations where data were available were exclusively assigned to one of three groups. Discrimination between populations allocated to different groups was high: almost all pairwise comparisons of population PC scores (on one or both axes) were significant between populations from different clusters. As is commonly the case (Jolicoeur and Mosimann 1960), the first PC was a size vector, describing the length of the skull. In their study of North American wapiti, Schonewald-Cox et al. (1985) found size variation of the skull was the major factor discriminating between populations. Similar work on Czechoslovakian roe deer found that, despite differences in absolute size, skulls from a variety of populations were assigned to a single group on the basis of shape alone (Zejda and Koubek 1988). However, skull shape seems to be equally important to distinguish between populations of roe deer in Britain. The second and third PC's, both shape vectors, explained a total of 37% of the overall variation, a similar proportion to that explained by PC 1 alone. Furthermore, pairwise comparisons of the PC scores found significant differences between populations from different clusters along axis 2, the shape axis.

The three distinct morphological groups were mainly discriminated on the basis of the length of the premaxillae in relation to the overall length of the skull:

Group 1. Thetford, Ringwood, Pickering, Stanford. The longest skulls with disproportionately long frontal portions, mainly due to very long premaxillae.

Group 2. Alice Holt, Kershope, Salisbury, Lulworth, Inshriach Forest. These skulls were shorter because of a much smaller premaxillae.

Group 3. Spadeadam, Craigellachie, Queens Forest. The shortest skulls but the premaxillae was not particularly short.

Skulls from South Strome and Kirkcudbright occurred in both groups 2 and 3.

At first sight it is difficult to understand the genetic associations between populations implied by these groupings. However, the ancestry of British roe deer is complex and the number and origin of reintroduced animals often poorly documented (see Chapter 1).

There were numerous reintroductions of roe deer to southern Britain in the nineteenth century, however, only in the case of the East Anglian roe is the origin of the animals well established. A small number of German roe were released around Thetford towards the end of the century. It is presumed that these animals formed the basis of the currently thriving population, but there is no empirical evidence to support or refute this assumption. Certainly, as expected the two East Anglian populations, Thetford and Stanford, which are geographically contiguous, show a high degree of similarity in cranial morphology. It has been suggested that the population at Pickering, North Yorkshire also has affiliation with German stock. Although this is unsubstantiated, the coincidence of Pickering skulls and East Anglian skulls would seem to support this hypothesis. It is more difficult to envisage a genetic identity between the roe at these sites and the population at Ringwood, Hampshire, which is also included in group 1. However, it seems quite likely that continental roe deer were imported to Britain's south coast during the last hundred years (R. Prior *pers. comm.* in Higgins not dated).

The myriad of releases and translocations of animals of unknown origin throughout southern Britain complicates the picture still further. However, it is certain that roe of Scottish origin were released at various points and this may help explain the coincidence in skull morphology of the Scottish populations, Kershope and Inshriach Forest with the southern populations of Alice Holt, Salisbury Plain and Lulworth within group 2.

Although the distinction is not widely recognised, Lonnberg (1910) described what he thought to be a race of roe deer indigenous to Britain (*Capreolus capreolus thotti*) in Craigellachie, Morayshire (Staines and Ratcliffe 1991). The composition of group 3 lends qualitative support to the hypothesis that the roe of this area are genetically distinct. The group is composed of the Scottish populations from the central northern highlands and includes the population at Craigellachie. Skulls from Craigellachie and Spadeadam are significantly different to skulls from each of the populations which comprise groups 1 and 2. The west coast populations of South Strome and Kirkcudbright also overlap this group to some extent and may therefore be of native stock. This group is clearly morphologically distinct from the purportedly German skulls and, unlike group 2, there is no overlap with skulls from any southern populations.

5.3.2 Genetic Variation in Relation to Reproductive Success

It is rather problematical to relate electrophoretic variation to correlates of reproductive success. Studies that have correlated between population differences of allozyme markers (e.g. Ramsey et al. 1979) with variation in reproductive performance, have rarely fully characterised the potential influence of environmental factors which may confound the interpretation of these relationships. One approach to overcome this limitation, is to determine how the genotype of individuals within the same population affects their fitness. However, such an approach requires data from a large number of individuals of the same population and is usually only feasible in long term studies.

