Phenotypic plasticity and population genetic structure in a wild vertebrate population

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Submitted for the degree of Doctor of Philosophy

University of Edinburgh

October 2005

Declaration:

I have composed this thesis. All analyses presented this thesis are my own work, with the exception of the spatial autocorrelation analysis presented in Figure 5.3 and discussed through Chapter 5, which was performed by Dr Dave Coltman. This work has not been submitted for any other degree or professional qualification.

Acknowledgements:

I'll begin by expressing my immense thanks to Loeske Kruuk, my supervisor, for her instruction, support and encouragement throughout my Ph.D. Loeske somehow managed to balance insightful and careful commentary on all the work I've done with genuine kindness and support whenever it was needed. I cannot imagine how I could possibly have been supervised better. I'm also extremely grateful to have had the support of Josephine Pemberton, as a second supervisor, and Steve Albon, as a CASE supervisor. Josephine's academic support has been much appreciated, particularly during Loeske's maternity leave. Steve's contribution to the plasticity work has improved the quality and rigour of the analyses presented here immensely. I've learnt a great deal from both of you. Thanks also to NERC for funding my time in Edinburgh, and to CEH Banchory and the Macaulay Institute which have provided CASE funding.

None of the work presented here would be possible without the vision of Tim Clutton-Brock in Cambridge, in starting up and managing the Kilmory deer project, or without the hard work and skill of Fiona Guinness and all the Kilmory field research assistants. I am especially grateful to Ali Donald and Sean Morris, the present and previous field researchers for the deer project on Rum, for everything they've taught me about deer and how to go about doing fieldwork properly, and for all the great times I've had on Rum during these three years. Comments and support from Tim Clutton-Brock, Tim Coulson and Dave Coltman have been instrumental in much of the work presented in this thesis. Ian Stevenson's remarkable red deer database has almost certainly shaved months off the date of this thesis' submission. I owe a great many people in Josephine's lab thanks too: Kate Byrne helped me find my feet in the lab when I first arrived, and Barbara Wimmer, Floss Jones, Silvia Perez-Espona and Dario Bernaldi have always been on hand whenever my ignorance of all things molecular has become too apparent. I have to thank Kev Connaghan, Keith Allan and Jon Slate for red deer DNA extractions which I managed to steal over the course of my PhD. The work in this thesis has also benefited immensely from discussion with David Elston, Dany Garant, Jon Brommer, Anne Charmantier, and Alastair Wilson. And thanks to everyone at the Netherlands Institute of Ecology, particularly Erik Postma, Phillip Gienapp, Kate Lessels and Marcel Visser, for putting up with me for a month and for many stimulating discussions about my work.

On a more personal note, I'd like to thank my parents for their unwavering support. I've had an incredible time during my three years in Edinburgh, and that's really down to the fantastic bunch of people I've spent that time with. First, to Eva Jacobs: thanks for all your kindness and support in the two-and-a-half years we shared in Edinburgh. And a huge thank you to Ron Nussey, Sapna Patel, Steve Roe, Kev Connaghan, Kate Henderson, Becky Coe, Helen Simcox, Floss Jones, Culum Brown, Alastair Wilson, Amie Fulton, Darren Obbard, Jamie Moore, Tracey Reynolds, Kath Baldock, Kathy Foerster, Chloe Scott, and Louisa Tempest for their valued friendship and the great times shared over the last three years. And finally, thanks to Sarah Reece for making these last few weeks of thesis writing - my PhD's "*death zone*" supposedly - some of the best I can remember.

Abstract:

My thesis focuses on maternal phenotypic plasticity in two neonatal traits and population genetic structure at different spatial scales in a wild red deer (*Cervus elaphus*) population on the Isle of Rum, Scotland. Life history plasticity represents a vital mechanism allowing populations to respond rapidly to environmental change, yet it is rarely examined in natural settings. At the same time, fine-scale genetic structure may lead to spatial variation in evolutionary potential or kin selection within natural populations. Empirical exploration of the presumably dynamic relationship between ecological or demographic processes and spatial genetic patterns in wild vertebrates is lacking. In the five data chapters comprising my thesis I present analyses and discussion of life-history plasticity in female red deer on Rum; I also document fine-scale spatial genetic patterns across the population and link these to ecology, demography and recent human management practices. Specifically, I present:

(i) An analysis of offspring birth weight – spring temperature plasticity in female red deer using linear regression to measure individual reaction norms. I found evidence of variation in plasticity between females and show that early experiences of high population density reduce female plasticity.

(ii) The description of a mixed-effects linear model approach to analysing phenotypic plasticity from a reaction norm perspective, and application of this model to birth date in the Rum deer population. I use the model to examine variation in phenotypic plasticity between females and selection on plasticity at different population density levels.

(iii) An examination of population history and structure in red deer from across the Isle of Rum using mitochondrial DNA and microsatellite markers. Analysis revealed that deer in this introduced population came from geographically isolated ancestral populations, and there was genetic evidence for strongly male-biased dispersal. Recent management practices on the island may have led to spatial variation in effective male dispersal on Rum.

(iv) A comparison of fine-scale spatial genetic structure between male and female deer in the North Block study area using microsatellite markers and census data. There was evidence of structure at extremely fine spatial scales amongst females but not males, and a decline in the structure amongst females over time. I relate this change in female spatial genetic structure to concurrent changes in population size and mating system.

(v) An analysis of the spatial distribution of different mtDNA haplotypes in male and female red deer across the North Block. There was evidence for spatial structuring of haplotypes in both sexes.

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Chapter 1:

General Introduction

The dependence of micro-evolutionary dynamics on the environmental conditions experienced by a population is becoming increasing clear from empirical research (Hoffmann and Merila, 1999, Charmantier and Garant, 2005). Long-term individual based studies of wild vertebrate populations have provided an excellent means of assessing the impact of the environment on natural selection and levels of additive genetic variation in a variety of traits (e.g. Coulson *et al*, 2003, McAdam and Boutin, 2003, Charmantier *et al*, 2004, Garant *et al*, 2004, Garant *et al*, 2005, Wilson *et al*, 2005c).

In this thesis, I use data collected during a long-term study of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland, to address two important aspects of any vertebrate species' evolutionary ecology: phenotypic plasticity in life history traits and fine-scale spatial genetic structure. Phenotypic plasticity, defined as the ability of a single genotype to express different phenotypes in different environments, represents an important mechanism by which populations can respond rapidly to changes in ecological conditions. Fine scale spatial genetic structure, the non-random distribution of alleles across relatively short geographic distances, can dictate the spatial scale on which selection can act and underpins a population's evolutionary potential.

Life history plasticity and fine-scale spatial genetic structure are expected in many vertebrate systems, and recent research interest in these topics has improved

our understanding of when and why they might vary between populations and species (Sugg *et al*, 1996, Pigliucci, 2001). Analytical techniques and theory are available to allow us to both measure and understand these important mechanisms in the wild (e.g. Via *et al*, 1995, Sugg *et al*, 1996, Pinheiro and Bates, 2000, Vekemans and Hardy, 2004). Researchers have begun to document the presence of maternal phenotypic plasticity and spatial genetic structure at very fine spatial scales (<1km) in naturally occurring populations (e.g. Przybylo *et al*, 2000, Stow *et al*, 2001, Brommer *et al*, 2003, Coltman *et al*, 2003b, Peakall *et al*, 2003, Réale *et al*, 2003). However, we still lack empirical data showing that they can vary as a function of the environment in wild vertebrate populations, even though we would expect them to do so. In this thesis, I utilise the 30-year time series of individual life histories, census and genetic data collected from the North Block red deer study population on Rum to assess the impacts of ecological change on life history plasticity and spatial genetic structure.

In this section I introduce key concepts and ideas with regard to phenotypic plasticity in maternal life history traits (1.1) and spatial genetic structure (1.2) in wild populations. I then briefly discuss the study population (1.3) and outline the questions addressed in this thesis regarding the red deer population on Rum (1.4).

1.1 The evolutionary ecology of phenotypic plasticity

The ability of any population to respond to environmental change depends on two mechanisms: phenotypic plasticity and microevolution. The former represents a rapid response at the level of the individual phenotype, the latter a much slower population

level genetic shift across generations in a trait under selection. These two mechanisms underpin the short-term evolutionary and ecological dynamics of natural populations. There is a growing body of research addressing the potential for microevolution in morphological and life history traits in wild vertebrate populations (Kruuk *et al*, 2000, Réale and Festa-Bianchet, 2000, Kruuk *et al*, 2002, Sheldon *et al*, 2003, Kruuk, 2004, Postma and van Noordwijk, 2005, Wilson *et al*, 2005a). However, despite the current consensus that "*plastic responses to heterogeneous environmental conditions... represent one of the most common phenomena characterizing the living world*" (Pigliucci, 2005), we know little about the evolutionary or ecological forces affecting plasticity in the wild (Pigliucci, 1996, 2001) or the ways in which plasticity effects population dynamics and ecosystem function (Miner *et al*, *in press*).

A great deal of laboratory work on phenotypic plasticity has been undertaken and there are now a variety of excellent model animal systems. These include predator induced defences in invertebrates such as *Daphnia* and variation in rates of metamorphosis in amphibians (Luning, 1992, Newman, 1992). For example, juveniles of the invertebrate *Daphnia pulex* alter their morphology by producing defensive 'neckteeth' as well as increasing body size in the presence of predatory midge larvae, making them less vulnerable to predation (Tollrian, 1995). Spadefoot toads (*Scaphiopus hammondii*) breed in temporary ponds, and their tadpoles need to metamorphose before these ponds dry out. Laboratory research shows that tadpoles whose aquarium water levels were reduced metamorphosed at an earlier age and smaller size and could reverse this process to some degree if water levels were replenished (Denver *et al*, 1998). In the wild this responsiveness to water levels

would presumably allow more tadpoles to metamorphose successfully when pond desiccation rates vary.

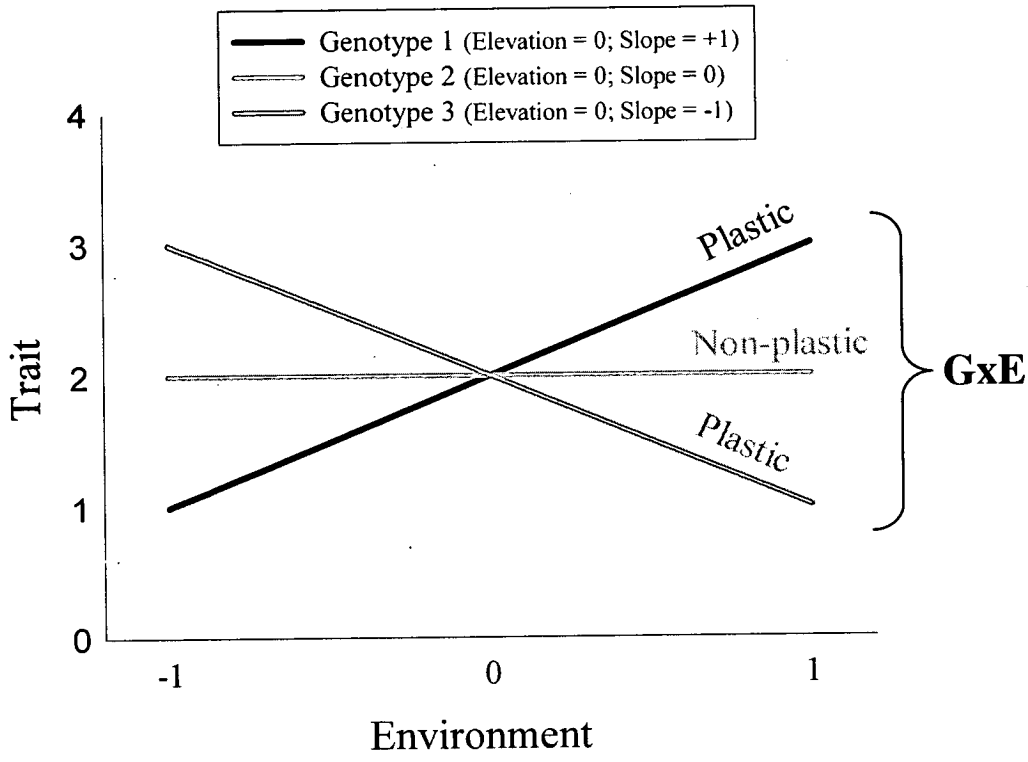
These are two classic examples of adaptive plasticity. However, phenotypic plasticity need not be adaptive (Via *et al*, 1995, Pigliucci, 2001). Many examples of life history plasticity are likely to be be the result of physiological condition-dependence (Gotthard and Nylin, 1995). Environmental variation may alter the levels of resources available to an individual and this may cause it to alter its phenotype. For example, an unusually cold winter might impose large thermoregulatory costs on a pregnant female deer, constraining the amount of resources she had available to invest in the growth and development of her offspring, and leading her to give birth to a small calf. The dependence of life history traits on environmental variables such as temperature and food availability is well documented in wild vertebrate populations, and is typically explained in terms of individual condition-dependent responses to the environment (i.e. phenotypic plasticity; Albon and Clutton-Brock, 1988, McCleery and Perrins, 1998, Stevenson and Bryant, 2000, Both *et al*, 2004). If plasticity is the result of physiologically condition-dependent responses to the environment, they themselves can presumably be mediated by other environmental factors that affect individual physiological condition. Selection on plasticity may also vary as a result of environmental conditions (Nussey *et al*, 2005c). However, such possibilities are very rarely explored in naturally occurring animal populations. Given that many species are currently experiencing anthropogenically-driven environmental change, a widening of our understanding of the environmental dependency of life history plasticity is imperative (Stenseth *et al*, 2002, Walther *et al*, 2002, Root, 2005).

1.1.1 Reaction norms and phenotypic plasticity

The reaction norm, a function or series of functions describing the relationship between a genotype's phenotypic state and the environmental conditions it experiences, is a central concept in current research into the evolutionary biology of plasticity (Via and Lande, 1985, Via *et al*, 1995, Scheiner, 2002). In their simplest and most widely applied form reaction norms are modelled as coefficients from a linear regression of a genotype's phenotype on an environmental covariate. This approach generates a coefficient estimating the phenotype-environment slope (phenotypic plasticity) and also the elevation of a genotype's slope, which – when the environmental covariate is mean-centred – can be taken to represent the genotype's phenotypic state in the average environment.

Figure 1.1 shows such linear regression based reaction norms for three genotypes. All three genotypes have the same elevation (they all cross the environmental average (zero) at a trait value of two). However, their slopes are different: genotype 1 responds positively to the environment (slope = +1), genotype 2 does not respond to the environment (slope = 0), whilst genotype 3 responds negatively (slope = -1). The slopes in Figure 1.1 highlight the subtle but important conceptual difference between a reaction norm and phenotypic plasticity. The elevation and slope estimates for genotypes 1-3 are all reaction norms (functions describing the trait-environment relationship), but only genotypes 1 and 3 show phenotypic plasticity (a change in phenotype over an environmental gradient). Genotype 2 does not alter its phenotype in response to the environment, and is therefore not considered plastic.

Figure 1.1: The differences between a reaction norm, phenotypic plasticity, and a genotype-by-environment interaction (GxE). Each of the three lines represents the reaction norm (or function(s) describing the environment – trait relationship) of a different genotype, only two of which demonstrate phenotypic plasticity. The presence of variation amongst the three reaction norm slopes is an example of GxE.



Another important concept, the genotype by environment interaction (GxE), refers to a population of reaction norms which vary in the way their phenotype changes across environments, i.e. they vary in slope as in Figure 1.1. The evolution of reaction norms has been approached using quantitative genetic theory as a framework (Via and Lande, 1985, Via *et al*, 1995, Pigliucci, 2001). The micro-evolutionary potential of plasticity in any population can be assessed using this framework by answering the following questions:

(i) Do individual reaction norms vary in their reaction norm slope (i.e. is there variation in phenotypic plasticity)?

(ii) Is there an additive genetic component to this variation in plasticity?

(iii) Is there selection on plasticity?

Quantitative genetics theory would lead us to expect that, where a GxE exists (as in Figure 1.1) and individuals that respond strongly to the environment by increasing their phenotypic trait value (plastic; genotype 1 in Figure 1.1) have higher reprodctive success than those that do not respond to the environment (non-plastic; genotype 2) or those that respond in the opposite direction (plastic; genotype 3), a micro-evolutionary response will occur in this population towards reaction norms like that of genotype 1.

Debate has existed for some years concerning whether genetic variation for reaction norm based estimates of plasticity can actually exist, or whether the evolution of plasticity occurs via selection acting on a trait in different environments (the 'character state' approach; Via and Lande, 1985, Via, 1993, Via *et al*, 1995). This theoretical debate has now been superseded by a wealth of empirical laboratory data that shows unequivocally that additive genetic variance in plasticity exists and that it can therefore respond to selection (Pigliucci, 1996, Scheiner, 2002, Pigliucci, 2005). Such laboratory studies have typically compared the phenotypes of replicate clones or sibship groups in several discrete environmental conditions to examine genetic variation in reaction norms (Via *et al*, 1995). However, we can readily apply the same approaches to model variation in, and selection on, the plasticity of individual iteroparous organisms that show within-individual variation in life history trait expression. Maternal reproductive traits, such as offspring size or timing of

breeding, are excellent examples. Iteroparous females express these traits repeatedly, whilst also experiencing variation in environmental conditions, across their life spans. It is just this type of life history plasticity that I address here, using the reaction norm approach, in a wild population of red deer.

1.1.2 Maternal life history plasticity in the wild

Many studies have documented correlations between maternal life history traits and environmental conditions such as population density and climate at the population level in natural vertebrate systems (Albon *et al*, 1987, Clutton-Brock *et al*, 1987b, Post *et al*, 1997, McCleery and Perrins, 1998, Post and Stenseth, 1999, Forchhammer *et al*, 2001, Both *et al*, 2004). Such life history reaction norms are frequently explained in terms of condition-dependent trait expression at the level of the individual (Albon and Clutton-Brock, 1988, Stevenson and Bryant, 2000), and maternal phenotypic plasticity is frequently the implicit mechanism behind these population level trends (Both *et al*, 2004, Visser *et al*, 2004). The presence of variation between individual females in their plastic responses to the environment is very rarely examined. This is hardly surprising, as detailed analyses of within-population variation in individual life history plasticity require data from large numbers of individuals breeding repeatedly across their lifetimes, and such data sets from wild populations are rare. As a result, we know little about how prevalent between-individual variation, at either phenotypic or genetic level, is in this type of plasticity, or how ecological conditions or selection act on this variation in the real world.

There are now several long-term individual based studies of ungulates and box-nesting passerines that are capable of addressing the issue of between-individual variation in maternal life history plasticity. For example, in several European box-nesting populations of great tits (*Parus major*) the annual average egg laying date advances with warming of spring temperature (McCleery and Perrins, 1998, Visser *et al*, 2002). However, there also is substantial variation in the laying date of females within populations in any given year as well as variation in the average laying date – spring temperature relationship between populations (Visser *et al*, 2002, Visser *et al*, 2004). The evolutionary or ecological basis of such variation is only likely to be understood from the perspective of female laying date reaction norms (Visser *et al*, 2004). Until very recently, only population-level average laying date relationships with spring temperature have been considered (see Brommer *et al*, 2005, Nussey *et al*, 2005c).

Many maternal traits in ungulates also vary with climate conditions during the mating season or gestation period (Albon and Clutton-Brock, 1988, Post and Stenseth, 1999, Forchhammer *et al*, 2001). For example, the population average birth weight of red deer calves in Scotland increases with spring temperature (Albon *et al*, 1987, Albon and Clutton-Brock, 1988). The foetus grows rapidly, at the expense of maternal resources, during the few months prior to parturition in late May or June (Albon *et al*, 1987). April temperatures determine the timing and extent of the spring flush, and thus the amount of food available to a female red deer in the final stages of her gestation period (Albon and Clutton-Brock, 1988). Warm April and May temperatures presumably increase the amount of resources available to female red deer during the last trimester of pregnancy, and this can then be invested in the

offspring, resulting in increased foetal growth and heavier calves at parturition. Again, though this relationship is well documented at the population level, it has yet to be explored at the level of individual females' birth weight reaction norms.

Why should individual females' reaction norms vary in their slope if the trait-environmental relationship is the result of condition-dependence? If individuals vary in overall quality then some may be in better physiological condition throughout their lifetimes and thus have more available resources. Whilst such variation in quality would certainly be expected to result in between-individual variation in the elevation component of reaction norms (the female's trait value in the average environment) it would not necessarily be expected to affect slope (plasticity). However, if plastic responses are condition-dependent then environmental factors other than the one on the x-axis of a female's reaction norm could also affect condition, and potentially change plasticity. For example, in red deer increased competition for food might reduce female condition. This could mean that a female changes her resource allocation strategy, investing any resources gained as a result of warm spring temperatures and increased grazing quality in her own maintenance and not her developing offspring. This would lead to a reduction in birth weight plasticity with respect to spring temperatures.

The question of whether females within a population vary in their plastic responses to the environment is relevant from both evolutionary and ecological perspectives. From an evolutionary viewpoint, we wish to understand whether plasticity serves an adaptive function and, if variation in plasticity exists, to understand how selection and heritability in plasticity might shape its future evolutionary trajectory. From an ecological perspective, understanding individual

variation in plasticity is vital to our ability to predict population and ecosystem responses to current or future climate changes. Most current studies are based on correlations between population level trait annual means and climate measures, ignoring possible within population variation in plasticity (Albon *et al*, 1987, Visser *et al*, 2002, Both *et al*, 2004). Yet systematic variation in individual plasticity – as a result of temporal change in an unmeasured second environmental variable for example – could undermine predictions regarding phenotypic responses to climate change and their consequences for ecosystem function.

1.2 Spatial genetic structure in wild mammal populations

Spatial genetic structure (SGS), defined as the non-random spatial distribution of alleles, is both predicted and frequently observed at fine spatial scales within naturally occurring populations of plant and animal species (Sugg *et al*, 1996, Storz, 1999, Ross, 2001, Vekemans and Hardy, 2004). Population genetic structure is more typically considered in terms of genetic differences between species or populations, often driven by adaptation to different environments. However, recently interest in SGS within populations, often at extremely fine spatial scales (<1km), in both plant and animal species has grown substantially. The significance of such fine-scale SGS for evolutionary biology is manifold. The presence, and scale, of SGS can affect the spatial scale at which selection operates (Garant *et al*, 2005), as well as the potential for kin selection processes (Chesser, 1998, Coltman *et al*, 2003b). It can also influence demographic processes and hence population dynamics (Lambin and Krebs, 1991, Piertney *et al*, 1999) and the rates of inbreeding and outbreeding. The

non-random spatial distribution of alleles has the potential to confound QTL and allelic association studies and to introduce error into quantitative genetic estimates of additive genetic variance and heritability (Coltman *et al*, 2003b). The male-biased dispersal, female philopatry and polygynous mating system typical of mammalian social systems (Greenwood, 1980, Clutton-Brock, 1989) lead to the expectation of SGS amongst females but not males, even at very fine spatial scales (Chesser, 1998, Storz, 1999, Coltman *et al*, 2003b).

The population- and individual-level processes underpinning population genetic structure are themselves dependent on environment conditions, of which human activity now forms a major part. However, the spatial and temporal dynamics of SGS, and the demographic and behavioural parameters influencing it, that are likely to occur as a result of environmental variation are rarely explored in wild populations. Whilst a growing number of studies have documented fine-scale SGS in vertebrate populations, very few have examined how this might vary as a function of environmental conditions.

1.2.1 Mechanisms and measurement

Classic population genetic models consider population genetic structure in terms of networks of sub-populations connected by gene flow, within which random mating with respect to genotype occurs. Four fundamental mechanisms influence population genetic structure: gene flow, random genetic drift, mutation and selection. Mutation represents the source of genetic variation, whilst selection, drift and gene flow are all capable of altering the distribution of this variation within and between sub-

populations. Most present day studies of population genetic structure utilise neutral molecular markers (such as microsatellites or the mitochondrial control region), removing the influence of selection (although see Chapters 6 and 7 for further discussion). Gene flow, in the case of mobile organisms like vertebrates, describes the rate of movement and subsequent successful mating of individuals from one sub-population to another. Gene flow tends to reduce levels of genetic structure between sub-populations. Random genetic drift is the rate of loss of alleles from the population by chance, and is inversely proportional to the effective population size. Drift increases genetic differentiation between sub-populations, especially where the population size is small. Thus, two fundamental demographic parameters, population size and dispersal rates, are intimately linked to population genetic structure.

The 'fixation index', F_{ST}, (Wright, 1965) is the parameter typically used to assess population genetic structure. F_{ST} measures the proportion of heterozygosity lost due to genetic differentiation between sub-populations, relative to that expected without population sub-division. Non-zero F_{ST} values therefore indicate structuring of genotypes between sub-populations. Recently, the roles of behavioural ecological processes such as mating tactics, dispersal and social structure have been explored within population genetic frameworks (Chesser, 1991, Sugg *et al*, 1996, Perrin and Mazalov, 1999). Under this approach, the social group becomes the sub-population unit for examination using F-statistics, and evident genetic structure between groups is considered to be the result of social associations between adults of the philopatric sex or the mating system (Chesser, 1991, Sugg *et al*, 1996, Chesser, 1998).

Studies of mammalian genetic structure using this approach have understandably tended to focus on species with strongly defined social group

structure (e.g. Pope, 1992, Dobson, 1998, Surridge *et al*, 1999b). Typical observations of strong between group structure amongst females but weak or absent structure amongst males is explained in terms of the female philopatry and matrilineal social system, male-biased dispersal and polygynous mating system typical of most mammal species (Chesser, 1991, 1998, Storz, 1999). Comparing sub-population or social group genetic structure between maternally- and bi-parentally inherited molecular markers represents another widely applied method of assessing structure driven by sex-biased dispersal. Here, in the typical mammalian social system, maternally inherited markers (such as mitochondrial DNA) are expected to show far higher levels of SGS than bi-parentally inherited nuclear markers because the former capture only the dispersal of highly philopatric females (Prugnolle and de Meeus, 2002). Many mammal species show fission-fusion societies, in which discrete social units or groups are not easily identifiable or vary over time, making the division of a population in sub-populations or social groups difficult. In such cases, genetic spatial autocorrelation techniques provide an extremely useful, albeit rather qualitative, method of assessing SGS across fine spatial scales and have now been applied in a variety of mammal species (Wasser and Elliott, 1991, van Staaden *et al*, 1996, Richardson *et al*, 2002, Coltman *et al*, 2003b, Peakall *et al*, 2003, Hazlitt *et al*, 2004).

1.2.2 The roles of the anthropogenic activity, environmental conditions, demography, and behaviour

One crucial element that is missing from most empirical studies of SGS in mammal, and indeed animal, populations is the environment (Bossart and Pashley Prowell, 1998). Human activity represents an important aspect of the environment experienced by wild populations, as a result of management or conservation practices, habitat destruction, alteration or fragmentation, physical disturbance, or climate change. We know that biotic and abiotic environmental conditions can change rapidly, as the result of either stochastic or deterministic processes, and are known to influence population demographic parameters such as dispersal and population growth, as well as individual behaviour and phenotypic quality (Stenseth *et al*, 2002, Walther *et al*, 2002). Long-term individual based studies of ungulates have greatly improved our understanding of such effects at demographic levels (Clutton-Brock *et al*, 1997, Grenfell *et al*, 1998, Coulson *et al*, 2001, Coulson *et al*, 2004). However, to date we have little empirical evidence linking such environment-demography interactions to changes in SGS, even though we would certainly expect them (Bossart and Pashley Prowell, 1998).

Although the consequences of anthropogenic activity for SGS and evolution of natural populations are under increasing scrutiny (e.g. Stow *et al*, 2001, Coulon *et al*, 2004), spatial or temporal variation in SGS is rarely explored in vertebrate systems. Where such variation has been investigated, the environment, population and individual processes driving it are typically inferred from the genetic data (Pope, 1998, Surridge *et al*, 1999a). Data sets allowing us to link anthropogenic or natural

variation in the environment to SGS via its effects on dispersal, population size, mating system or phenotype are extremely rare. The red deer population on the Isle of Rum represents one such rare system in which individual spatial, genetic, demographic, and behavioural data is available against a backdrop of systematic temporal and spatial variation in environmental conditions related to changes in deer management strategy (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 2002).

1.3 The Study Population

The work presented in this thesis is all based on data collected on red deer (*Cervus elaphus*) on the Isle of Rum, Scotland (Clutton-Brock *et al*, 1982). Although all relevant details of the population, data collection and analysis are given in the methods sections of each chapter, in this section I present brief details of the species' life history and annual reproductive cycle, as well as the history of the Rum red deer population and the long-term study of red deer in the North Block region of Rum.

Red deer are the largest extant wild land mammal in Great Britain, and are widely distributed through Scotland (Whitehead, 1964). They show marked sexual dimorphism: males are typically up to 70% heavier than females as adults. Females typically begin breeding at three years of age (although records exist of earlier breeding in well nourished populations), and will go on to produce a single calf in most years up until the age of twelve, after which fecundity declines (Clutton-Brock *et al*, 1982). Female red deer (hinds) in Scotland typically give birth between late May and June. Their calves remain concealed in long grass or heather when not being suckled by their mother for the first few weeks of life (Figure 1.2 A). Females

suckle their offspring until September / October of the same year if they conceive during the rut, or may continue to lactate into the following year if not. The mating season or rut begins in late September and runs through to November. At other parts of the year, the sexes show strong habitat segregation, but during the rut adult males (> 5 years old) move into normally female dominated areas of high quality grassland and compete to dominate harems, and mate with hinds as they come into oestrous (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 1997). The mating system is highly polygynous, and a handful of males gain the majority of paternities (Clutton-Brock *et al*, 1982, Rose *et al*, 1998). Outside of the breeding season females tend to be found in loosely matrilocal groups; they typically remain close to their mother's home range throughout their lives, whilst males typically disperse between the ages of 2 and 5. Physiological condition is thought to fluctuate over the year as a result of seasonal changes in the weather and food availability (Mitchell *et al*, 1976). Neonatal mortality of calves is not uncommon (Clutton-Brock *et al*, 1987b). However, most mortality of calves and adults occurs between January and April, when physiological condition reaches its lowest ebb, prior to the spring flush and increased vegetation availability (Mitchell *et al*, 1976, Clutton-Brock *et al*, 1982).

The Isle of Rum is a 10,600 ha member of the Inner Hebrides, located 19 km off the west coast of the Scottish mainland (Figure 1.3A). The island represents mountainous (up to 810m) and exposed habitat, dominated by *Culluna-Molinia-Trichophorum* vegetation interspersed with smaller, more fertile areas of *Agrostis-Festuca* grassland (Mitchell *et al*, 1976). The number of red deer on Rum is thought to have varied in the last half-century between 1100 and 1800, based on counts undertaken in early spring (Mitchell *et al*, 1976, Clutton-Brock *et al*, 2002). Red

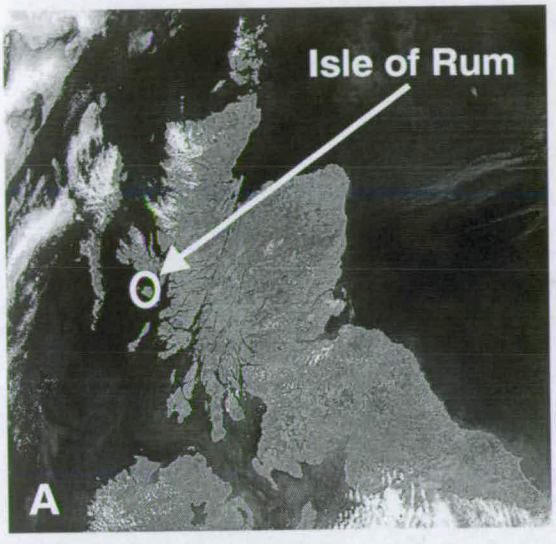
deer native to the island are thought to have been driven to extinction at some point in the eighteenth century, and the current population is the result of a series of introductions made by the island's owners from mainland UK deer populations between approximately 1840 and 1920 (see Chapter 4; Marshall, 1998). Since government agencies took over the running of the island in the 1950s, the Isle of Rum has been divided into five deer management blocks (1-5), with an area around Kinloch village in the east surrounded by deer fence (Clutton-Brock *et al*, 2002; Figure 1.3 B). Until 1972, the Rum red deer population was maintained at a density of about 14 deer per km^2 and was subject to an approximately 14% annual cull (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 2002). In 1972, the North Block (Block 4; Figure 1.3 B, C) was released from culling. The consequent changes in population density, sex ratio, fitness, dispersal, and spatial distribution of female and male deer in the North Block are discussed extensively in the chapters that follow (see also Clutton-Brock *et al*, 1982, Albon *et al*, 1992, Clutton-Brock *et al*, 1997, Kruuk *et al*, 1999a, Kruuk *et al*, 1999b, Albon *et al*, 2000, Clutton-Brock *et al*, 2002, Catchpole *et al*, 2004, Coulson *et al*, 2004). Between 1991 and 2001, culling regimes in Blocks 1-3 were also changed. These changes and their demographic consequences are discussed in Chapter 4 (see Clutton-Brock *et al*, 2002).

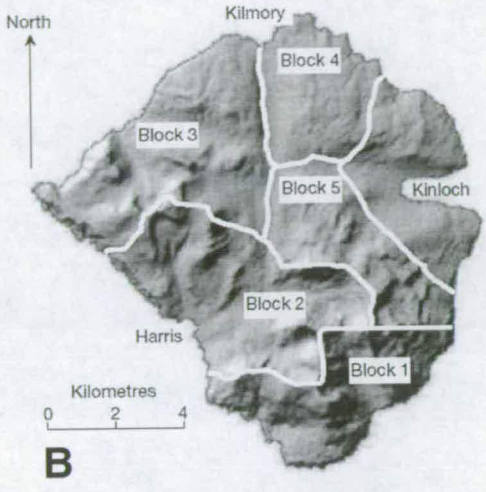
Red deer in the North Block (Figure 1.3 C) have been subject to individual based study since the late 1960s. Individual deer are recognisable to field researchers as a result of artificial and natural markings (Clutton-Brock *et al*, 1982; Figure 1.2 B). The vast majority of red deer currently resident to the study area are descended from females that were identified in the North Block when the study began. Since 1974, five censuses a month of the North Block study area have been undertaken

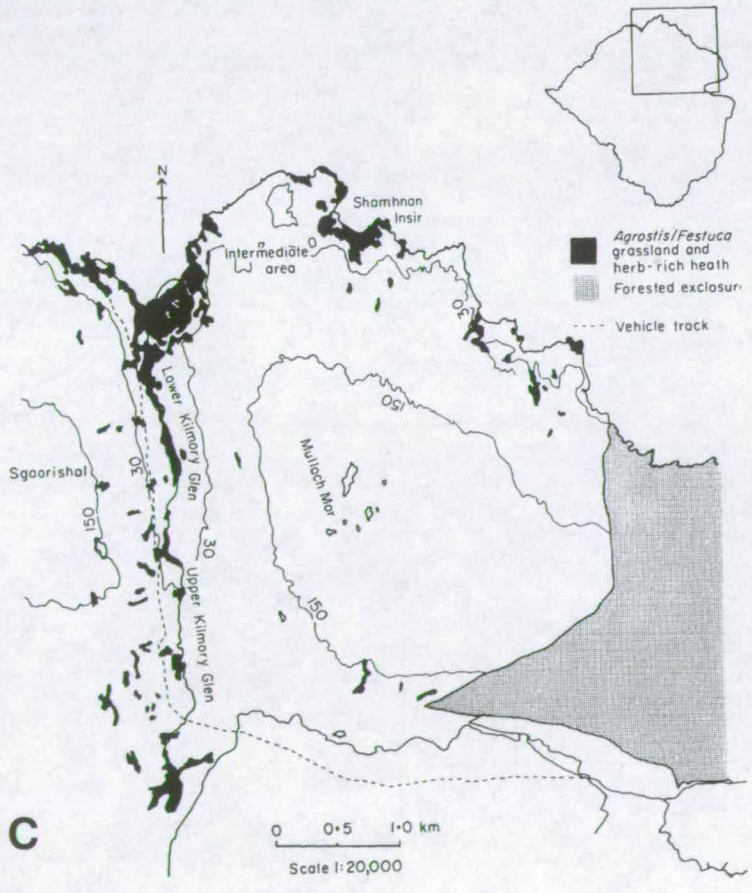
throughout the year. Censuses involve a field researcher walking a specified route through the North Block, identifying all red deer observed along with their approximate location (Coulson *et al*, 1997). In the spatial analyses conducted in Chapters 4-6, I used only census data from January to May to avoid changes in location associated with either calving or mating. In addition to regular censuses, during the calving season, intensive observation of heavily pregnant and recently calved hinds means that the date of birth for most calves is known to within a few hours. The majority of calves born each year are caught, whereupon they are weighed, sexed and marked and, since 1982, have also been blood and tissue sampled. Tissue sampling and marking of deer is also undertaken by immobilisation on an opportunistic basis throughout the year. During the rut, the identities and oestrous state of females in each male's harem are observed on a daily basis. Paternities can be assigned based on behavioural observations made during the rut and through genetic paternity analysis (see Chapter 5). During the winter months mortality searches are undertaken regularly throughout the North Block. This ongoing field research effort has generated a database containing over 3,500 deer, with almost complete life history, census and genetic information for approximately 1,500 individuals in this wild mammal population, spanning over three decades.

Figure 1.2: (A, above) Red deer calf (DTN04) from the North Block study population on the Isle of Rum hidden in long grass. Calves remain hidden like this when not being suckled by their mothers for the first few weeks of their lives (photograph courtesy of Sean Morris). (B, below) An adult female red deer (YTA74) and her yearling daughter (THO84) from the North Block study population, with typical artificial markings including collars, ear tags, flashes and punches (photo courtesy of Josephine Pemberton).

Figure 1.3: Isle of Rum maps. (A, top left) Map showing location of Isle of Rum within Scotland; (B, top right) Map of Rum showing divisions between Management Blocks 1 to 5; (C bottom; right) Detailed map of North Block (Management Block 4) study are of Rum including areas of high quality grassland shaded in black (from Guinness *et al*, 1978b).







1.4 The Objectives of this Thesis

In Chapters 2 and 3 I utilise the reaction norm approach to model maternal phenotypic plasticity in two life history traits in a wild population of red deer in the North Block of the Isle of Rum, Scotland. This population has been subject to over 30 years of individual based study, generating a substantial sample of females with repeated measures of life history traits across their lifetimes. In Chapter 2, I examine variation between females in their plastic responses of offspring birth weight to spring temperatures using simple linear regression of phenotype on environment across lifetimes. I specifically address the question of whether early experience of a measure of environmental quality (female population density), which has declined across the study period, has influenced maternal plasticity. In Chapter 3, a more complex and robust statistical approach is taken to modelling maternal reaction norm variation for a different trait-environment relationship (calving date-autumn rainfall), using random coefficients models. Here, the pattern of maternal plasticity is examined in two population density phases across the study period and selection on reaction norm components is investigated.

In Chapter 4, I examine red deer spatial genetic structure (SGS) across the whole of the Isle of Rum using samples collected from adult female red deer that were alive in early 2001. This represents the first genetic analysis of any kind that encompasses the whole of the Isle of Rum, and not just the North Block study area. I undertake phylogeographic analysis of sequence variation in the mitochondrial DNA control region in this cross-island sample with the aim of elucidating the population's geographic history. I then compare mitochondrial and microsatellite SGS, using both

fixation indices and spatial autocorrelation techniques, to infer levels of male-biased dispersal. I investigate evident patterns in light of recent changes to the culling regime in different parts of the island, and the known demographic consequences of these changes.

In Chapters 5 and 6, I present SGS analysis of red deer resident to the North Block study area (Block 4) of the Isle of Rum (see Figures 1.3 B, C). Spatial, genetic, and life history data are available for individual red deer resident or mating in this region of the island from the late 1970s. I compare fine-scale SGS in male and female red deer using microsatellite markers (Chapter 5) and mtDNA haplotypes (Chapter 6) using spatial autocorrelation techniques, and examine temporal variation in SGS in both sexes. I relate temporal changes in population genetic structure to changes in population size, levels of polygyny, and dispersal that have occurred since the North Block population was released from culling for the nuclear DNA markers (Chapter 5). Temporal shifts in spatial autocorrelation of mitochondrial haplotype are considered in terms of changes in the spatial distribution of these haplotypes within the North Block and how these might have been influenced by the environmental consequences of the release from culling (Chapter 6). I also use available life history data to test for selection on mitochondrial variation in this population (Chapter 6).

Chapter 2:

Constraints on plastic responses to climate variation in red deer

This chapter has been accepted for publication as: Daniel H. Nussey, Tim H. Clutton-Brock, Steve D. Albon, Josephine Pemberton & Loeske E. B. Kruuk (*in press*) Constraints on plastic responses to climate change in red deer. *Biology Letters.*

2.1 Summary

Influences of climate on life history traits in natural populations are well documented. However, the implications of between-individual variation in phenotypic plasticity underlying observed trait-environment relationships are rarely considered due to the large, long-term data sets required for such analysis. Studies typically present correlations of annual trait means with climate or assume that individual phenotypic responses are constant. Here, we examine this additional level of variation and show that, in a red deer population on the Isle of Rum, Scotland, changes in climate generate changes in phenotype only amongst individuals who have experienced favourable ecological conditions. Examination of offspring birth weight – spring temperature relationships within the lifetimes of individual females revealed that the tendency to respond to climate declined as the population density experienced early in life increased. The presence of such systematic variation in individual plasticity is rarely documented in the wild, and has important implications

for our understanding the environmental dependencies of traits under varying ecological conditions.

2.2 Introduction

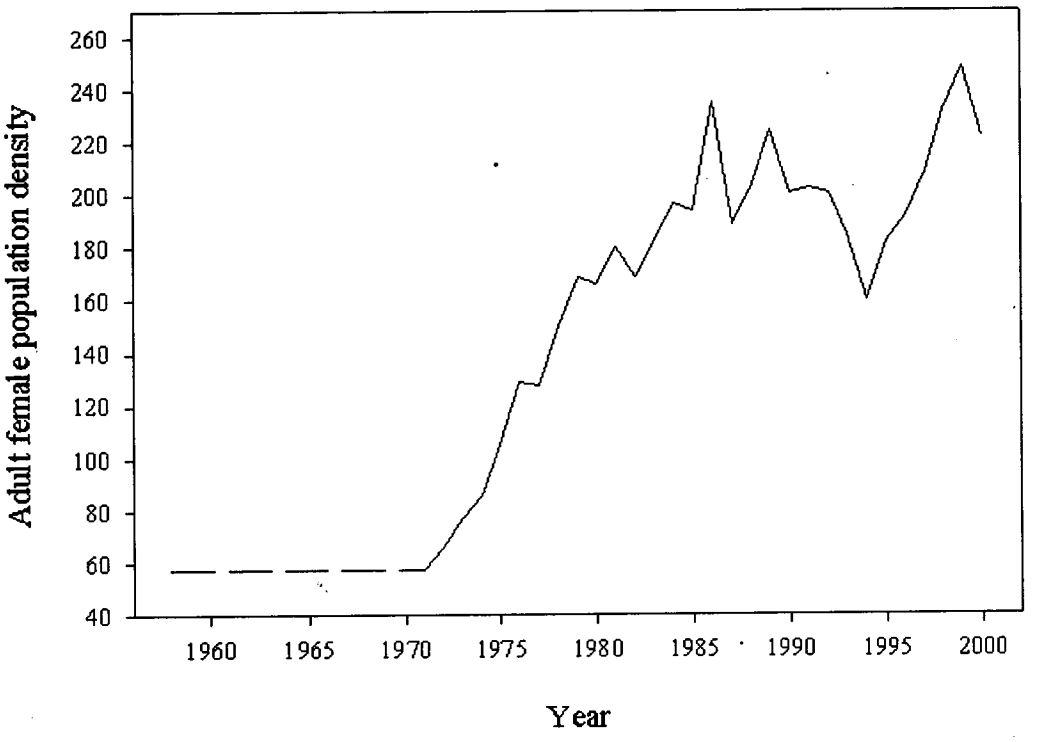
The influence of climatic variation on life history traits in natural populations is well documented (Stenseth *et al*, 2002). Many observed relationships between life history traits and the environment can be attributed to individual organisms expressing different phenotypes across their lifetimes in response to the conditions they experience (or phenotypic plasticity; e.g. Both *et al*, 2004). Such life history plasticity at the individual level may be the result of condition dependence: climate influences an individual's physiological condition, constraining the expression of costly traits or altering life history decisions (Stevenson and Bryant, 2000). Changing environmental circumstances, such as increased resource competition, may also impact on individual physiological condition and thus alter individual responses to climate. However, the effect of environmental deterioration on individual responses to the environment in wild vertebrate populations remains unexplored.

To date, studies of vertebrate life-history responses to the environment have typically assessed the correlation between annual population means for a given trait with an environmental variable (Post and Stenseth, 1998, Both *et al*, 2004) or utilised models that assume a constant response of all individuals in the population (Przybylo *et al*, 2000). The presence of and reasons for individual variation in life history responses to climate are rarely examined, however this variation will underpin any

population's ability to track environmental change over a prolonged period (Nussey *et al*, 2005a).

Here, we explore individual variation in a maternal life history trait (offspring birth weight) - climate (spring temperature) relationship in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland (Clutton-Brock *et al*, 1982). Previous studies of this population, treating birth weight as a trait of the offspring, have revealed that annual average birth weights are heavier following warm springs (Albon *et al*, 1987). This relationship is presumably driven by condition-dependence in maternal investment late in gestation: warm temperatures improve grazing conditions and hence pregnant females' physiological condition, allowing greater investment in foetal growth (Albon *et al*, 1987). A female's physiological condition may also be affected by the environmental conditions she experiences, and early experiences of environmental quality are known to have persistent effects on performance later in life (Post and Stenseth, 1998, Kruuk *et al*, 1999b). One of the key determinants of the quality of the environment in the Rum study area is adult female population density. Density has steadily increased through the 1970s following the cessation of culling in 1973, reaching carrying capacity in the early 1980s around which it has since fluctuated (Figure 2.1). This increase in resource competition has generated declines in numerous measures of performance such as juvenile survival and adult fecundity (Clutton-Brock *et al*, 1987b, Kruuk *et al*, 1999b). We examined the effects of this increase in population density and corresponding decline in environmental quality on the strength of the birth weight-spring temperature relationship within females.

Figure 2.1: Increasing female population density in the North Block study population. The plot shows the female population density (number of resident females observed in more than 10% of January to May censuses of the study area over one year of age), an indicator of environmental quality in the study area, over time. Females born before regular censusing begun were assumed to have experienced densities equal to those in 1971 (dashed line).