Two such studies, one on white-tailed deer the other on red deer, have shown significant correlation of a wide variety of fitness measures of reproductive success and survival with multi-locus heterozygosity (Johns et al. 1977; Cothran et al. 1983; Chesser and Smith 1987; Maffei and Woolf 1987) and with specific allozyme markers (Pemberton et al. 1988; Pemberton et al. 1991) of individuals. It was not possible to make such comparisons within populations in this study as both time and resources dictated that a maximum of twenty individuals per population were screened for each enzyme system. Furthermore, genotype frequencies from a single locus and average heterozygosity calculated over two loci did not provide sufficient discriminatory power to distinguish individuals, or even populations, using the electrophoretic data.

The genetic relationships of populations can be explored using cranial morphometrics. However, the differences revealed by this technique have previously not been related to variation in the average fitness of individuals within the population in a statistical manner. The application of canonical ordination techniques allowed the observed variation in skull morphology to be related to the parameters of reproductive success at the population level.

All four parameters of population reproductive success were significantly related to the average skull morphology, and hence genetic type, of the population, even when the influence of environmental factors was accounted for. Approximately one third of the underlying pattern of variation in skull morphology was significantly correlated with reproductive success (R>0.70).

From the coincidence of populations in the above groups of genetical similarity, derived from the cranial morphometric data, and the groups given in Chapter 3, which describe relative reproductive performance, it is possible to make some tentative conclusions about the genetic basis of the variation in reproductive success between populations of roe deer in Britain. It seems that the populations of Thetford, Ringwood, Pickering and Stanford (group 1 above) are of a genetic type that is relatively fecund. Three out of four of these are high performance populations and the other, Ringwood, is a medium performance population. In contrast, the three populations of group 3, Spadeadam, Craigellachie and Queens Forest are all low performance. This suggests that there is a heritable difference in reproductive performance of the populations of these two groups which may be due to genetic differences between native British deer (group 3) and imported continental animals (group 1). Certainly, reported figures on the reproductive performance of continental roe populations are usually equivalent of, and most often exceed, those of even the most productive populations investigated in this study (Borg 1970; Strandgaard 1972b; Bobek et al. 1974; Kaluzinski 1982a; Lovari et al. 1991; Gaillard at al. 1992).

The composition of group 2 is less easy to define, containing high (Alice Holt, Lulworth and Salisbury), medium (Kershope and possibly South Strome) and low (Inshriach Forest) performance populations. A speculative suggestion is that this group is a mixture of populations, with a variety of genetic provenances, resulting from the reintroductions and translocations of roe during the nineteenth century. These populations may, therefore, perform differentially under different environmental conditions.

These questions can only be answered more fully with extensive molecular genetic data. Comprehensive screening of samples from all populations for more enzyme loci, or the use of single locus DNA fingerprinting, may more fully elucidate the genetic provenance and relationships of roe deer in Britain. Intensive sampling within a single population may reveal to what extent reproductive success is modulated by selection acting on different genotypes of roe deer.

CHAPTER 6

FINAL DISCUSSION

It has been suggested by some authors that the reproductive performance of roe deer varies little and population regulation in this species occurs mainly through social factors acting during territory formation in the spring (Klein and Strandgaard 1972; Bobek 1977). This thesis has identified very substantial variation in reproductive performance of roe over only a small part of the geographical range of the species. Furthermore, density-dependent, density-independent and genetic factors have all been shown to have a significant influence on performance in British roe deer populations.

Certainly, little significant variation was detected in any of the four measures of reproductive performance between years within each population. A few long term studies on roe deer have identified significant variation in reproductive performance between years within a single population (Strandgaard 1972b; Maizeret et al. 1989; Vincent and Bideau 1991). The data on which the present study were based spanned an equivalent time period at only Alice Holt and Thetford, and indeed, half the significant year to year variations in the performance measures were at these two sites. However, as Gaillard et al. (1992) also found, variation in reproductive performance between years was explained by correlated variation in maternal body weight.

Some authors have noted a decline in performance among older age animals (Borg 1970; Kaluzinski 1982a), however, in this study, all does of more than two years old in a single population, on average, exhibited a uniform level of reproductive output. However, reproductive performance among the juvenile age classes was markedly lower. In agreement with the observations of Ratcliffe and Mayle (1992), fertile kids were not uncommon in some populations, however, from the data presented in Chapter 3, it seems that such precocious reproductive activity rarely results in successful implantation of a foetus. The differences in reproductive performance between juveniles and older animals within the same population are often attributed to the difference in body weight between these age classes (Gaillard et al. 1992), and indeed, this was found to be the case in this study. The very significant variation in performance between kids and yearlings and between yearlings and adults can be entirely explained by variation in the body weight of the female, suggesting that there may be a critical threshold weight for the onset of puberty in this species (see Mitchell and Brown 1974). However, as is the case for red deer (Clutton-Brock and Albon 1989), any such thresholds seem to be specific to each population.

Average jaw length (e.g Suttie and Mitchell 1983) was identified as the best index of habitat quality in relation to animal density, and was significantly correlated with each of the other such measures (average diastema height, rumen liquor quality, body weight and the density index, dung counts). The significant correlations of average jaw size of a cohort and reproductive performance in the year of that cohort's birth within a site were almost all in these same two populations, Alice Holt and Thetford. In contrast, there were very few significant relationships between reproductive performance and the climatic conditions in a particular year at these, or any other sites. This suggests that habitat quality rather than climate may cause this year to year variation in maternal body weight which, in turn, influences reproductive performance.

The main variation in reproductive output within a single population identified in this study involved manipulation of the sex of offspring prior to parturition. In accordance with the local resource competition theory (Clark 1978), the costs of rearing roe doe kids, which are philopatric (Strandgaard 1972b; Bideau et al. *in press*) and, therefore, subsequently compete for resources with their mother, may be substantially higher than those associated with rearing buck kids, which tend to disperse. Indeed, in each population, does carrying male foetuses tended to be significantly lighter than does carrying the same number of female foetuses. A series of increasing reproductive cost to potential mothers of one male, one female, two males, one male and one female, two females, was proposed by Verme (1985) for the similarly multiparous white-tailed deer, where daughters are also philopatric. The observed pattern of average maternal body weight in relation to the number and sex of the embryos carried in roe deer follows this same general sequence, with animals in relatively poor condition tending to selectively produce males rather than females.

There was also a significant variation in the embryo sex ratio between the fifteen study populations, which supported the hypothesis that roe does manipulate the sex of their offspring in response to the environmental conditions they experience. Ellenberg (1978) found that, on average, roe populations produced more males in resource limiting conditions. In this study, three of the four populations with the worst reproductive performance, presumably suffering such resource limitation, produced an excess of male embryos. In fact, the proportion of male embryos produced by does between populations was significantly inversely correlated with the average population reproductive performance. A similar relationship has been identified in white-tailed deer, and was found to relate to nutritional factors (Verme 1969).

Between population variation in adult reproductive performance was also highly significant in three of the four quantitative measures investigated (percentage of does pregnant, average number of corpora lutea per ovulating doe and average number of foetuses per pregnant doe, but not in percent ovulation, which approached 100% in all cases). Loudon (1987) suggested that variation in reproductive success between roe populations in northern Britain was entirely explained by variation in maternal body weight. However, in this study, although each of the three measures above was highly correlated with average maternal body weight, this relationship was not sufficient to explain the very significant degree of variation in reproductive performance between populations.