2.3 Methods

All data were collected on red deer in the North Block of the Isle of Rum, Scotland (a 12km^2 study area located 57° 01′ N, 06° 17′ W) between 1971 and 2000. Female deer give birth to a maximum of one offspring per year, usually in late May or June. Extensive daily surveys of the study population during this period meant that the timing of births was well known and most calves were caught and weighed within a few days of birth. Birth weights were calculated as follows: birth weight = capture weight (kg) – 0.01539 * age at capture (hours) (see Clutton-Brock *et al*, 1982 for

further details). Weather variables were obtained from a Meteorological Office weather station on Rum, and spring temperature was defined as the average daily maximum temperature through the months of April and May (as in Albon *et al*, 1987). There was no evidence of a linear change in spring temperatures across the study period ($F_{1,28} = 0.63$, $P = 0.44$).

Variation in offspring birth weights attributable to the significant effects of female's reproductive history (as a five level factor, see Section 3.3.2 for definitions) and age (as a quadratic), as well as offspring sex and date of birth was removed, and residual birth weights used in the analyses that follow. An individual female's plasticity was defined as the slope of a linear regression of her offspring's residual birth weight measurements on the spring temperatures she experienced in the year of each birth. Only females with measurements on four or more offspring were included in the analysis (190 out of 414 females). Mean plasticity and its coefficient of variation (as a percentage) were calculated.

We examined the effects of early experiences of population density on the strength of the birth weight – spring temperature relationship by grouping females according to the density in their year of birth (see Figure 2.2), and regressing plasticity estimates on density. Population density was defined as the number of females older than one year of age that were present in greater than 10% of January to May North Block censuses that year. Density estimates are available from 1971, however many reproductive females in the data set were born earlier than this. Since the main cause of change in density over the study period has been the demographic consequences of a release from culling in 1973 (Clutton-Brock *et al*, 1982), we assume that density has been constant prior to 1971 (Figure 2.1). In addition, we ran

a mixed-effects model in S-PLUS v. 6 (Insightful) on all available birth weight data (1,557 observations from 414 different females). We included the terms used in the calculation of residual birth weights described above as fixed-effects and female identity as a random effect. The inclusion of the random effect for female means the model assesses the significance of fixed effects against variation in birth weight within females. We tested for changes in the dependency of birth weight on spring temperature with density by fitting spring temperature, density in a female's year of birth and their interaction to the model as fixed effects and assessing their direction and significance.

2.4 Results

Estimates of individual plasticity confirmed that on average females gave birth to heavier offspring following warm springs: the average female offspring birth weight – spring temperature slope was 0.17 $kg°C^{-1}$. There was considerable variation between females in the strength of their plastic response to spring temperature (coefficient of variation (%) = 469.07).

There was a significant negative effect of density in year of birth on a female's plasticity ($F_{1,\ 8}$ = 12.55, $P < 0.01$). Individual responses to spring temperatures declined as females experienced higher population densities early in life (Figure 2.2). Plasticity decreased by 36% of its mean value for each additional 20 individuals present in the population in a female's year of birth. The generality of this reduction in plasticity with population density is substantiated by the presence of a highly significant negative interaction between spring temperature and population

density in the mixed-effects model of birth weight including all available data ($F_{1, 1133} = 21.52$, $P < 0.001$; see Table 2.1).

Figure 2.2: The tendency for individual female red deer to give birth to heavier offspring following warm springs declines with deteriorating environmental conditions. The plot shows mean regression slope estimates (± SE) of individual females' residual offspring birth weight – spring temperature relationship (in kg $°C^{-1}$) grouped by the densities females experienced in their year of birth with sample sizes for each density grouping indicated above the error bars. The regression line through these means is plotted (b = -0.0030 kg $°C^{-1}$ $female^{-1}$ +/- 0.0009 SE; $P < 0.01$; $r^2 = 0.61$).

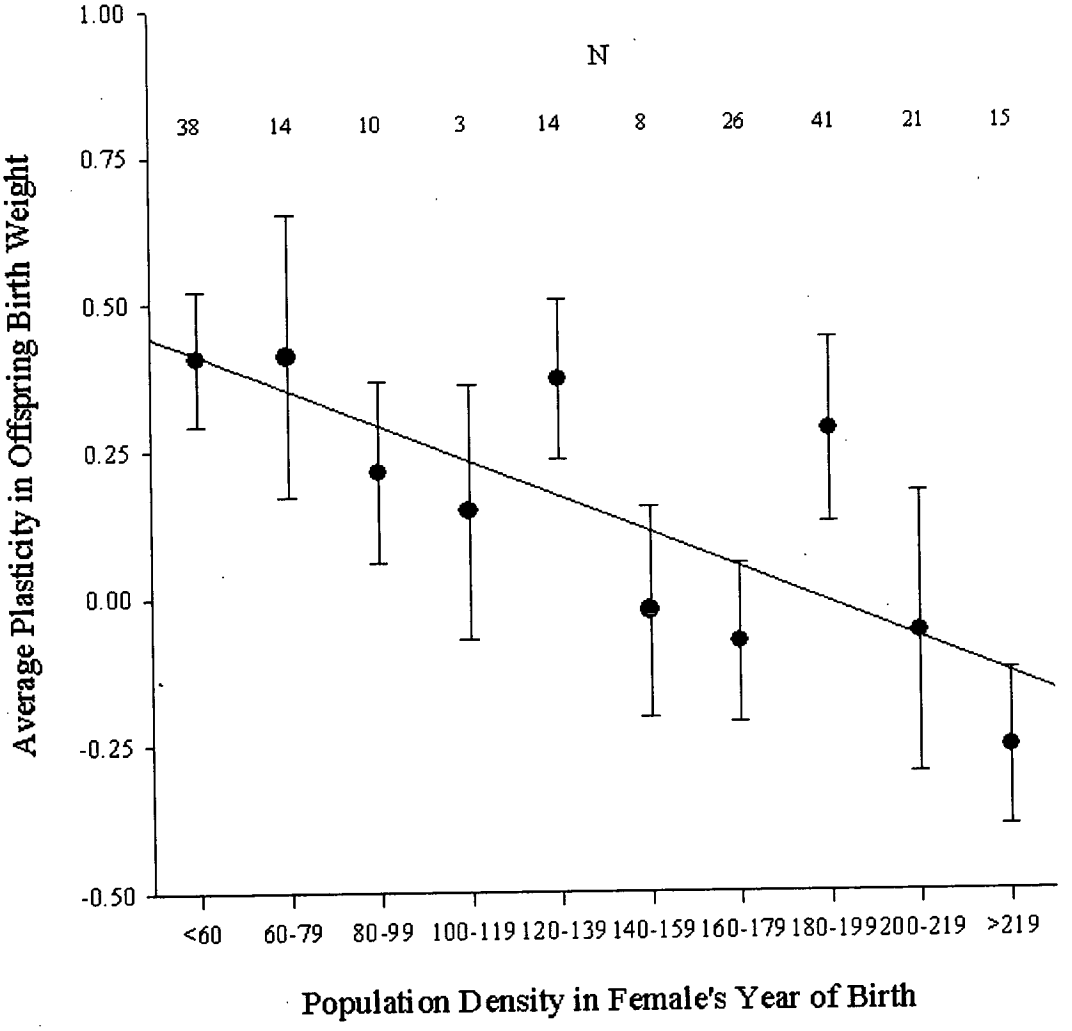


Table 2.1: A linear mixed-effects model of offspring birth weight in red deer on the Isle of Rum, Scotland. Fixed effects terms are included for factors and covariates known to influence offspring birth weight independent of climate conditions (offspring sex, date of birth, female's reproductive status and female's age as a quadratic). The random effect for female identity ensures that all fixed effects are tested against variance in birth weight within females. Estimates of effect size and direction for fixed-effects are shown for covariates. The significance of the negative spring temperature by density in female's year of birth interaction confirms the finding using regression estimates for individual females (Figure 2.2): the tendency for birth weights to increase following warm springs declines as population density increases. The model includes 1,557 birth weight observations from 414 different females.

Random Effects			
Term		Standard Deviation	
Female		0.81	
Residual		0.93	
Fixed Effects			
Term	F	P	Estimate
Offspring sex	45.14	<0.001	
Female's reproductive status	37.42	<0.001	
Female's age	19.67	<0.001	0.324
Female's age^2	35.77	<0.001	-0.020
Offspring date of birth	34.84	<0.001	0.011
Spring temperature	23.96	<0.001	0.593
Density in female's year of birth	0.03	0.86	0.028
Spring temperature x Density in female's year of birth	21.52	<0.001	-0.003

2.5 Discussion

We have shown here that plastic responses to climatic variation may vary between individuals experiencing different ecological conditions. As the density of female red deer in the Rum study area increased to carrying capacity (Figure 2.1), the relationship between offspring birth weight and spring temperature within individual

females declined to zero (Figure 2.2). There are several, non-exclusive mechanistic explanations for the changes in birth weight-spring temperature correlations amongst individuals. Changes in individual plastic responses could explain the observed trend: reductions in birth weight plasticity with increasing density could be the result of adaptive changes in female investment, which reduce the risks to mothers of sustaining large foetuses when density is high and food is scarce. Also, reductions in plasticity may occur because high density reduces the variability of condition in females, limiting the ability of superior females to increase pre-natal investment in warm springs. An alternative explanation could be that individual responses to spring temperature are reduced because, as a result of increased grazing pressures, warm springs do not generate significant increases in primary productivity when density is high.

A variety of alternative explanations exist, but whatever the underlying mechanisms, the presence of systematic variation in individuals' plastic responses to climate has implications for the way we interpret trait-environment relationships in long-lived organisms. Correlation of annual mean offspring birth weights with spring temperatures in our study population would suggest that, should springs get warmer, offspring birth weights would increase. Analysis conducted here at the individual level implies that, as long as the population density remains high, systematic changes in spring temperatures will not affect offspring birth weights. Many studies examining annual trait means have likewise shown density-independent effects in wild vertebrate populations (Post and Stenseth, 1998, Both *et al*, 2004). Findings presented here at the individual level suggest that our ability to detect such

environmental dependencies in life history traits may depend on the ecological conditions experienced by the population in question.

Analysis of variation in individual phenotypic plasticity in wild populations requires detailed information on individuals over long time series, and few studies will be able to meet these requirements. However, our analyses illustrate that the presence or absence of trait-climate relationships at the population level under one set of ecological conditions (such as rising population density) may not hold true under different conditions (such as a population at carrying capacity). Recent studies documenting variation in trait – climate correlations between geographically isolated populations (e.g. Visser *et al*, 2002) should note that whilst an effect of local ecological conditions on individual plasticity may explain observed variation between populations, it would also make extrapolation of results from one population to another problematic. Furthermore, in circumstances where there are adaptive benefits to environmental dependency in a given trait, a reduction in overall plasticity may be ultimately detrimental to population viability. By implication, populations may have to rely on the slower process of microevolution through a shift in their genetic composition to provide adaptation to changing environmental conditions.

Chapter 3:

Phenotypic plasticity in a maternal trait in red deer

This chapter has been published as: Daniel H. Nussey, Tim H. Clutton-Brock, David A. Elston, Steve D. Albon & Loeske E. B. Kruuk (2005) Phenotypic plasticity in a maternal trait in red deer. *Journal of Animal Ecology*, **74**, 387-396.

3.1 Summary

Phenotypic plasticity and microevolution represent the two processes by which phenotypic traits in a population can track environmental change. Whilst there is a growing literature documenting microevolution in reproductive traits in naturally occurring animal populations, few studies to date have examined either between-individual variation in levels of plasticity or how selection acts on plasticity. We present here mixed-effect linear models analysing changes in calving date in relation to autumn rainfall observed over a 30-year study of 2,147 red deer on the Isle of Rum, Scotland. The study period is characterised by a phase of low and rising population density (up to and including 1980), followed by a phase of high and fluctuating population density (1981 to present). Variation within individual females explained a population-level trend of delayed calving dates following years of high autumn rainfall. There was significant variation between females both in their average calving dates and in their individual plastic responses of calving date to autumn rainfall. Females born in the low population density phase were, on average, phenotypically plastic for the calving date - autumn rainfall relationship, and showed

significant variation in plasticity. Selection favoured individuals with early average calving dates amongst these females. Amongst females born at high population density, there was on average no significant plasticity for calving date, but variation in plastic responses was still present. Selection favoured females with increasingly positive plastic responses of calving date to autumn rainfall. We argue that early experience of high population density affects the physiological condition of females, making an environmental response (calving early following dry autumns) in later life physiologically untenable for all but a few high quality individuals. These same few individuals also tend to be fitter and have higher reproductive success.

3.2 Introduction

Phenotypic plasticity, defined as the expression of multiple phenotypic states by a single genotype under different environmental conditions (Bradshaw, 1965, Houston and McNamara, 1992), is a ubiquitous and widely documented phenomenon in naturally occurring animal populations (Gotthard and Nylin, 1995). Within-individual phenotypic plasticity represents one important means by which populations can track environmental changes. The other is microevolution: a change in genotypes across generations in response to selection on a trait. Assessing the relative importance of these two processes is crucial to our understanding of the evolutionary and ecological dynamics of populations, and depends on the development and application of suitable techniques capable of distinguishing them. However, we know very little about between-individual variation in phenotypic plasticity, or how selection acts on plasticity where such variation exists, in wild

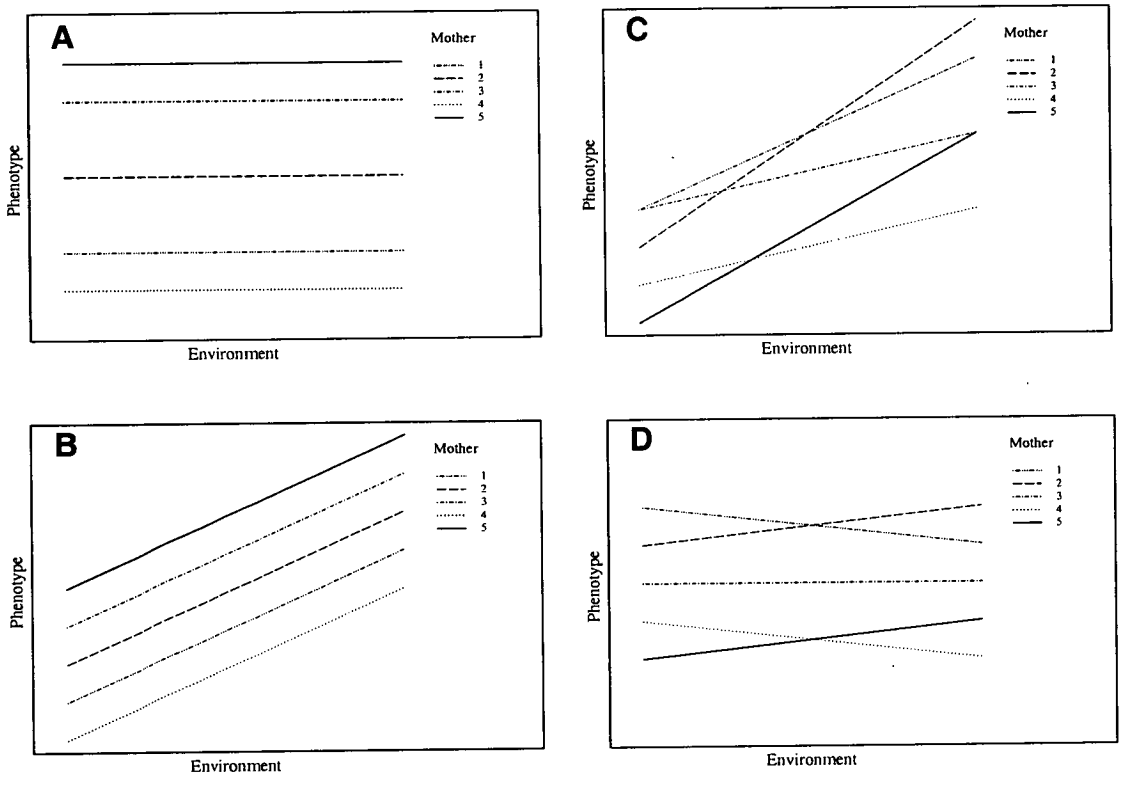
animal populations. Furthermore, the effects of environmental conditions or physiological state on individual phenotypic plasticity in natural populations are largely unknown. Long-term data sets on individually marked and monitored animals of relatively long-lived species provide an ideal opportunity to investigate these issues. We present here an analysis of phenotypic plasticity in a maternal trait in red deer.

An individual's response to the environment can be estimated using regression coefficients to describe changes in the value of a phenotypic trait expressed in different environments (see Chapter 2). Analysis would generate estimates of an individual's elevation (reflecting the expected trait value in the average environment) and slope (the plastic response to the environmental gradient). Whilst this approach has been developed within the theoretical framework of quantitative genetics following the reaction norm approach (Via *et al*, 1995), it is also applicable to studies that apply the individual optimisation or life-history approach to phenotypic plasticity (Smith, 1991). Under this framework, an individual's response to the environment is the result of condition-dependent decision making, and each individual is considered to be following its optimal trait-environment trajectory (Roff, 1992).

Pigliucci (2001) described four general and distinct patterns of phenotypic plasticity (Figure 3.1). Assuming there is variation between individuals in their mean phenotypic value for a given trait (i.e. individual estimates of elevation), a population might, on average, show a plastic response in the phenotype to an environmental gradient (Figures 3.1 B & C) or not (Figures 3.1 A & D). A population showing no average plasticity can still contain individuals that are plastic

if there is variation in plasticity (as in Figure 3.1 D). Distinguishing between these patterns in any population is important for our understanding of a population's ability to respond to the environment.

Figure 3.1: Phenotypic reaction norms across an environmental gradient for five maternal genotypes, illustrating the three main patterns of plasticity (adapted from Pigliucci 2001): (A) Variation in elevation (trait means) but no average plasticity or variation in plasticity; (B) Average plastic response without variation in plasticity; (C) Average plastic response with variation in plasticity; (D) No overall plastic response but variation in plasticity.



The reaction norm approach to modelling phenotypic plasticity has already been applied to maternal traits such as breeding date or clutch size that occur repeatedly within individual females across varying environmental conditions. Przybylo *et al* (2000) investigated phenotypic plasticity of laying date within female collared flycatchers (*Ficedula albicollis*) in response to climatic variation. Using a

similar approach, in which a female's identity was fitted as a random effect within a mixed model, Réale *et al* (2003) generated estimates of a negative linear relationship between parturition date and food availability in female red squirrels (*Tamiasciurus hudsonicus*) with multiple breeding records. The presence of a significant breeding time - environment relationship within these models indicated that the trend was present within individual females, and was explained, to a large degree, by maternal plasticity (see also Schiegg *et al*, 2002).

The studies above demonstrated that females within the respective populations showed, on average, a plastic response to environmental variation, but they did not assess the degree to which females varied in their plasticity. Thus patterns of plasticity shown in Figure 3.1 A and 3.1 D could be discounted, but the analyses could not distinguish which of the remaining two patterns best described the data: a population in which all individuals are effectively showing identical plastic responses, or one in which they vary in their response. To address this issue, a random coefficients model (Brown and Prescott, 1999) could be used. Such a linear mixed-effects model would include a random effect for the female identity-by-environment interaction, as well as for female identity. The random effect for female identity would assess variation in individual elevations, whilst the female-environment interaction would estimate variation between female reaction norm slopes. Significant variance between individual slopes would indicate variation in the plasticity of females, allowing discrimination between the patterns illustrated in Figures 3.1 B, C.

Plasticity itself can be regarded as a phenotypic trait on which selection may act. Selection can only occur on plasticity if there is variation in the phenotypic

response of individuals to the environment (as in Figures 3.1 C, D). Where variation exists, fitness differences between individuals of differing plasticity levels will generate selection on plasticity. The pattern of selection on plasticity in a population is theorised to be dependent on the amount of environmental variation experienced by the organisms in question. Where environmental variation is large, we might expect there to be selection on plasticity, whilst under constant conditions selection should act on individuals with favourable average trait values (de Jong, 1995, 1999). Levels of ecological stress experienced by individuals can also affect selection on life-history traits. Under taxing environmental conditions, the expression of plasticity or any phenotypic trait is more likely to be constrained by its physiological cost to an individual, and may therefore show a correlation with fitness not apparent under favourable conditions (Mueller, 1997, Pigliucci, 2001).

Selection on individual estimates of elevation (the individual's expected trait value in the average environment) and slope (the individual plasticity in the trait in response to the environmental variable) can be assessed if a suitable measure of individual fitness is available (Weis & Gorman 1990). Brommer *et al* (2003) examined individual female reaction norms for clutch size – laying date relationships in Ural owls (*Strix uralensis*). They found significant variation in both coefficients (elevation and slope) and, using lifetime reproductive success as a maternal fitness measure, showed that selection favoured females with larger clutch sizes but was not acting on plasticity of clutch size with respect to laying date. This represents the first published study, to our knowledge, that has used this approach to look for selection on individuals' plastic responses in a reproductive trait in a wild animal population.

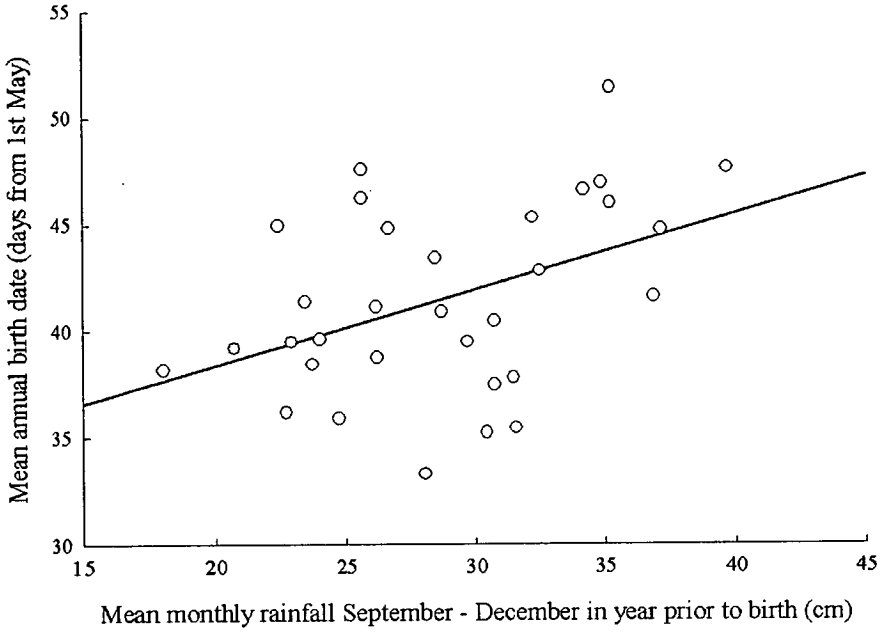
There are empirical data suggesting that within population variation in plastic responses exists and can be important (Lorenzon *et al*, 2000, Paschke *et al*, 2003). Furthermore, theoretical and laboratory work suggests that individual plasticity may be influenced by the experiences of an individual in development and early life (Pigliucci, 2001) and may alter in response to environmental conditions experienced by an individual (Tammaru *et al*, 2000, Van Kleunen and Fischer, 2003). However to date few studies have investigated changes in phenotypic plasticity and selection on it within a natural population experiencing environmental change (see also Nussey *et al*, 2005c).

3.2.1 Phenological plasticity in red deer

The present study examines patterns of female phenotypic plasticity and selection on plasticity for calving date in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland, in which female reproductive behaviour and success have been monitored extensively for over 30 years. In this population, significant correlations between calving date and both population density and climatic conditions around the time of conception and early pregnancy have been documented (Clutton-Brock *et al*, 1982, Figure 3.2). Variation in offspring birth date reflects variation in both oestrous date, which is entirely under maternal control, and gestation length, which is partly determined by both mother and offspring. Studies of parturition date in mammals have therefore varied in assignment of the trait to the mother (e.g. Réale *et al*, 2003) or the offspring (e.g. Clutton-Brock *et al*, 1987b).

Here, because we wish to examine within-female variation in response to different environmental conditions, we treat calving date as a maternal trait.

Figure 3.2: Mean monthly rainfall between September and December in year prior to birth plotted against annual average annual calving date (days after 1st May), with regression line (b = 0.36, +/- 0.10 SE).



Increasing population density and worsening weather conditions are thought to result in environmental deterioration and reduced food availability, and hence decreased female physiological condition in red deer (Guinness *et al*, 1978a). If an individual female's physiological condition determines her timing of oestrus and gestation length – and ultimately her calving date – then the observed correlations between environment and calving date at the population level are most likely the result of condition-dependent responses to the environment by those females. A previous study has examined selection on neonatal traits in this population at the level of the offspring, and revealed that parturition date affects offspring fitness in a

complex manner, via variable pathways (Coulson *et al*, 2003). However, the role of within-female variation in explaining trends in parturition date has yet to be explored or discussed in this population.

Two distinct phases of population density have been observed in the study population between the time regular censusing began (1973) and the present: a low density phase (-1980), followed by a high density phase (1981-present) during which the population density has fluctuated about an average value from year to year, suggesting it has reached habitat carrying capacity (Albon *et al*, 2000). There is strong evidence that conditions early in life influence individual life histories in this population (Albon *et al*, 1987, Langvatn *et al*, 1996), and that these effects are especially notable for females (Kruuk *et al*, 1999b). Experimental work from other systems suggests that phenotypic plasticity may vary with the conditions experienced by an individual across its lifespan (Tammaru *et al*, 2000). With this in mind, we investigated the effects of individual females' early lifetime experiences of population density on observed patterns of maternal plasticity.

The aims of this study were therefore:

(i) To examine the role of within-individual variation in explaining known environmental trends in calving date amongst breeding females in the Rum red deer population.

(ii) To investigate natural selection on maternal plasticity.

(iii) To assess any differences in patterns of plasticity and selection on maternal responses of calving date to the environment between females experiencing low and high population densities.

3.3 Methods

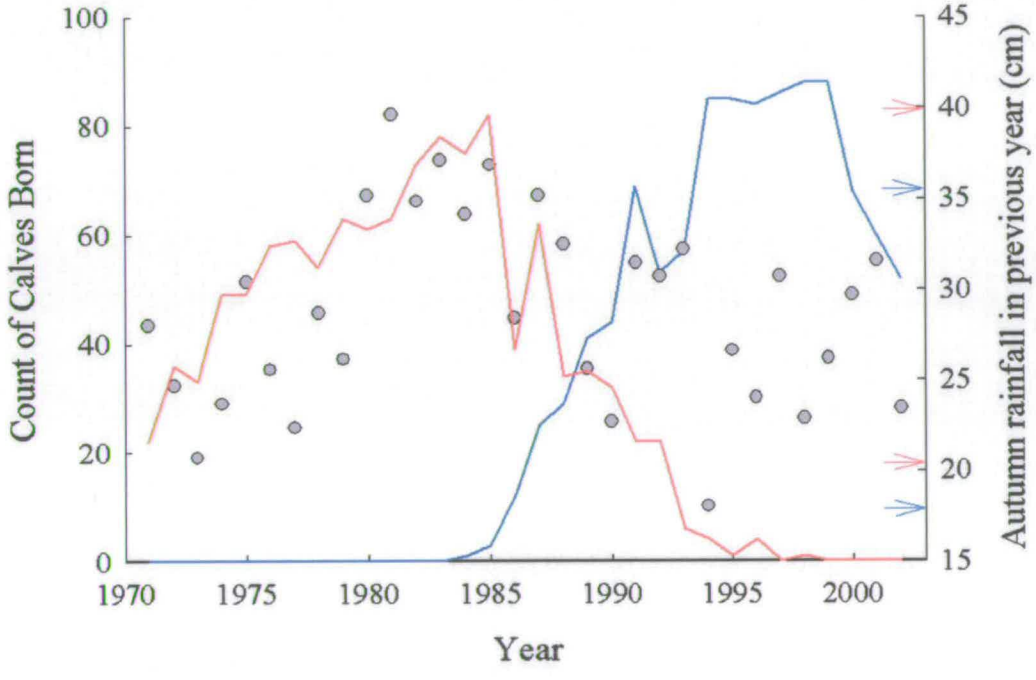
3.3.1 Study population

All data used were collected in the North Block study area of the Isle of Rum, Scotland, between 1971 and 2002. The red deer population within this area has been extensively monitored since the 1960's, and culling of the population within the confines of the study area stopped completely in 1973 (Clutton-Brock *et al*, 1982). Females in the population do not necessarily breed every year, and can produce a maximum of one calf per year. Females come into oestrous and conceive from late September onwards with most conceptions occurring in October (Guinness *et al*, 1978a). Calves are usually born in late May or June, and are generally weaned by October (Clutton-Brock *et al*, 1982).

After the cessation of culling, the density of resident adult females in the study area steadily increased to around 180 individuals in 1981. Since then, growth has stabilised and annual density has fluctuated between 160 and 249 females (Albon *et al*, 2000). Females were grouped according to which population growth phase they were born in, either up to and including 1980 ("low density") or after 1980 ("high density"). Whilst the reproductive lives of these females are not discrete (Figure 3.3), there is extensive evidence that this split divides females meaningfully in terms of their experience of resource competition. Previous studies have shown that females born in the high-density population phase, that is after 1980, have reduced longevity, less chance of reproducing during their lifetime, and lowered

fecundity, when compared to females born up to and including 1980 (Kruuk *et al*, 1999b).

Figure 3.3: The number of calves born per year of the study period to low (born up to and including 1980, red line) and high (born after 1980, blue line) density females, and mean monthly rainfall between September and December of the previous year ('autumn rainfall', grey circles). Red arrows indicate the range of autumn rainfall conditions experienced by low density females, blue arrows the range experienced ten high density females (an autumn rainfall value was included within these ranges if >10 individuals experienced it).



3.3.2 Monitoring and measurement

Females that breed regularly within the study area can be recognised from artificial and natural markings (Clutton-Brock *et al*, 1982). Regular censuses of the area, throughout the year, provide information on population density, as well as adult and juvenile mortality and hence individual survival and reproductive success. Daily

censuses are conducted throughout the calving period to provide records of birth date, whilst continuous monitoring of the study area during these months means that the vast majority of calves born can be caught, sexed and weighed, and ensures that neonatal mortalities are recorded (Clutton-Brock *et al*, 1982).

The life-history and environmental variables used in this study are described below. Variables refer to conditions in years in which individual calves were born, unless otherwise stated. Available data from between 1971 and 2002 were used.

Calving Date: Estimated date of calving, expressed in number of days after 1st May.

Female's Age: The age of female in years at a given breeding event, determined either through knowledge of the mother's year of birth or, for cases where females were born before monitoring began (approximately 2% of individuals), from tooth wear analysed post-mortem (Clutton-Brock *et al*, 1982).

Female's Reproductive Status: Categorised as follows according to a female's reproductive status in the year previous to a given breeding event (Coulson *et al.* 2003):

Milk: Female had given birth the previous year and her calf was still alive on 15th May of following year.

Naïve: Female had not bred previously.

Summer Yeld: Female had given birth the previous year and her calf had died before 1st October of that same year.

True Yeld: Female had bred before but had not given birth the previous year.

Winter Yeld: Female had given birth the previous year and the calf had died over the following winter (defined as between 1st October and 15th May).

Climate Variables: Mean monthly precipitation levels (cm) and temperatures (°C) were obtained from a Met Office weather station on Rum.

Female Lifetime Reproductive Success (LRS): The total number of offspring that survived to two years of age produced by a given female.

3.3.3 Mixed-effects linear models of calving date

We analysed data from females with available records for at least two breeding events (2,147 events for 406 females over 32 years), since individuals with a single data point provide considerably less information about individual specific slopes than individuals with multiple observations. We repeated the analyses described below restricting the data to females with five or more breeding records. This yielded very similar results to those presented. All continuous explanatory variables were centred on their mean value prior to inclusion in the analysis (Pinheiro and Bates, 2000), and linear mixed models (LMMs) were fitted using the restricted maximum likelihood (REML) method. All data analysis was conducted using GenStat v. 6.1 (VSN International).

We used the same fixed effects terms as Coulson *et al* (2003) to generate a maximal model for calving date, but with an expanded data set (including four more years of data). Climate covariates were estimated as comparisons between years, and so should be tested against unexplained year-to-year variation (Kruuk *et al*, 1999a, Milner *et al*, 1999). Consequently, a random effect for offspring's year of birth was added to this initial maximal model, which was used to test the significance of fixed-effects terms before moving onto the more advanced models. The significance of

fixed effects terms was assessed by referring Wald statistics divided by their degrees of freedom against quantiles of appropriate F-distributions. Non-significant fixed effects were dropped from the final model in a step-wise fashion until only those significant at the 5% level remained.

The final model of calving date used in the analyses that follow contained offspring's year of birth as a random effect and the following fixed effects terms: reproductive status, female's age and its quadratic term, and mean monthly rainfall between September and December prior to a calving (henceforth, 'autumn rainfall'). Autumn rainfall was the only climate variable found to be a significant predictor of calving date (as in Coulson *et al.* 2003). Across the study period examined here (1971-2002) calving dates were positively correlated with autumn rainfall (see Figure 3.2).

The mixed model structure described above was then extended to test patterns of variation in individual plasticity of calving date, keeping the fixed effects model unchanged. The significance of adding terms to the random effect model was assessed by referring changes in the model deviance to chi-squared distributions, with degrees of freedom determined by the number of additional parameters in the random effect model (Self and Liang, 1987). In models with both individual-specific elevations and slopes, we allowed for the potential correlation between these, to ensure BLUP estimates produced by the models were not affected by the method used to centre covariates.

The fixed-effects estimate for an environmental covariate produced by a LMM including female identity as a random effect is indicative of the average plastic response to that variable within all females. A significant fixed-effect for

autumn rainfall, for example, would indicate that, on average, individual females are plastic for calving date with respect to climatic conditions (i.e. that the population is showing the pattern of plasticity shown in Figures 3.1 B, C). A significant difference in deviance between LMMs with and without a random slope term for rainfall would indicate significant between-female variation in their plastic response of calving date to rainfall (i.e. patterns shown in Figures 3.1 C, D, as opposed to 3.1 A, B). This approach allows one to determine which of the four patterns of plasticity shown in Figures 3.1 best describes that observed in the population.

To identify the pattern of plasticity best describing females from each of the two population density groups in isolation, we ran separate LMMs with the final mixed effect structure determined for the entire population for each group.

3.3.4 Natural selection on phenotypic plasticity

Selection on phenotypic plasticity was assessed by the association between an individual female's elevation and slope and her lifetime reproductive success (LRS) (Weis and Gorman, 1990), which accounts for differential survival of offspring (although see Coulson *et al*, 1997 for a more detailed exploration of the pathways through which selection acts on birth date). Individual females were excluded from the analysis if they were still alive in 2002 or had been shot during culls in adjacent parts of the island (as in Kruuk *et al*, 1999a, Coulson *et al*, 2003), as under either scenario their LRS values would not be accurate representations of natural or complete individual fitness. However, re-running the analysis including living individuals did not affect the results obtained.

Since differences were found in the patterns of plasticity amongst females of the low and high density groups (see below), selection analyses were conducted on each of these groups of females separately. Estimates of elevation and slope for individual females were treated as separate but correlated traits. Best-linear unbiased predictors (BLUPs) for random effects for female identity and the female-by-autumn rainfall interaction within the mixed-effects models were used as estimates of individual elevations and slopes, these were standardised so that were in standard deviation units (following Lande and Arnold, 1983). BLUPs are estimates of random effects independent of other terms within a model, standardised to have a mean of zero. They are less sensitive to isolated extreme values within the data than separate regression estimates (Pinheiro and Bates, 2000). Selection on these estimates was measured by regressing relative female LRS on the standardised BLUP values for elevation and slope, their squares and their cross-product (Lande and Arnold, 1983).

3.4 Results

3.4.1 Patterns of plasticity for calving date

The final fixed effect model contained female's reproductive status, female's age and its quadratic and autumn rainfall. Tests comparing LMM deviances revealed that the random effect for female identity explained a significant amount of residual variation (Table 3.1). This indicated that there was significant variation in the average calving date of the 406 individual females within the data set. Further tests revealed that the addition of a random effect for each female's calving date-autumn

rainfall slope improved the model fit significantly. These results, shown in Table 3.1, imply that there was significant variation between individuals in the plastic responses of females' calving dates to autumn rainfall.

Table 3.1: The significance of adding random effects to the linear mixed-models of calving date, showing deviance estimates and log-likelihood ratio test statistics. Ticks indicate differences in the random effects fitted in respective models. All models were fitted with the following fixed-effects: female's reproductive status, female's age and its quadratic term, and mean monthly rainfall in centimetres between September and December in year prior to birth ('autumn rainfall'). Significant differences between models, based on χ^2 distributed log-likelihood test statistics (LRT), indicated in bold (*: P<0.05; **: P<0.01).

	Random variables included in mixed-effect model:						
		Maternal Variables:					
Model	Offspring year of birth	Identity	Identity x autumn rainfall	Model deviance	d.f.	Test	LRT
			Across study period (1971 – 2002)				
1	√			14385.85			
2	√	√		14344.87	1	1 vs 2	**40.98****
3	√	√	√	14329.91	2	2 vs 3	**14.96****
			Offspring of females born at low population density (≤1980)				
4	√	√		7457.19			
5	√	√	√	7446.65	2	4 vs 5	**10.54****
			Offspring of females born at high population density (1981-2002)				
6	√	√		6885.81			
7	√	√	√	6879.55	2	6 vs 7	**6.26***

In the model with all random effects, the fixed-effect estimate of autumn rainfall indicated that calving dates were delayed by an average of 0.41 (+/- 0.15 SE) days per centimetre of rain: there was an average within-female response

significantly greater than zero. It appears that the trend in calving date with autumn rainfall observed at the population level is largely explained by within-individual variation at the maternal level.

Across the study period (1971-2002), females: (i) varied in their average calving dates; (ii) showed, on average, plasticity of calving date with respect to autumn rainfall; (iii) varied in the magnitude of their plastic response to autumn rainfall, a pattern equivalent to that in Figure 3.1 C.

LMMs were run for each of the two female density groups separately. Table 3.2 shows that variation in individual calving date - autumn rainfall slopes was very similar in the high and low density groups of females (0.56 +/- 0.32 SE and 0.52 +/- 0.21 SE, respectively), whilst females in the high density group varied more in their calving date elevations that those at low density (35.13 +/- 9.97 SE compared with 23.80 +/- 7.87 SE). Correlations between elevations and slopes in the two groups were also different: both were negative, but females born at high densities showed a closer relationship between elevation and slope (r = -0.35 and -0.10). Females born at high density that showed early calving dates in the average environment were more likely to also show a strong positive response to autumn rainfall.

Females born up to and including 1980 (low density) showed an average positive plastic response to autumn rainfall of + 0.52 (+/- 0.20 SE) days per centimetre of rain per month (see Table 3.2). These females are giving birth earlier in response to dry autumn conditions, and vary significantly in their responses (χ^2 = 10.54, df = 2, $P < 0.05$, Table 3.1). This pattern of plasticity is illustrated by Figure 3.1 C.

Amongst females born at high density, there was a substantially reduced average response of calving date to autumn rainfall (+ 0.18 days per centimetre of rain per month +/- 0.21 SE, Table 3.2), which was not significantly different from zero. However, there was still variation between females in their plastic responses to autumn rainfall (χ^2 = 6.26, df = 2, P < 0.05, Table 3.1). This situation is described visually in Figure 3.1 D.

Table 3.2: Estimates of fixed and random effects produced by a linear mixed-model for calving dates (days after 1st May) for study data set split by mother's year of birth; (A) before 1980 (1117 calves, 185 mothers) and (B) between 1981 – 1997 (1030 calves, 221 mothers). Only calves of females with more than one calf available for analysis in each model were included. The key estimates to note with reference to the text are presented in bold font.

	(A) ≤1980		(B) 1981-2002	
Random Effects	Variance component	SE	Variance Component	SE
Offspring year of birth	18.0	7.8	9.4	5.6
Female elevation	**23.80**	**7.87**	**35.13**	**9.97**
Female slope on autumn rainfall	**0.52**	**0.21**	**0.56**	**0.32**
Residual	248.3	12.3	246.5	13.5
Fixed Effects	Estimate	SE	Estimate	SE
Reproductive Status				
Milk	44.70	1.38	46.37	1.47
Naïve	40.19	2.16	44.08	1.96
Summer Yeld	36.73	1.62	34.55	1.70
Winter Teld	46.95	2.06	44.94	1.91
Yeld	40.14	1.41	37.84	1.38
Female's age	-2.92	0.97	-2.26	1.21
Female's age^2	0.18	0.05	0.15	0.06
Autumn Rainfall	**0.52**	**0.20**	**0.18**	**0.21**

Variation exists in the calving date - autumn rainfall response of individual females in both the low and high population density phases of the study, and so selection may be acting on these traits.

3.4.2 Natural selection on phenotypic plasticity

Multiple regression analyses of female LRS revealed that selection pressures on females' calving date - autumn rainfall slopes differed between the two density groups (Table 3.3). Amongst females born at low density, there was directional selection on elevation, favouring earlier calving dates in the average environment, but no selection on slope (i.e. no direct selection on plasticity). The presence of a marginally non-significant interaction between elevation and slope in this model suggests that a slight fitness advantage is conferred to females responding to dry autumns by calving early if they also have early calves in the average environment, but this advantage declines with increasing female elevation. Amongst females born at high population density, a strong but marginally non-significant selection gradient was present on individual slope, suggesting that high density females giving birth early following dry autumns and late following wet autumns had higher LRS.

The magnitudes of the selection gradients on elevation amongst low density females and slope amongst high density females are notably high when compared with recent estimates of median selection gradients in natural populations, although the standard errors associated with these are large (-0.69 +/- 0.18 SE and 0.80 +/- 0.42 for low and high density groups respectively, compared to an absolute median of 0.17 from Kingsolver *et al.* 2001).

Table 3.3: Multiple regression of linear, quadratic, and interaction terms for best linear unbiased predictors for maternal elevation and slope produced by mixed-models of calving date shown in Table 3.2 for: (A) females born up to and including 1980 (n = 178); and (B) those born after 1980 (n = 87) on lifetime reproductive success. Intercepts were fitted in both regressions but are not shown.

	(A) Up to and Including 1980			(B) 1981 – 2002		
Coefficient	Estimate	SE	p-value	Estimate	SE	P value
β (elevation)	-0.69	0.18	<0.01	0.17	0.24	0.48
γ (elevation^2)	0.14	0.12	0.27	0.02	0.05	0.70
β (slope)	0.01	0.20	0.96	0.80	0.42	0.06
γ (slope^2)	0.10	0.08	0.22	0.30	0.19	0.12
γ (elevation x slope)	-0.23	0.13	0.09	0.15	0.16	0.36

A possible factor influencing the shift in selection on females' plastic responses between these two groups could be differences in their experiences of autumn rainfall. There was no evidence of a linear temporal trend in autumn rainfall across the study period (b = -0.03 centimetres per year +/- 0.10 SE, df = 32, t = 0.27, P > 0.05), or any difference in the mean autumn rainfall conditions experienced by the two groups (t-test comparing mean autumn rainfall in years in which 10 or more females from either low or high density groups bred (see Figure 3.3): t = 0.89, df = 32, P > 0.05). Whilst it does appear that the high density group of females experienced slightly lower variation in autumn rainfall, it is clear that there was still considerable variation in rainfall across their lifetimes (Figure 3.3).

3.5 Discussion

We have shown here that individual plasticity can explain the environmental trends observed between calving date and autumn rainfall amongst hinds in the Rum North

Block red deer population. Increased precipitation around the time of mating and early pregnancy results in environmental deterioration and reduced food availability for individual females (Clutton-Brock *et al*, 1987a). Increased rainfall may also impact directly on the physiological condition of females, through increased thermoregulatory costs for example. It has been hypothesised that the variation in calving date is largely due to physiological condition-dependent variation in a female's timing of oestrus and gestation length (Clutton-Brock *et al*, 1982). The finding that changes in birth date with environmental conditions occur at the level of the individual female supports this hypothesis. The few previous papers that have investigated maternal plasticity in naturally occurring animal populations (Przybylo *et al*, 2000, Brommer *et al*, 2003, Reale *et al*, 2003, Schiegg *et al*, 2002) have all shown it to have a role in observed phenotypic trends, and here we add further evidence that individual plasticity is an important and often over-looked component of variation in reproductive traits.

The observed variation between females in mean calving date is not surprising and can be ascribed to differences in genetic or non-genetic components of individual quality (Clutton-Brock *et al*, 1983). However, to date few studies have examined or discussed variation in responses to the environment between reproductive females in naturally occurring populations (although see Brommer *et al*, 2003). Across the study period we observed variation in females' response of calving date to autumn rainfall. If we assume the plastic response of a female in her lifetime's calving dates to autumn rainfall to be the result of physiological condition-dependent decision making, then we can see that the optimal response to rainfall may

vary depending on a female's physiological condition at the start of her reproductive cycle (i.e. August or September).

Females born at low population densities respond to dry autumns by giving birth earlier. Females born at high densities appear to show a weaker average response to autumn rainfall conditions. Whilst several papers have discussed possible evolutionary limitations to plasticity (Gotthard and Nylin, 1995, de Witt *et al*, 1998), to our knowledge ecologically or physiologically mediated shifts in phenotypic plasticity have not previously been observed in a naturally occurring animal population (see also Chapter 2; Nussey *et al*, 2005c). This result emphasises the importance of utilising long-term data sets for such analyses and the potential for ecological or environmental changes to influence phenotypic plasticity in the wild.

Why would female deer born at high population densities be less responsive to autumn rainfall conditions? Differences in patterns of rainfall experienced by the female density groups could be responsible. Reduced environmental variation might mean there is simply less scope for high density females to show plasticity. However, there was no evidence of a significant difference between the autumn rainfall conditions experienced by the two groups.

We argue that the patterns of plasticity observed are largely the result of a trade-off within females between the physiological costs of early calving and the costs imposed by high population density. There is extensive evidence of early environmental conditions affecting adult breeding behaviour and lifetime reproductive success (Albon *et al*, 1987, Kruuk *et al*, 1999b), and of high population density increasing the cost of reproduction for females (Clutton-Brock *et al*, 1983), in this population. It therefore seems likely that early experience of nutritional stress,

due to intense resource competition, can affect physiological condition later in life. The absence of differences in plasticity following categorisation of the data by offspring birth year rather than its mother's (data not shown) also suggests that consistent differences in plasticity between females are determined by conditions during early development.

Females born at higher population densities are likely to be in poorer condition at the start of the reproductive cycle compared to those born at low density. For many individuals this may mean that responding to favourable autumn conditions by calving early is physiologically out of the question. However, variation in individual quality and condition is still present, and it may be that only those few females in relatively good condition despite high population density can afford to give birth early following drier autumns. This would result in the substantially reduced, statistically non-significant response to the environment observed within females born at high density. The presence of increased variation in elevation and a stronger negative correlation between elevation and slope amongst high density females supports the argument that only a small number of these females are both breeding early in the average environment and responding to autumn rainfall.

Females giving birth early when autumns are dry are likely to reap fitness benefits as a result of early birth dates of their offspring (more time for calves to feed and grow before the winter) and the ability to invest more in their young (longer suckling period). At low densities, when most females are physiologically able to respond to favourable autumn conditions, selection on plasticity is only apparent in its marginally non-significant interaction with elevation. Females that give birth early

in the average environment are at a selective advantage, whilst females that also respond to favourable conditions by giving birth early have the highest LRS.

At high density, when few females can afford to advance calving dates even if autumn rainfall conditions are favourable, a direct fitness benefit of plasticity is apparent. The absence of selection on females' elevation of calving date amongst these females is surprising. However the standard errors associated with the estimated selection gradients are high, so the absence of significant selection does not mean that selection on elevation is not present. It should be noted that these findings do not necessarily mean there is a direct link between plasticity and LRS. Another unmeasured variable that affects fitness (e.g. female's physiological condition) and is correlated with plasticity may be the real target of selection.

We have discounted the possibility that changes in the amount of environmental variation experienced by the density groups were responsible for differences in the patterns of plasticity and selection on plasticity, a possibility suggested by de Jong, 1999) and others. Instead, we have argued that both early calving and experience of high population density impose physiological costs on females in this population. At low densities, most females appear to be able to meet the costs of early calving following favourable autumns, whilst at high population densities only those in the best condition can do so. Stressful environmental conditions are revealing the physiological cost of responding to the environment, and, in turn, a correlation between plasticity and fitness not detectable under favourable conditions. These findings are backed by tentative theoretical statements made by Pigliucci (2001) and research into density-dependent selection (Mueller, 1997). Further theoretical and empirical examination is now required to help

determine how ecological conditions are likely to affect the responses to individuals to the environment.

3.6 Conclusions

This study illustrates how mixed models can provide a valuable and readily available means of analysing patterns of plasticity in reproductive traits, and that maternal plasticity for calving date in red deer in response to autumn weather conditions is an important component of observed population trends in this trait. Examination of reproductive traits at the maternal level can reveal trends of ecological and evolutionary importance not apparent in analyses at the level of the offspring. We have shown that consistent changes in ecological conditions, often apparent in long-term population data sets, can influence patterns of plasticity and the way in which selection acts on plasticity. The findings presented here should encourage further exploration of plasticity for reproductive traits under some degree of maternal control, leading to a better understanding of its role within observed population dynamics in the wild.

Chapter 4:

The genetic consequences of human management in an introduced island population of red deer

4.1 Summary

The role of behaviour, demography and the environment in determining population genetic structure is the focus of increasing empirical and theoretical research interest, but there remains little consensus regarding the genetic impact of anthropogenic management practices in free-living populations. We investigated mitochondrial phylogeny and spatial genetic structure in an island population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland, which experienced variation between sub-populations in management regime. Phylogenetic analysis showed that the five mitochondrial haplotypes identified in our sample of female red deer from Rum clustered with different red deer sequences from across Europe and Africa. One haplotype – labelled "RUM A" – clustered with samples from Sardinia and North Africa, while four other "RUM B" haplotypes grouped with deer from Norwegian and Spanish populations. Recent and historical management practices explain this otherwise surprising result: the Rum population is descended from recent introductions from at least four different UK mainland populations, and translocation of red deer within the UK and across Europe is well-documented. There was significant spatial genetic structure across the island in both mtDNA haplotypes and microsatellite markers. Mitochondrial spatial structure was, as expected for a species

showing male-biased dispersal, over an order of magnitude greater than in the nuclear markers, and conformed to a pattern of isolation-by-distance. In contrast, the spatial structure in microsatellite genotypes did not follow this pattern, but was apparently dependent on differences between the high-density North Block sub-population and the rest of the island. This is surprising given the documented high levels of male emigration out of this sub-population. We suggest this pattern of genetic structure may be due to emigrating males from the North Block being of poor quality relative to males in other parts of the island, with whom they will have to compete for males, with the result that gene flow may be considerably less than expected based on behavioural observations of dispersal.

4.2 Introduction

Anthropogenic activity directly and indirectly influences the behaviour and demography of free-living animal populations and, as a result, may have a variety of consequences for spatial genetic structure (Harris *et al*, 2002). To date, the effects of human management practices on the population genetics of vertebrate game species has received little attention, despite their potential economic and conservation implications. The regular practice of human translocation of individuals between populations, often geographically distant from one another, in both domestic and game species can act to enhance genetic differentiation between populations, whilst blurring a species' phylogeographic structure and undermining expected patterns of isolation by distance, making native individuals or populations difficult to identify (Guiffra *et al*, 2000, Pereira *et al*, 2005). At the same time, selective culling to

maximise some phenotypic quality (e.g. antler or horn size in males) is likely to alter levels of genetic variation or specific allele frequencies associated with these traits (Harris *et al*, 2002, Coltman *et al*, 2003a). Hunting may also act to reduce spatial genetic structure if it is associated with disturbance to social structure leading to increased migration, or generates spatial differences in mating opportunities that may encourage dispersal (Harris *et al*, 2002). Anthropogenic activity may also isolate populations either through deliberate enclosure of managed stocks (e.g. fenced populations) or habitat fragmentation and human constructions preventing natural dispersal (Hartl *et al*, 1990), leading to increased genetic differentiation through genetic drift and mutation.

The red deer (*Cervus elaphus*) is one of the largest extant game species in Europe, and many populations are subject to human management and trophy hunting (Whitehead, 1964, Long, 2003). There is little doubt that "human intervention has drastically affected the natural genetic structure of red deer" (Gyllensten *et al*, 1983): the species has been the subject of regular human introductions from populations spread across the entire continent and beyond, since at least Roman times (Long, 2003). Indeed, records exist of introductions to UK populations from as far a field as North America with the aim of improving antler size (Long, 2003).

Analysis using mitochondrial DNA (mtDNA) has illuminated our understanding of phylogenetic relationships between species within the genus *Cervus* (Douzery and Randi, 1997, Polziehn and Strobeck, 1998, Randi *et al*, 2001), and the phylogeography of *Cervus elaphus* (Randi *et al*, 2001, Polziehn and Strobeck, 2002). This work suggests genetic separation of *Cervus elaphus* into Eastern (including European, Middle Eastern and African subspecies) and Western (including East

Asian and North American subspecies) clades or even species (Polziehn and Strobeck, 1998), with ancestral populations located in the Tarim region of southwest Asia (Ludt *et al*, 2004). Although seven geographic subspecies are currently recognised in Europe and Africa (Polziehn and Strobeck, 2002), genetic analysis has typically failed to support these taxonomic divisions (Gyllensten *et al*, 1983, Hartl *et al*, 1995). One notable exception is support from mitochondrial phylogenies for the taxonomic separation of red deer from the Tyrrhenian islands (Corsica and Sardinia) and North Africa from other mainland North and West European populations (Randi *et al*, 2001, Ludt *et al*, 2004; although see Zachos *et al*, 2003). However, given the widespread trafficking of *Cervus elaphus* across Europe for many centuries, the presence of highly divergent mtDNA haplotypes across restricted geographic ranges is to be expected (Feulner *et al*, 2004).

Red deer exhibit a polygynous mating system, with male-biased dispersal. As is the case in many mammal systems, females are typically philopatric and remain close to their maternal relatives throughout their lives whist males disperse from their natal area prior to reaching maturity (Clutton-Brock *et al*, 1982). Male-biased dispersal leads to the expectation of a greater than four-fold reduction in spatial structuring in nuclear genotypes relative to maternally inherited genetic material, such as mitochondrial DNA (Prugnolle and de Meeus, 2002). At finer spatial scales, female philopatry can result in clustering of related individuals and structuring of nuclear genotypes across continuous space (Coltman *et al*, 2003b, Nussey *et al*, 2005b). Studies of red deer using nuclear DNA or protein markers have tended to find significant genetic structure between proximate populations of red deer in Europe, whilst failing to find evidence of any relationship between genetic and

geographic distances between populations, which were often anthropogenically enclosed (Gyllensten *et al*, 1983, Hartl *et al*, 1990, Hartl *et al*, 1995). This is just the pattern expected amongst populations subject to physical isolation and regular introductions from diverse source populations. However, few studies have been able to compare the effects of different management practices on population genetic structure, as a result of either differences in ancestry, male-mediated gene flow or selective culling practices across unfragmented or unfenced habitat in vertebrate game species (although see Hartl *et al*, 1995).

The population of red deer on the Isle of Rum, Scotland, represents a rare opportunity to explore the population genetic consequences of human translocation and differences in culling regimes in a vertebrate game species. The natural population on the island is thought to have been hunted to extinction in the 1800s, and the current population is descended from animals introduced to the island from the UK mainland by different owners of the island between the 1840s and 1920s (Whitehead, 1964, Marshall, 1998). Existing records show deer were introduced to Rum from at least four different UK mainland populations: Windsor Great Park, Knowsley Park in Lancashire, Meggernie Estate in Perthshire, and Warnham Park in Sussex (Marshall, 1998). Since management of the island was handed over to government agencies in the 1950s, the red deer population on Rum has been subject to an annual 14% cull (Clutton-Brock *et al*, 2002). Since this period, the island has been separated into five 'management blocks', which are separated by physical structures such as valleys and mountain ridges, and a fenced area in the east around Kinloch village (see Figure 4.1). The physical features separating the management

blocks 1-5 (Figure 4.1) do not represent physical barriers to deer movement, indeed male dispersal between blocks often occurs (Clutton-Brock *et al*, 2002).

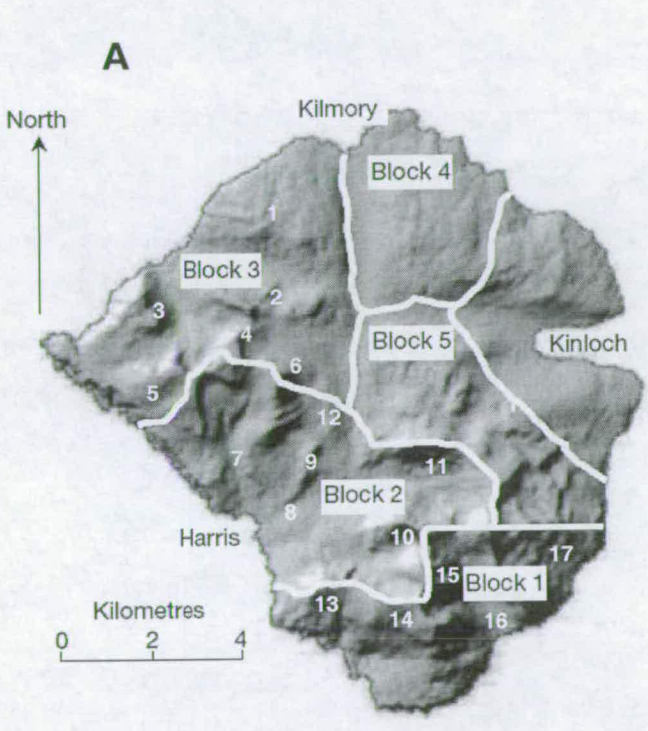
Since the late 1960s the red deer resident to the North Block (Block 4; Figure 4.1) have been subject to long-term individual based ecological, behavioural and genetic study (e.g. Guinness *et al*, 1978a, Clutton-Brock *et al*, 1984, Pemberton *et al*, 1988, Albon *et al*, 1992, Clutton-Brock *et al*, 1997, Kruuk *et al*, 2000, Coulson *et al*, 2004). Deer on the rest of the island have not been subject to individual based study, but regular censuses of numbers have been taken (Clutton-Brock *et al*, 2002), and collection of bone and tissue samples from culls has also occurred. In this study, the management blocks are treated as sub-populations; since the 1970s each block has been treated to different management regimes. In 1972, the North Block (Block 4) of the island was completely released from culling, and consequently the female population density in this sub-population increased while the number of males decreased (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 2002). Between 1991 and 2000, Blocks 1 (south) and 3 (west) (Figure 4.1) were subject to increased male and female culls, respectively (Clutton-Brock *et al*, 2002). The increase in population density following the release from culling in Block 4 is known to have increased male emigration from this sub-population, whilst increased male cull in Block 1 seems to have increased male immigration into this area (Clutton-Brock *et al*, 2002).

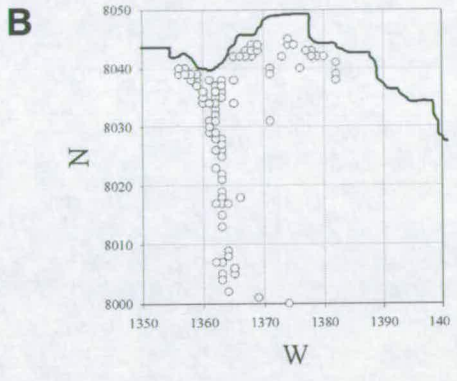
Here, we use tissue samples from both the North Block study population and from culled females from the rest of the island to:

(i) Explore mitochondrial sequence divergence in the present day Rum population and relate this to existing molecular phylogeographic research into European red deer.

(ii) Compare spatial genetic structure of red deer across Rum in both mtDNA and nuclear markers to examine patterns of population genetic structure and sex-biased dispersal and to relate these to differences in recent management regimes across the island.

Figure 4.1: (A) Map of the Isle of Rum showing boundaries between 4 culling blocks from which female red deer were sampled in this study. The numbers show locations of culls used in the analysis from Blocks 1 – 3; the location names and number of samples from each site are described in the key table. (B) Mean annual positions of female red deer aged one year or more and resident to block 4 in 2001.





Key to Map (A):		
No.	Location Name	Samples
1	Glen Shellester	3
2	Fionchra	2
3	Bloodstone Hill	5
4	Orval	4
5	Sgorr Reidh	1
6	Ard Nev	7
7	Gualainn Na Pairce	3
8	Glen Harris	21
9	An Dornabal	8
10	Trollaval	5
11	Hallaval	3
12	Long Loch	1
13	Ruinsival	15
14	Sandy Corrie	7
15	Bealach A Fhurain	5
16	Dibidil	23
17	Alt Nam Ba	31

4.3 Methods

4.3.1 Sample collection and selection

Tissue samples for genotyping were collected from red deer from management blocks 1-4 on the Isle of Rum. A previous analysis revealed evidence for spatial genetic structure amongst female red deer within the North Block, but no such structure amongst males (Nussey *et al*, 2005b). With this in mind, we examined spatial genetic structure across Rum using genetic data from only females in the present study.

Blocks 1 – 3: Tissue samples were collected from culled females on the Isle of Rum between October 2001 and January 2002. The location (to the nearest $1km^2$ Ordinance Survey grid square) and age of the animals shot were obtained along with tissue samples. 135 tissue samples from females aged one year or greater were obtained from a variety of localities across the island, from management Blocks 1 – 3. Figure 4.1 describes the approximate spatial distribution of the culls from which samples were obtained. The culling regime implemented in 2001 dictated the number of samples available from each Block: 74 samples from Block 1, 42 from Block 2, and 19 from Block 3 (Figure 4.1).

Block 4: Tissue samples from Block 4 (the 'North Block' study area) females were collected as part of the ongoing research programme in this part of the island, rather than from culled individuals. Red deer in the North Block have been subject to

individual based study since the late 1960s (Clutton-Brock *et al*, 1982). Individuals resident to the area are known from either artificial marks or natural idiosyncrasies (Clutton-Brock *et al*, 1982). Females in this population are matrilocal and rarely disperse beyond the block's boundaries; female immigration into the population is also extremely rare (Coulson *et al*, 1997, Catchpole *et al*, 2004). The 'matriline' of deer born in the North Block is defined based on their oldest known female ancestor. The vast majority of individuals can be traced back to female ancestors that were alive when the study began. Of 41 North Block matrilines with more than four female members, all matriarchs were born before 1975 and all but five of these matrilines can be traced back to females alive in the 1960s.

Since 1982 around 85% of calves born in Block 4 have been caught shortly after birth, and tissue samples taken. Tissue has also been taken from animals at immobilisation and post-mortem. From these samples the majority of deer in the North Block born from 1980 onwards have been genotyped at up to 15 microsatellite loci (Marshall *et al*, 1998, Nussey *et al*, 2005b). Detailed spatial data on the North Block animals has also been collected: since 1974 at least five censuses a month were undertaken between January and May, and the location (to the nearest $100m^2$ Ordinance Survey grid square) of each individual seen on a census was noted. From this data residency of red deer to the North Block was defined (based on appearance in greater than 10% of January – May censuses; see Coulson *et al*, 1997) and the mean annual position of each resident deer was calculated (truncated to allocate deer to a $100m^2$ OS grid square).

Twenty-four large to medium sized North Block matrilines (> 15 members of either sex throughout our study period), which had extant female members in 2001,

were selected for mitochondrial analysis. From each, a maximum of four individuals' tissue samples were chosen for mitochondrial control region sequencing, avoiding maternal half-sib and mother-daughter pairs. In three matrilines samples from less than four individuals were available (Table 4.1). Since mtDNA is maternally inherited and there was no evidence of different haplotypes within matrilines (see Results), we assumed identified mitochondrial haplotypes were shared within matrilines, and assigned haplotypes to all individuals from selected matrilines accordingly (Table 4.1).

In order to generate a contemporaneous and comparable sample to our cull samples from Blocks 1 – 3, we identified 143 female red deer from the selected matrilines (Table 4.1) that were alive, aged one year or more, and resident to Block 4 in 2001 using census (January – May 2001) and life history data. Since the majority of natural mortality in this population occurs between January and April (Clutton-Brock *et al*, 1982), these animals were likely to have been those resident to the North Block when the 2001 / 2002 hind cull was occurring on the rest of the island.

Table 4.1: The sampling regime for Block 4 deer on Rum for mitochondrial control region sequencing. The table includes the haplotype identified amongst matrilines members (there was no within-matriline variation) and the number of females from that matriline alive, aged one or more and resident to Block 4 in 2001.

Matriline	No. females sequenced	mtDNA haplotype	No. females alive in 2001
127	4	B1	4
132	4	A	13
133	4	B1	13
134	4	A	13
135	4	A	3
136	4	A	2
137	4	A	8
138	4	A	19
139	4	A	5
140	4	A	10
141	4	A	1
142	4	B2	3
143	4	A	30
144	4	A	13
147	4	A	29
148	4	A	6
151	4	A	12
152	3	A	2
153	4	A	15
155	4	A	7
156	4	A	3
158	4	A	2
159	1	A	1
165	2	A	3

4.3.2 Genetic data

Mitochondrial sequencing: A 922 bp region of the mitochondrial control region (mt CR) was sequenced from 91 samples from Block 4 (Table 4.1) and 40 randomly selected cull samples from the rest of the island (Blocks 1-3). DNA extraction was performed using either standard phenol-chloroform techniques or using Pharmacia™ cell and tissue extraction kits, or Qiagen™ DNAeasy tissue extraction kits. The

mitochondrial control region was PCR-amplified using PRO (5'–CACCATCAACCCCCAAAGCTGAAG–3') and PHE (5'–CAGTGCCTTGCTTTGGGTTAAGC–3') primers (Wood and Phua, 1996). Each sample was amplified using one unit of Taq polymerase, 1.5mM $MgCl^2$, 250μM dNTP, and 0.6μM of each primer. The following thermal cycle was used: 94°C for 2 minutes, followed by 30 cycles consisting of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, followed by 10 minutes at 72°C. Successfully amplified PCR products were purified prior to sequencing using a Sigma-Genosys GenElute™ PCR clean-up kit. Sequencing was performed using DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia™), using three different primers in order obtain full sequence coverage of the selected mt CR region: PRO (5'–CACCATCAACCCCCAAAGCTGAAG–3'), HC3 (5'–CAGACGGCCATAGCTGAGTCCAAG – 3') (Wood and Phua, 1996), and CST463 (5'– CTCGATGGACTAATGACTAA–3') (Polziehn *et al*, 1998). Thermal cycling conditions for the sequencing reactions were as follows: 25 cycles consisting of 95°C for 20 seconds, 50°C for 15 seconds, and 60°C for 1 minute. The fragments produced were analysed using either an ABI 377 or 3730 automated sequencer.

The sequences were aligned and consensus control region sequences produced for each individual using DNAStar™ Sequence Manager. The 131 consensus sequences produced were aligned and compared using BioEdit (Hall, 1999). Since two markedly different haplotype groups were evident from these initial alignments one of which was invariant across the samples, we identified a restriction enzyme (BstEII) that would digest sequences of this haplotype ("RUM A") but not sequence variants from the other ("RUM B") haplotype group. Extraction and PCR

of the remaining 94 samples from Blocks 1 – 3 were conducted as above. Restriction digests were then performed using 0.3µl of BstEII with 4µl of PCR product for 6 hours at 60°C. This digestion cut RUM A haplotypes at 350 base pairs from the 5' end of the amplified region but did not cut RUM B haplotypes, so digested PCR products of haplotype RUM A could be readily identifiable when run out on an agarose gel. Identified RUM B haplotype samples were then sequenced as above using only the PRO primer: the region amplified by this primer contained diagnostic sites for the RUM B haplotypes observed, as well as the majority of the variation in the mt CR (Douzery and Randi, 1997, Randi *et al*, 2001).

Microsatellite genotyping: Microsatellite genotypes from the selected 143 North Block females at 10 polymorphic loci (FCB304, INRA011, INRA035, JP15, JP27, JP38, MAF109, RT1, TGLA94, TGLA322) were obtained from ongoing genotyping, undertaken for paternity analysis. The 135 DNA extractions from culled Block 1 – 3 females were genotyped at the same 10 loci (see Appendix A for further details of microsatellite genotyping techniques). Length polymorphisms were identified using an ABI 3730 automated sequencer and GeneMapper™ software.

4.3.3 Data analysis

Phylogenetic analysis of mitochondrial haplotypes: Phylogenetic and cladistic analyses were conducted in PHYLIP (Felsenstein, 1991) and TCS (Clement *et al*, 2000). All mt CR sequence variants observed were included in the analysis along with sequences covering the same region from European red deer available on

GenBank (Table 4.2). UPGMA (based on Jukes-Cantor distances) and maximum parsimony trees were generated using the DNADIST, NEIGHBOUR and DNAPARS modules in PHYLIP. The reliability of the branching patterns of these trees was assessed using 1,000 bootstrap replicates of the sequence data. A haplotype network was also generated using statistical parsimony methods in TCS (Templeton *et al*, 1992).

Table 4.2: List of mitochondrial control region sequences from European and African red deer obtained from GenBank and used in phylogenetic analysis alongside Isle of Rum sequences presented here. The code for each sequence refer to those used in Figures 2 and 3.

Sub-species	Code	GenBank Code	Location	Source Reference
C. e. atlanticus	ATLANT	AF291888	Norway	Randi *et al.* (01)
C. e. hispanicus	HISPAN	AF291889	Spain	Randi *et al.* (01)
C. e. corsicanus	CORSIC	AF291885	Sardinia	Randi *et al.* (01)
C. e. hippelaphus	HIPPEL 86	AF291886	Southern Italy	Randi *et al.* (01)
C. e. hippelaphus	HIPPEL 87	AF291887	Southern Italy	Randi *et al.* (01)
C. e .barbarus	BARBAR	AF296808	Algeria[1]	Polziehn & Strobeck (02)

[1] The samples used for this sequence was actually from San Diego zoo and is described as the *C. e. barbarus* subspecies of red deer originating from Algeria in the source reference.

Analysis of Population Structure: Population genetic structuring of both mtDNA and microsatellite markers was assessed by treating management blocks as separate sub-populations. Estimates of global and pairwise F_{ST} between blocks were generated using ARLEQUIN (Schnieder *et al*, 2000: mtDNA data) and FSTAT (Goudet, 1995: microsatellite data). Global F_{ST} values significantly greater than zero indicate greater structuring of genetic variation between groups relative to within group variation. The significance of global F_{ST} estimates was assessed by randomising genotypes among sub-populations (Goudet, 1995). A four fold difference in F_{ST} values is

expected between maternally and bi-parentally transmitted markers, as a result of differences in effective population size (Prugnolle and de Meeus, 2002). However, structuring in a maternally transmitted marker greater than four times larger than that for nuclear markers is typically interpreted as indicating male-biased dispersal (Prugnolle and de Meeus, 2002).

We also investigated spatial genetic structure at finer spatial scales using data on the location of culls from Blocks 1 – 3 and mean annual positions in 2001 from Block 4 (Figure 4.1). Analysis of the relationship between genetic and geographic distances between individuals were then performed separately for nuclear and mitochondrial data using SPAGeDi (Hardy and Vekemans, 2002). Spatial and genetic data were used to generate Moran's I statistics (Hardy and Vekemans, 1999) at each of the following distance intervals between pairs of individuals: <1.5 km, 1.5 – 3 km, 3 – 6 km, 6 –9 km, and >9 km. Moran's I estimates the correlation between average gene frequencies between pairs of individuals at each distance interval (Hardy and Vekemans, 1999). The significance of Moran's I – ln(distance) relationships was assessed by permuting the spatial group locations of individuals among all spatial groups (Hardy and Vekemans, 2002).

Since we found evidence from the microsatellite data that observed patterns of association between genetic and geographic distance might be driven by block 4 alone (see Section 4.4), we re-ran the above analysis excluding data from block 4 to further examine isolation by distance over the rest of the island.

4.4 Results

4.4.1 Phylogenetic analysis of mitochondrial haplotypes

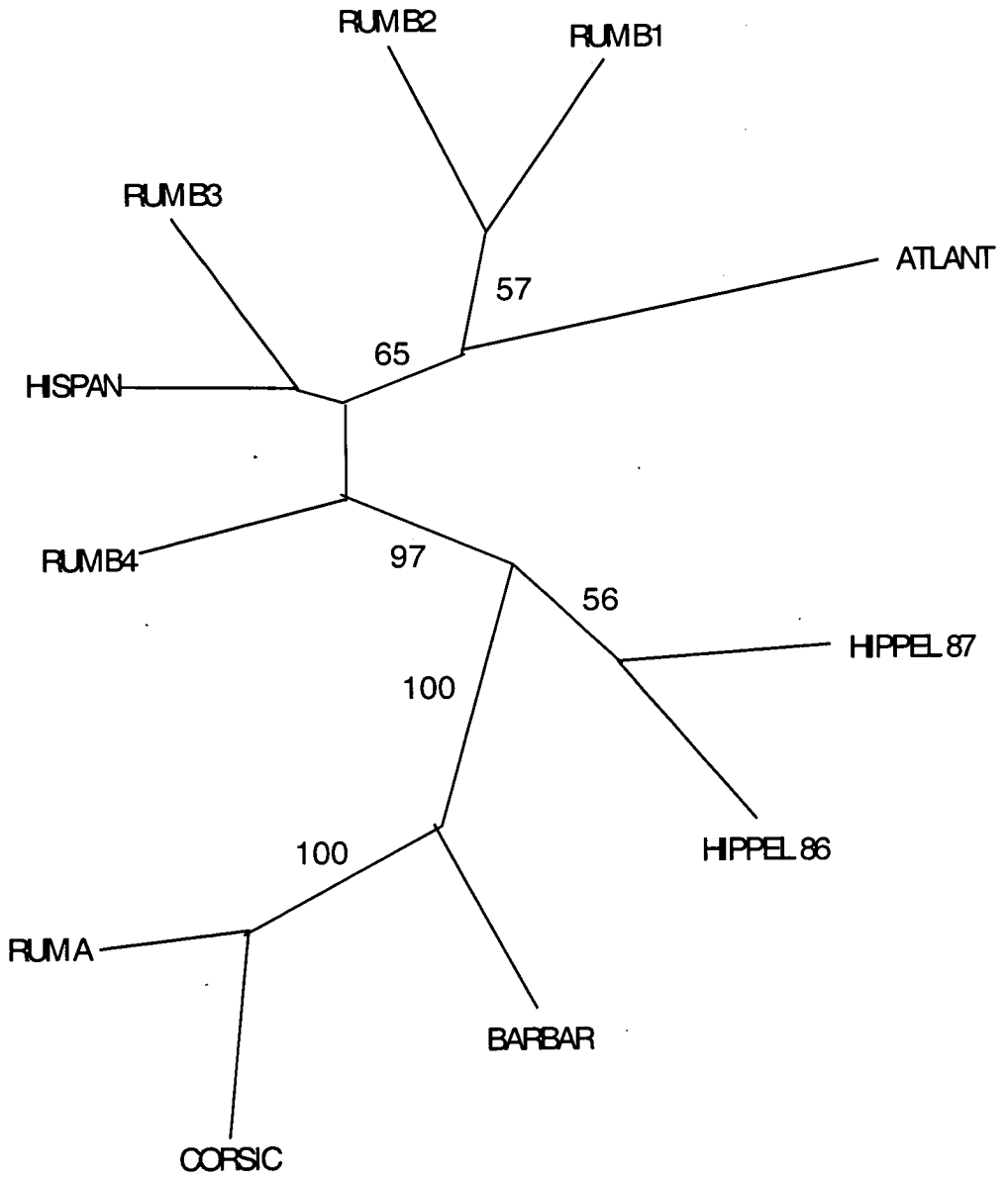
Five different mt CR haplotypes were found amongst the female red deer sequenced. Table 4.3 shows the proportion of sequence divergence between pairs of sequences. The haplotypes fell into two main groups. The first, a single haplotype, which diverged from the other Rum haplotypes by between 2.7 – 3.0% of the 911 bp region examined (11 sites with indels were excluded; see Table 4.3), was termed "RUM A". The second group included four more similar haplotypes, differing by 0.6% - 1.3%, were termed "RUM B1 – B4". The most divergent of the Rum haplotypes were RUM A and RUM B3 with 27 substitutions across the 922 mt CR region examined. The haplotype pairs B1 – B2, B1 – B4, and B3 – B4 all differed by only 5 nucleotide substitutions, the lowest divergence across the Rum haplotypes (see Appendix B for an alignment of complete RUM A and B mt CR sequences).

Table 4.3: The proportions of mitochondrial control region sequence divergence between 5 mtDNA haplotypes observed amongst female red deer on the Isle of Rum. The proportions of divergence were calculated having first excluded sites featuring indels (leaving 911 sites for comparison).

	Rum A	Rum B1	Rum B2	Rum B3	Rum B4
Rum A					
Rum B1	0.972				
Rum B2	0.971	0.994			
Rum B3	0.970	0.991	0.987		
Rum B4	0.971	0.994	0.991	0.994	

Phylogenetic analysis revealed three clusters amongst the Rum haplotypes and the sequences from European red deer obtained from GenBank (see Table 4.2): the RUMA haplotype grouped with the Sardinian and North African deer, the two Italian sequences grouped together, and the four RUM B haplotypes clustered with the Norwegian and Spanish red deer sequences (Figures 4.2, 4.3). Maximum parsimony analysis generated four equally likely trees, all of which featured these three basic clusters and differed only in the branching patterns within the RUM B / Norway / Spain group (Figure 4.2). A consensus parsimony tree generated 100% bootstrap support for the RUM A / Sardinia / North Africa group, and 97% support for the branch separating the RUM B / Norway / Spain cluster from the other haplotypes (Figure 4.2). The internal topography of this latter group was poorly resolved, and no branches were supported at over the 80% level by bootstrapping. The UPGMA tree (not shown) showed the same three main clades and a similar pattern of bootstrap support for branches. A parsimony network of these haplotypes (Figure 4.3), also supports the existence of three main groups, and illustrates the high levels of sequence divergence between these three groups of haplotypes. Figure 4.3 shows the single substitution separating the RUM A and Tyrrhenian sequences, and shows the high levels sequence divergence between these and the North African sequence.

Figure 4.2: Unrooted maximum parsimony tree (unscaled) based on red deer mtDNA control region sequences observed on Rum (RUM A and RUM B1 – RUM B4) and across Europe and North Africa (see Table 2 for descriptions of sequence labels). Analyses excluded sites featuring indels. Percentage consensus from 1,000 bootstrap replications of the data are shown for branches where these values were greater than 50%.



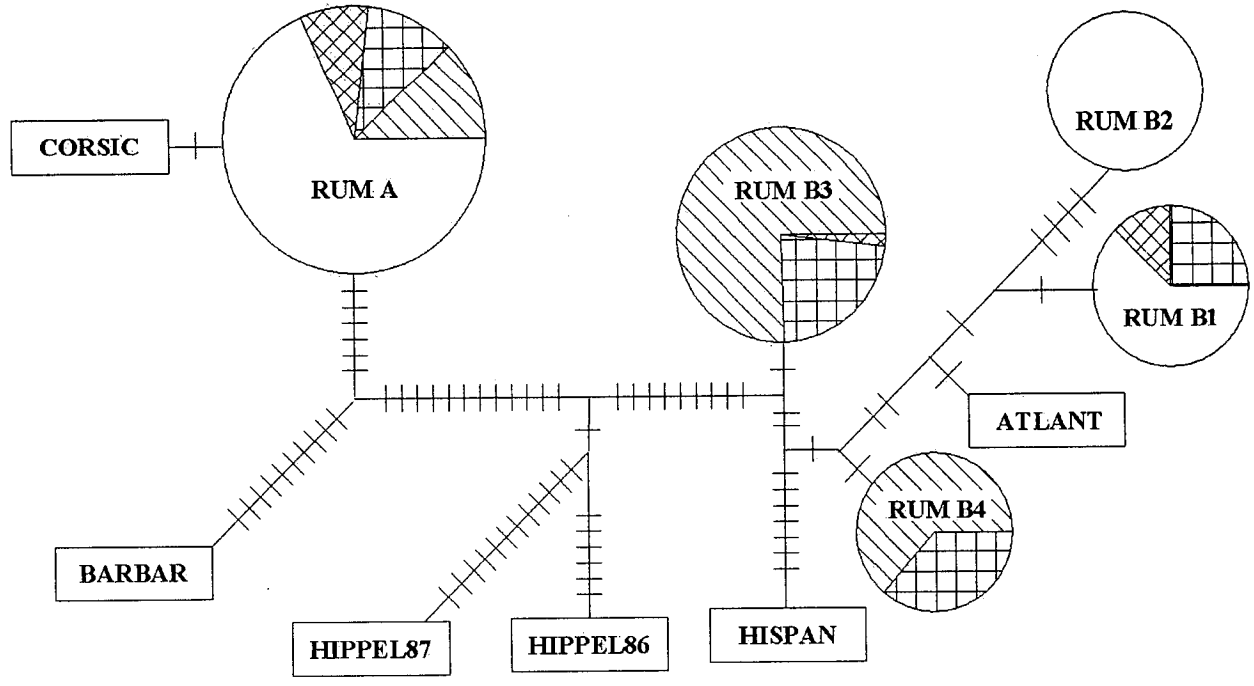


Figure 4.3: A parsimony network of red deer mtDNA control region sequences from Rum (pie charts) and across Europe and North Africa (rectangles; see Table 3 for descriptions of sequence labels). Sites including indels were treated as missing data. Each bar along a branch represents a single substitution. The size of the Rum haplotype pie charts correspond to the rank of their frequency across the island: RUM A was common (190), RUM B3 moderately common (57), and RUM B1, B2, and B4 were rare (16, 3, and 11 respectively). The pie slices represent the proportion of the total number of females of each haplotype found in each management block (Block 1: diagonal stripes; Block 2: squares; Block 3: diamonds; Block 4: empty).

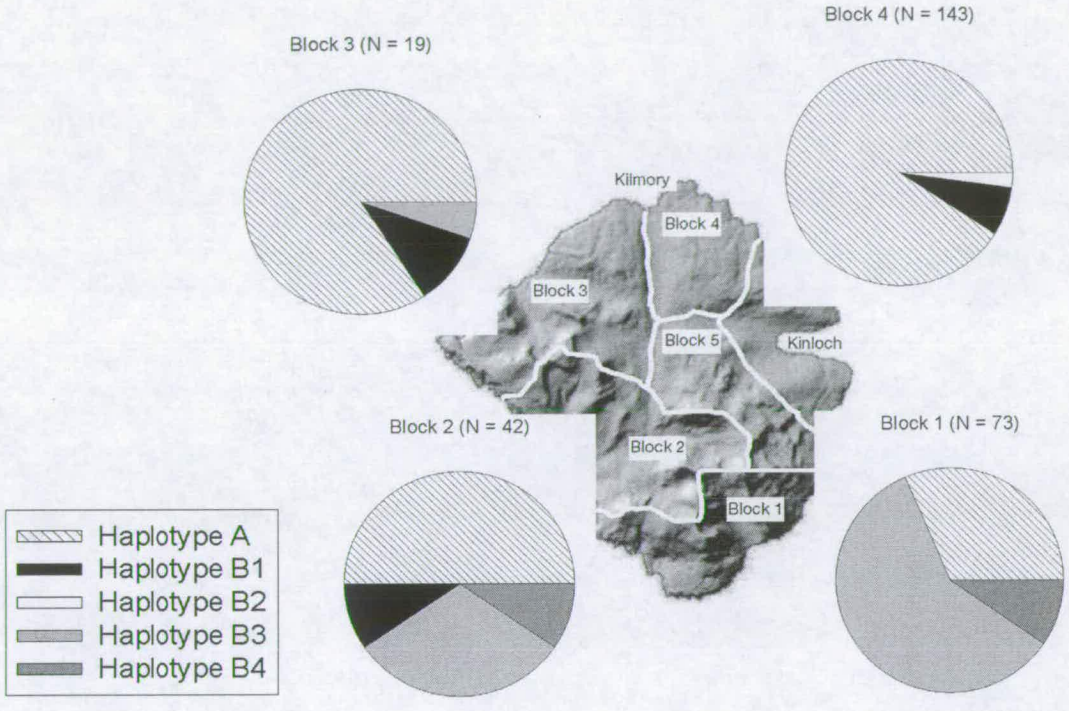
4.4.2 Analysis of population structure

Mitochondrial DNA: The global F_{ST} estimate for mitochondrial control region haplotype frequencies was high and significantly greater than zero (F_{ST} = 0.373; P < 0.001). Figure 4.4 shows the proportions of each mtDNA haplotype in each Rum management block. In Block 4, 21 of 24 sampled matrilines – 130 of 143 (91%) resident females in 2001 – were of the RUM A haplotype. 84% of culled females in Block 3 had RUM A haplotypes. In Block 2, 50% of cull samples were RUM A, but haplotype RUM B3 was also reasonably common (31%). These frequencies reversed in Block 3 with 59% of deer sampled being of RUM B3 haplotype and 32% of RUM A. Haplotype RUM B1 was found at low frequencies in Blocks 2, 3 and 4 (7 – 11%), B2 was present only in a single matriline (3 females alive in 2001) in Block 4 (Table 4.1), whilst B4 was only found in Blocks 1 and 2 (10% in both blocks).

The spatial structure evident in mitochondrial DNA stems from the prevalence of the RUM A haplotype through the north and west of Rum (Blocks 3 and 4) whilst RUM B4 becomes increasingly common to the west and predominates in the south (Blocks 1 and 2; see Figure 4.4). A pattern of isolation-by-distance in highly philopatric female red deer was supported by pairwise F_{ST} estimates (Table 4.4) and spatial autocorrelation analysis (Figure 4.5 A). Estimates in Table 4.4 revealed that neighbouring blocks had lower pairwise F_{ST} values than non-neighbouring blocks (e.g. neighbouring blocks: F_{ST} (4 vs 3) = -0.005, F_{ST} (1 vs 2) = 0.0078; non-neighbouring: F_{ST} (4 vs 2) = 0.323 , F_{ST} (3 vs 1) = 0.356). The correlogram for mtDNA in Figure 4.5 A shows the probability of haplotype sharing

decreases with the geographic distance between pairs of females: the estimated slope of Moran's I on ln(distance) was significantly negative (b = -0.192, P < 0.001).

Figure 4.4: Map and pie charts showing the proportions of each mitochondrial haplotype found amongst female deer sampled in each of the management blocks.



Microsatellites: Global F_{ST} for the 10 microsatellite loci was more than an order of magnitude lower than that for the mitochondrial marker, although it was still significantly greater than zero (F_{ST} = 0.011; P < 0.05). At finer spatial scales, the relationship between Moran's I and geographic distance was significantly negative (b = -0.011; P < 0.001), but again more than an order of magnitude lower than the estimate for the mtDNA marker (Figure 4.5 A).

Pairwise F_{ST} estimates between pairs of blocks show a different pattern to that observed for the mitochondrial marker (Table 4.4). Values for comparisons

involving Block 4 are an order of magnitude greater than estimates involving other blocks (Table 4.4): the majority of structure observed across blocks in the nuclear markers may be driven by genetic differences between Block 4 and the rest of the island. To test this possibility we re-analysed both global F_{ST} and isolation by distance patterns without Block 4 genotypes. There was still evidence of significant genetic spatial structure in mtDNA (F_{ST} = 0.172, $P < 0.05$; b = -0.207, $P < 0.01$, Figure 4.5 B), but no evidence of structure or isolation by distance in the microsatellite markers (F_{ST} = 0.003, $P > 0.05$; b = -0.0016; $P > 0.05$, Figure 4.5 B).

Table 4.4: Pairwise F_{ST} estimates between female red deer in management blocks 1 – 4 on Rum using mtDNA control haplotype frequencies (upper diagonal) and 10 polymorphic microsatellite loci (lower diagonal).

	Block 1	Block 2	Block 3	Block 4
Block 1		0.078	0.356	0.543
Block 2	-0.001		0.122	0.323
Block 3	0.007	0.005		-0.005
Block 4	0.014	0.013	0.013	

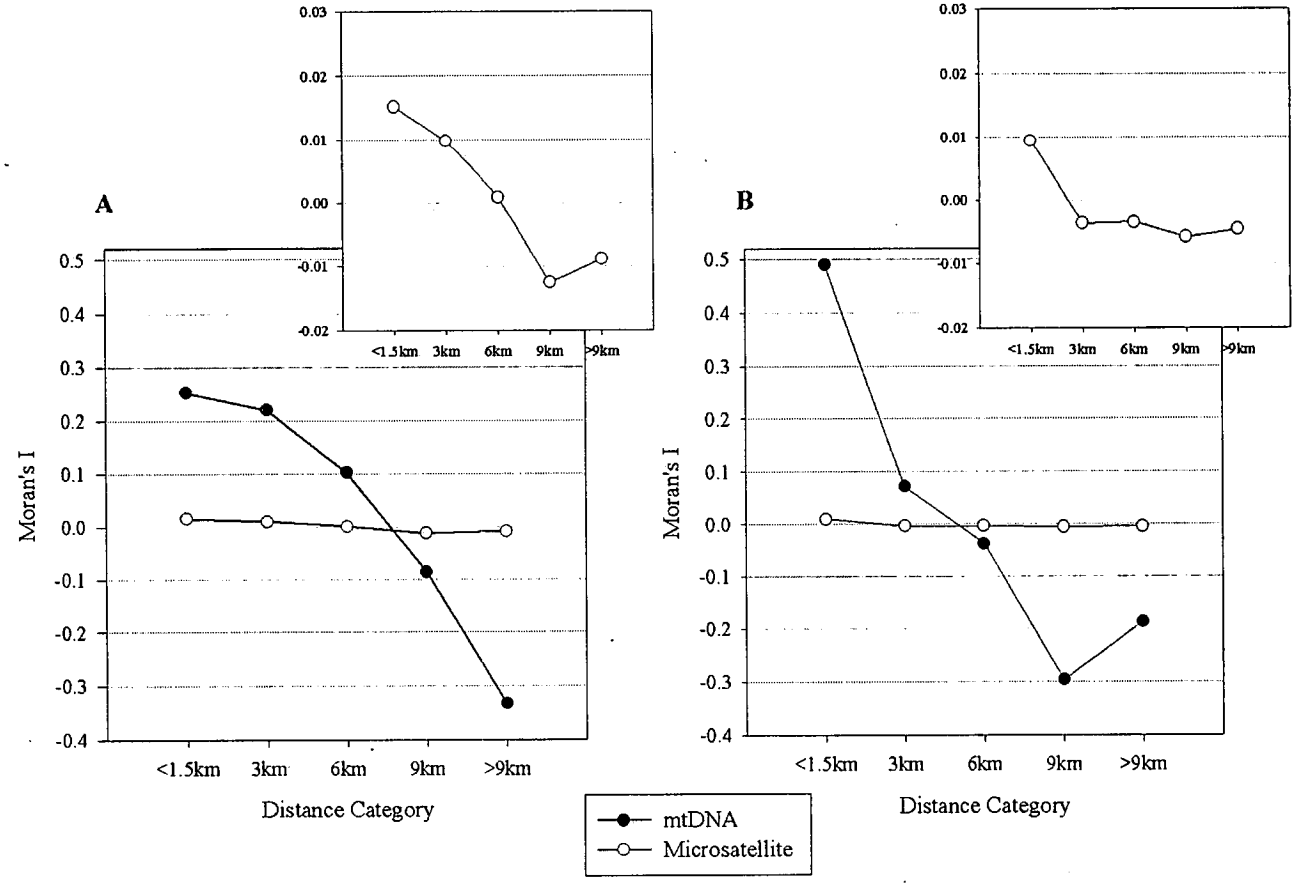


Figure 4.5: Correlograms, using Moran's I statistic as a measure of genetic spatial autocorrelation, for mtDNA and microsatellite data from female red deer on the Isle of Rum. (A): Correlogram based on all data for mtDNA (filled circles) and microsatellite markers (open circles, and inset with finer y-axis scaling). (B): as (A) except with females from block 4 removed. The Moran's I – ln(distance) relationship was significantly negative in both cased in (A), but only significant for the mtDNA marker in (B).

4.5 Discussion

4.5.1 Phylogeography of red deer on Rum: Genetic evidence of wide scale translocation of female red deer.

The levels of mtDNA sequence divergence observed between red deer on the Isle of Rum would be startling if this were a natural, isolated island population. However, given that the population is descended from numerous introductions between the 1840s and 1920s from at least four mainland populations across the U.K. (Marshall, 1998), and that red deer have been the subject of extensive human translocation throughout the UK and Europe for many centuries (Whitehead, 1964, Hartl *et al*, 2003, Long, 2003), the high levels of divergence between mtDNA haplotypes on Rum is not surprising. Similar patterns of mitochondrial divergence within restricted geographic ranges have been observed in both this species (Feulner *et al*, 2004), and within breeds of domestic species (Guiffra *et al*, 2000, Pereira *et al*, 2005). Furthermore, there is good reason to expect similar levels of divergent matrilineal ancestry in other Scottish populations of red deer: records show regular introductions from populations in English parks accord across the mainland and islands throughout the last three centuries (Whitehead, 1964).