The average jaw size of the population was positively correlated with each of the same three reproductive performance parameters, suggesting that at least some of the variation in performance (and body weight) was due to differences between populations in resource availability per individual. However, a series of climatic variables were also significantly related to the percentage of animals pregnant and the average number of foetuses per pregnant doe, but not to the average number of corpora lutea per doe in each population. When variation in habitat quality indicators (body weight and jaw size) and climatic variables were considered simultaneously, body weight was identified as the factor that best explained the variation in ovulation (percent and/or number of corpora lutea) between populations among both adults and yearlings. In contrast, climatic variables were generally selected as best able to explain variation in both adult and yearling pregnancy (percentage and/or number of foetuses) between populations, although body weight explained an additional proportion of the variation in the number of foetuses produced per doe.

The level of genetic variability among British roe deer populations revealed by electrophoresis was substantially lower than that found for roe in central Europe (Hartl et al. 1991a), but similar to the estimates of other authors for this species (Baccus et al. 1983; Sokolov et al. 1986b), and typical for mammals in general (Baccus et al. 1983). Two out of fifteen loci were identified as polymorphic, but it was only possible to screen individuals reliably for one of these two loci. At this locus, two alternative alleles were identified in five populations, while the remaining nine populations were fixed for the slow allozyme. It was not possible to relate such a low level of genetic variability to differences in reproductive performance within or between populations.

On the basis of cranial morphometrics (Rees 1969), three groups of genetically related populations were identified. Some authors have only found absolute size differences in such analyses of roe populations (Zejda and Koubek 1988), however, in this study, discrimination was based on both size and shape differences. Furthermore, the analysis explained a large proportion of the observed variation in skull morphology (82%), and populations belonging to different genetic clusters were significantly different from one another in both size and shape. This genetic variation revealed by skull morphology was significantly correlated with variation in population reproductive performance. In particular, average potential litter size was linked to differences in both size and shape of the skull, suggesting some significant influence of genotype on variation in reproductive performance between populations of roe deer in Britain.

The various factors that may affect reproductive performance independently and/or interactively were outlined in Chapter 1 and presented in Fig. 1.2. This thesis has investigated the effects of both environmental and genetic influences on the reproductive performance of roe deer. The fitness of a particular genotype can vary according to environmental conditions (Hedrick et al. 1976), and therefore, genetic and environmental factors may have an interactive effect that is not purely additive. However, in practice, to quantify such an effect would require extremely detailed information that is only generally available from experimental systems.

It is, however, desirable to try to assess the influence of genetic factors on performance, having already accounted for environmentally generated effects. The degree of multi-locus heterozygosity has been shown to be positively correlated with a number of fitness parameters in North American white-tailed deer (Johns et al. 1977; Cothran et al. 1983; Maffei and Woolf 1987), but these studies have often given very little attention to environmental factors which may confound such analysis. In contrast, Pemberton et al. (1988) examined differences in juvenile survival between different genotypes at three enzyme loci in a herd of red deer. The environmental influences affecting juvenile survival in this herd, such as birth weight, birth date, year of birth, sex and location have been thoroughly investigated (see Clutton-Brock et al. 1989), therefore, these factors were also considered in the analysis. Fitness differences (the probability of survival) between genotypes were still detected, even when this 'phenotypic' variation was accounted for. The extent of biochemical genetic variation detected in roe deer in this study was insufficient to allow such analyses.

Although cranial morphometrics has been widely used to investigate genetic relationships in deer (Rees 1969; Lowe and Gardiner 1974; Sokolov 1985a; Zima et al. 1989), few such studies have subsequently related the observed genetic variation to fitness correlates. Rees (1970) found that differences in environmental factors such as climate and range quality were insufficient to explain the difference in skull morphology between two reproductively isolated populations of white-tailed deer. However, fitness differences between the populations were not investigated. In this study, the significant relationship between skull shape and reproductive performance of British roe deer populations remained, even when the potential environmental influences of body weight, condition and climate on skull morphology had been accounted for.