The phylogenetic analyses presented here suggest that red deer on Rum are descended from at least two geographically separate ancestral stocks (Figures 4.2, 4.3). All three analyses conducted imply that the RUM A haplotype is very closely related to Tyrrhenian red deer, and also clusters with North African sequences. A recent phylogeographic study of red deer using the mtDNA cytochrome b region,

suggested that North African red deer colonised the Tyrrhenian islands and represent a taxonomic unit – an 'African' subspecies – discrete from mainland European populations (Ludt *et al*, 2004). The phylogeny presented here (Figure 4.2) provides further support for Ludt et al's (2004) suggestion of separate 'African' and 'Western European' taxonomic groups, utilising both a previously unanalysed combination of mt CR sequences (Table 4.2), and a different mtDNA region. The four RUM B haplotypes clustered with mainland European sequences and presumably are descended from several different European stocks. It is impossible to be more specific until more detailed phylogeographic analysis has been conducted, and even then extensive human translocation of this species may obfuscate any meaningful phylogeographic structure amongst European populations of red deer.

More detailed phylogeographic analyses of red deer in Europe and Africa are warranted to better understand their geographic origins and the extent of human translocation between regions. Furthermore, the presence of a close descendant of Tyrrhenian populations in a managed island population in the UK is potentially interesting from a conservation biology perspective. The Tyrrhenian subspecies, *C. e. corsicanus*, is currently listed as endangered by CITES (Jabbour *et al*, 1997) and was recently described as "one of world's most endangered mammals" (Zachos *et al*, 2003). Although RUM A individuals have presumably undergone extensive introgression with other European red deer sub-species, it may be possible to use historical records and mtDNA sequence analysis to trace the source of this haplotype and identify extant populations of *C. e. corsicanus* descendants within UK populations which may have been less interbred with other red deer stocks.

4.5.2 Population structure and sex-biased dispersal: A consequence of different culling regimes?

The degree of genetic differentiation observed between culling blocks was almost 34 times higher in our mtDNA marker than in the microsatellite markers. The higher effective population size of nuclear DNA relative to maternally inherited DNA leads us to expect an approximately four-fold reduction in genetic structure from bi-parentally to uni-parentally inherited genetic markers (Prugnolle and de Meeus, 2002). The well-documented strong male-bias in dispersal in this species (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 2002) would account for the differences in spatial structure between nuclear and mtDNA markers, over and above the expected four-fold difference. Whilst differences in effective population size and sex-biased dispersal are sufficient to account for the observed inequality of magnitudes of the estimates of genetic structure between management blocks and at finer spatial scales between the markers types, other mechanisms may have contributed to the observed high levels of mtDNA spatial structure. Firstly, the spatial structuring of mtDNA haplotypes may have been the result of a non-random pattern of introduction to the island: different source populations, composed of different haplotypes may have been introduced to different parts of the island. A second possibility would be post-introduction segregation of haplotypes, as a result of spatial variation in selection on different matrilines (see Chapter 6 for further discussion of selection on mtDNA variation).

There were differences in the pattern of genetic isolation by distance of management blocks between mtDNA and microsatellite markers. Pairwise F_{ST}

estimates suggested that individuals in Block 4 were responsible for most of the structure evident in the microsatellites, but that mtDNA showed a pattern expected under isolation by distance. Removal of block 4 females from the data set backed this contention, suggesting that females from this area were responsible for much of the genetic structure in both global estimates of structure between blocks and finer-scale patterns of spatial autocorrelation in the microsatellite data.

The observed demographic consequences of recent changes in management practices in the four blocks provide a plausible explanation for this observation. Following a release from culling in the North Block in 1973, the female population density in the block has increased to carrying capacity around which it has fluctuated since the early 1980s (Clutton-Brock *et al*, 2002). The male population in the North Block concurrently declined, as a result of increased male emigration, decreased male immigration, and an increase in the male-bias of juvenile mortality (Clutton-Brock *et al*, 2002). The increasing population density and competition for food within Block 4 over the last three decades years has resulted in declines in a variety of female and male fitness parameters (Clutton-Brock *et al*, 1987b, Kruuk *et al*, 1999b) and is likely to have resulted in a decrease in the phenotypic quality of males born in this region relative to the rest of the island. Whilst levels of emigration of males from the North Block into other areas have certainly increased over this period (Clutton-Brock *et al*, 2002, Catchpole *et al*, 2004), gene flow from this block could have decreased if these dispersing males are in poor physiological condition relative to the males in neighbouring blocks, as they may be unable to successfully compete to hold harems and hence get access to oestrous females during the rut. It is also possible that rising grazing pressure and resource competition, or increasing

difficulties maintaining harems when so many females are present, in the North Block may discourage males from immigrating into the area during the rut to mate, although there is currently no data to support this contention.

Changes to culling regimes across the rest of the island may also have contributed to the observed pattern in the microsatellite data. The experimentally increased cull of males in Block 1 since 1991 is thought to have attracted young males from neighbouring areas (Blocks 2 and 5) into the area, as overall male numbers in Block 1 proved hard to reduce (Clutton-Brock *et al*, 2002). This is likely to have increased gene flow across the south of Rum – young males from neighbouring blocks would presumably have excellent chances of mating with females in Block 1 given the reduced local male population – and is likely to have reduced any existing population genetic structure between Blocks 1 and 2.

Human management practices are often hypothesised to reduce gene flow and hence lead to enhanced effects of genetic drift, and an eventual reduction in overall genetic variability. Whilst it is not possible to exclude population processes that occurred before population monitoring began as an explanation for observed differences in spatial structuring of microsatellite genotypes across the island, recent changes in culling regimes on Rum represent a plausible explanation for the observed patterns of nuclear genetic structure amongst females. A recent study linked temporal changes in fine-scale population genetic structure amongst North Block females to changes in male and female effective population size, but not to changes in dispersal (Nussey *et al*, 2005b). Across the Isle of Rum, differences in effective dispersal of males from and into Blocks 1 and 4 associated with recent changes in culling regimes appear to best explain patterns of female spatial genetic

structure. This highlights the potentially subtle interactions between human management strategies, environment, behaviour and phenotype affecting spatial genetic structure, which itself is a key factor determining the future evolutionary trajectory of any isolated population. Continued research examining cross-island population structure using older cull samples (e.g. before the experimental management changes began in 1991) or those collected from future culls are likely to further illuminate our understanding of how management practices and environmental quality affect dispersal behaviour and hence patterns of spatial genetic structure.

Chapter 5:

Rapidly declining fine-scale spatial genetic structure in female red deer

This chapter has been published as: Nussey, D.H., Coltman, D. W., Coulson, T., Kruuk, L.E.B., Donald, A., Morris, S. J., Clutton-Brock, T.H. & Pemberton, J. (2005) Rapidly declining fine-scale spatial genetic structure in female red deer. *Molecular Ecology*, **14**, 3395-3405.

5.1 Summary

A growing literature now documents the presence of fine-scale genetic structure in wild vertebrate populations. Breeding population size, levels of dispersal and polygyny – all hypothesised to affect population genetic structure – are known to be influenced by ecological conditions experienced by populations, but the possibility of temporal or spatial variation in fine-scale genetic structure as a result of ecological change is rarely considered or explored. Here we investigate temporal variation in fine-scale genetic structure in a red deer population on the Isle or Rum, Scotland. We document extremely fine-scale spatial genetic structure (< 100m) amongst females but not males across a 24-year study period during which resource competition has intensified and the population has reached habitat carrying capacity. Based on census data, adult deer were allocated to one of three sub-populations in each year of the study. Global F_{ST} estimates for females generated using these sub-populations

decreased over the study period, indicating a rapid decline in fine-scale genetic structure of the population. Global F_{ST} estimates for males were not different from zero across the study period. Using census and genetic data, we illustrate that, as a consequence of a release from culling early in the study period, the number of breeding females has increased whilst levels of polygyny have decreased in this population. We found little evidence for increasing dispersal between sub-populations over time in either sex. We argue that both increasing female population size and decreasing polygyny could explain the decline in female population genetic structure.

5.2 Introduction

An understanding of the processes underlying population structure at fine spatial scales is central to evolutionary biology. Fine-scale population structure may facilitate kin or localised selection processes, as well as potentially confounding population and quantitative genetic research (Coltman *et al*, 2003b). Recently, studies have shown such fine scale spatial structure within populations of a variety of vertebrate taxa using genetic techniques (Shorey *et al*, 2000, Taylor *et al*, 2001, Lampert *et al*, 2003). Theoretical and empirical studies have explained such genetic structuring in terms of mating systems and dispersal patterns (Chesser, 1991, Sugg *et al*, 1996, Dobson, 1998). Where limited dispersal results in close spatial associations between relatives, fine-scale structure will arise (Chesser, 1998). Highly polygynous breeding systems, where only a handful of unrelated males father offspring, enhance

structure, as many offspring receive paternal genes from the same source and local coancestry will be increased (Chesser, 1991).

In mammals, male-biased dispersal and female philopatry are the norm (Greenwood, 1980, Clutton-Brock, 1989). Females tend to remain close to their maternal relatives throughout their lives, often forming matrilineal social groups (Greenwood, 1980). Male-biased dispersal may have evolved in concert with female philopatry and polygyny to avoid costs of inbreeding associated with bisexual philopatry (Chesser, 1991). Studies of mammalian social structure through population genetics have utilized F-statistics (Wright, 1965) to examine partitioning of genetic variance and levels of inbreeding in species showing discrete social groups. Using the social group as the sub-population unit, numerous studies have documented F_{ST} values significantly different from zero, indicating genetic structuring between groups, as well as negative F_{IS} values, indicating less inbreeding than expected under complete breeding within groups (see Storz, 1999 for review). In mammal species with fission-fusion societies showing less discrete group structure, examination of the correlation of genetic relatedness estimates with distances between pairs of individuals has also revealed structure at fine spatial scales (Coltman *et al*, 2003b, Hazlitt *et al*, 2004).

The presence and implications of temporal and spatial variation in mammalian population structure has been largely ignored. However, recent research has shown that population genetic parameters may vary spatially within species or populations, specifically where habitat fragmentation differs (Peacock and Smith, 1997, Stow *et al*, 2001). There is also evidence of temporal instability in such parameters (Viard *et al*, 1997, Piertney *et al*, 1999, Garant *et al*, 2000), which could

itself represent an important intrinsic factor in population dynamic patterns (Lambin and Krebs, 1991). Changes in the number of breeding individuals, dispersal patterns, and levels of inbreeding and polygyny would be expected to influence population genetic parameters such as fixation indices (Chesser, 1991, 1998, Balloux, 2004). There is evidence that variation in resource competition can alter group composition, ranging and spacing behaviour in mammals (Albon *et al*, 1992, Kilpatrick *et al*, 2001), and ultimately influence population genetic structure (Pope, 1998, Aars and Ims, 2000), as well as influencing male emigration and the distribution of male mating success (Clutton-Brock *et al*, 1997, Pemberton *et al*, 1999). Here, we explore temporal variation in fine-scale genetic structure in a wild red deer population and relate this specifically to variation in population size, dispersal patterns and polygyny associated with the population's recent release from culling.

5.2.1 Previous research on the study population

The red deer (*Cervus elaphus L.*) in the North Block study area of the Isle of Rum, Scotland have been the subject of intensive individual based study since 1973 (Clutton-Brock *et al*, 1982). The feeding habitat within the North Block consists of areas of high quality *Agrostis – Festuca* grassland and poorer quality regions of heath and *Molinia* grassland (Clutton-Brock *et al*, 1982). The population's mating system is polygynous, with males competing to dominate harems of oestrous females between September and November each year (Clutton-Brock *et al*, 1997). Male emigration is common between the ages of two and five and is density-dependent, with many males returning to the North Block later in life to rut (Clutton-Brock *et al*,

1997, Clutton-Brock *et al*, 2002, Catchpole *et al*, 2004). Female emigration is rare and depends mainly on the distance between their natal area and the study area's boundaries (Catchpole *et al*, 2004). Females are loosely matrilocal, and several studies have observed close spatial and social associations between maternal relatives (Clutton-Brock *et al*, 1982, Coulson *et al*, 1997).

Following release from culling in 1973, the number of resident adult females in the population increased throughout the 1970s and early 1980s (Clutton-Brock *et al*, 1982). The population has been at or close to carrying capacity since the mid-1980s (Albon *et al*, 2000). Previous studies have shown rising density to be associated with reductions in female fecundity, reproductive success, and over-winter calf survival (Clutton-Brock *et al*, 1987b, Kruuk *et al*, 1999b, Albon *et al*, 2000), as well as increased spacing between female maternal relatives (Albon *et al*, 1992). Rising female density has also been associated with increased male juvenile mortality and early emigration, and with decreased permanent male immigration into the North Block (Clutton-Brock *et al*, 1997). The ratio of adult resident females: males in the North Block has increased sharply, resulting in an almost complete absence of males from high quality grazing areas in the recent years (Coulson *et al*, 2004). Clutton-Brock *et al* (1997) showed that the number of males obtaining successful matings increased with adult sex ratio, and argued that rising female population density and resource competition have lead to decreased competition for mates amongst males.

5.2.2 The present study

Given the general pattern of female philopatry and male dispersal evident in this population, we expected to find fine-scale structuring of genotypes only amongst female red deer. However, as a consequence of the population's release from culling, changes in population size and mating system have occurred that would be expected to alter such fine-scale structure. The increase in the number of reproductive females, increasing dispersal of both sexes, and decreasing levels of polygyny might be expected to lead to a decrease in genetic structure amongst females over time. Here, we examined overall fine-scale genetic structure in males and females, changes in population structure over time, and we relate any observed changes to analyses of temporal variation in breeding population size, dispersal and polygyny using the long-term census and genetic data collected from the North Block red deer population.

5.3 Methods

5.3.1 Field data

All individual red deer in the North Block study area are recognisable as a result of either artificial marks (collars, ear tags or ear punches attached as calves or following immobilisation) or natural markings. Since 1973, censuses of the North Block study area have been conducted at least five times a month between January and May (Coulson *et al*, 1997). On each census all individuals observed were identified and

their position, to the nearest 100m Ordinance Survey (OS) grid square, was noted. Individuals were included in the analyses that follow if they were seen in at least 10% of censuses between January and May (termed 'resident' animals; see Coulson *et al.* (1997) for further discussion and justification) and were of reproductive age (≥ 3 years). Only census data from between 1978 and 2001 was analysed, unless specifically stated, to complement the available genetic data set.

We used the following parameters in our analyses:

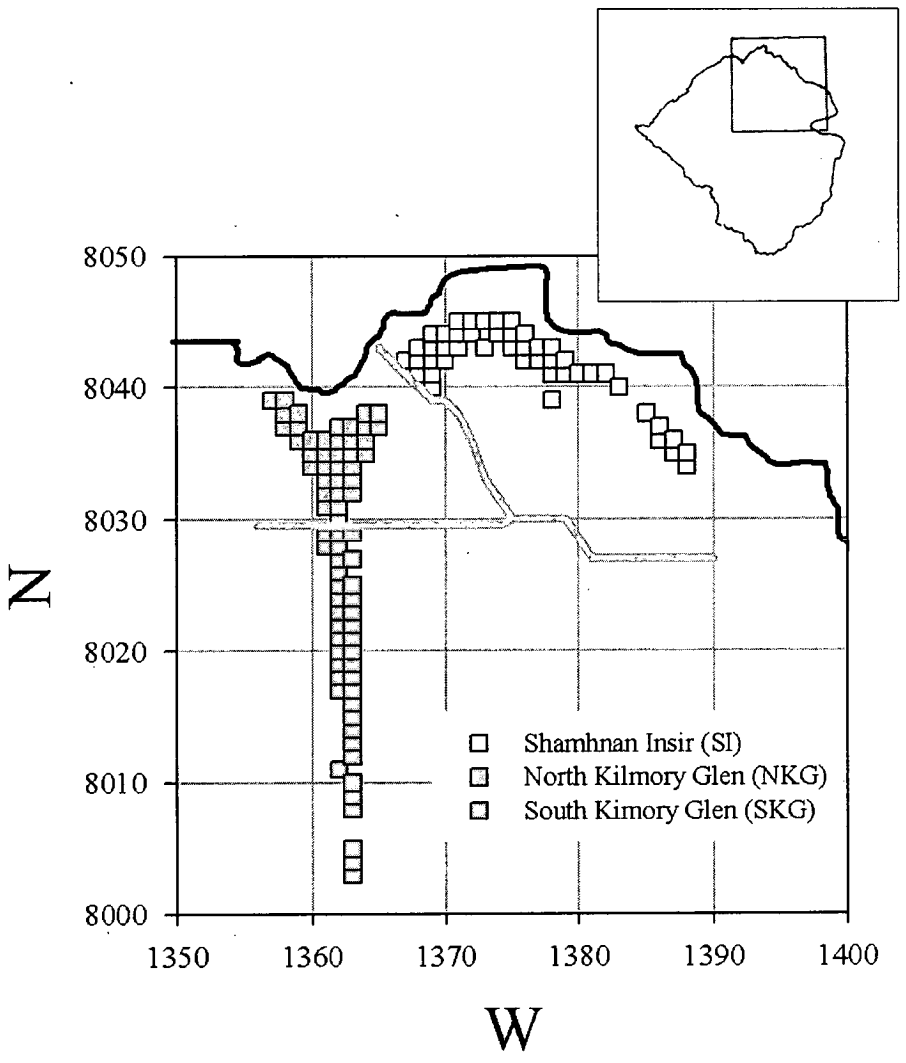
Mean annual position: Average x and y coordinates from January to May census positions for an individual in a given year. Averages were truncated to allocate each individual to the nearest 100m OS grid square.

Between-year movement: For individuals resident in consecutive years, the distance between current and previous years' mean annual positions was calculated.

Population sub-divisions: Many studies of the North Block red deer have treated the study area as a single unit, but there is evidence that fitness and behaviour vary spatially within the North Block (Clutton-Brock *et al*, 1982). Sub-division of the study area based on habitat types and ranging behaviour has improved explanatory power in models of over-winter calf survival (Coulson *et al*, 1997), spacing behaviour (Albon *et al*, 1992) and other aspects of fitness (Guinness *et al*, 1978b, Conradt *et al*, 1999). Following these studies, we split the study area into three sub-divisions (Figure 5.1): Samhnan Insir (SI), North Kilmory Glen (NKG) and South Kilmory Glen (SKG). Individuals were assigned to one of the three sub-divisions in

each year they were resident to the population, according to their mean annual position.

Figure 5.1: Plot showing North Block study area, Isle of Rum (boxed in inset map of Rum), split into three sub-divisions based on previous research. The grey lines indicate boundaries between the sub-divisions. Squares indicate the regions most intensely used by adult female red deer, defined as $100m^2$ OS grid squares in which 9 or more females' mean annual positions were located across the study period (1978 – 2001).



Natal sub-division: An individual's natal sub-division was defined as the sub-division of his or her mother in the year following that individual's birth. Sub-divisions were allocated based on January – May census data but calving takes place from May onwards, so this would represent maternal sub-division in the first year of life. Where natal sub-division could not be allocated in this fashion – typically because the individual was born before regular censusing began – the modal sub-division across a mother's lifetime was used. This allowed natal sub-divisions to be assigned to 92% of females and 87% of males resident to the study area at some point in their lifetimes.

5.3.2 Genetic data

Since 1982, approximately 85% of calves born in the study area have been caught shortly after birth and tissue and blood samples taken for genotyping. Additionally, almost 300 animals born prior to this date were sampled by chemical immobilisation or post-mortem. The analyses that follow include genetic data for resident adult deer that were alive between 1978 and 2001, however restriction of the data set to 1982 onwards does not affect the nature of the findings presented here.

Individuals were genotyped at up to 8 microsatellite loci: JP15, JP27, JP38, CP26, FCB193, FCB304, MAF109, TGLA94 (see Appendix A for further details). Not all individuals were genotyped at all loci, but individuals with genotypes at fewer than four loci were excluded from the analysis. These loci have been previously shown to assort randomly and not to show evidence of deviance from Hardy-Weinberg equilibrium (Marshall, 1998).

Analysis of population structure: Analysis was conducted on individuals aged three years and older to exclude pre-reproductive juveniles and calves from the analyses. To assess differences between the sexes in fine-scale population genetic structure, geographic distances between the mean annual positions of pairs of individuals were compared to an index of genetic relatedness. Genetic relatedness coefficients (R; Lynch and Ritland, 1999) and geographic distances between pairs of resident individuals were examined using SPAGeDi (Hardy and Vekemans, 2002). Average R estimates were taken for pairs of individuals separated by distance intervals of 100m (from <100m to >2km), in each year of the study. The analysis was conducted separately for pairs of females and pairs of males. R coefficients for each distance interval were averaged across years for pairs of males and females to examine overall fine-scale population genetic structure. The significance of spatial genetic structuring within each sex was assessed using linear regression of mean R estimates over all years on geographic distance (Hardy and Vekemans, 2002). In addition to this genetic analysis of differences between the sexes in population structure, we examined the differences between males and females in movement behaviour. We compared average between-year movement for males and females at different ages (2 – 10 years).

Changes in spatial partitioning of genetic variance and inbreeding were assessed using F-statistics (Wright, 1965) in which the three population sub-divisions were treated as sub-populations. In each year all resident deer were assigned to a sub-division based on their mean annual position. Separate estimates of global F_{ST} and F_{IS}, as well as pairwise F_{ST} values, were generated for females and males in each year

of the study period using FSTAT (Goudet, 1995). Temporal trends in these estimates were assessed using a linear regression of the F-statistic on year.

Global F_{ST} values significantly greater than zero indicate greater partitioning of genetic variance between than within groups, whilst pairwise F_{ST} values represent estimates of genetic differentiation between sub-divisions. Negative F_{IS} values, typical of mammalian systems, imply lower than expected inbreeding within sub-populations relative to random mating. The significance of these terms was assessed using permutation tests. FSTAT assesses global F_{ST} significance by randomising genotypes among sub-divisions and global F_{IS} by permuting alleles among individuals (Goudet, 1995).

Analysis of breeding population size and mating system: Using genotypic and census data we investigated the possibility of temporal trends in the breeding population size, dispersal between population sub-divisions and levels of polygyny. In all cases, changes in indices over time were assessed by means of a linear regression on year. The following parameters were investigated:

Female breeding population size: The number of resident females giving birth to a calf in each year of the study period was taken as an index of the size of the breeding population. Since the main increase in both female population size and the number of breeding females occurred in the decade following release from culling, and data was available from 1974 on female breeding behaviour and population size, this index was examined from 1974 to 2001. Note that even for females these figures are

substantially lower that the absolute count of breeding age deer alive in any one year, since not all individuals breed each year.

Dispersal: Dispersal between population sub-divisions was assessed by comparing adults' natal sub-division with their assigned sub-division in a given year. If individuals had moved from their natal sub-division then the direction of dispersal was classified by their natal sub-division followed by their current sub-division (i.e. a female natal to SI, but assigned to NKG in 1980 would be classified as "SI-NKG" for 1980). Females and males of each dispersing category were counted for each year of the study.

Male breeding population size and levels of polygyny: Estimates of these parameters were based on paternity assignment of individuals born in the North Block between 1978 and 2001, using all available microsatellite data, with CERVUS (Marshall *et al*, 1998). All males observed holding harems during a rut year were considered as potential candidate fathers. Genetic paternities were assigned where the confidence score given by CERVUS was 80% or greater. 35.4% of calves born 1978 – 2001 were successfully assigned genetic paternities. If possible, males were assigned behavioural paternity where genetic paternity could not be determined. Behavioural paternities were assigned to males if they held the calf's mother in their harem for more days during the 11-day window around estimated conception than any other male (see Clutton-Brock *et al*, 1997, Kruuk *et al*, 2000, Slate *et al*, 2000, Kruuk *et al*, 2002 for further details and discussion of these genetic and behavioural approaches

to paternity assignment). Using combined methods, 62.8% of calves were assigned paternities.

For all males observed holding harems in a given year, we calculated annual breeding success (ABS). For each study year we used this information to calculate the number of breeding males (i.e. the number of males with one or more assigned paternities) as well as the maximum and variance in ABS in each year. Variance in male ABS was used as an index of polygyny. A previous study of this population highlighted the large number of progeny produced by a single male, named MAXI (Slate *et al*, 2000). To assess the dependence of temporal trends in polygyny on the handful of such males we identified four males that had been assigned the most paternities across their lifetimes and investigated the effect of removing them from our data set. The identity codes of these four males, the number of offspring they sired and the years in which they bred, respectively follow: RED77, 48 offspring, 1983–1991; MAXI, 36 offspring, 1978–1982; BRF76, 30 offspring, 1982–1989; BASIL, 29 offspring, 1997–2001. These individuals represented the top 0.5% of breeding males.

Temporal trends in the variance of male ABS could reflect improvements in the quality of the genetic data set in this population: the proportion of calves born that were assigned paternities each year increased over the study period ($F_{1,22}$ = 26.27, $P < 0.001$). To ensure any change in polygyny was independent of this improvement we ran a multiple regression of variance in male ABS including both year and the proportion of paternities successfully assigned in that year.

5.4 Results

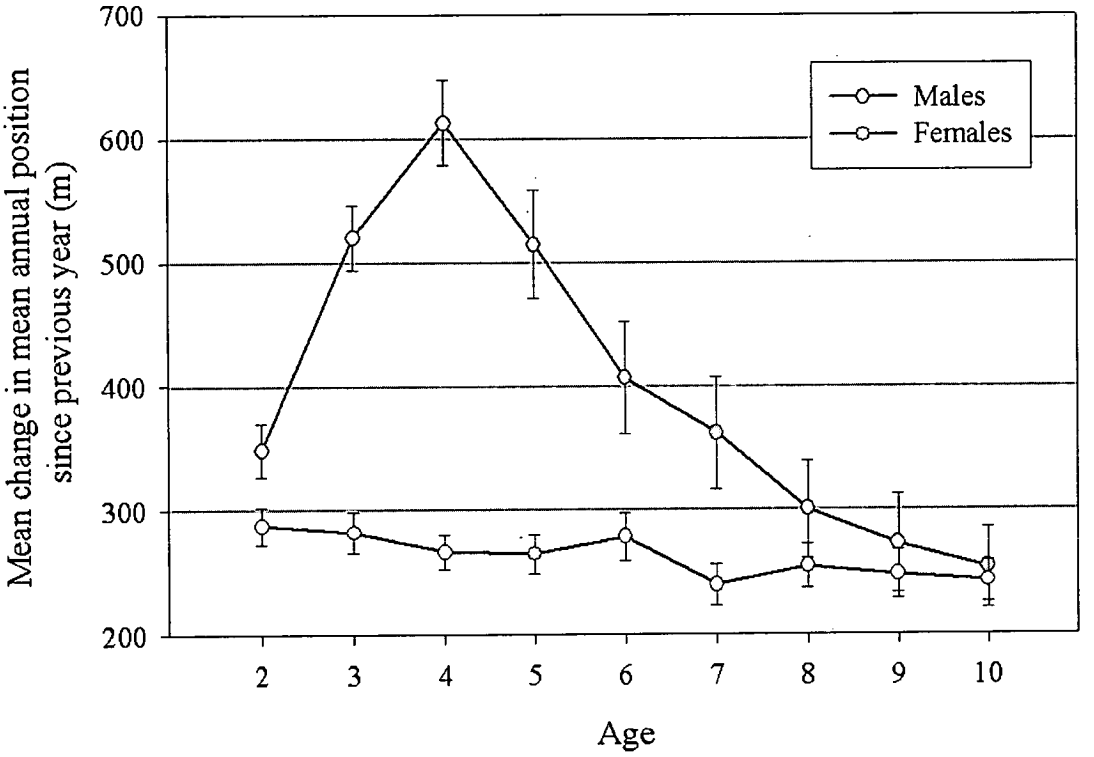
5.4.1 Differences between the sexes in population structure

Field and genotypic data analysis across the study period both imply that male deer are highly dispersive, whilst females are generally philopatric and remain in close spatial proximity to relatives of the same sex (Figures 5.2, 5.3 & 5.4). Females tended to move relatively little across their lifetimes, and in general had lower means and standard errors for between year movements than males aged two to ten (Figure 5.2). Males born in the North Block moved increasing distances between years from ages two to four (Figure 5.2). Both these data and previous work on the population show that males are more dispersive than females, and many surviving males have dispersed from the study area by the time they reach sexual maturity (Clutton-Brock *et al*, 1982, Catchpole *et al*, 2004).

As expected from these general differences in dispersal between the sexes, the genetic relatedness between a pair of females decreased as the distance between them became greater, but pairs of males were unrelated across the distance range examined (Figure 5.3). Females with mean annual positions 100m or less from one another were related, on average, at R = + 0.06, and this decreased to a relatedness of zero at around 900m (Figure 5.3). This decline in relatedness with distance was significant (linear regression of mean R on distance amongst pairs of females: intercept = 0.053 +/- 0.004 SE, slope = -0.025 +/- 0.002 SE, $r^2 = 0.93$, $P < 0.001$). For pairs of males, there was no evidence of any change or difference from zero in

relatedness across the distance range examined (intercept = 0.002 +/- 0.03 SE, slope = -0.006 +/- 0.005 SE, $r^2 = 0.06$, P = 0.51).

Figure 5.2: Between year movements of resident deer plotted against age of individual (at second mean annual position measured). Circles represent means for each age with standard error bars. Separate averages are shown for females (white circles) and males (grey circles). Males disperse considerably more between years than females between the ages of three and five.

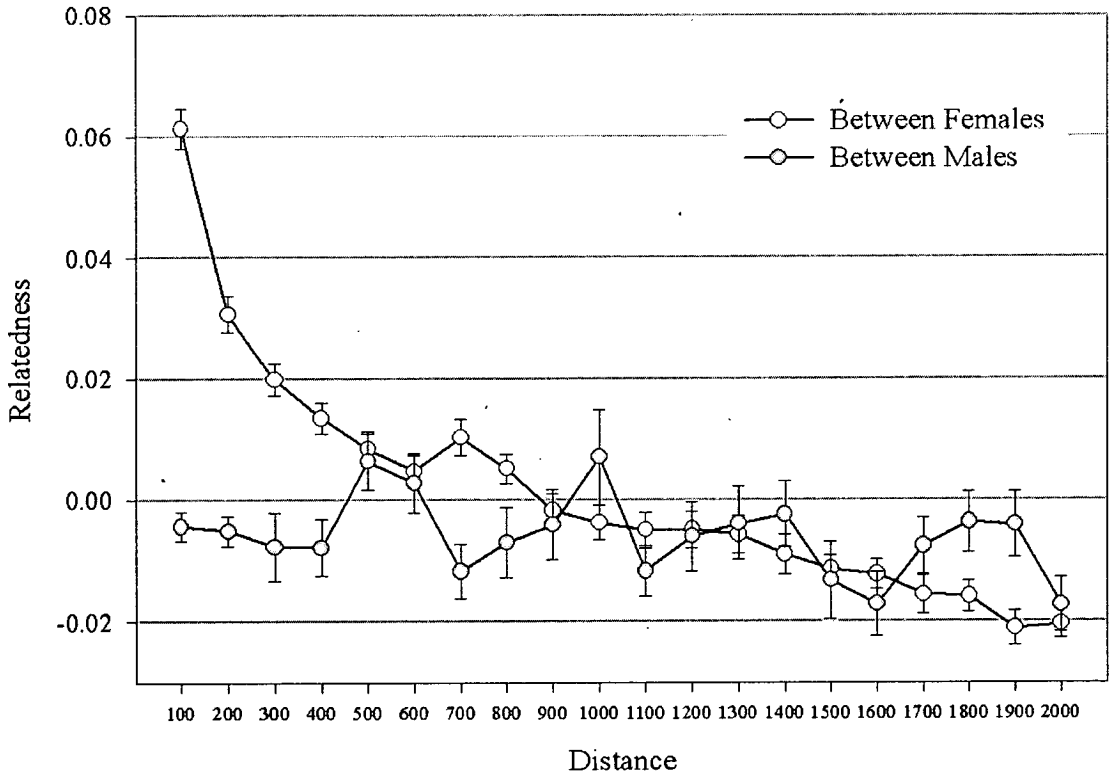


5.4.2 Temporal variation in fine-scale genetic structure

Fixation indices for the North Block adult females revealed strong partitioning of genetic variation between sub-divisions, however there was no evidence of such structure amongst males. Global F_{ST} values for females were significantly greater than zero in all years of the study period for females (female mean annual F_{ST} =

0.021), whilst F_{IS} values were significantly less than zero in all years except 2000 (mean annual F_{IS} = -0.058). Amongst males, F_{ST} estimates were not significantly greater than zero, except in 1980 (male mean annual F_{ST} = 0.002), and F_{IS} estimates were not different from zero except in 2000 and 2001 (mean annual F_{IS} = -0.022). F_{ST} estimates significantly greater than zero suggest that there is structuring of allelic variance between the three sub-divisions. Negative F_{IS} values imply an excess of heterozygosity relative to random mating.

Figure 5.3: The relationship between genetic relatedness and geographic distance amongst pairs of females (white circles) and males (grey circles). Circles represent pairwise relatedness comparisons at each distance averaged across years (1978 – 2001), with standard error bars. Relatedness between pairs of females is high at short distances and decreases with distance, but between pairs of males relatedness does not deviate from zero.



There was a significant decline in global F_{ST} estimates for females from around 0.03 to 0.01 across the study period ($F_{1,22} = 201.6$, $P < 0.001$, Figure 5.4 A). There was no temporal trend in male F_{ST} estimates ($F_{1,22} = 0.58$, $P > 0.05$; Figure 5.4 B). This indicates a decline in fine-scale genetic structure amongst females, but not males, over the course of the study period. Female F_{IS} estimates increased significantly over the study period from approximately –0.08 to –0.03 ($F_{1,22} = 17.45$, $P < 0.001$), whilst estimates for males showed no temporal trend ($F_{1,22} = 2.19$, $P > 0.05$).

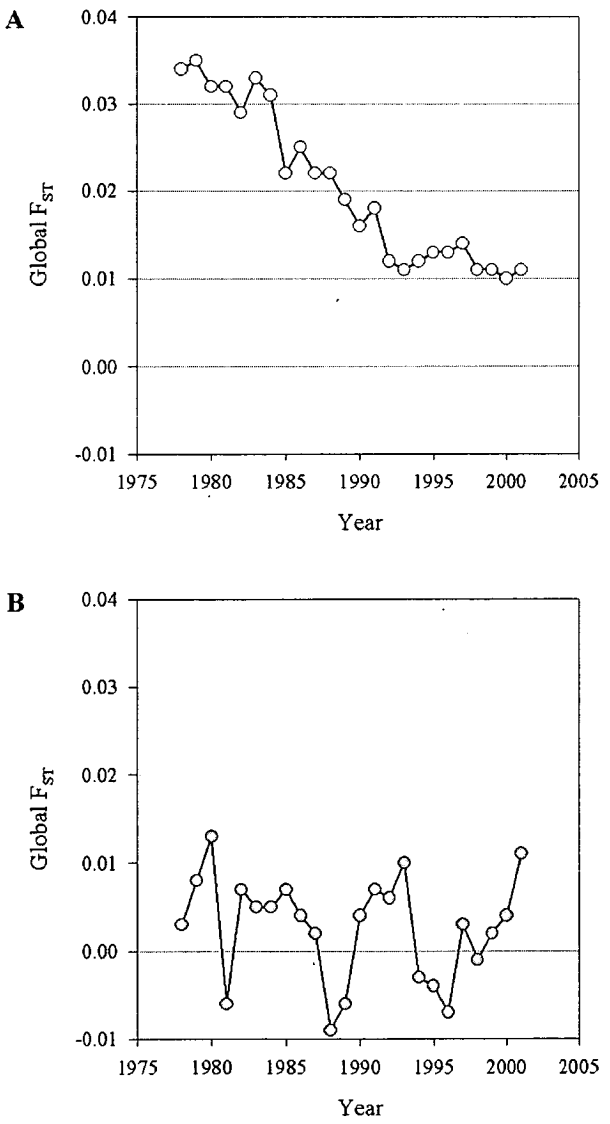


Figure 5.4: Changes in global F_{ST} estimates over time, based on adult deer only with population sub-divisions (see Figure 5.1) treated as sub-populations. (A) Females; Global F_{ST} declines significantly over the study period (b = -0.0012 +/- 0.0001 SE, $P < 0.001$) showing partitioning of genetic variance between the sub-divisions has decreased. (B) Males; Global F_{ST} shows no significant relationship with year (b = -0.0001 +/- 0.0002 SE, $P = 0.46$) and was only significantly greater than zero in 1980, illustrating an absence of population structure amongst males.

Pairwise F_{ST} estimates comparing female genotypes from SI and NKG, and SI and SKG, both show significant negative trends with time (SI-NKG: $F_{1,22} = 68.70$, SI-SKG: $F_{1,22} = 224.0$, both $P < 0.001$; Figure 5.5 A). In the early stages of the study genetic distances between females in SI and SKG were around 0.06 but had declined to just over 0.01 by the mid-1990s. F_{ST} estimates between SI and NKG declined from around 0.03 to 0.01 over a similar period (Figure 5.5 A). The pairwise F_{ST} comparison of NKG and SKG females showed no significant temporal trend ($F_{1,22} = 0.31$, $P > 0.05$): it fluctuated between 0.015 and zero throughout the study period (Figure 5.5 A). None of the pairwise F_{ST} estimates for males showed a significant relationship with year (all regressions: $F_{1,22} < 2.3$, $P > 0.05$, Figure 5.5 B).

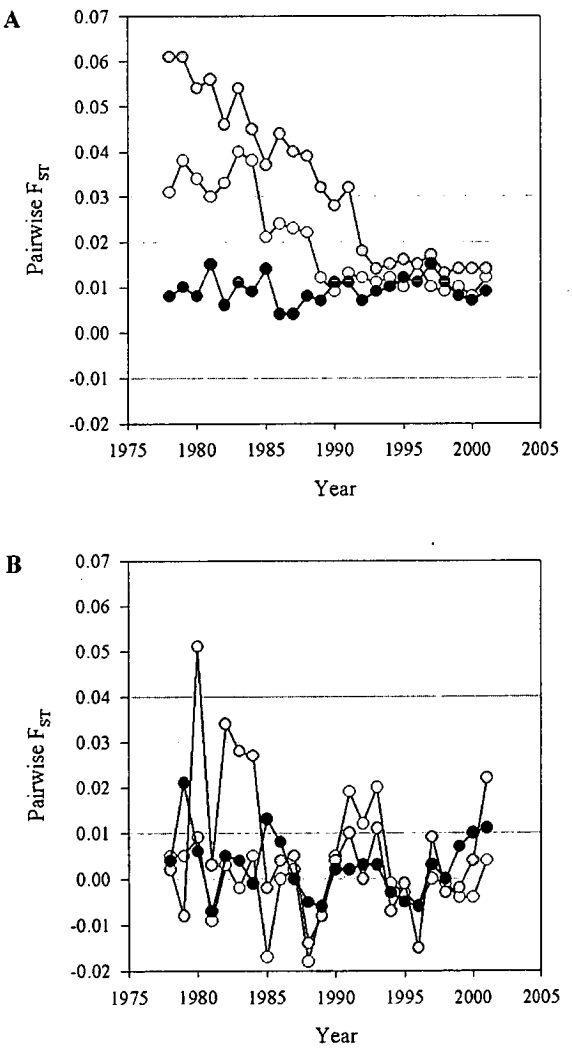


Figure 5.5: Pairwise F_{ST} estimates comparing genetic differentiation between pairs of population sub-divisions over time. (A) Females; the pairwise F_{ST} estimates between Shamhnan Insir (SI) and North Kilmory Glen (NKG) declines over time (white circles; b = -0.0013 +/- 0.0002 SE, $P < 0.001$), as do the SI – South Kilmory Glen (SKG) estimates (grey circles; b = -0.0024 +/- 0.0002 SE, $P < 0.001$). The NKG – SKG pairwise F_{ST} estimates remain constant and low across the study period (black circles; b = 0.0000 +/- 0.0001 SE, $P > 0.05$). (B) Males; no temporal trends apparent in either SI – NKG (white circles), SI – SKG (gray circles), or SKG – NKG (black circles) in pairwise F_{ST} estimates.

5.4.3 Temporal variation in breeding population size and mating system

Breeding Population Size: Since the population's release from culling in 1973, the number of females breeding in a given year has increased from around 55 in 1974 to fluctuate between 70 and 100 from the mid-1980's onwards ($F_{1,25} = 15.2$, $P < 0.001$, Figure 5.6 A). The number of different males assigned paternities has also increased from around 20 in the late 1970's to between 30 and 40 in the last five years of the study ($F_{1,22} = 45.35$, $P < 0.001$, Figure 5.6 B).

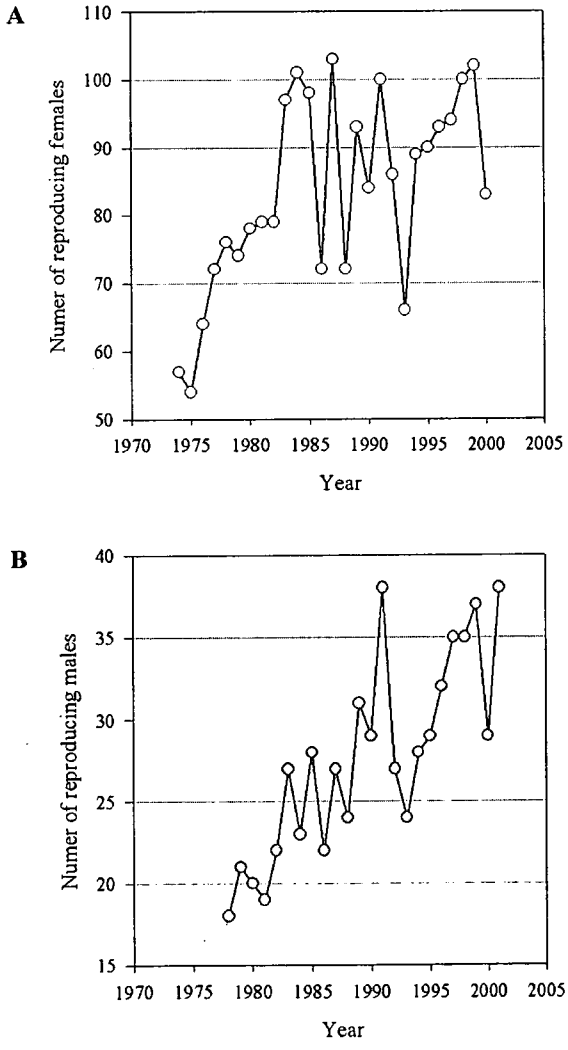


Figure 5.6: (A) The number of females reproducing in each year has significantly increased since 1974 as overall female population density has increased in the population (b = 1.10 +/- 0.28 SE, $P < 0.001$). Note that this graph includes additional data for 1974 – 1977, as the main increase in female population size occurred immediately following the release from culling in 1973. (B) The number of different males assigned at least one paternity in a given year has increased across the study period (b = 0.70 +/- 0.10 SE, $P < 0.001$).

Dispersal: On average, only 11.7% of resident adult females were located outside their natal sub-division in a given year, compared to 30.0% for adult males. The numbers and direction of males and females dispersing from their natal sub-division are shown in Figure 5.7. Movement of either sex between SI and SKG appears to be rare, as does movement from NKG to SI. Whilst higher levels of dispersal are observed between NKG and SKG and, for males at least, from SI to NKG, there is little indication of increases in dispersal capable of explaining the decline in female genetic structure. The only significant positive temporal trend in dispersal was for males dispersing from NKG to SI ($F_{1,22} = 28.87$, $P < 0.001$), however no more than three males had dispersed in this direction in any year (Figure 5.7). There were significant declines over time in male dispersal from SI to NKG ($F_{1,22} = 6.88$, $P < 0.05$) and SI to SKG ($F_{1,22} = 14.36$, $P < 0.01$), and in female dispersal from SI to NKG ($F_{1,22} = 5.31$, $P < 0.05$).

Polygyny: Variance in male annual breeding success (ABS) decreased over time ($F_{1,22} = 12.14$, $P < 0.01$, Figure 5.8). This decline in the level of polygyny is most likely the result of the increase in the number of males gaining at least one paternity increased (Figure 5.6 B), rather than a change in the maximum ABS, which showed no relationship with year ($F_{1,22} = 0.59$, $P > 0.05$). The removal of the four males with the largest lifetime breeding success from the data set resulted in a marginally non-significant decline in the variance in male ABS ($F_{1,22} = 3.14$, $P = 0.09$). The decline in polygyny was independent of the increase in the proportion of calves assigned paternities over time. In a multiple regression, the proportion of paternities was not a significant predictor of the variance in male ABS ($F_{1,21} = 2.96$, $P = 0.10$), whilst year

had a significant negative effect ($F_{1,21}$ = 11.33, P < 0.01) on this measure of polygyny.

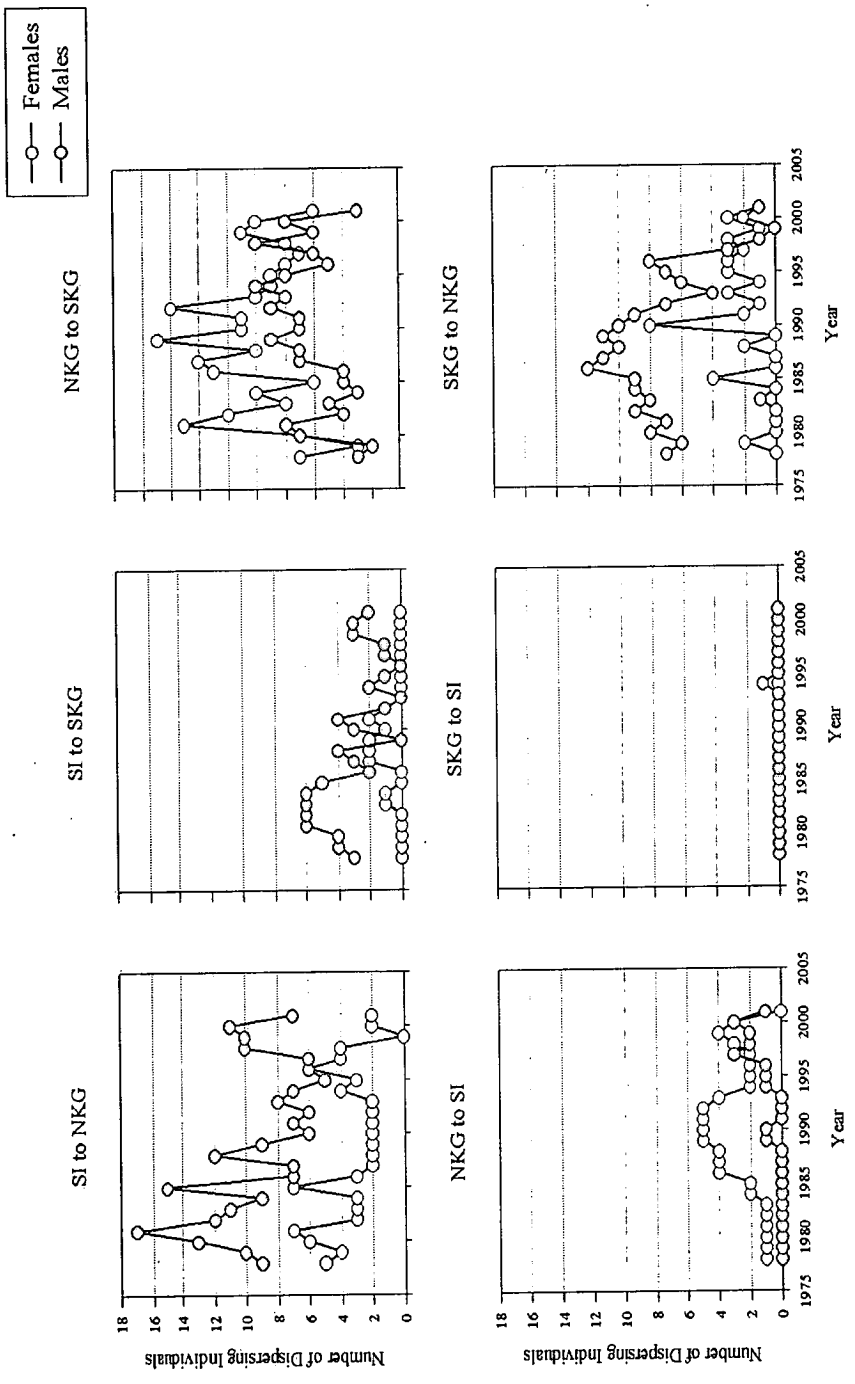
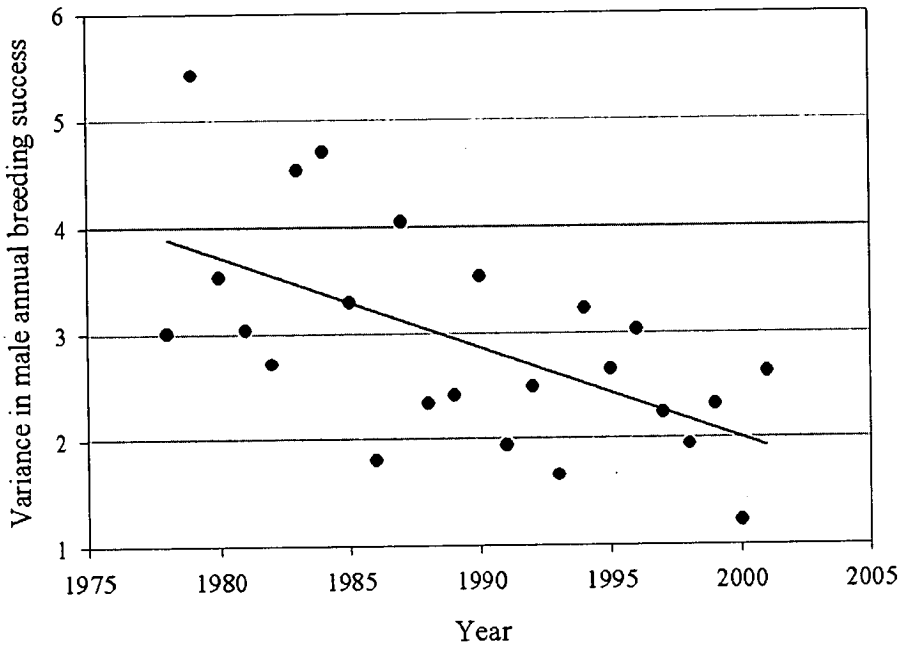


Figure 5.7: Dispersal patterns in the North Block over time. Y-axes on each of the panels represent the number of adult deer found outside of their natal population sub-division for each of the six possible dispersal direction categories. Legends above each panel indicate dispersal direction, with natal sub-division followed by current sub-division. Numbers of dispersing females are indicated by white circles, numbers of males by grey circles. Sub-division abbreviations are as follows, SI: Shamhnan Insir, NKG: North Kilmory Glen, SKG: South Kilmory Glen.

Figure 5.8: Levels of polygyny amongst North Block red deer decreased over the study period. The plot shows the variance in male annual breeding success over time with a linear regression slope plotted (b = -0.09 +/- 0.02 SE, P < 0.01).



5.5 Discussion

Figure 5.3 clearly illustrates the presence of genetic structure at very fine spatial scales amongst female, but not male, red deer in the North Block study population. This was as predicted: a significant decline in genetic relatedness was observed over continuous space between pairs of the philopatric sex, and no spatial genetic structure was found between members of the dispersing sex. The presence of significantly positive F_{ST} and negative F_{IS} estimates across the study period imply population structure amongst females consistent with those in previous studies of mammalian population genetics (Storz, 1999). In the only other study to examine genetic structure at such a fine scale in ungulates, Coltman *et al* (2003) found similar

differences between the sexes in Soay sheep on St Kilda, although the degree of spatial structure in females was lower in general and declined to zero at around 150m. This difference between species could be explained by the fact that red deer possess a more obviously matrilocal social system (Clutton-Brock and Coulson, 2002). However, it may also be the result of differences in feeding habitat and foraging behaviour: red deer on Rum have larger home ranges than Soay sheep on St Kilda.

We found significant partitioning of genetic variance between population sub-divisions amongst females, but not males (Figure 5.4). However, across our 24-year study period we observed a decline in female genetic structure (Figure 5.4 A). Fixation indices may take many generations to reach mutation – drift equilibrium, and so it is possible that these temporal changes in genetic structure could be the product of events occurring before our study period began. F_{ST} estimates are influenced by factors such as dispersal, mating system, and effective population size. Previous work on the North Block red deer population suggests that these parameters have altered across our study period as a direct or indirect consequence of the cessation of culling (Clutton-Brock *et al*, 1982, Albon *et al*, 1992, Clutton-Brock *et al*, 1997). Increases in dispersal between population sub-division in either sex, increases in the breeding population size and decreasing polygyny levels would represent viable explanations for the observed decline in population genetic structure, although these would not represent mutually exclusive or exhaustive explanations for the observed trend.

Female population density in the North Block has increased three-fold since the population's release from culling the early 1970s. We have shown this to be

concurrent with an increase in the number of females breeding in a given year (Figure 5.6 A). Although this increase took place mainly in the early part of our study period, the observed decline in F_{ST} and increase in F_{IS} values observed among females could be a direct result of these recent increases in effective female population size (Chesser, 1991, Balloux, 2004). Furthermore, as female numbers have risen the population's female: male ratio has increased as the male bias in juvenile mortality and immigration have become more pronounced (Albon *et al*, 2000, Catchpole *et al*, 2004). As a consequence, competition for mates between males has decreased (Clutton-Brock *et al*, 1997). Our results showing a decrease in variance in male ABS, explained by an increase in the number of males obtaining at least one mating rather than an increase in maximal ABS, replicate the results for the period 1972 – 1990 of Clutton-Brock *et al* (1997). The outcome of these changes has been a decline in polygyny (Figure 5.8). Theoretical studies predict that reduced polygyny combined with increased numbers of reproducing females would substantially reduce coancestry within populations (Chesser, 1991, Perrin and Mazalov, 1999).

Increased dispersal of either sex from their natal population sub-division in response to rising resource competition would cause a decline in genetic structure (Slatkin, 1987). Whilst there is evidence of an overall density-driven increase in spacing between maternal relatives amongst females and in emigration amongst males in the population (Albon *et al*, 1992, Catchpole *et al*, 2004), our results do not support the hypothesis that an increase in dispersal between sub-divisions is responsible for the observed breakdown in female genetic structure. Figure 5.7 shows little evidence of increases in dispersal in either male or female red deer

within the North Block. Whilst dispersal patterns may not explain the decline in female global F_{ST}, the observation of relatively high levels of female NKG - SKG dispersal, compared to other possible directions, could explain the low and temporally stable pairwise F_{ST} estimates between NKG and SKG among females. This finding ties well with previous research suggesting a southward expansion of Kilmory Glen females as population density has increased to carrying capacity (Coulson *et al*, 2004). Such movement would make recent coancestry between females in the two sub-divisions likely. Previous studies treating these two areas of the North Block as one sub-population appear justified (Milner-Gulland *et al*, 2000).

5.6 Conclusions

Non-random spatial distribution of genotypes at small spatial scales can confound studies of allelic association and quantitative genetics, and may have important evolutionary consequences such as the facilitation of kin selection and localized selection (Coltman *et al*, 2003b). We found evidence of extremely fine scale spatial structure amongst female red deer but not males, as would be expected for a typical mammalian system showing male-biased dispersal and female philopatry. Spatial structuring of genotypes amongst females declined over the course of our study period. This rapid decline in structure could be explained by both changes in female population density or levels of polygyny that are the result of the population's release from culling, but do not appear to be related to density-dependent changes in dispersal. Furthermore, we cannot be certain that the observed trends are not long-term consequences of events that took place prior to the start of our study. The

analysis presented here is, to our knowledge, the first direct demonstration of a breakdown in the fine-scale genetic structure in a wild mammal population over time. The results represent an important step towards developing our understanding of the dynamic nature of population genetic structure and illustrate clearly that temporal stability in population genetic parameters cannot simply be assumed. Further research is now required to refine and expand our understanding of the interaction between the spatial distribution of genotypes, behaviour and the environment in free-living populations.

Chapter 6:

Fine-scale spatial structuring of mtDNA variation in a wild red deer population: the role of ecological conditions and selection on haplotype

6.1 Summary

Environmental conditions, demography and selection may all influence population genetic structure, but data sets from wild populations with the ecological, life history, spatial and genetic data required to examine such influences are extremely rare. The red deer population in the North Block study area of the Isle of Rum represents just such a data set. Following the analysis of cross-island spatial genetic structure using nuclear and mitochondrial markers in Chapter 4, and of fine-scale structure within the North Block of the island using nuclear markers in Chapter 5, I present here an analysis of fine-scale structure in mtDNA amongst red deer in the North Block. I tested for non-random spatial distribution of red deer natal to the study area with respect to their mtDNA haplotypes, using spatial autocorrelation techniques for both males and females across a 24-year time period. Temporal trends in the patterns of mtDNA spatial autocorrelation were also assessed in both sexes, and evidence for selection on mtDNA haplotype was examined. There was fine-scale spatial structuring of mtDNA haplotypes in females, which declined over time. This decline was explained in terms of the spatial expansion of North Block females in response to increased grazing pressure in the north and east of the study area. Surprisingly,

there was some evidence of spatial genetic structure in highly dispersive male red deer, particularly late in the study period and at intermediate geographic distances. Finally, there was no evidence of selection favouring the predominant RUM A haplotype in terms of either matrilineal extinction patterns or individual fitness parameters across the study period.

6.2 Introduction

Mitochondrial DNA, considered as selectively neutral, non-recombinant maternally inherited genetic material, has been utilised for the analysis of population genetic structure in a disparate range of mammal species (e.g. Girman *et al*, 1997, Nagata *et al*, 1998, Gerloff *et al*, 1999, Charif *et al*, 2005). There is evidence from many mammalian systems of strong spatial structuring of mtDNA variation across broad geographic scales (Lyrholm *et al*, 1999, Rueness *et al*, 2003, Schaschl *et al*, 2003). The male-biased dispersal typical of mammalian systems leads to the expectation of fine-scale spatial structuring of mitochondrial haplotypes amongst females but not males. In several mammal species, mtDNA sequence variation has been examined within social groups or across continuous space, typically showing strong spatial clustering of philopatric females of the same mtDNA variant with both more mtDNA variation and a lack of spatial structure in dispersive males (Kappeler *et al*, 2002, Wimmer and Kappeler, 2002, Fredsted *et al*, 2004).

Genetic analyses have proved extremely useful in elucidating the power of both ecological conditions and anthropogenic activity to affect population structure across relatively broad spatial scales (Fuller *et al*, 1997, Ehrich *et al*, 2001, Rueness

et al, 2003, Coulon *et al*, 2004) and at finer spatial levels both between and within populations (Pope, 1992, Nyakaana *et al*, 2001, Charif *et al*, 2005, Nussey *et al*, 2005b). The individual-based study of red deer in the North Block of the Isle of Rum represents a rare system for which both extensive life history and genetic data are available for a wild population over an extended time series. Genetic analyses presented in the previous two chapters have revealed high levels of spatial structuring of mtDNA haplotypes in female red deer from across the Isle of Rum (Chapter 4), whilst microsatellite genotypes showed fine-scale spatial structure amongst female, but not male, deer in the North Block of Rum (Chapter 5). This microsatellite data also revealed a rapid temporal decline in female population structure, concurrent with the demographic consequences – specifically, increased effective population size – of a release from culling in the early 1970s, that could explain this decline (Chapter 5). In this chapter, the mtDNA sampling regime for North Block deer used in Chapter 4 (described in Section 4.2.1) was extended, increasing the number of matrilines sampled and extending the time series analysed from a single year to a 24-year period, in order to explore overall patterns of spatial autocorrelation of mtDNA haplotypes in the population, and to relate any temporal trends in these to known demographic or environmental changes.

Although female philopatry, coupled with the low effective population size of uni-parentally transmitted genetic material, adequately explains most examples of spatial structuring of variation in mtDNA in wild mammal populations (Lyrholm *et al*, 1999, Prugnolle and de Meeus, 2002, Fredsted *et al*, 2004), there is growing evidence challenging the assumption of selective neutrality in mtDNA (Rand *et al*, 1994, William *et al*, 1995, Gemmell *et al*, 2004). Selection affecting mitochondrial

variation can operate via differences in respiratory function that are a direct consequence of mutations in mtDNA, cytonuclear fitness associations, and genetic hitchhiking of mtDNA variants linked to nuclear genes associated with fitness (William *et al*, 1995, Rand *et al*, 2001, James and Ballard, 2003). Whilst few studies of wild vertebrate populations have considered the role of selection in observed distributions of mtDNA variation (see Nevo and Beiles, 1992 for a rare example), a recent review by Gemmell *et al* (2004) argued that wherever possible genetic research should test for selection on mitochondrial markers rather than simply assuming selectively neutrality.

In Chapter 4, strong spatial structuring of mtDNA haplotypes was revealed amongst female red deer from across the whole of the Isle of Rum. In the North Block (Block 4) of the island, RUM A haplotype was predominant (91% of sampled females), whilst in the southernmost Block (Block 1) the RUM B3 haplotype was most prevalent (59%; see Figure 4.4). The most likely explanations for this pattern are that differences in haplotype frequencies between Blocks are, at least in some part, historical and that strong female philopatry in red deer coupled with the low effective population size of mtDNA have lead to genetic drift, strengthening spatial structure, since the deer were introduced to Rum between 80 and 150 years ago. Selection on mtDNA haplotype may be detectable either through differential survival of matrilines of different haplotype (since all members of a matriline share their mtDNA haplotype) or through differences in fitness parameters between individuals from different haplotypes.

In this chapter, I used census and life history data from the North Block red deer study population, along with mtDNA analysis from tissue samples collected from the population, to:

(i) Assess the spatial autocorrelation of mtDNA haplotypes amongst males and females natal to the North Block, and examine temporal trends in this autocorrelation in the two sexes.

(ii) Test for selection favouring the predominant RUM A haplotype at both matrilineal and individual levels.

6.3 Methods

6.3.1 The Study Population & Field Data

Red deer in the North Block of the Isle of Rum, Scotland, were released from culling in 1973, and have been subject to individual based study since the early 1970s (Clutton-Brock *et al*, 1982). All individuals resident to the area are recognisable as a result of either artificial or natural markings. The 'matriline' of deer natal to the North Block was defined, following Chapter 4, based on their oldest known female relative. Matrilines can be traced back to one female alive when regular censusing began in 1974, or to a handful (16 females; members of these immigrant matrilines comprised 2% of the total female population) of female immigrants to the population since then. Regular censusing of the study area, alongside mortality searches during winter and observation during the calving season, has meant that accurate life history

data are available for the majority of individuals (Clutton-Brock *et al*, 1982). Females in this population are known to be matrilocal and rarely disperse beyond the block's boundaries or immigrate into the population; males tend to disperse from their mother's home range prior to reaching maturity and often disperse beyond the study area's boundaries (Nussey *et al*, 2005b; Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 2002, Catchpole *et al*, 2004).

Red deer were typically located along the north and west edges of the North Block (see Figures 6.2 or 5.1). Previous analysis has shown variation in habitat quality (Clutton-Brock *et al*, 1982) as well as associated spatial variation in demographic rates (Milner-Gulland *et al*, 2000, Coulson *et al*, 2004), fitness parameters (Guinness *et al*, 1978a, Guinness *et al*, 1978b) and nuclear genotypes (Nussey *et al*, 2005b) across the North Block. In particular, differences have been observed between red deer resident in the glen running north-south along the western edge of the study area ('Kilmory Glen') and those dwelling in the area known as 'Samhnan Insir' in the north-east of the block (see Figure 5.1).

Since 1974, study area censuses have been conducted at least five times a month between January and May (Coulson *et al*, 1997). Following the approach described and justified in Chapters 4 and 5, we calculated the 'mean annual position' for all adult ($\geq$ 3 years old) residents of the population in each year from 1978 to 2001 for use in spatial autocorrelation analyses of mtDNA variation. The 'natal sub-division' – either 'Samhnan Insir' (SI), 'North Kilmory Glen' (NKG) or 'South Kilmory Glen' (SKG) – of each deer was also assessed as described in Chapter 5. In addition, 'matrilineal sub-divisions' were defined based on the proportion of January to May census sightings (1978 – 2001) of females from each matriline that were

located either side of 8030N and 1370E, which correspond to the approximate borders between the three North Block sub-divisions used in Chapter 5. Matrilines with greater than or equal to 50% sightings north of 8030N and east of 1370E were assigned to SI, those with 50% or more north of 8030N and west of 1370E were assigned to NKG, whilst the remainder (≥ 50% south of 8030N) were assigned to SKG (see Table 6.1, Figure 6.2).

6.3.2 Genetic Data

We selected individuals for mtDNA sequencing by identifying 35 large to medium sized North Block matrilines (> 15 members of either sex throughout our study period), for which tissue samples were likely to be available for several individuals. Following the sampling regime described in Chapter 4, a maximum of four individuals' tissue samples per matriline was chosen for sequencing, avoiding maternal half-sib and mother-daughter pairs. For several matrilines less than four individuals' tissue samples were available (see Table 6.1). In addition to the individuals from the 24 North Block matrilines sequenced in Chapter 4, the mitochondrial control region (mt CR) of individuals from a further 11 matrilines were sequenced following the protocols described in Section 4.3.2.

The mt CR haplotypes of 117 female red deer from 35 different matrilines were obtained. We found no evidence of sequence variation within matrilines and therefore assigned the haplotypes identified from the sequenced deer to all remaining, unsequenced members of the 35 matrilines in our life history database (as in Chapter 4; see Table 6.1).

Table 6.1: Summary information for matrilines included in mtDNA control region haplotype analysis. Matrilineal sub-division was defined based on the proportion of all January - May census sightings of female deer from a matriline that were either side of 8030N and 1370E: matrilines with ≥50% of sightings south of 8030N and west of 1370E were 'South Kilmory Glen' (SKG), those with ≥50% of sightings north of 8030N and east of 1370E were 'Samhnan Insir' (SI), and all others were 'North Kilmory Glen' (NKG). The number of extant females indicates the number alive in 2001 (extant matrilines in bold).

Matriline	mt CR Haplotype	Number of deer sequenced	Sub-division	Number of extant females
101	A	3	SKG	0
106	B1	2	SKG	0
120	B5	3	SKG	0
123	B1	1	SKG	0
124	B5	1	NKG	0
127	**B1**	**4**	**SKG**	**4**
130	A	2	SKG	0
131	A	4	SKG	0
132	**A**	**4**	**SKG**	**16**
133	**B1**	**4**	**SKG**	**14**
134	**A**	**4**	**NKG**	**16**
135	**A**	**4**	**SKG**	**4**
136	**A**	**4**	**NKG**	**3**
137	**A**	**4**	**NKG**	**9**
138	**A**	**4**	**NKG**	**20**
139	**A**	**4**	**NKG**	**5**
140	**A**	**4**	**NKG**	**11**
141	**A**	**4**	**NKG**	**1**
142	**B2**	**4**	**NKG**	**4**
143	**A**	**4**	**NKG**	**38**
144	**A**	**4**	**NKG**	**13**
145	A	1	NKG	0
147	**A**	**4**	**NKG**	**35**
148	**A**	**4**	**NKG**	**9**
150	A	2	NKG	0
151	**A**	**4**	**SI**	**15**
152	**A**	**3**	**SI**	**2**
153	**A**	**4**	**SI**	**16**
154	A	4	SI	0
155	**A**	**4**	**SI**	**7**
156	**A**	**4**	**SI**	**4**
157	A	4	SI	0
158	**A**	**4**	**SI**	**3**
159	**A**	**1**	**SI**	**1**
165	**A**	**2**	**NKG**	**3**

6.3.3 Relative frequencies of mtDNA haplotypes

The number of living female and male deer from each of the 35 haplotyped matrilines in each year from 1978 to 2001 was calculated. We did not use individual residency to the North Block as our inclusion criterion, as in the spatial genetic analysis conducted here and in Chapters 4 and 5, because non-residency does not necessarily mean an animal has died. Instead we assessed the number of living deer within the matrilines that had been mtDNA haplotyped based on known dates of birth and death. The year of birth of all individuals from the haplotyped matrilines from the 1978 cohort onwards was known. Individuals without known death dates – those classified as being of 'missing' or 'unknown' status – were assumed to have died in the year after their last census sighting. The proportion of living deer from each mtDNA haplotype relative to the total number of living haplotyped deer was calculated for each year and each sex. Temporal trends were assessed by regression of the haplotype proportions on year.

6.3.4 Fine-scale spatial structuring of mtDNA haplotypes

Since only four haplotypes were found within the North Block population (see Results), we explored spatial distributions of females and males of each haplotype visually by plotting the locations of all mean annual positions of each sex separately for the time periods: 1978-1983, 1984-1989, 1990-1995, and 1996-2001. Spatial autocorrelation analysis was conducted using SPAGeDi (Hardy and Vekemans, 2002). Mean annual positions of North Block red deer were used to estimate

geographic distances between pairs of individuals. Moran's I statistic (computed in SPAGeDi following Hardy and Vekemans, 1999) was used to estimate the degree of spatial autocorrelation between haplotypes in males and females. Correlograms were generated across a distance range from <100m to >2km, by calculating I statistics at distance intervals of 100m. Moran's I statistic estimates the degree of haplotype sharing at each spatial interval. A decline in I as the spatial distance interval between pairs increase would indicate that individuals found in close proximity are more likely to share haplotypes than individuals that are distant from one another, i.e. that there is fine-scale spatial structuring of mtDNA haplotypes.

The analysis was conducted separately for each sex in each year of the study. Moran's I coefficients for each sex at each distance interval were averaged across study years to assess overall spatial genetic structure. To investigate variation in spatial structure over time, all Moran's I estimates for each sex were fitted as response variables in a multiple regression including year, distance class and their interaction in SPLUS version 6 (Insightful) and dropping terms from the regression until only those that were significant ($P < 0.05$) remained (following Crawley, 2002). Since we found evidence of curvature in the Moran's I – distance slope amongst males (see Results), a quadratic term for distance and its interaction with year was also fitted for this sex. We also examined trends in Moran's I in each sex visually by plotting the raw values over distance and time and apply loess smoothing techniques to the data in SigmaPlot 2000 (SPSS Inc.).

6.3.5 Matrilineal extinction and mtDNA haplotype

The effect of mtDNA haplotype on matrilineal survival was assessed using a generalised linear model (GLM), with a binomial error structure, of extinction or survival (coded 0 or 1) in the 35 haplotyped North Block matrilines. Extinction was defined as a matriline possessing no living female members in 2001. Main effects for haplotype and matrilineal sub-division were fitted to the model. Sub-division was fitted to control for spatial variation in habitat quality. Haplotype was fitted as a two-level factor, with the predominant RUM A haplotype as one level and all other RUM B haplotypes as the other, as we were interested in assessing differences in the probability of survival of RUM A matrilines relative to other haplotypes that could explain its prevalence in the North Block.

6.3.6 Individual fitness and mtDNA haplotype

Differences in four fitness parameters between individuals from RUM A matrilines and RUM B matrilines were assessed using generalised linear mixed-effect models (GLMMs), run in GenStat v. 7.2 (VSN International, 2003). We used all available data from the study's inception through to 2001. In all cases, individuals whose year of death was unknown or those who were shot were excluded from analyses (following Kruuk *et al*, 1999b). Neonatal and first winter survival of red deer calves are major contributors to overall mortality levels in this population (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 1987b). We took these two survival measures along with longevity and fecundity (lifetime breeding success) as our individual fitness

parameters. There were used as response variables in four GLMMs and are defined below (with the error structures used in the relevant GLMM in brackets):

Neonatal calf survival (binomial error structure): Each individual was scored according to whether it survived from birth (typically in May or June) until 1st October in the same year ('1') or not ('0').

Winter survival (binomial error structure): Individuals that died between 1st October of their year of birth and 30th April of the following years were scored '0', those that survived into May of the year after birth were scored '1'. Individuals scoring '0' in the summer survival parameter were excluded.

Longevity (normal error structure): An individual's age at death. Individuals still alive in 2004 were excluded from the analyses. Individuals that did not survive their first summer or winter were not included in the analysis.

Lifetime breeding success (LBS; poisson error structure): Defined as the number of offspring assigned to each individual. Paternities were assigned using genotypic and behavioural analyses (as described in Section 5.3.4). Maternities are known because calves remain in close proximity to their mothers for the first six months of life at least. Individuals were only included in this analysis if they had died before 2001 and had survived to at least three years of age (see Kruuk *et al*, 1999b).

In each GLMM we fitted sex, birth weight and (for neonatal and winter survival only) mother's reproductive status (see Sections 2.3 and 3.3 for definitions) to account for known the effects of these variables on fitness parameters in this population (Clutton-Brock *et al*, 1987b, Kruuk *et al*, 1999b). We also fitted

individual's natal sub-division to control for spatial variation in fitness, along with mtDNA haplotype (again, as a two-level factor). The significance of these fixed effects terms within the GLMMs was tested by comparing their Wald statistics divided by their number of degrees of freedom to the appropriate critical F value (following Nussey *et al*, 2005a). We included year of birth as a random effect in these models (following Milner *et al*, 1999) to account for well documented effects of population density and climate on development and early growth in this population (Albon *et al*, 1987, Clutton-Brock *et al*, 1987b). Additional random effects for matriline and mother's identity (nested within matriline) did not alter the results of any of the GLMMs, and were never statistically significant. In several cases, these extra random effects caused GLMMs to fail to converge, and so they were omitted from the final models.

6.4 Results

Table 1 summarises the mtDNA haplotypes found in sequenced individuals from 35 North Block matrilines for which tissue samples were available, along with the sub-division of the matriline, and whether it went extinct during the study period. The same 'RUM A', 'RUM B1' and 'RUM B2' haplotypes described in Chapter 4 were found amongst the 117 North Block red deer mt CR sequences. An additional haplotype, 'RUM B5', was also identified. This was more similar to the other 'RUM B' haplotypes than to the 'RUM A' haplotype. It differed from 'RUM B1' by six substitutions across the 922 bp sequence analysed, 11 substitutions from 'RUM B2', and by 29 substitutions from 'RUM A' (see Appendix B). Addition of 'RUM B5' to

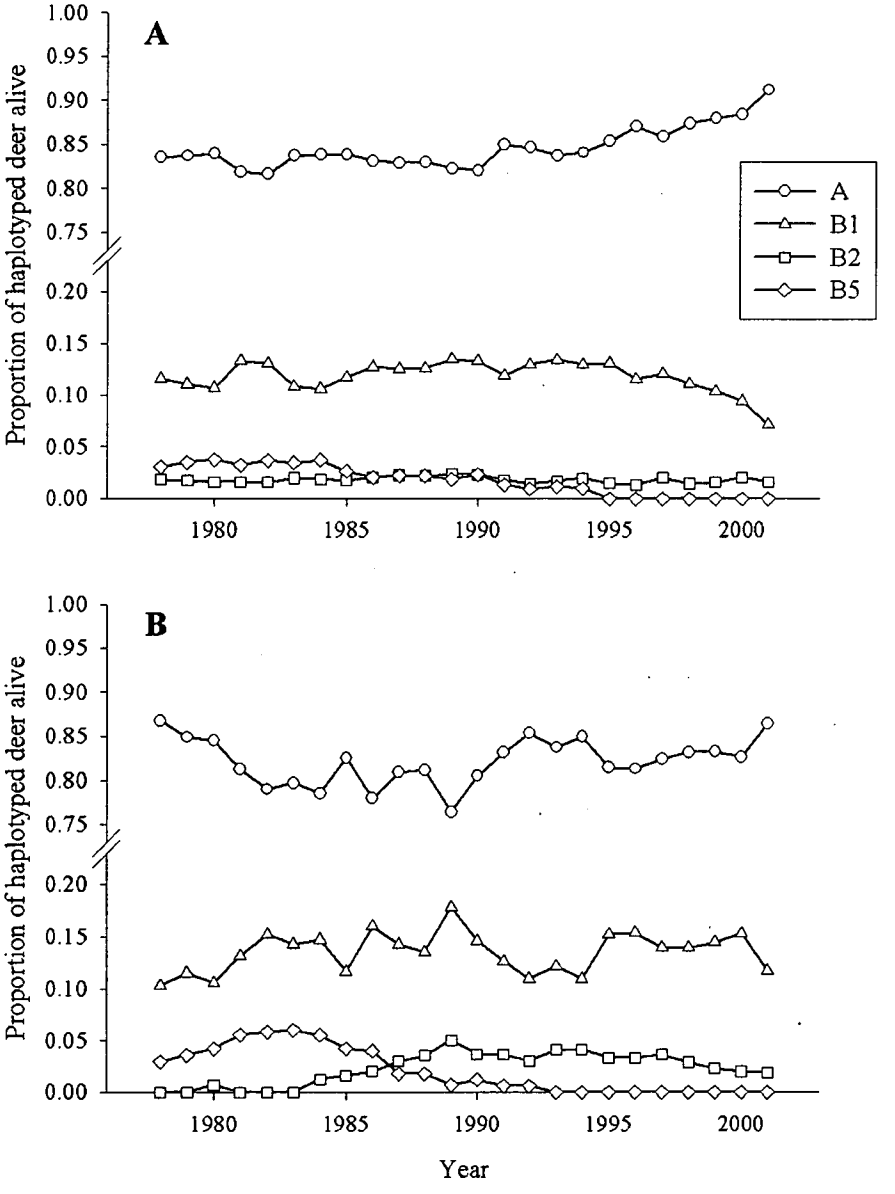
the phylogenetic analyses analysis presented in Chapter 4 showed that the new haplotype clustered with the poorly resolved group which included all 'RUM B' haplotypes and red deer from Spain and Norway.

6.4.1 Relative frequencies of mtDNA haplotypes

Figure 6.1 shows the relative proportions of each haplotype amongst males and females from the haplotyped matrilines that were alive in the years 1978 – 2001. RUM A was by far the most prevalent haplotype: it accounted for between 76% and 91% of North Block deer of either sex (Figure 6.1) and was present in 28 matrilines, 7 of which went extinct during the study period (Table 6.1). The proportion of females of RUM A in the North Block increased significantly over time ($F_{1, 22}$ = 33.51, $P < 0.001$) from around 80% to 90% by 2001, whilst the proportion of males showed no temporal trend and fluctuated between 75% and 85% ($F_{1, 22} = 0.70$, $P > 0.05$). RUM B1 haplotypes accounted for 7 - 17% of living male or female deer (Figure 6.1) and was present in four matrilines, only two of which were extant in 2001 (Table 6.1). There were no significant temporal trends in either sex for RUM B1 (females: $F_{1, 22} = 2.04$, males: $F_{1,22} = 1.24$, both $P > 0.05$). The RUM B2 haplotype was found in only a single matriline that did not go extinct (Table 6.1). There was evidence of an increase in the proportion of RUM B2 males over time ($F_{1, 22} = 15.00$, $P < 0.001$) from zero in the late 1970s to fluctuation between 2 – 5% in the last 15 years of the study. No temporal trend was present in RUM B2 females ($F_{1,22} = 0.62$, $P > 0.05$). Finally, the two RUM B5 matrilines (between 0 - 6% of the haplotyped population) went extinct in both sexes by 1995 (Figure 6.1), leading to

significantly decreased relative frequencies over time (females: $F_{1,22} = 229.5$, males: $F_{1,22} = 57.29$, both $P < 0.001$). Evident declines in both sexes of the RUM B5 haplotype were companied by increases in the relative frequency of RUM A in females and RUM B2 in males.

Figure 6.1: The relative frequencies of red deer from each mtDNA haplotype between 1978 and 2001 (circle: RUM A; triangle: RUM B1; square: RUM B2; diamond: RUM B5). Proportions were assessed separately for (A): females, and (B): males.



6.4.2 Fine-scale spatial structuring of mtDNA haplotypes

The mean annual positions occupied by females and males of the four mtDNA haplotypes in four time periods are presented in Figure 6.2. Deer were most commonly observed along the western and north edges of the study area, within the NKG, SKG and SI regions (illustrated in Figures 6.2 and 5.1): these low-lying areas contain the highest quality grazing and the most shelter (Clutton-Brock et al, 1982). The distribution of males (Figures 6.2 E-H) is markedly more scattered than that of females (Figures 6.2 A-D), with many more males located in the more marginal habitats in the south-central and south-east of the North Block. This is expected given the dispersive nature of males and the matrilocal social structure shown by female red deer (Clutton-Brock et al, 2002, Nussey et al, 2005b), as well as evidence for both habitat and spatial segregation of the sexes in this population (Conradt, 1999, Conradt *et al*, 1999, Conradt *et al*, 2001) Figure 6.2 suggests that, across the study period, there has been southward and eastward expansion in females (Figure 6.2 A vs. D), and a general tendency for males' locations to be less spatially clustered across the study area (Figure 6.2 E vs. H). This is concurrent with increased female population density and decreased male density in the North Block (Clutton-Brock et al, 2002, Coulson et al, 2004).

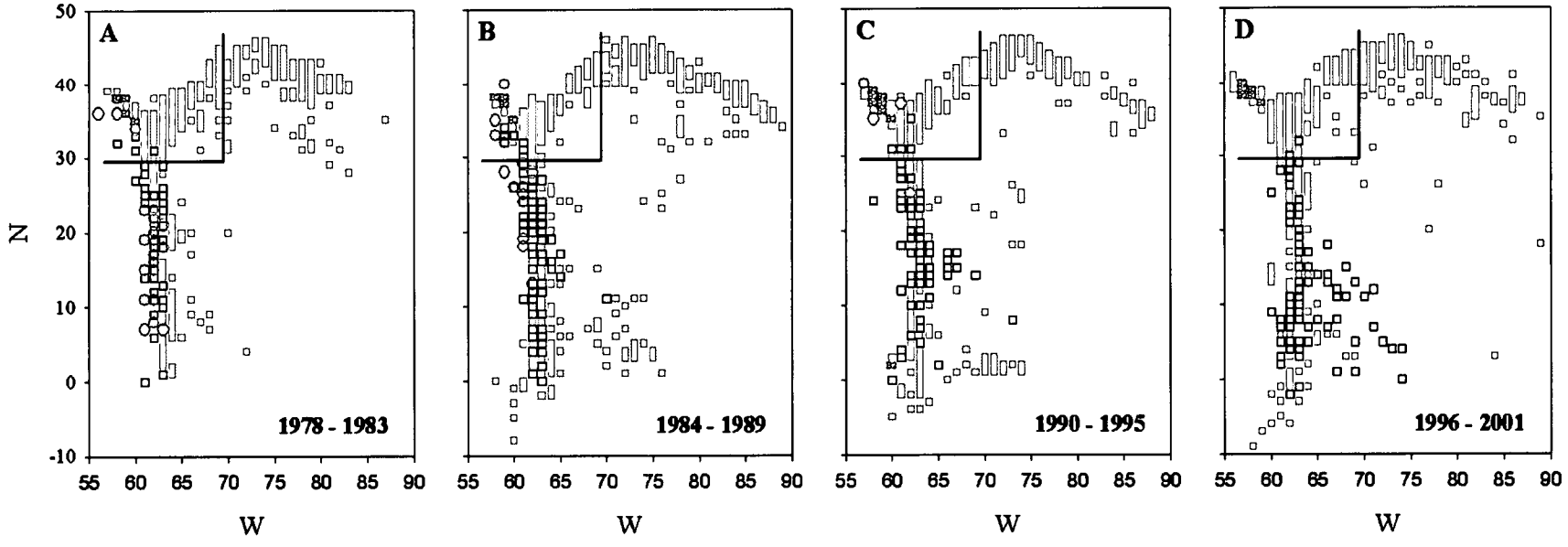


Figure 6.2 (A-D): Plot of locations of mean annual positions for adult female red deer resident to the North Block study area from different mtDNA haplotypes (RUM A: gray filled squares; RUM B1: empty, black edged squares; RUM B2: blue 'B2' text; RUM B5: empty, red edged circles), in four time periods: 1978-83 (A); 1984-89 (B); 1990-95 (C); 1996-2001 (D). The lines represent the approximate boundary between population sub-divisions: South Kilmory Glen in the southwest, North Kilmory Glen in the northwest, and Samhnan Insir in the northeast.

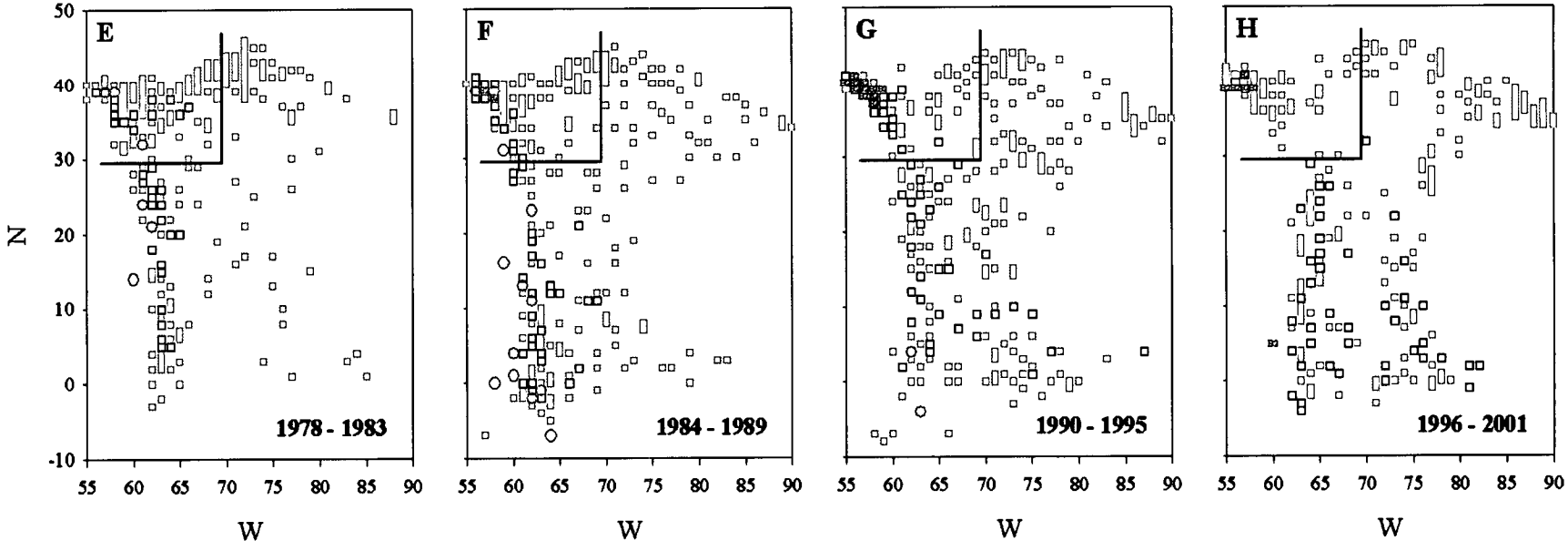


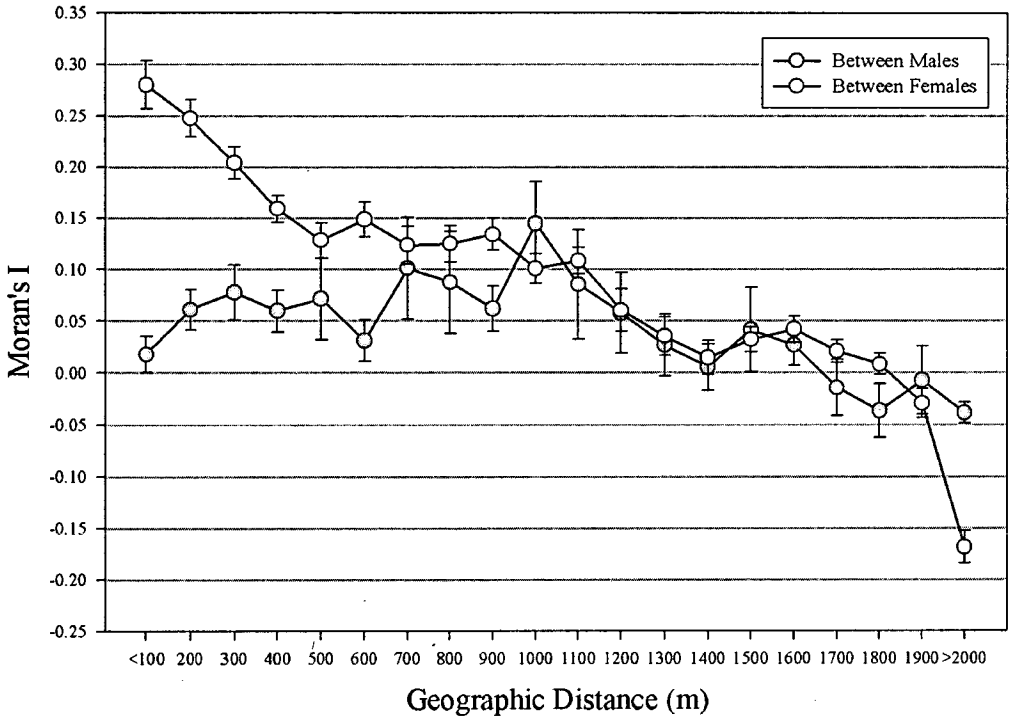
Figure 6.2, continued (E-H): Plot of locations of mean annual positions for adult male red deer resident to the North Block study area from different mtDNA haplotypes (RUM A: gray filled squares; RUM B1: empty, black edged squares; RUM B2: blue 'B2' text; RUM B5: empty, red edged circles), in four time periods: 1978-83 (E); 1984-89 (F); 1990-95 (G); 1996-2001 (H).

At the level of specific mtDNA haplotypes, RUM A individuals followed the broad population trends discussed above (Figure 6.2). However, RUM B males and females were located only in Kilmory Glen: the SI sub-division in the northeast was entirely composed of individuals from RUM A matrilines (see Figure 6.2). RUM B1 males and females were found throughout North and South Kilmory Glen. However, in the later part of study very few RUM B1 deer were found in North Kilmory Glen, instead they occupied a wider range of localities in South Kilmory Glen and the south-east of the study area (Figures 6.2 A & E vs. 6.2 D & H). RUM B2 deer of both sexes were confined almost entirely to the extreme northwest of NKG (Figure 6.2). The few RUM B5 individuals were scattered through Kilmory Glen, with the haplotype extinct from the North Block by 1996 (Figure 6.2).

The across-year correlogram examining spatial autocorrelation of mtDNA haplotypes (Figure 6.3) shows the expected pattern for females: as distances between pairs of females increased, the likelihood of haplotype sharing decreased ($F_{1,18}$ = 128.5, $P < 0.001$). However, there was also an unexpected, weak but significant decrease in haplotype sharing as distances between males increased ($F_{1,18} = 10.1$, $P < 0.01$). Visual inspection of the across-year correlogram for males (Figure 6.3) suggested that Moran's I estimates declined in a non-linear fashion, with a possible increase up to distances of 1000m followed by a decline thereafter. Addition of a quadratic term for distance to the across-year regression of Moran's I on distance for males significant improved the model fit (model including quadratic: $F_{2, 18} = 18.7$, $P < 0.001$; comparison of models with and without quadratic term: $F = 17.53$, $df = 1$, $P < 0.001$). The overall expectation of fine-scale spatial structuring of mtDNA

variation amongst females was met, but the prediction of an absence of such structure amongst males was not.

Figure 6.3: Correlogram based on mtDNA control region haplotype data showing Moran's I spatial autocorrelation coefficients against geographic distances between females (empty circles) and males (filled circles). Circles represent the mean kinship coefficient at each distance class across study years (1978 – 2001), with standard error bars.



Linear models of Moran's I coefficients from all 24 study years revealed patterns of temporal change in the spatial autocorrelation of mtDNA haplotypes in both sexes (Table 6.2, Figure 6.4). For females, the full model could not be simplified: there were significant effects for distance ($F_{1, 476} = 599.32$, $P < 0.001$) and for the distance-by-year interaction ($F_{1, 476} = 16.15$, $P < 0.001$), although the main effect for year was marginally non-significant ($F_{1, 476} = 2.82$, $P = 0.09$). Examination of the Moran's I estimates following parametric smoothing, alongside the linear

model's predicted correlogram slopes in different years (Figure 6.4 A), showed that the across-year negative slope of I on geographic distance between female pairs (Figure 6.3) was becoming more positive over time: i.e. the strength of fine scale structure amongst females is decreasing across the study period.