The potential interactions of environmental factors such as population density, food availability and climate have been introduced in previous chapters. The relative impact of each of these factors was investigated in Chapter 4, using multiple regression analysis. In order to further explore the relative influences of body weight, habitat quality in relation to density (jaw size), climate and absolute density (dung counts) on reproductive performance of different populations, a further set of regression equations were constructed under relaxed criteria, for each measure of population reproductive success, for both adults and yearlings. The inclusion of a particular variable in these equations does not necessarily imply a statistically significant relationship. However, this approach provides an indication of the relative contribution of all the factors considered towards explaining the variation in reproductive performance between populations, and can be used for purely descriptive purposes. By relaxing the criteria for inclusion of an independent variable in the multiple regression equation (i.e. the correlation between the dependent variable -the reproductive success measure- and the independent variable -the explanatory factor- need only be significant at only the 20% level, rather than the 5% level used in the main analysis), the summary equations presented in Table 6.1 were produced. Although many of the relationships between the reproductive performance measures and a particular environmental variable appear not to be significant, when the primary explanatory variable(s) is removed from the analysis, this relationship, with the secondary variables, often becomes significant.

In British roe deer, maternal body weight is the primary determining factor regulating the percentage of does that ovulate (effectively 100% among adults) and the number of ovulations that are successfully fertilised in both adults and yearlings. Similar findings are described by other authors for this species (Blant 1987; Loudon 1987). Even within a single population, does with two corpora lutea are, on average, heavier than does with one, who are, on average, heavier than those animals that fail to ovulate at all. This pattern is evident among both adults and yearlings.

However, body weight appears to be of lesser importance subsequently in the reproductive cycle. While there is a significant positive relationship across populations between body weight and the number of foetuses implanted among adults (body weight is the primary explanatory variable, Table 6.1), it seems likely that this reflects, at least in part, the positive carry-over effect of body weight on the number of ovulations successfully fertilised. The proportion of animals which go on to implant and become pregnant seems rather to be related primarily to winter climatic conditions. Harsh winter conditions have been implicated in causing high levels of mortality in roe deer,

Table 6.1: Multiple regressions (relaxed criteria - see text) of reproductive performance (four measures) of a population against climatic variables (average monthly mid-winter temperature, average monthly winter rainfall and average monthly summer rainfall), habitat quality indices (body weight, average jaw length and average minimum diastema height) and the density index, dung counts for a. adults (2+ years) and b. yearlings. (Significance levels: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

Reproductive Performance	**Adults**			**Yearlings**		
	Variable Selected	Cumm. R^2	Sig.	**Variable Selected**	Cumm. R^2	Sig.
Percentage Ovulation	Dung Counts	0.28		Body Weight	0.62	*
				Mid-Win Temp	0.78	
				Diastema Ht.	0.89	
				Dung Counts	0.96	
Av. No. of Corp. Lutea	Body Weight	0.67	**	Body Weight	0.80	**
	Jaw Length	0.77		Mid-Win Temp	0.96	**
	Summer Rain	0.91		Diastema Ht.	0.98	
	Dung Counts	0.95		Dung Counts	0.99	
				Jaw Length	0.99	
Percentage Pregnancy	Mid-Win Temp	0.35	*	Winter Rain	0.58	*
	Winter Rain	0.46		Body Weight	0.75	
Av. No. of Foetuses	Body Weight	0.73	***	Mid-Win Temp	0.80	**
	Winter Rain	0.84	*	Dung Counts	0.94	***
	Summer Rain	0.88		Diastema Ht.	0.97	
	Jaw Length	0.90		Summer Rain	0.99	

particularly among juveniles (Borg 1970; Cederlund and Lindstrom 1983; Ratcliffe and Mayle 1992), but such a pronounced direct climatic impact on reproductive performance has not been observed previously in this species. In particular, the average monthly mid-winter temperature at a site is positively correlated with percentage pregnancy among adults. High mid-winter temperatures indicate mild conditions which will reduce the energetic costs of exposure (Grace and Easterbee 1979), and may encourage the onset of the growing season, replenishing the available forage (Gloyne 1968). Low winter rainfall is significantly associated with an increase in the proportion of yearlings that become pregnant, and is also the second contributory factor explaining variation in adult percentage pregnancy between populations (Table 6.1). Heavy rainfall during winter can exacerbate the exposure problems created by low temperatures and high winds, but can also cause water logging and leaching of soil nutrients (Young 1982).