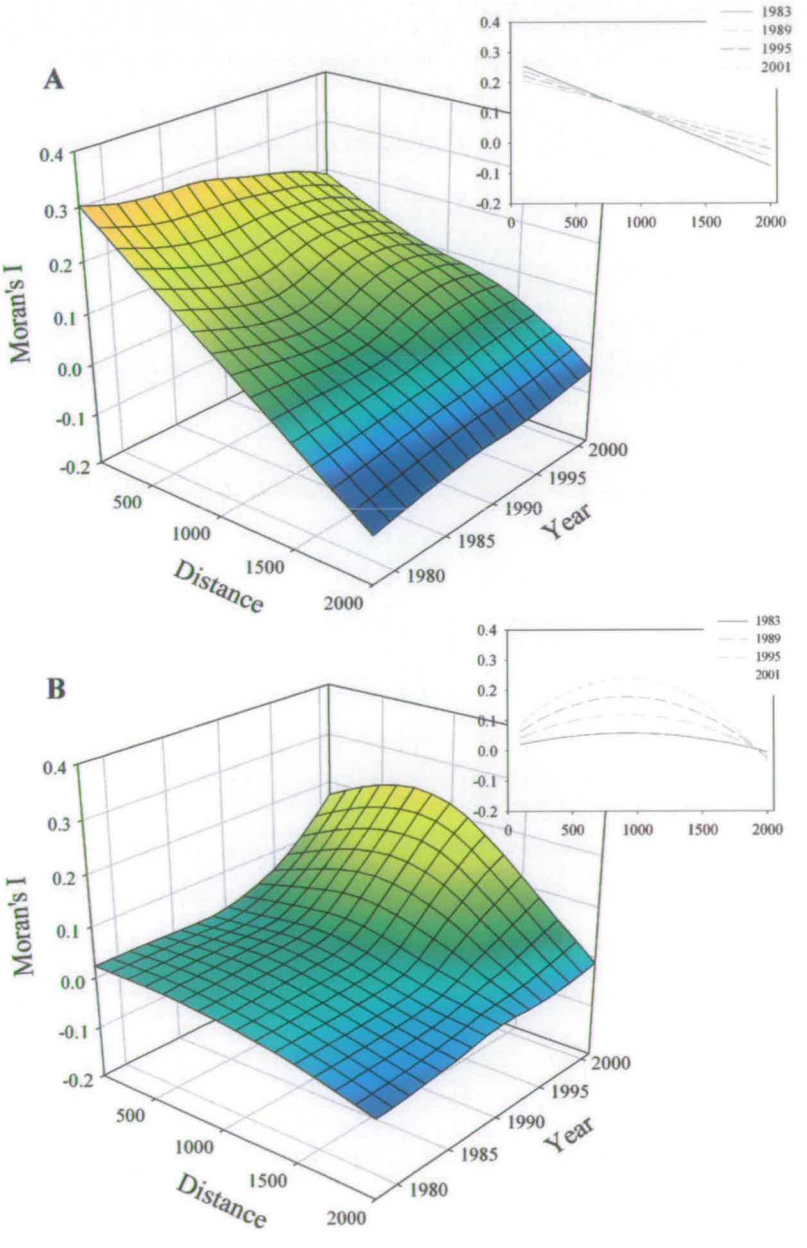
Table 6.2: Minimum linear models of Moran's I spatial autocorrelation coefficients between mtDNA haplotypes, estimated separately in years 1978-2001 and at 100m distance intervals from 100m to ≥2km.

Term	df	MS	F	P	Estimate
		Females			
Distance	1	4.13	599.3	<0.001	-0.0078
Year	1	0.019	2.82	<0.1	-0.0031
Distance * Year	1	0.11	16.15	<0.001	$3.8 * 10^{-6}$
Residual	476	0.069			
		Males			
Distance	1	0.17	7.63	<0.01	-0.035
Distance^2	1	0.52	22.99	<0.001	0.000019
Year	1	0.98	42.85	<0.001	0.0020
Distance * Year	1	0.13	5.85	<0.05	0.000018
Distance^2 * Year	1	0.12	5.22	<0.05	$9.7 * 10^{-9}$
Residual	474	0.023			

An additional quadratic term for distance, and its interaction with year, were included in the full model of male Moran's I coefficients to examine curvature of the correlogram and how it might be changing across the study period. The final model could not be simplified (Table 6.2): there were significant year-by-distance ($F_{1, 474}$ = 5.85, $P < 0.05$) and year-by-distance^2 ($F_{1, 474}$ = 5.22, $P < 0.05$) interactions. Main effects for distance ($F_{1, 474}$ = 7.63, $P < 0.01$), distance^2 ($F_{1, 474}$ = 22.99, $P < 0.001$) and year ($F_{1, 474}$ = 42.85, $P < 0.001$) were also highly significant. Figure 6.4 B presents these interactions visually, and revealed that the curvature in the across-year male correlogram (Figure 6.3) appeared to be driven by increased spatial autocorrelation

estimates at low and mid-range distances in the last decade of the study. Prior to the mid-1990s there was little spatial structuring of mtDNA haplotypes amongst males.

Figure 4: Mesh plots of Moran's I spatial autocorrelation coefficients for mtDNA haplotypes across distance and year in female (A) and male (B) red deer smoothed using loess parametric techniques. Inset: predicted Moran's I – distance relationships at four different years from the estimated effects in the linear models in Table 6.2.



6.4.3 Matrilineal extinction and mtDNA haplotype

Eleven of the 35 matrilines sequenced for mtDNA control region variation went extinct during the study period. The GLM of matrilineal extinction revealed that matrilines possessing RUM B haplotypes were not significantly more likely to go extinct than RUM A matrilines ($F_{1, 31} = 1.88$, $P > 0.05$), and that a matriline's sub-division also did not affect extinction ($F_{2, 31} = 1.35$, $P > 0.05$).

6.4.4 Individual fitness and mtDNA haplotype

There was no evidence for differences in fitness between individuals with RUM A haplotypes and RUM B haplotypes (see Appendix C for full GLMMs). Wald statistics were non-significant for mtDNA haplotype in GLMMs of summer (Wald statistic / df = 0.03, $P > 0.05$) and winter (Wald / df = 0.01, $P > 0.05$) calf survival. The geographic sub-division term in both GLMMs was highly significant, however (Wald / df = 13.67 and 8.26, respectively; both $P < 0.001$). Intriguingly, the SKG sub-division seemed to be causing these significant effects: the sub-division effect on summer survival was driven by low survival of SKG calves, whilst increased winter survival in SKG relative to other sub-divisions was responsible for the effect in that GLMM (see Appendix C). Although not pertinent to the questions addressed in this chapter, the causes of this variation in neonatal survival parameters in SKG surely merits further research and clearly demonstrate spatial variation in selection within the North Block. Haplotype effects were also non-significant in GLMMs of longevity (Wald / df = 1.86, $P > 0.05$) and LBS (Wald / df = 0.12, $P > 0.05$).

6.5 Discussion

6.5.1 Fine scale structuring of mtDNA variation in males and females: overall patterns and temporal trends

The main difference between the spatial distribution of RUM A and RUM B mtDNA haplotypes, in both sexes, was the complete prevalence of RUM A in the north-eastern region of the North Block (Samhnan Insir; Figure 6.2). Fine-scale spatial structuring of mtDNA haplotypes was expected and demonstrated amongst philopatric females in the population (Figure 6.3). However, no such structure was predicted in males, which usually disperse from their mother's home range before reaching maturity, and often leave the study area completely (Clutton-Brock *et al*, 2002, Catchpole *et al*, 2004). Although male mean annual positions were considerably less spatially clustered (Figure 6.2) and male spatial autocorrelation coefficients at small distances (100 – 600m) were lower than those for females (Figure 6.3), spatial autocorrelation analysis revealed some degree of spatial structure in males, most strongly apparent between pairs at intermediate (800 – 1100m) distances, in males (Figures 6.3 and 6.4).

The presence of fine-scale structure in the dispersive sex, against our predictions, may be attributable to some degree to limitations in the sampling regime for males. Our sampling regime completely excluded immigrant males, from unknown matrilines, that make up a proportion of the resident population of the North Block (on average 14% of the annual resident male population) – unlike the microsatellite analysis presented in Chapter 5. This, coupled with the far lower

variability in mtDNA compared to microsatellite genotypes, may inflate estimates of spatial autocorrelation observed in males. However, as already mentioned, the complete absence of RUM B males from SI (Figures 6.2 E-H) argues against a completely random spatial distribution of haplotypes in males, at least amongst those natal to the North Block.

In Chapter 5, analysis revealed that both male and female natal dispersal from Kilmory Glen to Samhnan Insir is extremely rare (see Figure 5.7). It is worth noting that movement from KG to SI is well within the dispersal capability of male red deer, making the complete absence of 'RUM B' haplotypes from this area rather puzzling. An ecological explanation for this lack of male eastward dispersal lies in the fact that red deer in Samhnan Insir have been at or close to carrying capacity for longer than animals in the rest of the North Block (Milner-Gulland *et al*, 2000). This is both because of the area's relatively few, closely aggregated patches of high quality grazing, and the possibly also because it may have been subject to less intensive culling since re-introduction of deer than KG because of its less accessible location (T. Coulson, *pers. comm.*). Males might be avoiding eastward dispersing into an area of high resource competition and preferentially moving west, out of the study area. Indeed, increasing westward emigration of males from KG has been observed in recent years (Clutton-Brock *et al*, 2002).

The presence of temporal trends in the spatial autocorrelation of mtDNA haplotypes in both sexes (Figure 6.4) also requires explanation. The declining spatial autocorrelation of haplotypes amongst females (Figure 6.4 A) mirrors the decline in microsatellite F_{ST} estimates amongst females (Figure 5.4) across the same 24-year study period. However, the decline in mitochondrial spatial autocorrelation is likely

to be directly related to temporal changes in the spatial distribution of females of different haplotypes, and not to the changes in effective population size discussed with reference to microsatellite F_{ST} estimates in Chapter 5. The south- and east-ward expansion of RUM A and RUM B1 females (Figure 6.2 A-D) – previously explained in terms of increasing resource competition in NKG and SI following cessation of culling in 1973 (Coulson *et al*, 2004) driving females away from the best grazing habitat – may account for the declining mtDNA spatial autocorrelation. Spatial expansion of females with the common RUM A haplotype would make the presence of identical haplotypes amongst pairs of females at intermediate and large distances more likely, thereby flattening the slope of the correlogram, and reducing the Moran's I on distance slope amongst females.

The evident temporal trends in the mtDNA spatial autocorrelation in males (Figure 6.4 B) – with more positive autocorrelation estimates emerging only late in the study period and at intermediate distances – are puzzling. The temporal increase in scatter of male mean annual positions, evident from visual inspection of Figures 6.2 E-H, is one possible explanation. The almost complete confinement of RUM B1 males to SKG, along with their eastward expansion within the sub-division, by 1996 (Figure 6.2 H), could also have contributed to the relative increase in haplotype sharing at intermediate distances driving the pattern in Figure 6.4 B. However, evidence of spatial structure in males must be regarded cautiously until immigrant residents can be included in the sample.

6.5.2 Mechanisms driving mtDNA spatial structure: environment, behaviour, drift and selection

Whilst recent patterns in haplotype spatial distributions can be explained in terms of recent changes in ecological conditions in the North Block and differences in dispersal between the sexes, more general questions remain regarding the mechanisms driving the overall prevalence of the RUM A haplotype amongst North Block red deer (Figure 6.1), and its complete predominance in the SI sub-division with the North Block (Figure 6.2). As already mentioned, female philopatry and genetic drift are sufficient explanations for this level of spatial structure in mtDNA variation (Prugnolle and de Meeus, 2002, Fredsted *et al*, 2004). An additional geographical explanation exists for the observed predominance of RUM A deer in SI, which would make rare female immigration events – that could potentially introduce rare or novel haplotypes to the sub-division – very unlikely. Immigration to SI would involve negotiating either the high, unfavourable ground of Mullach Mor to the south, the Kinloch village deer fence to the east (challenging but not impossible, *pers. obs.*), or passing over the superior grazing habitat of NKG to the west (extremely unusual, see Figure 5.7).

In Chapter 4, cross-island population genetic analysis revealed that the red deer North of Rum were predominantly RUM A, while the majority of those in the south we of a RUM B haplotype. The question of whether selection favouring RUM A haplotypes in the North of Rum could be in some way responsible for the prevalence of this haplotype in the North Block was addressed in this chapter. The analysis suggested that matrilineal extinction patterns observed within our 24-year

study period were random with respect to mtDNA haplotype, and furthermore that there was no evidence of fitness differences between individuals of RUM A and RUM B haplotypes across the same period. Whilst these analyses only crudely control for possible spatial variation in fitness or matrilineal extinction and do not consider growth and extinction patterns of individual matrilines in detail, they do suggest that selection has not favoured the RUM A haplotype in deer resident to the North Block in recent years, and that fine-scale spatial structure of mtDNA haplotypes in the study area is better explained by philopatry and random drift than selection favouring RUM A matrilines.

It is certainly worth bearing in mind that the data set does not allow us to conclude anything about selection on mtDNA haplotype prior to 1978. Although we are unable to ascertain the degree of admixture between Rum haplotypes, either since introduction to the island or within managed mainland populations prior to it, this is likely to have been extensive. This would have lead to the removal of any previously existing associations between mtDNA variants and fitness – present as a result of direct effects on fitness, mitochondrial hitchhiking or cytonuclear fitness associations – from the population (William *et al*, 1995, Rand *et al*, 2001). However, the analyses presented here represent an extremely rare test for difference in fitness between mtDNA haplotypes in a wild vertebrate population (Nevo and Beiles, 1992, Gemmell et al, 2004).

6.6 Conclusions

In this chapter, evidence for a temporal decline in the spatial autocorrelation of mtDNA haplotypes amongst female red deer was explained in terms of population expansion in response to changing ecological conditions. The result further emphasises the importance long-term genetic and life history data sets such as the Rum North Block red deer population in our understanding of the relationship between environment, behaviour and population genetics (Sugg *et al*, 1996, Bossart and Pashley Prowell, 1998). Although there was no evidence of any selection on mtDNA haplotypes in the North Block population, the assumption of neutrality – and concurrent invocation of philopatry and drift as the only mechanisms likely to be driving spatial structure – is clearly violated in certain cases and requires further testing in wild systems (Gemmell *et al*, 2004).

Chapter 7:

General Discussion

In this section I discuss the findings presented in this thesis relating to maternal life history plasticity (Section 7.1) and spatial genetic structure (7.2) in the red deer population on the Isle of Rum. In each case I briefly summarise the key results, and then discuss their wider evolutionary and ecological implications and avenues for future research.

7.1 Maternal life history plasticity in the wild

The analysis presented in Chapter 2 (Nussey *et al*, *in press*) represents a rare attempt to investigate the effects of environmental quality on maternal life history plasticity in a wild red deer population on the Isle of Rum, Scotland. The results show that the early experience of poor environmental quality, as indicated by high population densities, was related to a reduction in the plastic response of individual females. In Chapter 3 (Nussey *et al*, 2005a) I presented a novel application of a random coefficients model (RCM; Brown and Prescott, 1999), a particular type of linear mixed-effects model (LMM), to investigate patterns of phenotypic plasticity using data from the North Block study population. Considering a different maternal trait–environment relationship to Chapter 2, I found significant variation in plasticity between females and a reduction in plasticity at increased population densities. Selection on best linear unbiased predictors (BLUPs) for elevation and slope

components of individual reaction norms were investigated, revealing changes in the target of selection, from elevation to slope, between high and low population density phases. Both analyses were discussed in terms of maternal life history trait expression showing physiological condition-dependence, and their ecological consequences were considered.

7.1.1 The implications of variation in life history plasticity in wild populations

Maternal reaction norms are an important but often overlooked component of variation in vertebrate life history traits. The handful of studies to date that have analysed variation in individual reaction norms in wild vertebrate systems, using LMM approaches, have found evidence for significant variance in both individual elevation (phenotype in the average environment) and slope (phenotypic plasticity) components of reaction norms (Brommer *et al*, 2005, Nussey *et al*, 2005a, Nussey *et al*, 2005c). The RCM, discussed and applied in Chapter 3, represents a useful method for assessing the pattern of individual plasticity using data sets from wild populations. Variance components estimates from these models do not appear to be strongly influenced by the large number of reaction norms containing only two or three data points, which are inevitable in most data sets from natural populations (Section 3.3.3; Brommer *et al*, 2005). One major problem with using simple linear regression (as in Chapter 2) to estimate reaction norm slopes in data sets where some individuals have only a few life history measurements arises when measurements within individuals occur in very similar environmental conditions. This will lead to

slope estimates of extremely large (and presumably spurious) magnitude. The RCM deals with such potential outliers by weighting their BLUPs towards to the population mean. A recent study using a RCM to investigate maternal phenological plasticity in a Swedish collared flycatcher (*Ficedula albicollis*) population found that slope BLUPs were robust to manipulations of the number of data points per female (Brommer *et al*, 2005). I would argue that the novel application of the random coefficients model presented here represents a major step forward in our ability to assess the pattern of individual phenotypic plasticity using data sets collected from individual based studies of wild vertebrates.

Where data sets are of sufficient detail and depth, these models allow new insight into the ecological dependence and consequences of phenotypic plasticity in nature. In particular, the importance of plasticity in our understanding of the effects of climate change should not be overlooked. Plasticity represents the main mechanism by which populations can respond rapidly to either natural or anthropogenic changes in their environment. While many studies have documented ecological responses to recent climate change in natural systems (Stenseth *et al*, 2002, Walther *et al*, 2002), particularly in life history traits like phenology, they have rarely considered the role of individual plasticity, and its variation within populations (Fitter and Fitter, 2002, Both *et al*, 2004, Edwards and Richardson, 2004). In Chapters 2 and 3, maternal plasticity was shown to decline with as female population density increased for two different trait–climate relationships. In both cases, the maternal plasticity in the Rum red deer population is thought to be the result of condition-dependence. The climate variable on the reaction norm x-axis causes variation in female physiological condition at vital junctures in the reproductive

cycle, resulting in changes in investment in offspring development or growth. Environmental deterioration associated with increasing population density may affect condition independently of the reaction norm climate variable, reducing the apparent condition-dependent response to climate. Assuming either temporal or spatial constancy in trait-climate relationships when considering the predictions of long-term climate models ignores the ecologically dynamic nature of individual plasticity.

The application of selection analysis and, more recently, the 'animal model' to RCM-generated BLUPs for individual female elevation and slope has provided insights into the causes of variation in life history reactions norms as well as their potential micro-evolutionary dynamics (Brommer *et al*, 2005, Nussey *et al*, 2005a, Nussey *et al*, 2005c). There is now evidence from several natural systems for selection on maternal plasticity in phenology (Brommer *et al*, 2005, Nussey *et al*, 2005a, Nussey *et al*, 2005c). A very recent study of a Dutch population of great tits (*Parus major*) presented the first evidence for the heritability of maternal phenological plasticity (Nussey *et al*, 2005c). In cases where both additive genetic variance and selection for plasticity exist, quantitative genetics theory would lead us to predict a micro-evolutionary response to selection (Lynch and Walsh, 1998).

One of the leading lights in recent phenotypic plasticity research, Massimo Pigliucci, stated in a recent review that "*some areas of research, such as the study of the quantitative genetic underpinning of plasticity, have been either settled in broad outline or superseded by new approaches and questions*" (Pigliucci, 2005). Pigliucci seems laudably keen draw plasticity research away from the potential quagmire of theoretical debate and the recent explosion of lab-based quantitative genetic studies, towards a molecular understanding of plasticity. It's difficult to argue with this point

of view in broad principle: hard fought theoretical debate and an onslaught of empirical data now seem to have settled the argument over whether plasticity itself can be heritable (Pigliucci, 1996, 2001, 2005). Simply presenting evidence of genetic variance for reaction norm slopes can hardly be considered novel.

However, there is a subtle distinction between the genotypic reaction norms typical of laboratory systems in empirical plasticity research – where repeated measures of clones or full- or half-sib families are available – and plasticity analysis undertaken at the level of the individual, as presented here. Significant variation in reaction norm slopes evident from the RCM presented in Chapter 3 (and illustrated in Figure 3.1 C, D) is not evidence of GxE interaction (Figure 1.1). An additional analytical step (e.g. via the 'animal model') is required to show genetic variation for plasticity if we are considering individual (rather than genotypic) reaction norms (see Brommer *et al*, 2005). We still have limited data from which to judge the prevalence of genetic variation or selection on individual plasticity in wild vertebrate populations. The continued examination of the quantitative genetics of reaction norms in natural settings provides an opportunity to understand when and why they are present, rather than whether their presence is theoretically possible.

Further research into the quantitative genetics of maternal plasticity in wild populations is surely warranted. Analyses of the short-term evolutionary dynamics of any kind of plasticity in the wild, where organisms experience naturally occurring ranges of environmental conditions (rather than those imposed in the lab) against real and complex ecological backdrops, are still extremely rare. We have already illustrated clearly that selection on naturally-occurring maternal life history reaction norms can change with deteriorating ecological conditions (Chapter 3) and through

interactions between trophic levels of an ecosystem (Nussey *et al*, 2005c). Further quantitative genetic work on maternal phenological plasticity in systems where ecological conditions are actually improving (e.g. the red squirrel population described in Réale *et al*, 2003) or comparing individual plasticity between populations of the same species showing different patterns of response to ecological change (e.g. great tit populations described in McCleery and Perrins, 1998, Visser *et al*, 1998) are likely to prove extremely illuminating.

7.1.2 Future Directions

Whilst quantitative genetic and condition-dependence based approaches to explaining the evolutionary and ecological dynamics of life history plasticity in wild population seem complementary (Smith, 1991), the development of a predictive theoretical framework would be extremely useful. Such theory is likely to require an interweaving of quantitative genetic (specifically, the polynomial reaction norm) and life history (specifically, individual optimisation / condition-dependence) approaches. Empirical work is beginning to elucidate some of the conditions under which we should expect plasticity across the population (Figures 3.1 B, C), and variation in plasticity (Figures 3.1 C, D). Theoretical or simulation models based on these ideas are likely to both clarify expectations under varying ecological conditions and focus future research efforts in this field. Such models may actually represent straightforward extensions of those that already exist in the plasticity or life history literature.

Recent developments in the modelling of genetic (co)variance matrices across ontogeny in wild populations using random regression (RR; Wilson *et al*, 2005b) represent an intriguing means of assessing variation in the heritability of life history traits as a function of an environmental variable. Random coefficient and random regression models will surely prove complementary in our understanding of the role of environmental variation in trait evolution in natural systems. However, the two approaches smack worryingly of the troublesome 'character state' versus 'polynomial' approach debate in the plasticity literature (Via *et al*, 1995). The RCM can be used to assess genetic variation in individual trait-environment functions, whilst RR could be applied to model changes in population-level genetic variation in a trait across environments. The former is similar to the 'polynomial' approach to plasticity, with the minor difference, already mentioned, that it models reaction norms at the level of the individual not genotype. The latter is identical to the 'character state' approach, except that it attempts to simplify the genetic (co)variance matrix of traits in each environment using polynomial functions (Wilson *et al*, 2005b). Only further consideration and complementary application will elucidate the relevance of each of these approaches. The fundamental difference between them – and the source of most of the controversy in the original 'polynomial' versus 'character state' debate – is likely to reside in the degree to which one considers gene action driving observed genetic variation to occur at the individual or developmental stage (RCM) or for gene action to vary depending on environment through ontogeny (RR).

Finally, the RCM and RR analyses can be readily applied to other areas of evolutionary biology by removing environment from the x-axis of the reaction norm

and considering other aspects of life history instead. For example, fitting age along the reaction norm x-axis would allow exploration of the quantitative genetics of senescence in wild populations of long-lived organisms. One recent study used 'animal models' for individuals of different age classes to estimate age-specific heritabilities of laying dates in mute swans (*Cygnus olor*; Charmantier *et al*, *in press*). Another used a random regression to assess changes in the heritability of body size in bighorn sheep (*Ovis canadensis*; Wilson *et al*, 2005b). Both studies present compelling evidence for changes in additive genetic variance with age. Further exploration of how additive genetic variance changes with age in other wild vertebrate populations and traits is surely warranted. Ultimately, the use of both RR and RCM approaches may permit very rare tests of competing theories of aging (antagonistic pleiotropy versus mutation accumulation) within naturally occurring populations.

In a similar vein, life history theory generally assumes trade-offs between traits are optimised by selection and do not vary between individuals, but one study examining individual variation between clutch size – laying date reaction norms in a wild bird population suggests otherwise (Brommer *et al*, 2003). Fitting two related life history traits as reaction norm axes in an RCM analysis could elucidate between-individual variation in life-history trade-offs occurring in natural populations that breed repeatedly across their lifetimes.

7.2 Spatial genetic structure in a wild mammal population

In Chapter 4, mitochondrial sequence analysis of red deer from across the Isle of Rum revealed phylogenetic clustering of one Rum haplotype with sequences from Sardinia and North Africa while other Rum haplotypes clustered with red deer sequences from mainland Europe. This finding was explained in terms of the high levels of historical and recent human translocation of red deer across Europe and the UK, and the fact that deer on Rum are descended from introductions from a variety of managed mainland populations. Levels of mitochondrial spatial structure across the island were an order of magnitude higher than those found in nuclear genetic markers, as expected given the male-biased dispersal in this species. However, evident spatial genetic structure in the nuclear markers, but not mitochondrial DNA, was predominantly driven by differentiation of deer in a single region of the island: the North Block. The high population density of female deer in the North Block, caused by the cessation of culling in this area of the island, may explain this. Increased competition for food may reduce the phenotypic quality of males born in the North Block and mean that North Block males are unable to compete successfully for mates with males from other areas. Thus, even though physical dispersal of males from the North Block has increased since the 1970s, gene flow from this region to the rest of the island may actually have been reduced.

In Chapter 5, I examined fine-scale spatial genetic structure (SGS) in microsatellite genotypes amongst deer in the North Block study area. There was evidence for extremely fine scale (<100m) structure amongst females, but no evidence for spatial genetic structure across the same distance range (0-2000m) in

males. A temporal decline was observed in the genetic structure amongst females over the 24-year study period. An increase in the number of breeding females and a decrease in polygyny, both linked to the North Block population's release from culling in 1972 and subsequent increase in population density to carrying capacity, could explain this genetic trend. Natal dispersal of deer within the North Block could not explain the trend. Using similar spatial autocorrelation techniques to those applied in Chapter 5, I went on to assess spatial distributions of mitochondrial haplotypes amongst red deer natal to the North Block study area in Chapter 6. I found evidence of SGS in females and, to a lesser degree, in males. The spatial autocorrelation of haplotypes in females declined over time, and this was explained by spatial expansion of the population as the population size increased in response to the release from culling. I also explored the possibility of selection acting on mitochondrial haplotype, but found no evidence for selection on haplotype at either matrilineal or individual level.

7.2.1 Spatio-temporal variation in genetic structure linked to demography, behaviour and the environment

The results in Chapters 4 to 6 of this thesis present clear evidence of the spatially and temporally dynamic nature of fine scale SGS. The power of the anthropogenic, biotic and abiotic environment to influence population demography and individual behaviour and phenotype is well known, but it is rare to be able to investigate its consequences for genetic structure in a natural setting (Bossart and Pashley Prowell, 1998). Our ability to do so here is attributable to the remarkably detailed long-term

life history, census and genetic databases available for the North Block red deer population. The quite remarkable "open air laboratory" approach to deer management between the early 1970s and 2001 on Rum, which examined demographic consequences of variation in culling regime, has also presented unusual opportunities to relate these changes to spatial genetic structure across the island, and within the North Block study area specifically (Clutton-Brock *et al*, 1982, Clutton-Brock *et al*, 2002). The evolutionary consequences of such temporal changes in fine scale SGS are unknown, but their presence should be noted in future quantitative genetic (especially QTL) studies and may influence the spatial scale at which selection occurs, and the potential for kin selection (Coltman *et al*, 2003b, Garant *et al*, 2005).

The analysis presented here illustrates the profound effects human activity can have on phylogeographic structure in managed vertebrate populations. Although well documented now in domestic species, such effects have rarely been shown using mtDNA-based phylogenies in game species. The results in Chapter 4 are certainly the starkest molecular demonstration to date of the extent of human translocation of red deer across Europe in recent and historical times. The introductions of red deer to Rum in the last century and a half involved, presumably unbeknownst to the island's owners, the genetic mixing of ancestrally highly divergent matrilines. Broad-scale phylogeographic analysis of European red deer presented to date show relatively neat geographic separation of haplotypes (e.g. Randi *et al*, 2001, Ludt *et al*, 2004). More detailed analysis based on extensive sampling within smaller spatial regions (of the kind undertaken by Zachos *et al*, 2003, Feulner *et al*, 2004) is revealing a more

muddled phylogeographic picture, in which the profound effects of human introductions are becoming clear.

The genetic evidence for male-biased dispersal both across the entire Rum deer population (Chapter 4) and within the North Block study area (Chapters 5 and 6) is no surprise. However, spatial autocorrelation techniques proved a useful means of revealing the extremely fine-scale spatial genetic structure produced by matrilocal behaviour amongst females in the North Block (Figures 5.3 and 6.3). We already know that management strategy has affected both male dispersal (Clutton-Brock *et al*, 2002) and female social behaviour (Albon *et al*, 1992), via its effects of environmental conditions (specifically levels of competition for mates or food). However, results in Chapter 4 illustrate how population genetic analysis can reveal the potential discrepancies between levels physical dispersal and gene flow. If the hypothesis of restricted effective male dispersal between the north and south of Rum is true, then there is increased potential for both random drift and selection acting on genetically structured sub-populations to result in micro-evolutionary divergence of the deer on Rum in future.

The complete release of the North Block red deer population from culling in 1972 has had a variety of demographic, phenotypic and ecological consequences, which are discussed throughout this thesis. These changes appear to have had significant consequences for spatial genetic structure amongst female deer in the population, which I have been able to document over a 24-year study period. I have been able to discount natal dispersal within the North Block as a force driving temporal change in the spatial structuring of microsatellite genotypes, which would be impossible in a population lacking such detailed individual-based data. I instead

argued that changes in the male and female breeding population size, or historical events prior to the study commencing, were responsible for the change. The potential role of historical events in these changes in SGS is worth considering in further detail. The introduction of red deer to Rum may have involved genetically differentiated groups of red deer arriving on the island at various points over an 80 year period. The time taken for the genetic structure between deer from different introduced mainland populations to break down as a result of male dispersal and interbreeding once on Rum would depend on a number of unmeasured factors. These include the number of deer of each haplotype introduced, the degree of nuclear genetic differentiation at introduction, and the degree of interbreeding subsequent to introduction. Mitochondrial spatial structure across the island (Figure 4.4) and in the North Block (Figure 6.2) is strong and suggests very limited female movement since introduction. The temporal changes in genetic structure documented in Chapter 5 could represent ongoing island-wide breakdown in structure associated with original introduction events.

The analyses in Chapters 4 to 6 should illustrate clearly that assuming constancy in any parameter likely to influence population genetic structure (e.g. environment, demography, or behaviour) is problematic. I have shown temporal variation in spatial genetic structure in the red deer population on Rum and explained these in terms of known changes in environment, demography, behaviour and phenotype. "Snapshot" studies of mammalian population structure, using genetic samples taken at a single time point, are prevalent and provide useful insights into the evolutionary biology and ecology of these species (Sugg *et al*, 1996, Storz, 1999). However, given the highly dynamic nature of the relationship between

environment, demography and behaviour in generating spatial genetic structure, the generality of the findings in such studies across space and time certainly requires careful consideration.

7.2.2 Future directions

Further work regarding the phylogeography of red deer in both the UK and Europe would be interesting. It would be useful to know the source of the 'RUM A' haplotype within the UK and its prevalence in the rest of Scotland, given its potential importance from a conservation genetics standpoint. Ongoing work investigating mitochondrial variation in red deer across the west of mainland Scotland has yet to identify any mtDNA haplotypes similar to the RUM A or 'African' type sequences (Pérez-Espona, *pers. comm.*). Further research may lead to an understanding of the extent of human translocation of red deer across Britain and Europe in recent and historical times and potentially locate native British haplotypes that might be of conservation interest.

Analysis of spatial genetic autocorrelation at fine scales in other mammal species would also be useful. Currently, only a handful of species have been investigated in this way (Wasser and Elliott, 1991, van Staaden *et al*, 1996, Richardson *et al*, 2002, Coltman *et al*, 2003b, Peakall *et al*, 2003, Hazlitt *et al*, 2004). Autocorrelation techniques represent qualitative descriptions of spatial structure: variation in spatial scale, marker type, and autocorrelation measure used makes comparison between studies problematic. However, a statistic recently developed and used by Vekemans and Hardy (2004) for a comparative analysis in plants ('*Sp*')

represents a quantification of spatial genetic structure based on spatial autocorrelation analysis that is readily derived from published studies. Meta-analyses of mammalian spatial autocorrelation analyses in the literature using this statistic are likely to shed light on the evolutionary role of SGS on kin selection, cooperation, dispersal and social structure. These autocorrelation techniques have been developed with reference to plant population genetics (Hardy and Vekemans, 2002, Vekemans and Hardy, 2004). Unfortunately, red deer (and mammals in general) are usually a little more mobile than the average plant and as such the use of spatial measures like mean annual position or capture location for individuals that have home ranges of variable sizes and shapes is questionable, although in many case unavoidable (e.g. Coltman *et al*, 2003b, Hazlitt *et al*, 2004, Nussey *et al*, 2005b). Collaborative work currently underway will seek to investigate SGS in the North Block study population with respect to individual annual or lifetime home ranges using GIS techniques.

Selection on mitochondrial variation in the North Block was considered in Chapter 6. The further possibility that selection is involved in the changes observed in the spatial distribution of microsatellite alleles over time in the North Block represents a rather more difficult analysis to undertake. In a similar vein to the work questioning the neutrality of mtDNA discussing in Chapter 6, research is increasingly pointing to the same assumption being invalid in some cases for microsatellites through linkage with genes associated with fitness, and several studies of the North Block red deer population on Rum have revealed associations between microsatellite variation and fitness (Coulson *et al*, 1998, Coulson *et al*, 1999). Further work, utilising novel analytical methods ('de-lifing'; Coulson *et al*, *in press*), is planned to investigate selection on microsatellite alleles in this population.

Finally, the possible role of historical events at an island-wide scale in the observed changes in female SGS within the North Block, as well as further genetic investigation of the consequences of spatial variation in management regime, may be addressed by repeating the analysis undertaken in Chapter 4 using older tissue or bone samples from previous culls across the island. Evidence for stability in spatial structuring of nuclear genotypes across the island in previous decades would argue against a cross-island breakdown in structure following introduction events as a mechanism underlying the temporal trends described in Chapter 5. Samples from culls in 1991 or earlier might also reveal a great deal more about the effects of the ten year deer management experiment (Clutton-Brock *et al*, 2002) on effective male dispersal across the island.

7.3 Final Thoughts

Analyses presented in Chapters 2-6 of this thesis clearly demonstrate the importance of long-term data sets from wild populations in evolutionary ecology. To fully understand micro-evolutionary and ecological dynamics, we need to be able to complement laboratory and theoretical research initiatives with analyses of natural populations that experience complex ecological interactions that vary over space and time. The environment affects individual life histories and population genetic structure in complex ways under natural conditions, through subtle interactions with individual physiology, behaviour and population demography. Long-term individual based studies, like that of the North Block red deer population on Rum, allow us to detect the impacts of spatio-temporal environmental variation on important

evolutionary mechanisms such as phenotypic plasticity and fine-scale spatial genetic structure. More importantly, they also reveal the ecological parameters that underpin these effects of environmental variation.

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Appendix A:

Red deer microsatellite markers

Fifteen microsatellite markers are now regularly used for genotyping red deer in the North Block study area on Rum as part of ongoing paternity analysis. The table shows the sequence data for each primer for each microsatellite marker. Various markers from this list were used for genotyping in Chapters 4-6 of this thesis.

Marker		Sequence	Reference
FBC304	F	CCCTAGGAGCTTTCAATAAAGAATCGG	(1)
	R	CGCTGCTGTCAACTGGGTCAGGG	
JP27	F	GCAAATCAGAAATAGACCCACAGAC	(2)
	R	GATCCCCTCCTTGTGCCAC	
CP26	F	GGCCTAACAGAATTCAGATGATGTTGC	(3)
	R	GTCACCATACTGACGGCTGGTTCC	
VH54	F	ATGGTTACTGAATGGCTGCCTAACCC	(4)
	R	CCTTAGGAACTAATGTGCACTTGTATGTG	
JP38	F	CTGCACAGAGTCGGACACAAC	(2)
	R	CCAGATTATTCCAGTGTATTGCC	
JP15	F	GGAAATACCTTATCTTTCATTCTTGACTGTGG	(2)
	R	CCTTCTTTCTCATTGCTAACTTATATTAAATATCC	
FCB5	F	GACCTGACCCTTACTCTCTTCACTC	(1)
	R	AAGTTAATTTTCTGGCTGGAAAACCC	
RT1	F	TGCCTTCTTTCATCCAACAA	(5)
	R	CATCTTCCCATCCTCTTTAC	
MAF109	F	GGAAGATTAGAACTTTCATATATCTTTAAACTC	(6)
	R	TAATTGAATTTGAAGTGTATATGCCTAAATGC	
FCB193	F	GCTTGGAAATAACCCTCCTGCATCCC	(7)
	R	TTCATCTCAGACTGGGATTCAGAAAGGC	
TGLA94	F	CATCAAAACAGTGAAGGATGATTGCCAG	(8)
	R	CGAATCTCTTCTAGGGATTGAGACTGTG	
INRA011	F	CGAGTTTCTTTCCTCCTGGTAGGC	(9)
	R	GCTCGGCACATCTTCCTTAGCAAC	
TGLA322	F	CATGCCACCTCTTGTCTGAAA	(8)
	R	CTTTAACATGGTTTAAATGACTATT	
TGLA127	F	CAATTGTGTGGTAGTTTGGACATTC	(8)
	R	ACACTATTGCAAAAGGACCTCCAATT	
INTRA035	F	ATCCTTTGCAGCCTCCACATTG	(9)
	R	TTGTGCTTTATGACACTATCCG	

References for red deer microsatellite markers:

(1) Buchanan, FC, Galloway, SM and Crawford, AM (1994). Ovine Microsatellites at the Oarfcb5, Oarfcb19, Oarfcb20, Oarfcb48, Oarfcb129 and Oarfcb226 Loci. *Animal Genetics* **25**, 60-60.

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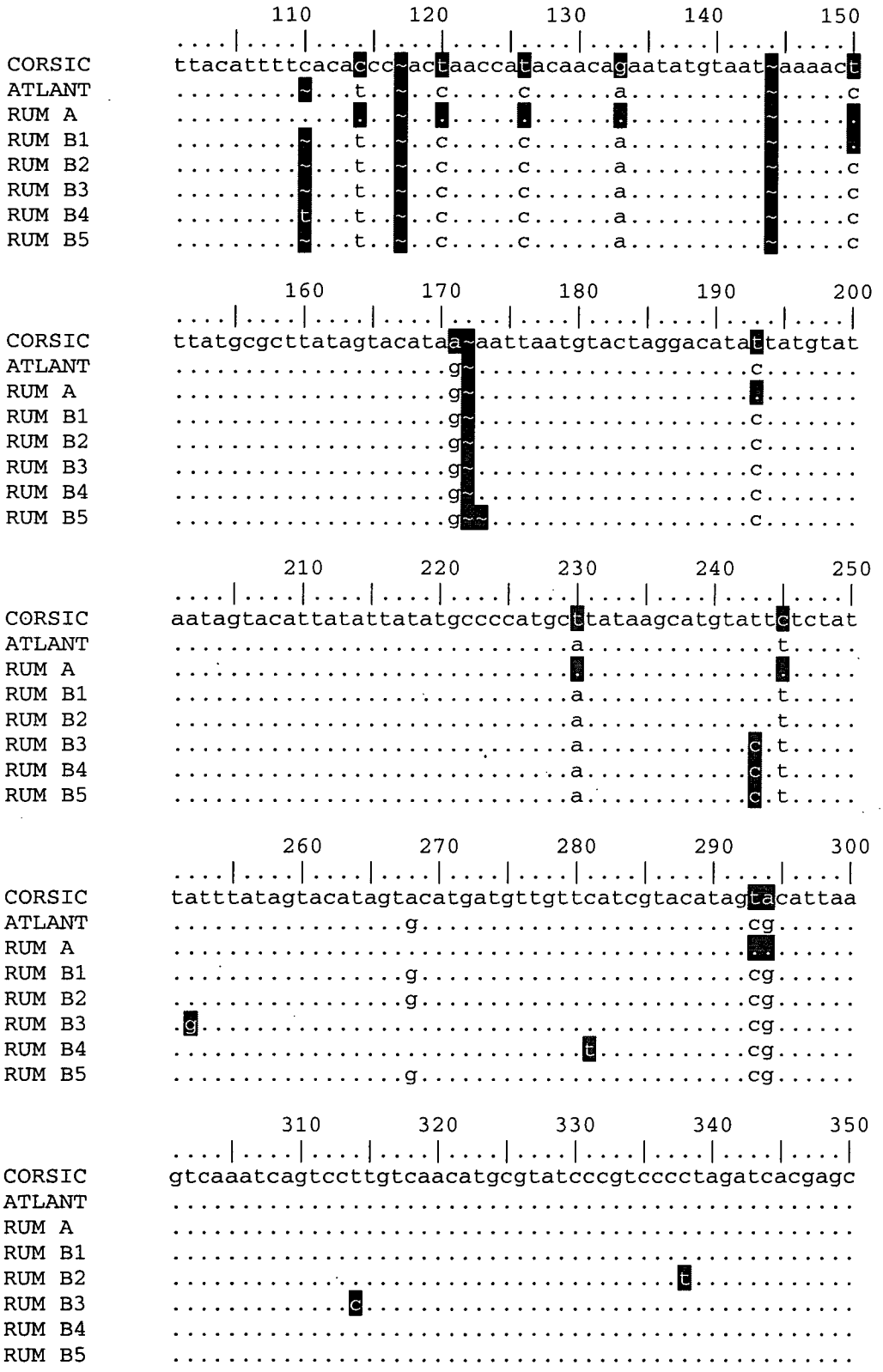
Appendix B:

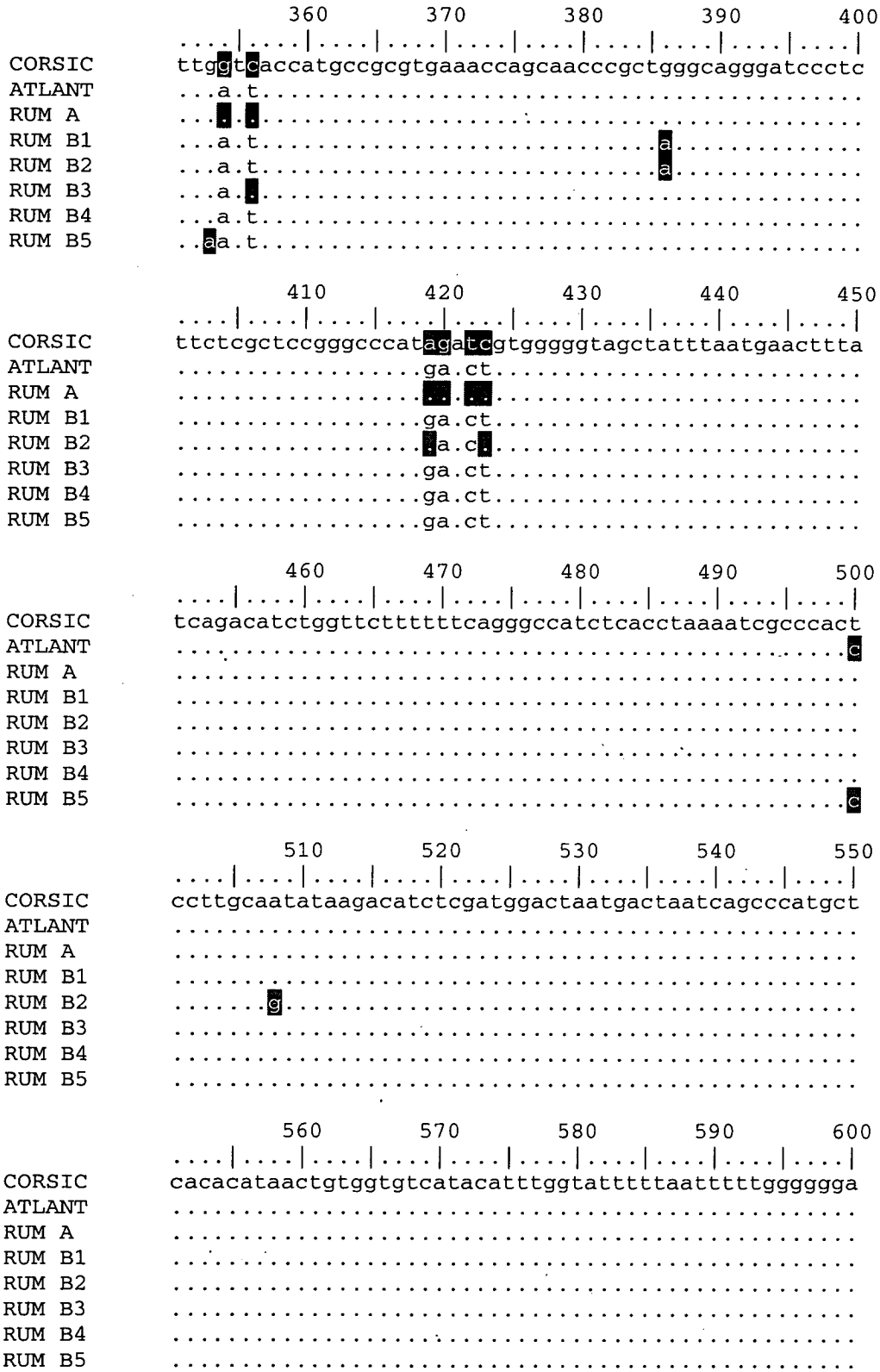
Alignment of red deer mitochondrial control region sequences

An alignment of the five 922 base pair sections mitochondrial control region haploptypes identified amongst red deer on the Isle of Rum in this thesis. Red deer sequences from GenBank from Sardinia (CORSIC) and Norway (ATLANT) are included (see Table 4.2 for more details). Sequences are aligned to the CORSIC sequence. Dots indicate nucleotides are shared with this sequence. Substitutions and deletions are highlighted.

```
                  10        20        30        40        50
            ....|....|....|....|....|....|....|....|....|....|
CORSIC      acgcttattaatatagttccataaaaatcaagaactttatcagtattaaa
ATLANT      .t..................................................
RUM A       .-..................................................
RUM B1      .t..................................................
RUM B2      .t..................................................
RUM B3      .t..................................................
RUM B4      .t..................................................
RUM B5      .t.........................c........................

                  60        70        80        90       100
            ....|....|....|....|....|....|....|....|....|....|
CORSIC      tttccaaaaagttttaatatttcaatacagctttccactcaatacccatt
ATLANT      ..........................................c.........
RUM A       ..........................................-.........
RUM B1      ..........................................c.........
RUM B2      ..........................................c.........
RUM B3      ..........................................c.........
RUM B4      ..........................................c.........
RUM B5      ..........................................c.........
```


110 120 130 140 150
CORSIC ttacattttcacaccc~actaaccatacaacagaatatgtaat~aaaact
ATLANT .....~...t..~..c.....c......a.....~.....c
RUM A .....~...t..~..c.....c......a.....~.....c
RUM B1 .....~...t..~..c.....c......a.....~.....c
RUM B2 .....~...t..~..c.....c......a.....~.....c
RUM B3 .....~...t..~..c.....c......a.....~.....c
RUM B4 .....~...t..~..c.....c......a.....~.....c
RUM B5 .....~...t..~..c.....c......a.....~.....c
160 170 180 190 200
CORSIC ttatgcgcttatagtacataa~aattaatgtactaggacatattatgtat
ATLANT .....g~.....c.....
RUM A .....g~.....c.....
RUM B1 .....g~.....c.....
RUM B2 .....g~.....c.....
RUM B3 .....g~.....c.....
RUM B4 .....g~.....c.....
RUM B5 .....g~~.....c.....
210 220 230 240 250
CORSIC aatagtacattatattatatgccccatgcttataagcatgtattctctat
ATLANT .....a.....t.....
RUM A .....a.....t.....
RUM B1 .....a.....t.....
RUM B2 .....a.....t.....
RUM B3 .....a.....c.t.....
RUM B4 .....a.....c.t.....
RUM B5 .....a.....c.t.....
260 270 280 290 300
CORSIC tatttatagtacatagtacatgatgttgttcatcgtacatagtacattaa
ATLANT .....g.....cg.....
RUM A .....
RUM B1 .....g.....cg.....
RUM B2 .....g.....cg.....
RUM B3 .g.....cg.....
RUM B4 .....t.....cg.....
RUM B5 .....g.....cg.....
310 320 330 340 350
CORSIC gtcaaatcagtccttgtcaacatgcgtatcccgtcccctagatcacgagc
ATLANT .....
RUM A .....
RUM B1 .....
RUM B2 .....t.....
RUM B3 .....c.....
RUM B4 .....
RUM B5 .....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RUM A .....
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RUM B3 .....ga.ct.....
RUM B4 .....ga.ct.....
RUM B5 .....ga.ct.....
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RUM B2 .....
RUM B3 .....
RUM B4 .....
RUM B5 c
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RUM B2 .....g.....
RUM B3 .....
RUM B4 .....
RUM B5 .....
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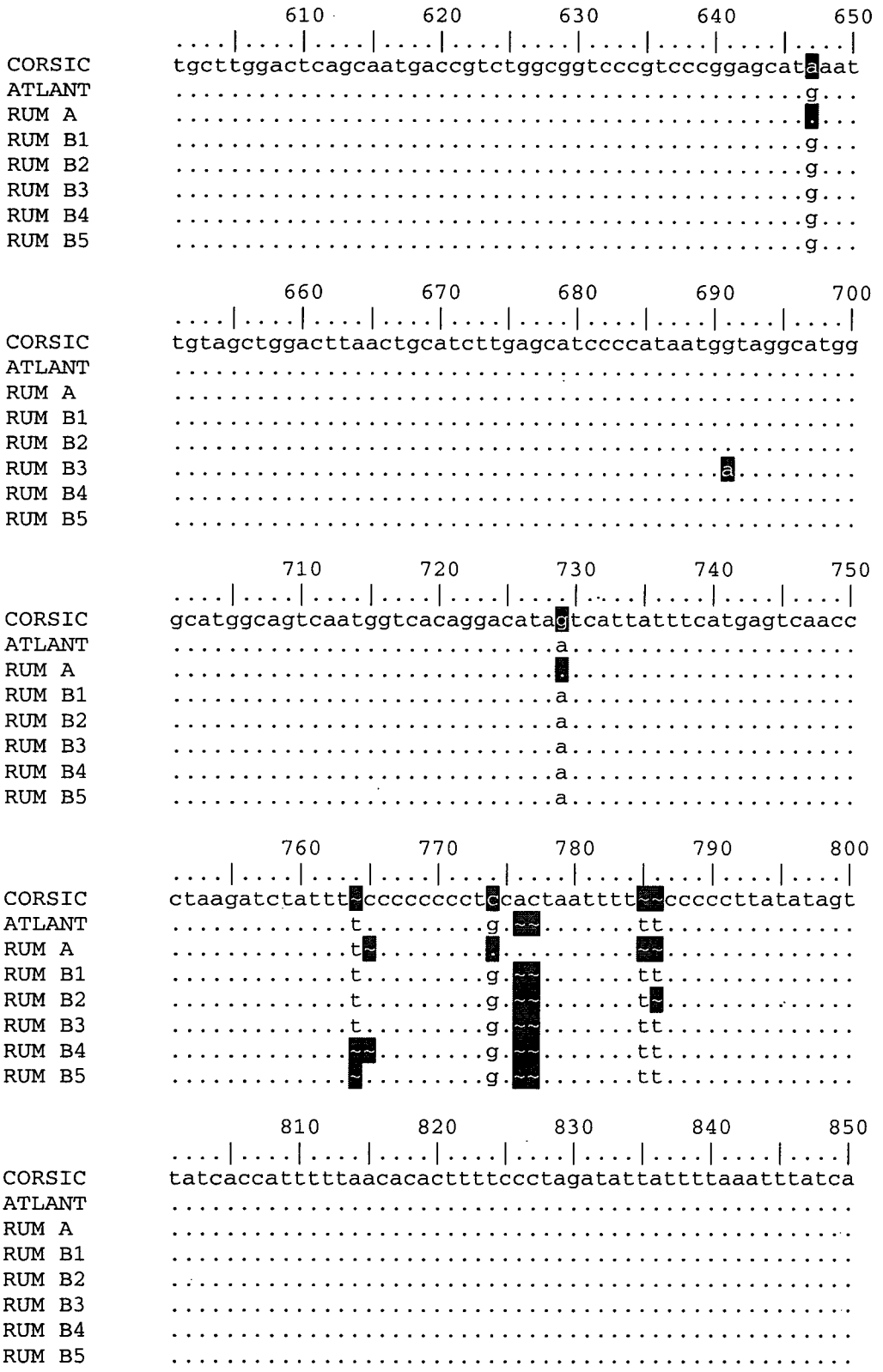
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RUM B4 ...g...
RUM B5 ...g...
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RUM A ....
RUM B1 ...a...
RUM B2 ...a...
RUM B3 ...a...
RUM B4 ...a...
RUM B5 ...a...
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RUM B3 ...t...g...tt...
RUM B4 ...g...tt...
RUM B5 ...g...tt...
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ATLANT ...
RUM A ...
RUM B1 ...
RUM B2 ...
RUM B3 ...
RUM B4 ...
RUM B5 ...

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RUM B4     ..................c.....................c..........
RUM B5     ..................c.....................c..........

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RUM B1     ..................t...
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RUM B3     ..................t...
RUM B4     ..................t...
RUM B5     ..................t...
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Appendix C:

Generalised linear mixed effects models for individual fitness traits in red deer

Generalised linear mixed effects models for four individual fitness traits in red deer natal to the North Block study area on the Isle of Rum (see Section 6.3.6 for details). Samples sizes and error structures used are listed above the table for each trait. Fixed-effects back transformed estimated effects for factors are only listed if the factor was significant in the model, except for mtDNA haplotype for which all effects are listed. Significant terms are in bold.

C.1 Neonatal Survival (N = 1,042; binomial error structure)

Random Effects			
Term		Standard Deviation	
Year of birth		0.96	
Residual		0.96	
Fixed Effects			
Term	Wald / df	P	Estimate
Sex	0.09	0.77	
Mother's reproductive status	1.21	0.30	
Birth weight	1.53	0.22	
Natal sub-division	**13.76**	**<0.001**	
Samhnan Insir			0.76
North Kilmory Glen			0.76
South Kilmory Glen			0.51
mtDNA haplotype	0.03	0.86	
RUM A			0.69
RUM B			0.68

C.2 Winter Calf Survival (N = 690; binomial error structure)

Random Effects			
Term		Standard Deviation	
Year of birth		2.56	
Residual		0.87	
Fixed Effects			
Term	Wald / df	P	Estimate
Sex	**16.78**	**<0.001**	
Female			0.76
Male			0.60
Mother's reproductive status	**4.74**	**<0.001**	
Milk			0.72
Naïve			0.67
Summer Yeld			0.82
True Yeld			0.69
Winter Yeld			0.48
Birth weight	**20.55**	**<0.001**	0.35
Natal sub-division	**8.26**	**<0.001**	
Samhnan Insir			0.56
North Kilmory Glen			0.61
South Kilmory Glen			0.84
mtDNA haplotype	0.01	0.92	
RUM A			0.68
RUM B			0.69

C.3 Longevity (N = 367; normal error structure)

Random Effects				
Term		Standard Deviation		
Year of birth		6.38		
Residual		19.28		
Fixed Effects				
Term		Wald / df	P	Estimate
Sex		**17.96**	**<0.001**	
	Female			
	Male			
Birth weight		2.22	0.14	0.29
Natal sub-division		1.49	0.23	
mtDNA haplotype		0.87	0.35	
	RUM A			7.42
	RUM B			8.43

C.4 Lifetime breeding success (N = 247; poisson error structure)

Random Effects				
Term		Standard Deviation		
Year of birth		0.07		
Residual		3.57		
Fixed Effects				
Term		Wald / df	P	Estimate
Sex		**29.70**	**<0.001**	
	Female			5.03
	Male			2.63
Birth weight		**4.50**	**<0.05**	0.10
Natal sub-division		0.59	0.56	
mtDNA haplotype		0.12	0.73	
	RUM A			3.50
	RUM B			3.77

Appendix D:

Publications arising from this thesis

The following pages contain material arising from this thesis that has been published or is currently in press:

Daniel H. Nussey, Tim H. Clutton-Brock, Steve D. Albon, Josephine Pemberton & Loeske E. B. Kruuk (*in press*) Constraints on plastic responses to climate change in red deer. *Biology Letters.* [Online early version]

Daniel H. Nussey, Tim H. Clutton-Brock, David A. Elston, Steve D. Albon & Loeske E. B. Kruuk (2005) Phenotypic plasticity in a maternal trait in red deer. *Journal of Animal Ecology*, **74**, 387-396.

Nussey, D.H., Coltman, D. W., Coulson, T., Kruuk, L.E.B., Donald, A., Morris, S. J., Clutton-Brock, T.H. & Pemberton, J. (2005) Rapidly declining fine-scale spatial genetic structure in female red deer. *Molecular Ecology*, **14**, 3395-3405.

Nussey, D. H., Postma, E., Gienapp, P., & Visser, M. E. (2005) Selection on heritable phenotypic plasticity in a wild bird population. *Science*, **310**, 304-306.

biology letters

Biol. Lett.
doi:10.1098/rsbl.2005.0352
Published online

Constraints on plastic responses to climate variation in red deer

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Influences of climate on life history traits in natural populations are well documented. However, the implications of between-individual variation in phenotypic plasticity underlying observed trait–environment relationships are rarely considered due to the large, long-term datasets required for such analysis. Studies typically present correlations of annual trait means with climate or assume that individual phenotypic responses are constant. Here, we examine this additional level of variation and show that, in a red deer population on the Isle of Rum, Scotland, changes in climate generate changes in phenotype only amongst individuals who have experienced favourable ecological conditions. Examination of relationships between offspring birth weight and spring temperature within the lifetimes of individual females revealed that the tendency to respond to climate declined as the population density experienced early in life increased. The presence of such systematic variation in individual plasticity is rarely documented in the wild, and has important implications for our understanding of the environmental dependencies of traits under varying ecological conditions.

Keywords: phenotypic plasticity; constraint; life history; *Cervus elaphus*; climate

1. INTRODUCTION

The influence of climatic variation on life history traits in natural populations is well documented (Stenseth *et al.* 2002). Many observed relationships between life history traits and the environment can be attributed to individual organisms expressing different phenotypes across their lifetimes in response to the conditions they experience (or phenotypic plasticity; e.g. Both *et al.* 2004). Such life history plasticity at the individual level may be the result of condition-dependence: climate influences an individual's physiological condition, constraining the expression of costly traits or altering life history decisions (Stevenson & Bryant 2000). Changing environmental circumstances, such as increased resource competition, may also impact on individual physiological condition and thus alter individual responses to climate. However, the effect of environmental deterioration on individual responses to the environment in wild vertebrate populations remains unexplored.

To date, studies of vertebrate life history responses to the environment have typically assessed the correlation between annual population means for a given trait with an environmental variable (Post & Stenseth 1999; Both *et al.* 2004) or utilised models that assume a constant response of all individuals in the population (Przybylo *et al.* 2000). The presence of and reasons for individual variation in life history responses to climate are rarely examined; however, this variation will underpin any population's ability to track environmental change over a prolonged period (Nussey *et al.* 2005).

Here, we explore individual variation in a maternal life history trait (offspring birth weight)–climate (spring temperature) relationship in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland (Clutton-Brock *et al.* 1982). Previous studies of this population, treating birth weight as a trait of the offspring, have revealed that annual average birth weights are heavier following warm springs (Albon *et al.* 1987). This relationship is presumably driven by condition-dependence in maternal investment late in gestation: warm temperatures improve grazing conditions and hence pregnant females' physiological condition, allowing greater investment in foetal growth (Albon *et al.* 1987). A female's physiological condition may also be affected by the environmental conditions she experiences, and early experiences of environmental quality are known to have persistent effects on performance later in life (Kruuk *et al.* 1999; Post & Stenseth 1999). One of the key determinants of the quality of the environment in the Rum study area is adult female population density. Density has steadily increased through the 1970s following the cessation of culling in 1973, reaching carrying capacity in the early 1980s around which it has since fluctuated (figure 1). This increase in resource competition has generated declines in numerous measures of performance, such as juvenile survival and adult fecundity (Clutton-Brock *et al.* 1987; Kruuk *et al.* 1999). We examined the effects of this increase in population density and corresponding decline in environmental quality on the strength of the birth weight–spring temperature relationship within females.

2. METHODS

All data were collected on red deer in the North Block of the Isle of Rum, Scotland (a 12 km^2 study area located 57°01′ N, 06°17′ W) between 1971 and 2000. Female deer give birth to a maximum of one offspring per year, usually in late May or June. Extensive daily surveys of the study population during this period meant that the timing of births was well known and most calves were caught and weighed within a few days of birth. Birth weights were calculated as follows: birth weight = capture weight (kg) − 0.01539 × age at capture (hours) (see Clutton-Brock *et al.* 1982 for further details). Weather variables were obtained from a Meteorological Office weather station on Rum, and spring temperature was defined as the average daily maximum temperature through the months of April and May (as in Albon *et al.* 1987). There was no evidence of a linear change in spring temperatures across the study period ($F_{1,28}=0.63$, $p=0.44$).

Variation in offspring birth weights attributable to the significant effects of female's reproductive history (as a five level factor, defined

Received 17 May 2005
Accepted 26 May 2005

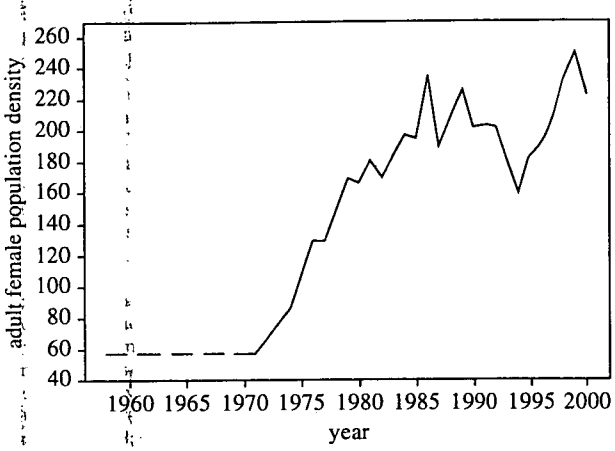

Figure 1. Line plot showing the female population density (number of resident females observed in more than 10% of January to May censuses of the study area over 1 year of age), an indicator of environmental quality in the study area, over time. Females born before regular censusing began were assumed to have experienced densities equal to those in 1971 (dashed line).

following Coulson *et al.* 2003) and age (as a quadratic), as well as offspring sex and date of birth, was removed, and residual birth weights used in the analyses that follow. An individual female's plasticity was defined as the slope of a linear regression of her offspring's residual birth weight measurements on the spring temperatures she experienced in the year of each birth. Only females with measurements on four or more offspring were included in the analysis (190 out of 414 females). Mean plasticity and its coefficient of variation (as a percentage) were calculated.

We examined the effects of early experiences of population density on the strength of the birth weight–spring temperature relationship by grouping females according to the density in their year of birth (see figure 2), and regressing plasticity estimates on density. Population density was defined as the number of females of over 1 year of age that were present in more than 10% of January to May North Block censuses that year. Density estimates are available from 1971; however, many reproductive females in the data set were born earlier than this. Since the main cause of change in density over the study period has been the demographic consequences of a release from culling in 1973 (Clutton-Brock *et al.* 1982), we assume that density has been constant prior to 1971 (figure 1). In addition, we ran a mixed-effects model in S-PLUS v. 6 (Insightful Corp.) on all available birth weight data (1557 observations from 414 different females). We included the terms used in the calculation of residual birth weights described above as fixed effects, and female identity as a random effect. The inclusion of the random effect for female means the model assesses the significance of fixed effects against variation in birth weight within females. We tested for changes in the dependency of birth weight on spring temperature with density by fitting spring temperature, density in a female's year of birth and their interaction to the model as fixed effects and assessing their direction and significance (see Electronic Appendix).

3. RESULTS

Estimates of individual plasticity confirmed that on average females gave birth to heavier offspring following warm springs: the average female offspring birth weight–spring temperature slope was 0.17 kg $°C^{-1}$. There was considerable variation between females in the strength of their plastic response to spring temperature (coefficient of variation (%) = 469.07).

There was a significant negative effect of density in year of birth on a female's plasticity ($F_{1,8} = 12.55$, $p < 0.01$). Individual responses to spring temperatures declined as females experienced higher population densities early in life (figure 2). Plasticity decreased by 36% of its mean value for each additional 20 individuals present in the population in a female's year of birth. The generality of this reduction in plasticity with population density is substantiated by the presence of a highly significant negative interaction between spring temperature and population density in the mixed-effects model of birth weight including all available data ($F_{1,1133} = 21.52$, $p < 0.001$; see Electronic Appendix).

4. DISCUSSION

We have shown here that plastic responses to climatic variation may vary between individuals experiencing different ecological conditions. As the density of female red deer in the Rum study area increased to carrying capacity (figure 1), the relationship between offspring birth weight and spring temperature within individual females declined to zero (figure 2). There are several non-exclusive mechanistic explanations for the changes in birth weight–spring temperature correlations amongst individuals. Changes in individual plastic responses could explain the observed trend: reductions in birth weight plasticity with increasing density could be the result of adaptive changes in female investment, which reduce the risks to mothers of sustaining large foetuses when density is high and food is scarce. Also, reductions in plasticity may occur because high density reduces the variability of condition in females, limiting the ability of superior females to increase pre-natal investment in warm springs. An alternative explanation could be that individual responses to spring temperature are reduced because, as a result of increased grazing pressures, warm springs do not generate significant increases in primary productivity when density is high.

A variety of alternative explanations exist, but whatever the underlying mechanisms, the presence of systematic variation in individuals' plastic responses to climate has implications for the way we interpret trait–environment relationships in long-lived organisms. Correlation of annual mean offspring birth weights with spring temperatures in our study population would suggest that, should springs get warmer, offspring birth weights would increase. Analysis conducted here at the individual level implies that, as long as the population density remains high, systematic changes in spring temperatures will not affect offspring birth weights. Many studies examining annual trait means have likewise shown density-independent effects in wild vertebrate populations (see, for example, Post & Stenseth 1999; Both *et al.* 2004). Findings presented here at the individual level suggest that our ability to detect such environmental dependencies in life history traits may depend on the ecological conditions experienced by the population in question.

Analysis of variation in individual phenotypic plasticity in wild populations requires detailed information on individuals over long time series, and few studies will be able to meet these requirements.

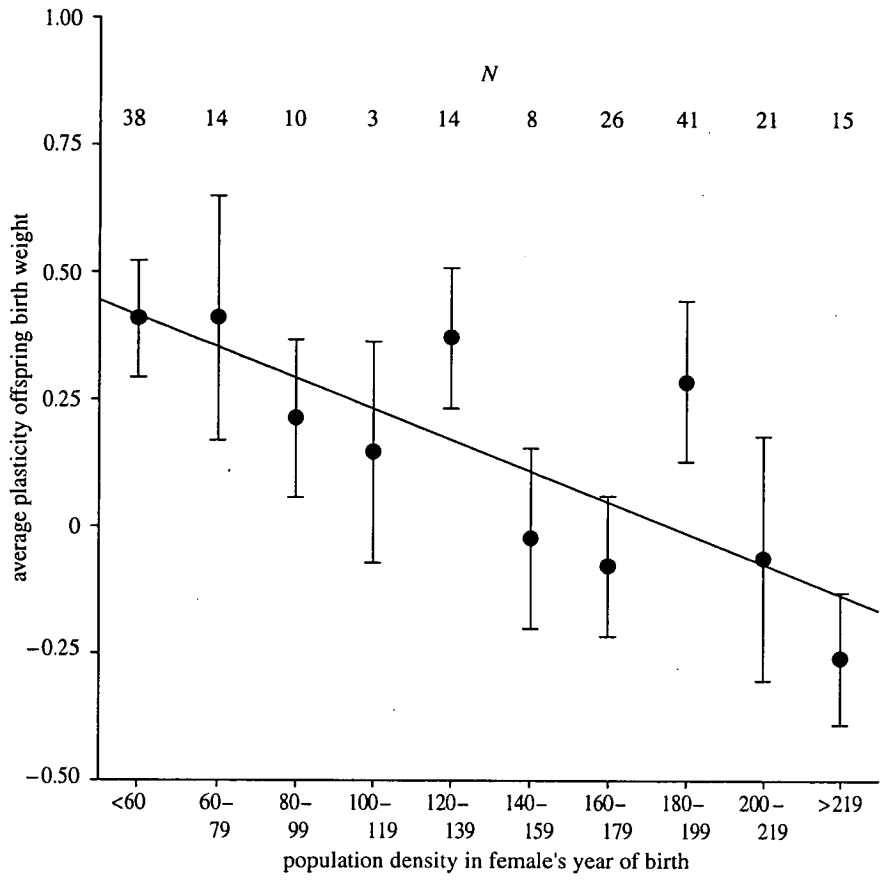

Figure 2. The tendency for individual female red deer to give birth to heavier offspring following warm springs declines with deteriorating environmental conditions. The plot shows mean regression slope estimates (±s.e.) of individual females' residual offspring birth weight–spring temperature relationship (in kg °C^{-1}) grouped by the densities that females experienced in their year of birth, with sample sizes for each density grouping indicated above the error bars. The regression line through these means is plotted ($b = -0.0030$ kg °C^{-1} female^{-1} ± 0.0009 s.e.; $p < 0.01$; $r^2 = 0.61$).

However, our analyses illustrate that the presence or absence of trait–climate relationships at the population level under one set of ecological conditions (such as rising population density) may not hold true under different conditions (such as a population at carrying capacity). Recent studies have documented variation in trait–climate correlations between geographically isolated populations (e.g. Both *et al.* 2004). Our findings hightight that whilst an effect of local ecological conditions on individual plasticity may explain observed variation between populations, it would also make extrapolation of results from one population to another problematic. Furthermore, in circumstances where there are adaptive benefits to environmental dependency in a given trait, a reduction in overall plasticity may be ultimately detrimental to population viability. By implication, populations may have to rely on the slower process of microevolution through a shift in their genetic composition to provide adaptation to changing environmental conditions.

The authors thank Scottish Natural Heritage for permission to work on Rum and their local staff for support. We thank Alison Donald, Sean Morris, and Fiona Guinness and many other field workers on the Kilmory deer project, as well as Tim Coulson, Alastair Wilson, and three anonymous referees for comments on earlier drafts of the manuscript.

This work was supported by NERC (via a Ph.D. studentship to D.H.N., and research grants to T.H.C.B. and J.P.) and the Royal Society (L.E.B.K.).

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The supplementary Electronic Appendix is available at http://dx.doi.org/10.1098/rsbl.2005.0352 or via http://www.journals.royalsoc.ac.uk.

Journal of Animal Ecology 2005
74, 387–396

Phenotypic plasticity in a maternal trait in red deer

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Summary

1. Phenotypic plasticity and microevolution represent the two processes by which phenotypic traits in a population can track environmental change. While there is a growing literature documenting microevolution in reproductive traits in naturally occurring animal populations, few studies to date have examined either between-individual variation in levels of plasticity or how selection acts on plasticity.

2. We present here mixed-effect linear models analysing changes in calving date in relation to autumn rainfall observed over a 30-year study of 2147 red deer on the Isle of Rum, Scotland. The study period is characterized by a phase of low and rising population density (up to and including 1980), followed by a phase of high and fluctuating population density (1981 to present).

3. Variation within individual females explained a population-level trend of delayed calving dates following years of high autumn rainfall. There was significant variation between females both in their average calving dates and in their individual plastic responses of calving date to autumn rainfall.

4. Females born in the low population density phase were, on average, phenotypically plastic for the calving date–autumn rainfall relationship, and showed significant variation in plasticity. Selection favoured individuals with early average calving dates among these females.

5. Among females born at high population density, there was on average no significant plasticity for calving date, but variation in plastic responses was still present. Selection favoured females with increasingly positive plastic responses of calving date to autumn rainfall.

6. We argue that early experience of high population density affects the physiological condition of females, making an environmental response (calving early following dry autumns) in later life physiologically untenable for all but a few high quality individuals. These same few individuals also tend to be fitter and have higher reproductive success.

Key-words: *Cervus elaphus*, natural selection, phenotypic plasticity, rainfall, timing of breeding.

Journal of Animal Ecology (2005) **74**, 387–396
doi: 10.1111/j.1365-2656.2005.00941.x

Introduction

Phenotypic plasticity, defined as the expression of multiple phenotypic states by a single genotype under different environmental conditions (Houston & McNamara 1992), is a ubiquitous and widely documented phenomenon in naturally occurring animal populations (Gotthard & Nylin 1995). Within-individual phenotypic plasticity represents one important means by which populations can track environmental changes. The other is microevolution: a change in genotypes across generations in response to selection on a trait. Assessing the relative importance of these two processes is crucial to our understanding of the evolutionary and ecological dynamics of populations, and depends on the development and

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application of suitable techniques capable of distinguishing them. However, we know very little about between-individual variation in phenotypic plasticity, or how selection acts on plasticity where such variation exists, in wild animal populations. Furthermore, the effects of environmental conditions or physiological state on individual phenotypic plasticity in natural populations are largely unknown. Long-term data sets on individually marked and monitored animals of relatively long-lived species provide an ideal opportunity to investigate these issues. We present here an analysis of phenotypic plasticity in a maternal trait in red deer.

An individual's response to the environment can be estimated using regression coefficients to describe changes in the value of a phenotypic trait expressed in different environments. Analysis would generate estimates of an individual's elevation (reflecting the expected trait value in the average environment) and slope (the plastic response to the environmental gradient). While this approach has been developed within the theoretical framework of quantitative genetics following the reaction norm approach (Via *et al.* 1995), it is also applicable to studies utilising individual optimization or life-history approach to phenotypic plasticity (Smith 1991). Under this framework, an individual's response to the environment is the result of condition-dependent decision making, and each individual is considered to be following its optimal trait–environment trajectory (Roff 1992).

Pigliucci (2001) described four general and distinct patterns of phenotypic plasticity (Fig. 1). Assuming there is variation between individuals in their mean phenotypic value for a given trait (i.e. individual estimates of elevation) a population might, on average, show a plastic response in the phenotype to an environmental gradient (Fig. 1b,c) or not (Fig. 1a,d). A population showing no average plasticity can still contain individuals that are plastic if there is variation in plasticity (as in Fig. 1d). Distinguishing between these patterns in any population is important for our understanding of a population's ability to respond to the environment.

The regression approach to modelling phenotypic plasticity has already been applied to maternal traits such as breeding date or clutch size that occur repeatedly within individual females across varying environmental condition. Przybylo, Sheldon & Merilä (2000) investigated phenotypic plasticity of laying date within female collared flycatchers (*Ficedula albicollis*) in response to climatic variations. Using a similar approach, in which a female's identity was fitted as a random effect within a mixed model, Réale *et al.* (2003) generated estimates of a negative linear relationship between parturition date and food availability in female red squirrels (*Tamiasciurus hudsonicus*) with multiple breeding records. The presence of a significant breeding time–environment relationship within these models indicated that the trend was present within individual females and was explained,

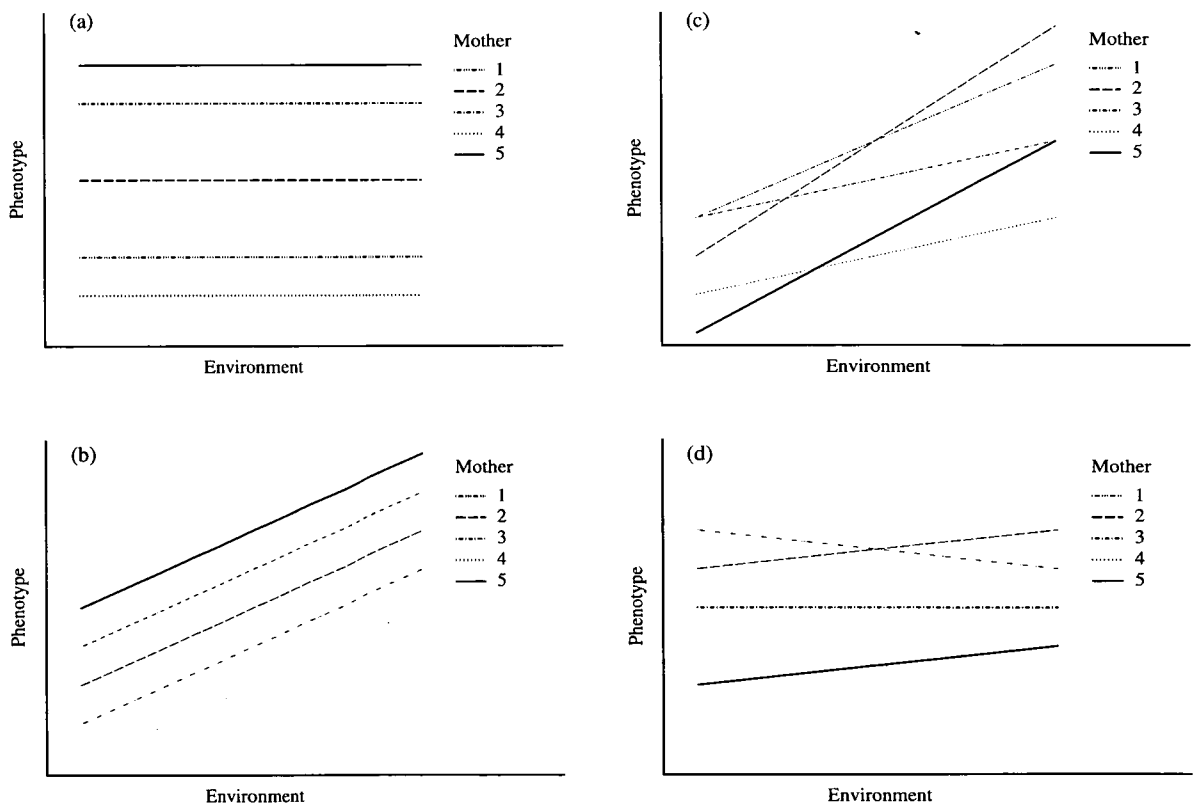

Fig. 1. Line plots of phenotypic trends across an environmental gradient for five maternal genotypes, illustrating the four main patterns of plasticity (adapted from Pigliucci 2001): (a) variation in elevation (trait means) but no average plasticity or variation in plasticity; (b) average plastic response without variation in plasticity; (c) average plastic response with variation in plasticity; (d) no overall plastic response but variation in plasticity.

to a large degree, by maternal plasticity (see also Schiegg *et al.* 2002).

The studies above demonstrated that females within the respective populations showed, on average, a plastic response to environmental variation, but they did not assess the degree to which females varied in their plasticity. Thus patterns of plasticity shown in Fig. 1a,d could be discounted, but the analyses could not distinguish which of the remaining two patterns best described the data: a population in which all individuals are effectively showing identical plastic responses, or one in which they vary in their response. To address this issue, a random effect for individual females' slopes of the phenotypic trait with an environmental variable can be fitted to a mixed model, in addition to female identity. Significant variance between individual slopes would indicate variation in the plasticity of females, allowing discrimination between the patterns illustrated in Fig. 1b,c.

Plasticity itself can be regarded as a phenotypic trait on which selection may act. Selection can occur on plasticity only if there is variation in the phenotypic response of individuals to the environment (as in Fig. 1c,d). Where variation exists, fitness differences between individuals of differing plasticity levels will generate selection on plasticity. The pattern of selection on plasticity in a population is theorized to be dependent on the amount of environmental variation experienced by the organisms in question. Where environmental variation is large, we might expect there to be selection on plasticity, while under constant conditions selection should act on individuals with favourable average trait values (de Jong 1995). Levels of ecological stress experienced by individuals can also affect selection on life-history traits. Under taxing environmental conditions, the expression of plasticity or any phenotypic trait is more likely to be constrained by its physiological cost to an individual, and may therefore show a correlation with fitness not apparent under favourable conditions (Mueller 1997; Pigliucci 2001).

Selection on individual estimates of elevation (the individual's expected trait value in the average environment) and slope (the individual's plasticity in the trait in response to the environmental variable) can be assessed if a suitable measure of individual fitness is available (Weis & Gorman 1990). Brommer, Pietiäinen & Kolunen (2003) adopted this approach to examine individual female reaction norms for clutch size–laying date relationships in Ural owls (*Strix uralensis*). They found significant variation in both coefficients (elevation and slope) and, using lifetime reproductive success as a maternal fitness measure, showed that selection favoured females with larger clutch sizes but was not acting on plasticity of clutch size with respect to laying date. This is the only study, to our knowledge, that has used this approach to look for selection on individuals' plastic responses in a reproductive trait in a wild animal population.

There are empirical data suggesting that within population variation in plastic responses exist and can be important (Lorenzon, Clobert & Massot 2000; Paschke, Bernasconi & Schmid 2003). Furthermore, theoretical and laboratory work suggests that individual plasticity may be influenced by the experiences of an individual in development and early life (Pigliucci 2001) and may alter in response to environmental conditions experienced by an individual (Tammaru, Ruohomaki & Montola 2000; Van Kleunen & Fischer 2003). However, to date no study has investigated changes in phenotypic plasticity and selection on it within a natural population experiencing environmental change.

PHENOLOGICAL PLASTICITY IN RED DEER

The present study examines patterns of female phenotypic plasticity and selection on plasticity for calving date in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland, in which female reproductive behaviour and success have been monitored extensively for over 30 years. In this population, significant correlations between calving date and both population density and climatic conditions around the time of conception and early pregnancy have been documented (Clutton-Brock, Guinness & Albon 1982; Fig. 2). Variation in offspring birth date reflects variation in both oestrous date, which is entirely under maternal control, and gestation length, which is determined partly by both mother and offspring. Studies of parturition date in mammals have therefore varied in assignment of the trait to the mother (e.g. Réale *et al.* 2003) or the offspring (e.g. Clutton-Brock *et al.* 1987a). Here, because we wish to examine within-female variation in response to different environmental conditions, we treat calving date as a maternal trait.

Increasing population density and worsening weather conditions are thought to result in environmental deterioration and reduced food availability, and hence decreased female physiological condition in red deer (Guinness, Albon & Clutton-Brock 1978). If an individual female's physiological condition determines her timing

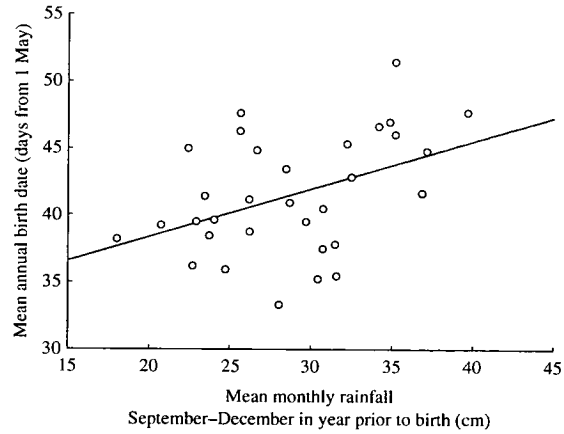

Fig. 2. Scatter-plot showing the mean monthly rainfall between September and December in year prior to birth and annual average birth date (days after 1 May), with regression line ($b = 0{\cdot}36 \pm 0{\cdot}10$ se).

of oestrus and gestation length – and ultimately her calving date – then the observed correlations between environment and calving date at the population level are most probably the result of condition-dependent responses to the environment by those females. A previous study has examined selection on neonatal traits in this population at the level of the offspring, and revealed that parturition date affects offspring fitness in a complex manner, via variable pathways (Coulson *et al.* 2003). However, the role of within-female variation in explaining trends in parturition date has yet to be explored or discussed in this population.

Two distinct phases of population density have been observed in the study population between the time regular censusing began (1973) and the present: a low-density phase (–1980), followed by a high-density phase (1981–present) during which the population density has fluctuated about an average value from year to year, suggesting it has reached habitat carrying capacity (Albon *et al.* 2000). There is strong evidence that conditions early in life influence individual life histories in this population (Albon, Clutton-Brock & Guinness 1987; Langvatn *et al.* 1996), and that these effects are especially notable for females (Kruuk *et al.* 1999a). Experimental work from other systems suggests that phenotypic plasticity may vary with the conditions experienced by an individual across its lifespan (Tammaru *et al.* 2000). With this in mind, we investigated the effects of individual females' early lifetime experiences of population density on observed patterns of maternal plasticity.

The aims of this study were therefore: (i) to examine the role of within-individual variation in explaining known environmental trends in calving date among breeding females in the Rum red deer population; (ii) to investigate natural selection on maternal plasticity; and (iii) to assess any differences in patterns of plasticity and selection on maternal responses of calving date to the environment between females experiencing low and high population densities.

Materials and methods

STUDY POPULATION

All data used were collected in the North Block study area of the Isle of Rum, Scotland, between 1971 and 2002. The red deer population within this area has been monitored extensively since the 1960s, and culling of the population within the confines of the study area stopped completely in 1973 (Clutton-Brock *et al.* 1982). Females in the population do not necessarily breed every year, and can produce a maximum of one calf per year. Females come into oestrous and conceive from late September onwards, with most conceptions occurring in October (Guinness *et al.* 1978). Calves are usually born in late May or June, and are generally weaned by October (Clutton-Brock *et al.* 1982).

After the cessation of culling, the density of resident adult females in the study area increased steadily to around 180 individuals in 1981. Since then, growth has stabilized and annual density has fluctuated between 160 and 249 females (Albon *et al.* 2000). Females were grouped according to which population-growth phase they were born in, either up to and including 1980 ('low density') or after 1980 ('high density'). While the reproductive lives of these females were not discrete (Fig. 3), there is extensive evidence that this split divides females meaningfully in terms of their experience of resource competition. Previous studies have shown that females born in the high-density population phase, that is after 1980, have reduced longevity, less chance of reproducing during their lifetime, and lowered fecundity, when compared to females born up to and including 1980 (Kruuk *et al.* 1999a).

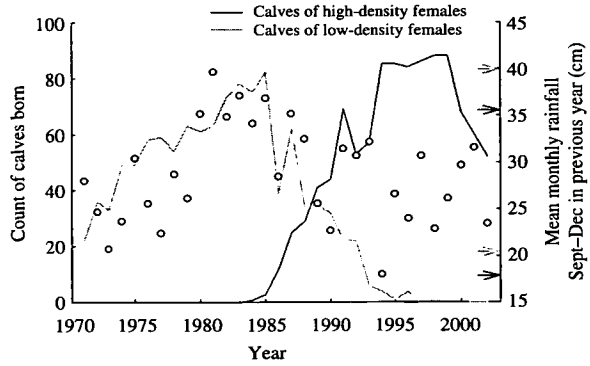

Fig. 3. Line and scatter-plot showing the number of calves born per year of the study period to low (born up to and including 1980, grey line) and high (born after 1980, black line) density females, and mean monthly rainfall between September and December of the previous year ('autumn rainfall', open circles). Grey arrows indicate the range of autumn rainfall conditions experienced by low-density females, black arrows the range experienced by high-density females (an autumn rainfall value was included within these ranges if > 10 individuals experienced it).

MONITORING AND MEASUREMENT

Females that breed regularly within the study area can be recognized from artificial and natural markings (Clutton-Brock *et al.* 1982). Regular censuses of the area, throughout the year, provide information on population density, as well as adult and juvenile mortality and hence individual survival and reproductive success. Daily censuses are conducted throughout the calving period to provide records of birth date, while continuous monitoring of the study area during these months means that the vast majority of calves born can be caught, sexed and weighed, and ensures that neonatal mortalities are recorded (Clutton-Brock *et al.* 1982).

The life-history and environmental variables used in this study are described below. Variables refer to conditions in years in which individual calves were born, unless otherwise stated. Available data from between 1971 and 2002 were used.

Calving date: estimated date of calving, expressed in number of days after 1 May.

Female's age: the age of the female in years at a given breeding event, determined either through knowledge of the mother's year of birth or, for cases where females were born before monitoring began (approximately 2% of individuals), from tooth wear analysed postmortem (Clutton-Brock *et al.* 1982).

Female's reproductive status: categorized as follows according to a female's reproductive status in the year previous to a given breeding event (see Coulson *et al.* 2003 for details).

- *Milk*: female had given birth the previous year and her calf was still alive on 15 May of following year.
- *Naïve*: female had not bred previously.
- *Summer yeld*: female had given birth the previous year and her calf had died before 1 October of that same year.
- *True yeld*: female had bred before but had not given birth the previous year.
- *Winter yeld*: female had given birth the previous year and the calf had died over the following winter (between 1 October and 15 May).

Climate variables: mean monthly precipitation levels (cm) and temperatures (°C) were obtained from a Meteorological Office weather station on Rum.

Female lifetime reproductive success (LRS): the total number of offspring that survived to 2 years of age produced by a given female.

Statistical analysis

We analysed data from females with available records for at least two breeding events (2147 events for 406 females over 32 years), because individual slope estimates cannot be derived for individuals with a single data point. We repeated the analyses described below restricting the data to females with five or more breeding records. This yielded very similar results to those presented. All continuous explanatory variables were centred on their mean value prior to inclusion in the analysis (Pinheiro & Bates 2000), and linear mixed models (LMMs) were fitted using the restricted maximum likelihood (REML) method.

All data analysis was conducted using GENSTAT version 6·1 (VSN International).

MIXED-EFFECTS LINEAR MODELS OF CALVING DATE

We used the same fixed-effects terms as Coulson *et al.* (2003) to generate a maximal model for calving date, but with an expanded data set (including 4 more years of data). Climate covariates were estimated as comparisons between years, and so should be tested against unexplained year-to-year variation (Milner, Elston & Albon 1999). Consequently, a random effect for offspring's year of birth was added to this initial maximal model, which was used to screen covariates before moving onto the more advanced models. The significance of fixed-effects terms was assessed by referring Wald statistics divided by their degrees of freedom against quantiles of appropriate *F*-distributions. Non-significant fixed effects were dropped from the final model in a step-wise fashion until only those significant at the 5% level remained.

The final model of calving date used in the analyses that follow contained offspring's year of birth as a random effect and the following fixed effects terms: reproductive status, female's age and its quadratic term and mean monthly rainfall between September and December prior to a calving (henceforth, 'autumn rainfall'). Autumn rainfall was the only climate variable found to be a significant predictor of calving date (as in Coulson *et al.* 2003). Across the study period examined here (1971–2002) calving dates were positively correlated with autumn rainfall (see Fig. 2).

The mixed model structure described above was then extended to test patterns of variation in individual plasticity of calving date, keeping the fixed effects model unchanged. The significance of adding terms to the random effect model was assessed by referring changes in the model deviance to χ^2 distributions, with degrees of freedom determined by the number of additional parameters in the random effect model (Self & Liang 1987). In models with both individual-specific elevations and slopes we allowed for the potential correlation between these, to ensure that BLUP estimates produced by the models were not affected by the method used to centre covariates.

The fixed-effects estimate for an environmental covariate produced by a LMM including female identity as a random effect can be taken to represent the average plastic response to that variable within all females. A significant fixed-effect for autumn rainfall, for example, would indicate that, on average, individual females are plastic for calving date (i.e. that the population is showing the pattern of plasticity shown in Fig. 1b or 1c). A significant difference in deviance between LMMs with and without a random slope term for rainfall would indicate significant between-female variation in their plastic response of calving date to rainfall (i.e. patterns shown in Fig. 1c or 1d, as opposed to 1a or 1b). This approach allows one to determine which of the four patterns of plasticity shown in Fig. 1 best describes that observed in the population.

To identify the pattern of plasticity best describing females from each of the two population density groups in isolation, we ran separate LMMs with the final mixed effect structure determined for the entire population for each group.

NATURAL SELECTION ON PHENOTYPIC PLASTICITY

Selection on phenotypic plasticity was assessed by the association between an individual female's elevation and slope and her lifetime reproductive success (LRS) (Weis & Gorman 1990), which accounts for differential survival of offspring (although see Coulson *et al.*

1997 for a more detailed exploration of the pathways through which selection acts on birth date). Individual females were excluded from the analysis if they were still alive in 2002 or had been shot during culls in adjacent parts of the island (as in Kruuk *et al.* 1999b; Coulson *et al.* 2003), as under either scenario their LRS values would not be accurate representations of natural or complete individual fitness. However, re-running the analysis including living individuals did not affect the results obtained.

Because differences were found in the patterns of plasticity among females of the low- and high-density groups (see below), selection analyses were conducted on each of these groups of females separately. Estimates of elevation and slope for individual females were treated as separate but correlated traits. Best-linear unbiased predictors (BLUPs) for random effects within the mixed-effects models were used as estimates of individual elevation and slope; these were standardized so that they were in standard deviation units and had a mean of zero (following Lande & Arnold 1983). BLUPs are estimates of random effects independent of other terms within a model, standardized to have a mean of zero. They are much less sensitive to extreme values within the data than separate regression estimates (Pinheiro & Bates 2000). Selection on these estimates was measured by regressing relative female LRS, which showed adequate normality of error structure, on the standardized BLUP values for elevation and slope, their squares and their cross-product (Lande & Arnold 1983).

Results

PATTERNS OF PLASTICITY FOR CALVING DATE

Tests comparing LMM deviances revealed that the random effect for female identity explained a significant amount of residual variation (Table 1). This indicated that there was significant variation in the average calving date of the 406 individual females within the data set. Further tests revealed that the addition of a random effect for each female's calving date–autumn rainfall slope improved the model fit significantly. These results, shown in Table 1, imply that there was significant variation in the plastic responses of females' calving dates to autumn rainfall.