The number of foetuses an individual adult implants is, therefore, the product of a combination of body weight and climatic factors. This is reflected by the fact that within each population, the relationship between body weight and litter size, observed in the corpora lutea data, is no longer apparent among pregnant animals. Although does carrying two foetuses are always heavier than those with one or zero, those carrying one foetus are sometimes heavier (seven populations) and sometimes lighter (six populations) than barren animals, but the difference is never very pronounced. Because the proportion of animals that become pregnant is essentially determined by winter climatic factors, independently of maternal body weight, the non-pregnant group will be composed of individuals which had nought, one or two corpora lutea, and consequently will represent the full range of maternal body weight in that population. Whether or not does with one foetus are, on average, heavier than those with no foetuses in a particular population will depend on the proportion of individuals that had nought, one or two successfully fertilised ovulations within the non-pregnant group.

In addition to influencing implantation, climatic variation also explains some of the variation in ovulation among yearlings (but not in adults), not explained by body weight. In particular, high average mid-winter temperature is associated with increased percentage juvenile ovulation, and high average numbers of successfully fertilised ovulations (the latter relationship is statistically significant in the multiple regressions,

Table 6.1). This additional influence of climate may indicate the higher sensitivity of juveniles to environmental factors. If low winter temperatures delay the onset of the growing season, this may significantly reduce habitat quality during a does first spring and summer, preventing these individuals from achieving the puberty body weight threshold which is necessary for successful ovulation as a yearling. The heightened sensitivity of juveniles to climatic influences is emphasised by the pattern of body weight in relation to the number of foetuses an individual yearling produces. Within each population, yearlings carrying two foetuses are always heavier than those with a one or none, but yearlings with a singleton are always lighter, on average, than animals which are not pregnant. This suggests that variation in percentage juvenile pregnancy is very strongly dependent on climatic factors, directly, and through the effect of climate on habitat quality and therefore body weight.

Although maternal body weight is important in determining ovulation, it is usually presumed that body weight itself reflects variation in animal condition, due to changes in range quality or the density of animals sharing that range (Klein and Strandgaard 1972). This is supported by the present study, as in each equation where body weight is positively associated with fecundity, jaw size, a condition index (e.g. Challies 1978), is a secondary explanatory factor (Table 6.1). In particular, average jaw length is the secondary factor which explains differences between populations in average number of corpora lutea per doe among adults. Furthermore, if body weight is removed from this equation, jaw length becomes the primary, and significant, determining factor. Large jaw size is also associated with high percentage ovulation and high average number of fertilised ovulations in yearlings (average diastema height is a secondary factor in the regression equations, Table 6.1).

Pellet group counts provide an index of population density (Neff 1968), and therefore, reflect one of the two aspects of food availability per individual (the other being range conditions). As described above, ovulation in roe deer responds to maternal body condition, and therefore, is density-dependent. Low deer density will often be associated with animals in generally good condition (Klein and Strandgaard 1972), and therefore, with high levels of fecundity. This is supported by the selection of pellet counts as a secondary factor explaining variation between populations in percentage ovulation and average number of corpora lutea per doe for both adults and yearlings (but not adult ovulation rate, which is effectively 100%). Low counts, and therefore low density, are associated with high ovulation performance in all age classes.

Roe deer exhibit delayed implantation (Aitken 1974), so the action of ovulation and fertilisation at the rut is temporally divorced from subsequent implantation and development of the foetus during the winter. It has been suggested that this is an adaptive mechanism, allowing roe deer to inhabit harsh northern climates (Loudon 1982). Certainly, from the data presented in this thesis, it seems that density-dependent and density-independent factors act at different stages in the reproductive cycle of roe. This may provide a mechanism for population regulation in this species which can respond to large seasonal variations in environmental conditions, and is less wasteful of reproductive effort than neo-natal mortality (Maynard Smith 1980). Furthermore, this period of embryonic diapause may also allow an individual doe to respond to the prevailing environmental conditions by adaptive manipulation of the sex of implanted foetus(es), according to her condition at implantation, and the presumed differential energetic costs of rearing male or female roe deer kids.

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