In the model with all random effects, the fixed-effect estimate of autumn rainfall indicated that calving dates were delayed by an average of 0·41 (± 0·15 SE) days cm^{-1} of rain: there was an average within-female response significantly greater than zero. It appears that the trend in calving date with autumn rainfall observed at the population level is largely explained by within-individual variation at the maternal level.

Across the study period (1971–2002), females: (i) varied in their average calving dates; (ii) showed, on average, plasticity of calving date with respect to autumn rainfall; (iii) varied in the magnitude of their plastic response to autumn rainfall, a pattern equivalent to that in Fig. 1c.

As predicted, females experiencing high population densities showed a reduced response to autumn rainfall. Low density females responded by 0.36 days cm^{-1} of rain more than high density females, a marginally non-significant difference (female density group * autumn rainfall interaction: $t = 1.44$, $P = 0.08$, one-sided t-test). LMMs were run for each of the two female density groups separately. Table 2 shows that variation in individual calving date–autumn rainfall slopes was very similar in the high- and low-density groups of females (0·56 ± 0·32 SE and 0·52 ± 0·21 SE, respectively), while females in the high-density group varied more in their calving date elevations than those at low density (35·13 ± 9·97 SE compared with 23·80 ± 7·87 SE). Correlations between

Table 1. The significance of adding random effects to the linear mixed models of calving date, showing deviance estimates and log-likelihood ratio test statistics. Ticks indicate differences in the random effects fitted in respective models. All models were fitted with the following fixed-effects: female's reproductive status, female's age and its quadratic term, and mean monthly rainfall in centimetres between September and December in year prior to birth ('autumn rainfall'). Significant differences between models, based on χ^2 distributed log-likelihood tests, indicated in bold type ($^*P < 0.05$; $^{**}P < 0.01$)

	Random variables included in mixed-effect model						
		Maternal variables					
Model	Offspring year of birth	Identity	Slope for autumn rainfall	Deviance of model	d.f.	Test	Log-likelihood test statistic
Across study period (1971–2002)							
1	√			14385·85			
2	√	√		14344·87	1	1 vs. 2	**40·98****
3	√	√	√	14329·91	2	2 vs. 3	**14·96****
Offspring of females born at low population density (up to and including 1980)							
4	√	√		7457·19			
5	√	√	√	7446·65	2	4 vs. 5	**10·54****
Offspring of females born at high population density (1981–2002)							
6	√	√		6885·81			
7	√	√	√	6879·55	2	6 vs. 7	**6·26***

Table 2. Estimates of fixed and random effects produced by a linear mixed-model for calving dates (days after 1 May) for study data set split by mother's year of birth; (a) before 1980 (1117 calves, 185 mothers) and (b) between 1981 and 1997 (1030 calves, 221 mothers). Only calves of females with more than one calf available for analysis in each model were included. 'Autumn rainfall' represents the mean monthly rainfall between September and December in the year prior to calving in centimetres. Estimates for reproductive status are factor level means. The key estimates to note with reference to the text are presented in bold type

	(a) Up to and including 1980		(b) 1981–2002	
Random effects	Variance component	SE	Variance component	SE
Offspring year of birth	18·0	7·8	9·4	5·6
Female elevation	**23·80**	**7·87**	**35·13**	**9·97**
Female slope on autumn rainfall	**0·52**	**0·21**	**0·56**	**0·32**
Residual	248·3	12·3	246·5	13·5
Fixed effects	Estimate	SE	Estimate	SE
Reproductive status				
Milk	44.70	1.38	46.37	1.47
Naïve	40.19	2.16	44.08	1.96
Summer yeld	36.73	1.62	34.55	1.70
Winter yeld	46.95	2.06	44.94	1.91
Yeld	40.14	1.41	37.84	1.38
Female's age	−2·92	0·97	−2·26	1·21
Female's age^2	0·18	0·05	0·15	0·06
Autumn rainfall	**0·52**	**0·20**	**0·18**	**0·21**

elevations and slopes in the two groups were also different: both were negative, but females born at high densities showed a closer relationship between elevation and slope ($r = -0{\cdot}35$ and $-0{\cdot}10$). Females born at high density that showed early calving dates in the average environment were more likely to also show a strong positive response to autumn rainfall.

Females born up to and including 1980 (low density) showed an average positive plastic response to autumn rainfall of $+0{\cdot}52 \pm 0{\cdot}20$ SE days cm^{-1} of rain per month (see Table 2). These females are giving birth earlier in response to dry autumn conditions, and vary significantly in their responses ($\chi^2 = 10{\cdot}54$, d.f. = 2, $P < 0{\cdot}05$, Table 1). This pattern of plasticity is illustrated by Fig. 1c.

Among females born at high density, there was a substantially reduced average response of calving date to autumn rainfall ($+0{\cdot}18 \pm 0.21$ SE days cm^{-1} of rain per month, Table 2), which was not significantly different from zero. However, there was still variation between females in their plastic responses to autumn rainfall ($\chi^2 = 6{\cdot}26$, d.f. = 2, $P < 0{\cdot}05$, Table 1). This situation is described visually in Fig. 1d.

Variation exists in the calving date–autumn rainfall response of individual females in both the low- and high-population density phases of the study, and so selection may be acting on these traits.

NATURAL SELECTION ON PHENOTYPIC PLASTICITY

Multiple regression analyses of female LRS revealed that selection pressures on females' calving date–autumn rainfall slopes differed between the two density groups (Table 3). Among females born at low density, there was directional selection on elevation, favouring earlier calving dates in the average environment, but no selection on slope (i.e. no direct selection on plasticity). The presence of a marginally non-significant interaction between elevation and slope in this model suggests that a slight fitness advantage is conferred to females responding to dry autumns by calving early if they also have early calves in the average environment, but this advantage declines with increasing female elevation. Among females born at high population density, a strong

Table 3. Multiple regression of linear, quadratic, and interaction terms for best linear unbiased predictor estimates for maternal elevation and slope produced by mixed models of calving date shown for: (a) females born up to and including 1980 ($n = 178$); and (b) those born after 1980 ($n = 87$) on lifetime reproductive success. Intercepts were fitted in both regressions but are not shown

	(a) Up to and including (1980)			(b) 1981–2002		
Coefficient	Estimate	SE	*P*-value	Estimate	SE	*P*-value
β (elevation)	−0·69	0·18	< 0·01	0·17	0·24	0·48
γ (elevation^2)	0·14	0·12	0·27	0·02	0·05	0·70
β (slope)	0·01	0·20	0·96	0·80	0·42	0·06
γ (slope^2)	0·10	0·08	0·22	0·30	0·19	0·12
γ (elevation × slope)	−0·23	0·13	0·09	0·15	0·16	0·36

but marginally non-significant selection gradient was present on individual slope, suggesting that high-density females giving birth early following dry autumns and late following wet autumns had higher LRS.

The magnitudes of the selection gradients on elevation among low-density females and slope among high-density females are notably high when compared with recent estimates of median selection gradients in natural populations, although the standard errors associated with these are large (–0·69 ± 0·18 SE and 0·80 ± 0·42 for low- and high-density groups, respectively, compared to an absolute median of 0·17 from Kingsolver *et al.* 2001).

A possible factor influencing the shift in selection on females' plastic responses between these two groups could be differences in their experiences of autumn rainfall. There was no evidence of a linear temporal trend in autumn rainfall across the study period ($b = -0{\cdot}03 \pm 0{\cdot}10$ SE cm per year, d.f. = 32, $t = 0{\cdot}27$, $P > 0{\cdot}05$), or any difference in the mean autumn-rainfall conditions experienced by the two groups (*t*-test comparing mean autumn rainfall in years in which 10 or more females from either low- or high-density groups bred (see Fig. 3): $t = 0{\cdot}89$, d.f. = 32, $P > 0{\cdot}05$). While it does appear that the high-density group of females experienced slightly lower variation in autumn rainfall, it is clear that there was still considerable variation in rainfall across their lifetimes (Fig. 3).

Discussion

We have shown here that individual plasticity can explain the environmental trends observed between calving date and autumn rainfall among hinds in the Rum North Block red deer population. Increased precipitation around the time of mating and early pregnancy results in environmental deterioration and reduced food availability for individual females (Clutton-Brock, Albon & Guinness 1987b). Increased rainfall may also impact directly on the physiological condition of females, for example through increased thermoregulatory costs. It has been hypothesized that the variation in calving date is due largely to physiological condition-dependent variation in a female's timing of oestrus and gestation length (Clutton-Brock *et al.* 1982). The finding that changes in birth date with environmental conditions occur at the level of the individual female supports this hypothesis. The few previous papers that have investigated maternal plasticity in naturally occurring animal populations (Przybylo *et al.* 2000; Schiegg *et al.* 2002; Brommer *et al.* 2003; Réale *et al.* 2003) have all shown it to have a role in observed phenotypic trends, and here we add further evidence that individual plasticity is an important and often overlooked component of variation in reproductive traits.

The observed variation between females in mean calving date is not surprising and can be ascribed to differences in genetic or non-genetic components of individual quality (Clutton-Brock, Guinness & Albon 1983). However, to date few studies have examined or discussed variation in responses to the environment between reproductive females in naturally occurring populations (although see Brommer *et al.* 2003). Across the study period we observed variation in females' response of calving date to autumn rainfall. If we assume the plastic response of a female's calving dates to autumn rainfall to be the result of physiological condition-dependent decision making, then we can see that the optimal response to rainfall may vary depending on a female's physiological condition at the start of her reproductive cycle (i.e. August or September).

Females born at low population densities respond to dry autumns by giving birth earlier. Females born at high densities appear to show a weaker average response to autumn rainfall conditions. While several papers have discussed possible evolutionary limitations to plasticity (Gotthard & Nylin 1995; de Witt, Sih & Wilson 1998), to our knowledge this is the first ecologically or physiologically mediated shift in phenotypic plasticity observed in a naturally occurring animal population. This result emphasizes the importance of utilizing long-term data sets for such analyses and the potential for ecological or environmental changes to influence phenotypic plasticity in the wild.

Why are female deer born at high population densities not responding to autumn rainfall conditions? Differences in patterns of rainfall experienced by the female density groups could be responsible. Reduced environmental variation might mean that there is simply less scope for high-density females to show plasticity. However, there was no evidence of a significant difference between the autumn rainfall conditions experienced by the two groups.

We argue that the patterns of plasticity observed are largely the result of a trade-off within females between the physiological costs of early calving and the costs imposed by high population density. There is extensive evidence of early environmental conditions affecting adult breeding behaviour and lifetime reproductive success (Albon *et al.* 1987; Kruuk *et al.* 1999a), and of high population density increasing the cost of reproduction for females (Clutton-Brock *et al.* 1983), in this population. It therefore seems likely that early experience of nutritional stress, owing to intense resource competition, can affect physiological condition later in life. The absence of differences in plasticity following categorization of the data by offspring birth year rather than its mother's (data not shown) also suggests that consistent differences in plasticity between females are determined by conditions during early development.

Females born at higher population densities are likely to be in poorer condition at the start of the reproductive cycle compared to those born at low density. For many individuals this may mean that responding to favourable autumn conditions by calving early is physiologically out of the question. However, variation in individual quality and condition is still present, and it may be that only those few females in relatively good condition despite high population density can afford to give birth early following drier autumns. This would

result in the substantially reduced, statistically non-significant response to the environment observed within females born at high density. The presence of increased variation in elevation and a stronger negative correlation between elevation and slope among high-density females supports the argument that only a small number of these females are both breeding early in the average environment and responding to autumn rainfall.

Females giving birth early when autumns are dry are likely to reap fitness benefits as a result of early birth dates of their offspring (more time for calves to feed and grow before the winter) and the ability to invest more in their young (longer suckling period). At low densities, when most females are physiologically able to respond to favourable autumn conditions, selection on plasticity is apparent only in its marginally non-significant interaction with elevation. Females that give birth early in the average environment are at a selective advantage, while females that also respond to favourable conditions by giving birth early have the highest LRS.

At high density, when few females can afford to advance calving dates even if conditions are good, a direct fitness benefit of plasticity is apparent. The absence of selection on females' elevation of calving date among these females is surprising. However, the standard errors associated with the estimated selection gradients are high, so the absence of significant selection does not mean that selection on elevation is not present. It should be noted that these findings do not necessarily mean there is a direct link between plasticity and LRS. Another unmeasured variable that affects fitness (e.g. female's physiological condition) and is correlated with plasticity may be subject to more intense selection.

We have discounted the possibility that changes in the amount of environmental variation experienced by the density groups were responsible for differences in the patterns of plasticity and selection on plasticity, a possibility suggested by de Jong (1995) and others. Instead, we have argued that both early calving and experience of high population density impose physiological costs on females in this population. At low densities, most females appear to be able to meet the costs of early calving following favourable autumns, while at high population densities only those in the best condition can do so. Stressful environmental conditions are revealing the physiological cost of responding to the environment and, in turn, a correlation between plasticity and fitness not detectable under favourable conditions. These findings are backed by tentative theoretical statements made by Pigliucci (2001) and research into density-dependent selection (Mueller 1997). Further theoretical and empirical examination is now required to help determine how ecological conditions are likely to affect the responses to individuals to the environment.

Conclusions

This study illustrates how mixed models can provide a valuable and readily available means of analysing patterns of plasticity in reproductive traits, and that maternal plasticity for calving date in red deer in response to autumn weather conditions is an important component of observed population trends in this trait. Examination of reproductive traits at the maternal level can reveal trends of ecological and evolutionary importance not apparent in analyses at the level of the offspring. We have shown that consistent changes in ecological conditions, often apparent in long-term population data sets, can influence patterns of plasticity and the way in which selection acts on plasticity. The findings presented here should encourage further exploration of plasticity for reproductive traits under some degree of maternal control, leading to a better understanding of its role within observed population dynamics in the wild.

Acknowledgements

We would like to thank Scottish Natural Heritage for permission to work on Rum, their staff for local support, Fiona Guinness, Ali Donald, Sean Morris and many other fieldworkers on the Kilmory deer project, as well as Denis Réale, Josephine Pemberton, Tim Coulson, Ben Sheldon, Nick Barton, Tim Benton, Stewart Plaistow and an anonymous referee for helpful comments and discussion. This work was funded by NERC (via a studentship to D.H.N. and a research grant to T.H.C.B.) and the Royal Society (L.E.B.K.).

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Received 10 February 2004; accepted 25 September 2004

Molecular Ecology (2005) 14, 3395–3405 doi: 10.1111/j.1365-294X.2005.02692.x

Rapidly declining fine-scale spatial genetic structure in female red deer

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Abstract

A growing literature now documents the presence of fine-scale genetic structure in wild vertebrate populations. Breeding population size, levels of dispersal and polygyny — all hypothesized to affect population genetic structure — are known to be influenced by ecological conditions experienced by populations. However the possibility of temporal or spatial variation in fine-scale genetic structure as a result of ecological change is rarely considered or explored. Here we investigate temporal variation in fine-scale genetic structure in a red deer population on the Isle or Rum, Scotland. We document extremely fine-scale spatial genetic structure (< 100 m) amongst females but not males across a 24-year study period during which resource competition has intensified and the population has reached habitat carrying capacity. Based on census data, adult deer were allocated to one of three subpopulations in each year of the study. Global F_{ST} estimates for females generated using these subpopulations decreased over the study period, indicating a rapid decline in fine-scale genetic structure of the population. Global F_{ST} estimates for males were not different from zero across the study period. Using census and genetic data, we illustrate that, as a consequence of a release from culling early in the study period, the number of breeding females has increased while levels of polygyny have decreased in this population. We found little evidence for increasing dispersal between subpopulations over time in either sex. We argue that both increasing female population size and decreasing polygyny could explain the decline in female population genetic structure.

Keywords: *Cervus elaphus*, density dependence, dispersal, polygyny, population structure

Received 10 June 2005; revision accepted 11 July 2005

Introduction

An understanding of the processes underlying population structure at fine spatial scales is central to evolutionary biology. Fine-scale population structure may facilitate kin or localized selection processes, as well as potentially confounding population and quantitative genetic research (Coltman *et al.* 2003). Recently, studies have shown such fine-scale spatial structure within populations of a variety of vertebrate taxa using genetic techniques (Shorey *et al.* 2000; Taylor *et al.* 2001; Lampert *et al.* 2003). Theoretical and empirical studies have explained such genetic structuring in terms of mating systems and dispersal patterns (Chesser 1991; Sugg *et al.* 1996; Dobson 1998). Where limited dispersal results in close spatial associations between relatives, fine-scale structure will arise (Chesser 1998). Highly polygynous breeding systems, where only a handful of unrelated males father offspring, enhance structure, as many offspring receive paternal genes from the same source and local co-ancestry will be increased (Chesser 1991).

In mammals, male-biased dispersal and female philopatry are the norm (Greenwood 1980; Clutton-Brock 1989). Females tend to remain close to their maternal relatives

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throughout their lives, often forming matrilineal social groups (Greenwood 1980). Male-biased dispersal may have evolved in concert with female philopatry and polygyny to avoid costs of inbreeding associated with bisexual philopatry (Chesser 1991). Studies of mammalian social structure through population genetics have utilized *F*-statistics (Wright 1965) to examine partitioning of genetic variance and levels of inbreeding in species showing discrete social groups. Using the social group as the subpopulation unit, numerous studies have documented F_{ST} values significantly different from zero, indicating genetic structuring between groups, as well as negative F_{IS} values, indicating less inbreeding than expected under complete breeding within groups (see Storz 1999 for review). In mammal species with fission-fusion societies showing less discrete group structure, examination of the correlation of genetic relatedness estimates with distances between pairs of individuals has also revealed structure at fine spatial scales (Coltman *et al.* 2003; Hazlitt *et al.* 2004).

The presence and implications of temporal and spatial variation in mammalian population structure have been largely ignored. However, recent research has shown that population genetic parameters may vary spatially within species or populations, specifically where habitat fragmentation differs (Peacock & Smith 1997; Stow *et al.* 2001). There is also evidence of temporal instability in such parameters (Viard *et al.* 1997; Piertney *et al.* 1999; Garant *et al.* 2000), which could itself represent an important intrinsic factor in population dynamic patterns (Lambin & Krebs 1991). Changes in the number of breeding individuals, dispersal patterns, and levels of inbreeding and polygyny would be expected to influence population genetic parameters such as fixation indices (Chesser 1991, 1998; Balloux 2004). There is evidence that variation in resource competition can alter group composition, ranging and spacing behaviour in mammals (Albon *et al.* 1992; Kilpatrick *et al.* 2001), and ultimately influence population genetic structure (Pope 1998; Aars & Ims 2000), as well as influencing male emigration and the distribution of male mating success (Clutton-Brock *et al.* 1997; Pemberton *et al.* 1999). Here, we explore temporal variation in fine-scale genetic structure in a wild red deer population and relate this specifically to variation in population size, dispersal patterns and polygyny associated with the population's recent release from culling.

Previous research on the study population

The red deer (*Cervus elaphus* L.) in the North Block study area of the Isle of Rum, Scotland, have been the subject of intensive individual-based study since 1973 (Clutton-Brock *et al.* 1982b). The feeding habitat within the North Block consists of areas of high quality *Agrostris–Festuca* grassland and poorer quality regions of heath and *Molinia* grassland (Clutton-Brock *et al.* 1982b). The population's mating system is polygynous, with males competing to dominate harems of oestrous females between September and November each year (Clutton-Brock *et al.* 1997). Male emigration is common between the ages of 2 and 5 years and is density dependent, with many males returning to the North Block later in life to rut (Clutton-Brock *et al.* 1997, 2002; Catchpole *et al.* 2004). Female emigration is rare and depends mainly on the distance between their natal area and the study area's boundaries (Catchpole *et al.* 2004). Females are loosely matrilocal, and several studies have observed close spatial and social associations between maternal relatives (Clutton-Brock *et al.* 1982a; Coulson *et al.* 1997).

Following release from culling in 1973, the number of resident adult females in the population increased throughout the 1970s and early 1980s (Clutton-Brock *et al.* 1982b). The population has been at or close to carrying capacity since the mid-1980s (Albon *et al.* 2000). Previous studies have shown rising density to be associated with reductions in female fecundity, reproductive success, and overwinter calf survival (Clutton-Brock *et al.* 1987; Kruuk *et al.* 1999; Albon *et al.* 2000), as well as increased spacing between female maternal relatives (Albon *et al.* 1992). Rising female density has also been associated with increased male juvenile mortality and early emigration, and with decreased permanent male immigration into the North Block (Clutton-Brock *et al.* 1997). The ratio of adult resident females to males in the North Block has increased sharply, resulting in an almost complete absence of males from high quality grazing areas in the recent years (Coulson *et al.* 2004). Clutton-Brock *et al.* (1997) showed that the number of males obtaining successful matings increased with adult sex ratio, and argued that rising female population density and resource competition have lead to decreased competition for mates amongst males.

The present study

Given the general pattern of female philopatry and male dispersal evident in this population, we expected to find fine-scale structuring of genotypes only amongst female red deer. However, as a consequence of the population's release from culling, changes in population size and mating system have occurred that would be expected to alter such fine-scale structure. The increase in the number of reproductive females, increasing dispersal of both sexes, and decreasing levels of polygyny might be expected to lead to a decrease in genetic structure amongst females over time. Here, we examined overall fine-scale genetic structure in males and females, changes in population structure over time, and we related any observed changes to analyses of temporal variation in breeding population size, dispersal and polygyny using the long-term census and genetic data collected from the North Block red deer population.

Materials and methods

Field data

All individual red deer in the North Block study area are recognizable as a result of either artificial marks (collars, ear tags or ear punches attached as calves or following immobilization) or natural markings. Since 1973, censuses of the North Block study area have been conducted at least five times a month between January and May (Coulson *et al.* 1997). On each census all individuals observed were identified and their position, to the nearest 100-m^2 ordinance survey (OS) grid square, was noted. Individuals were included in the analyses that follow if they were seen in at least 10% of censuses between January and May (termed 'resident' animals; see Coulson *et al.* 1997 for further discussion and justification) and were of reproductive age (≥ 3 years). Only census data from between 1978 and 2001 were analysed, unless specifically stated, to complement the available genetic data set.

We used the following parameters in our analyses.

Mean annual position. Average x and y coordinates from January to May census positions for an individual in a given year. Averages were truncated to allocate each individual to the nearest 100-m^2 OS grid square.

Between-year movement. For individuals resident in consecutive years, the distance between current and previous years' mean annual positions was calculated.

Population subdivisions. Many studies of the North Block red deer have treated the study area as a single unit, but there is evidence that fitness and behaviour vary spatially within the North Block (Clutton-Brock *et al.* 1982b). Subdivision of the study area based on habitat types and ranging behaviour has improved explanatory power in models of overwinter calf survival (Coulson *et al.* 1997), spacing behaviour (Albon *et al.* 1992), and other aspects of fitness (Guinness *et al.* 1978; Conradt *et al.* 1999). Following these studies, we split the study area into three subdivisions (Fig. 1): Shamhnan Insir (SI), North Kilmory Glen (NKG) and South Kilmory Glen (SKG). Individuals were assigned to one of the three subdivisions in each year they were resident to the population, according to their mean annual position.

Natal subdivision. An individual's natal subdivision was defined as the subdivision of his or her mother in the year following that individual's birth. Subdivisions were allocated based on January–May census data but calving takes place from May onwards, so this would represent maternal subdivision in the first year of life. Where natal subdivision could not be allocated in this fashion — typically because the individual was born before the regular censusing began — the modal subdivision across a mother's lifetime was used. This allowed natal subdivisions to be assigned to 92% of females and 87% of males resident to the study area at some point in their lifetimes.

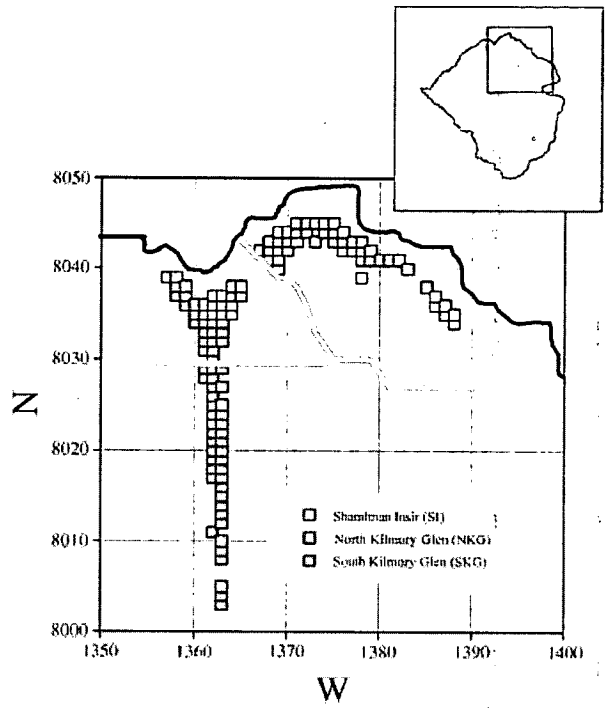

Fig. 1 Plot showing North Block study area, Isle of Rum (boxed in inset map of Rum), split into three subdivisions based on previous research. The grey lines indicate boundaries between the subdivisions. Squares indicate the regions most intensely used by adult female red deer, defined as 100-m^2 OS grid squares in which 9 or more females' mean annual positions were located across the study period (1978–2001).

Genetic data

Since 1982, approximately 85% of calves born in the study area have been caught shortly after birth and tissue and blood samples were taken for genotyping. Additionally, almost 300 animals born prior to this date were sampled by chemical immobilization or post-mortem. The analyses that follow include genetic data for resident adult deer that were alive between 1978 and 2001; however, restriction of the data set to 1982 onwards does not affect the nature of the findings presented here.

Individuals were genotyped at up to eight microsatellite loci: JP15, JP27, JP38, CP26, FCB193, FCB304, MAF109, TGLA94 (see Marshall *et al.* 1998 for further details). Not all individuals were genotyped at all loci, but individuals

with genotypes at fewer than four loci were excluded from the analysis. These loci have been previously shown to assort randomly and not to show evidence of deviance from Hardy–Weinberg equilibrium (Marshall *et al.* 1998).

Analysis of population structure

Analysis was conducted on individuals aged 3 years and older to exclude pre-reproductive juveniles and calves from the analyses. To assess differences between the sexes in fine-scale population genetic structure, geographical distances between the mean annual positions of pairs of individuals were compared to an index of genetic relatedness. Genetic relatedness coefficients (R; Lynch & Ritland 1999) and geographical distances between pairs of resident individuals were examined using SPAGEDI (Hardy & Vekemans 2002). Average *R* estimates were taken for pairs of individuals separated by distance intervals of 100 m (from < 100 m to > 2 km), in each year of the study. The analysis was conducted separately for pairs of females and pairs of males. *R* coefficients for each distance interval were averaged across years for pairs of males and females to examine overall fine-scale population genetic structure. The significance of spatial genetic structuring within each sex was assessed using linear regression of mean *R* estimates over all years on geographical distance (Hardy & Vekemans 2002). In addition to this genetic analysis of differences between the sexes in population structure, we examined the differences between males and females in movement behaviour. We compared average between-year movement for males and females at different ages (2–10 years).

Changes in spatial partitioning of genetic variance and inbreeding were assessed using *F*-statistics (Wright 1965) in which the three population subdivisions were treated as subpopulations. In each year, all resident deer were assigned to a subdivision based on their mean annual position. Separate estimates of global F_{ST} and F_{IS}, as well as pairwise F_{ST} values, were generated for females and males in each year of the study period using FSTAT (Goudet 1995). Temporal trends in these estimates were assessed using a linear regression of the *F*-statistic on year.

Global F_{ST} values significantly greater than zero indicate greater partitioning of genetic variance between groups than within groups, while pairwise F_{ST} values represent estimates of genetic differentiation between subdivisions. Negative F_{IS} values, typical of mammalian systems, imply lower than expected inbreeding within subpopulations relative to random mating. The significance of these terms was assessed using permutation tests. FSTAT assesses global F_{ST} significance by randomizing genotypes among subdivisions and global F_{IS} by permuting alleles among individuals (Goudet 1995).

Analysis of breeding population size and mating system

Using genotypic and census data, we investigated the possibility of temporal trends in the breeding population size, dispersal between population subdivisions and levels of polygyny. In all cases, changes in indices over time were assessed by linear regression on year. The following parameters were investigated.

Female breeding population size. The number of resident females giving birth to a calf in each year of the study period was taken as an index of the breeding population size. Since the main increase in both female population size and the number of breeding females occurred in the decade following release from culling, and data were available from 1974 on female breeding behaviour and population size; this index was examined from 1974 to 2001. Note that even for females, these figures are substantially lower that the absolute count of breeding age deer alive in any one year, since not all individuals breed each year.

Dispersal. Dispersal between population subdivisions was assessed by comparing adults' natal subdivision with their assigned subdivision in a given year. If individuals had moved from their natal subdivision then the direction of dispersal was classified by their natal subdivision followed by their current subdivision (i.e. a female natal to SI, but assigned to NKG in 1980 would be classified as 'SI–NKG' for 1980). Females and males of each dispersing category were counted for each year of the study.

Male breeding population size and levels of polygyny. Estimates of these parameters were based on paternity assignment of individuals born in the North Block between 1978 and 2001, using all available microsatellite data, with CERVUS (Marshall *et al.* 1998). All males observed holding harems during a rut year were considered as potential candidate fathers. Genetic paternities were assigned where the confidence score given by CERVUS was 80% or greater. Of the calves born in the period 1978–2001, 35.4% were successfully assigned genetic paternities. If possible, males were assigned behavioural paternity where genetic paternity could not be determined. Behavioural paternities were assigned to males if they held the calf's mother in their harem for more days during the 11-day window around estimated conception than any other male (see Clutton-Brock *et al.* 1997; Kruuk *et al.* 2000; Slate *et al.* 2000 for further details and discussion of these genetic and behavioural approaches to paternity assignment). Using combined methods, 62.8% of calves were assigned paternities.

For all males observed holding harems in a given year, we calculated annual breeding success (ABS). For each study year we used this information to calculate the number of breeding males (i.e. the number of males with

one or more assigned paternities) as well as the maximum and variance in ABS in each year. Variance in male ABS was used as an index of polygyny. A previous study of this population highlighted the large number of progeny produced by a single male, named MAXI (Slate *et al.* 2002). To assess the dependence of temporal trends in polygyny on the handful of such males, we identified four males that had been assigned the most paternities across their lifetimes and investigated the effect of removing them from our data set. The identity codes of these four males, the number of offspring they sired and the years in which they bred, respectively, are as follow: RED77, 48 offspring, 1983–1991; MAXI, 36 offspring, 1978–1982; BRF76, 30 offspring, 1982–1989; BASIL, 29 offspring, 1997–2001. These individuals represented the top 0.5% of breeding males.

Temporal trends in the variance of male ABS could reflect improvements in the quality of the genetic data set in this population: the proportion of calves born that were assigned paternities each year increased over the study period ($F_{1,22} = 26.27$, $P < 0.001$). To ensure any change in polygyny was independent of this improvement, we ran a multiple regression of variance in male ABS including both year and the proportion of paternities successfully assigned in that year.

Results

Differences between the sexes in population structure

Field and genotypic data analysis across the study period both imply that male deer are highly dispersive, while females are generally philopatric and remain in close spatial proximity to relatives of the same sex (Figs 2, 3 and 4). Females tend to move relatively little across their lifetimes,

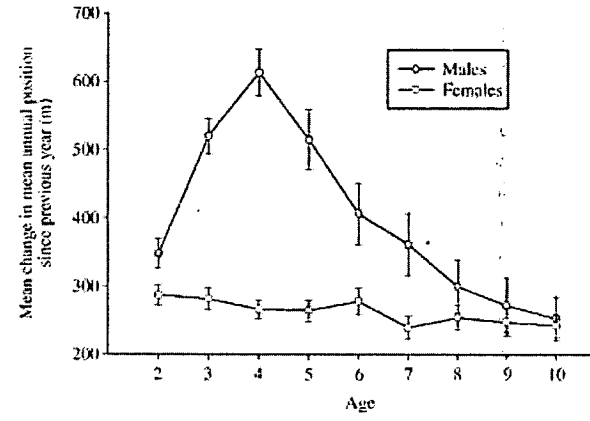

Fig. 2 Between-year movements of resident deer plotted against age of individual (at second mean annual position measured). Circles represent means for each age with standard error bars. Separate averages are shown for females (white circles) and males (grey circles). Males disperse considerably more between years than females between the ages of 3 and 5 years.

and in general had lower means and standard errors for between-year movements than males age 2 to 10 years (Fig. 2). Males born in the North Block moved increasing distances between years from ages 2 to 4 years (Fig. 2). Both these data and previous work on the population show that males are more dispersive than females, and many surviving males have dispersed from the study area by the time they reach sexual maturity (Clutton-Brock *et al.* 1982b; Catchpole *et al.* 2004).

As expected from these general differences in dispersal between the sexes, the genetic relatedness between a pair of females decreased as the distance between them became greater, but pairs of males were unrelated across the dis-

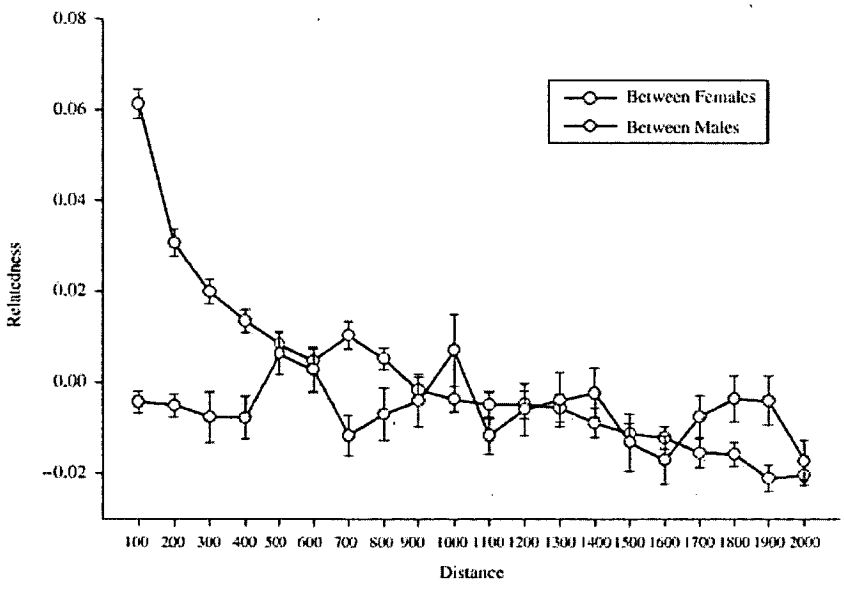

Fig. 3 Line and scatter plot showing the relationship between genetic relatedness and geographical distance amongst pairs of females (white circles) and males (grey circles). Circles represent pairwise relatedness comparisons at each distance averaged across years (1978–2001), with standard error bars. Relatedness between pairs of females is high at short distances and decreases with distance, but between pairs of males relatedness does not deviate from zero.

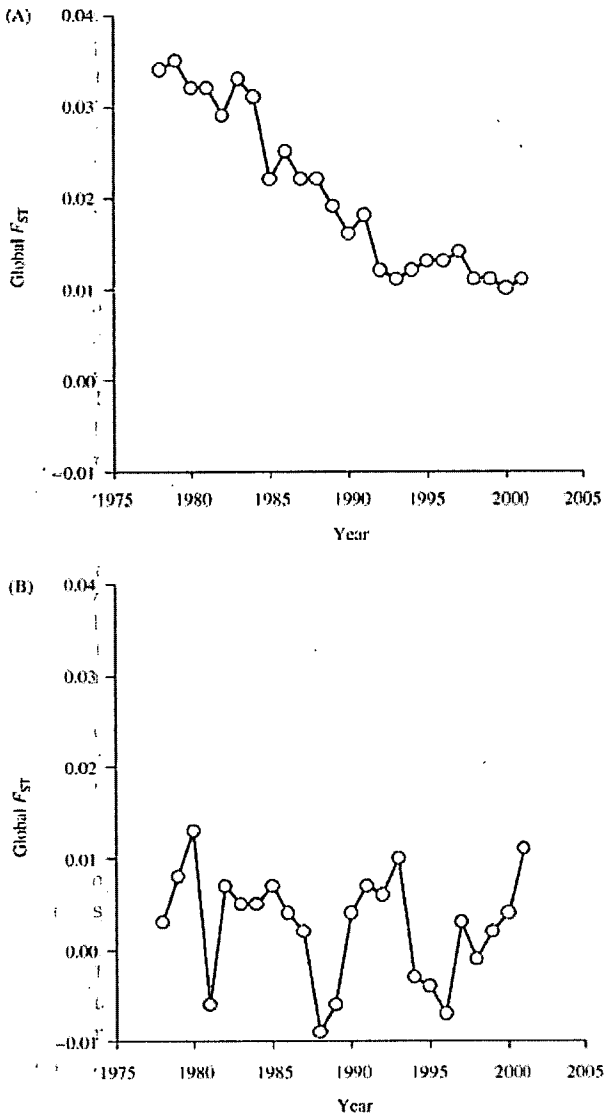

Fig. 4 Changes in global F_{ST} estimates over time, based on adult deer only with population subdivisions (see Fig. 1) treated as subpopulations. (A) Females; global F_{ST} declines significantly over the study period ($b = -0.0012 \pm 0.0001$ SE, $P < 0.001$) showing partitioning of genetic variance between the subdivisions has decreased. (B) Males; global F_{ST} shows no significant relationship with year ($b = -0.0001 \pm 0.0002$ SE, $P = 0.46$) and was only significantly greater than zero in 1980, illustrating an absence of population structure amongst males.

tance range examined (Fig. 3). Females with mean annual positions 100 m or less from one another were related, on average, at $R = +0.06$, and this decreased to a relatedness of zero at around 900 m (Fig. 3). This decline in relatedness with distance was significant (linear regression of mean R on distance amongst pairs of females: intercept = 0.053 ± 0.004 SE, slope = -0.025 ± 0.002 SE, $r^2 = 0.93$, $P < 0.001$). For pairs of males, there was no evidence of any change or difference from zero in relatedness across the distance range examined (intercept = 0.002 ± 0.03 SE, slope = -0.006 ± 0.005 SE, $r^2 = 0.06$, $P = 0.51$).

Temporal variation in fine-scale genetic structure

Fixation indices for the North Block adult females revealed strong partitioning of genetic variation between subdivisions; however, there was no evidence of such structure amongst males. Global F_{ST} values for females were significantly greater than zero in all years of the study period for females (female mean annual $F_{ST} = 0.021$), while F_{IS} values were significantly less than zero in all years except 2000 (mean annual $F_{IS} = -0.058$). Amongst males, F_{ST} estimates were not significantly greater than zero, except in 1980 (male mean annual $F_{ST} = 0.002$), and F_{IS} estimates were not different from zero except in 2000 and 2001 (mean annual $F_{IS} = -0.022$). F_{ST} estimates significantly greater than zero suggest that there is structuring of allelic variance between the three subdivisions. Negative F_{IS} values imply an excess of heterozygosity relative to random mating.

There was a significant decline in global F_{ST} estimates for females from around 0.03–0.01 across the study period ($F_{1,22} = 201.6$, $P < 0.001$, Fig. 4A). There was no temporal trend in male F_{ST} estimates ($F_{1,22} = 0.58$, $P > 0.05$; Fig. 4B). This indicates a decline in fine-scale genetic structure amongst females, but not males, over the course of the study period. Female F_{IS} estimates increased significantly over the study period from approximately −0.08 to −0.03 ($F_{1,22} = 17.45$, $P < 0.001$), while estimates for males showed no temporal trend ($F_{1,22} = 2.19$, $P > 0.05$).

Pairwise F_{ST} estimates comparing female genotypes from SI and NKG, and SI and SKG, both show significant negative trends with time (SI–NKG: $F_{1,22} = 68.70$, SI–SKG: $F_{1,22} = 224.0$, both $P < 0.001$; Fig. 5A). In the early stages of the study, genetic distances between females in SI and SKG were around 0.06 but had declined to just over 0.01 by the mid-1990s. F_{ST} estimates between SI and NKG declined from around 0.03–0.01 over a similar period (Fig. 5A). The pairwise F_{ST} comparison of NKG and SKG females showed no significant temporal trend ($F_{1,22} = 0.31$, $P > 0.05$); it fluctuated between 0.015 and zero throughout the study period (Fig. 5A). None of the pairwise F_{ST} estimates for males showed a significant relationship with year (all regressions: $F_{1,22} < 2.3$, $P > 0.05$, Fig. 5B).

Temporal variation in breeding population size and mating system

Breeding population size. Since the population's release from culling in 1973, the number of females breeding in a given year has increased from around 55 in 1974 to fluctuate between 70 and 100 from the mid-1980s onwards ($F_{1,25} =$

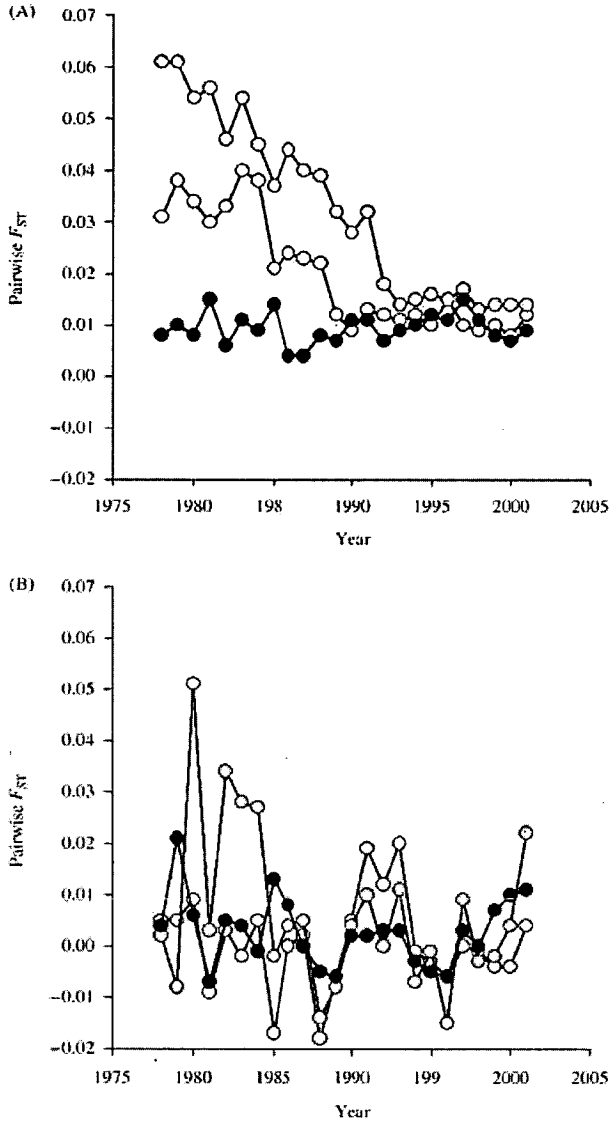

Fig. 5 Pairwise F_{ST} estimates comparing genetic differentiation between pairs of population subdivisions over time. (A) Females; the pairwise F_{ST} estimates between Shamhnan Insir (SI) and North Kilmory Glen (NKG) declines over time (white circles; $b = -0.0013 \pm 0.0002$ SE, $P < 0.001$), as do the SI–South Kilmory Glen (SKG) estimates (grey circles; $b = -0.0024 \pm 0.0002$ SE, $P < 0.001$). The NKG–SKG pairwise F_{ST} estimates remain constant and low across the study period (black circles; $b = 0.0000 \pm 0.0001$ SE, $P > 0.05$). (B) Males; no temporal trends apparent in either SI–NKG (white circles), SI–SKG (grey circles), or SKG–NKG (black circles) in pairwise F_{ST} estimates.

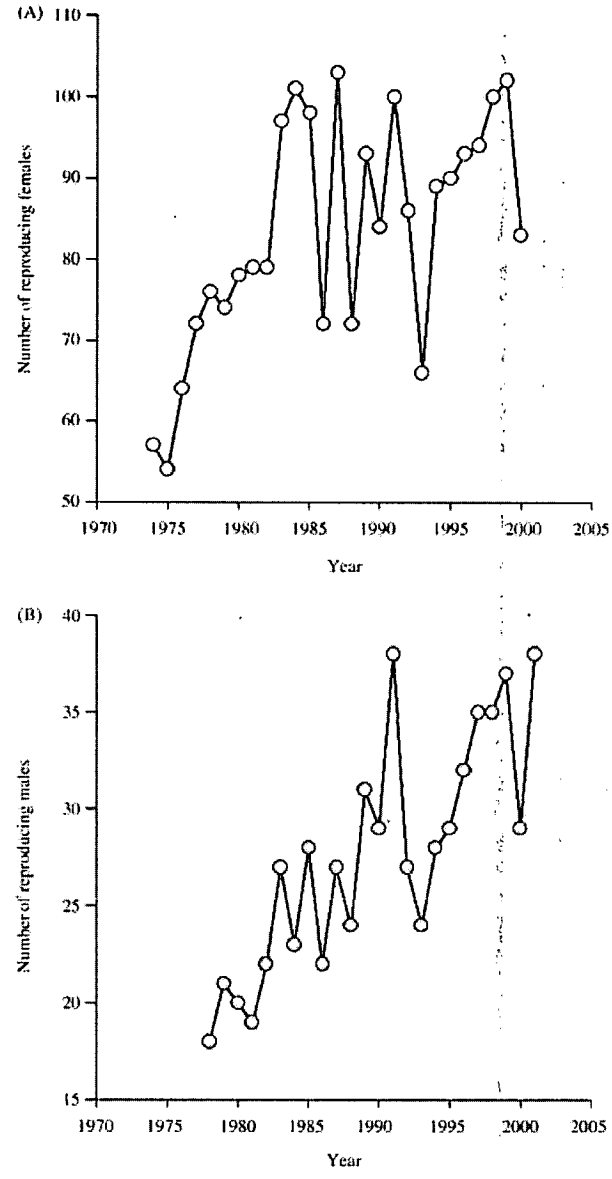

Fig. 6 (A) The number of females reproducing in each year has significantly increased since 1974 as overall female population density has increased in the population ($b = 1.10 \pm 0.28$ SE, $P < 0.001$). Note that this graph includes additional data for 1974–1977, as the main increase in female population size occurred immediately following the release from culling in 1973. (B) The number of different males assigned at least one paternity in a given year has increased across the study period ($b = 0.70 \pm 0.10$ SE, $P < 0.001$).

15.2, $P < 0.001$, Fig. 6A). The number of different males assigned paternities has also increased from around 20 in the late 1970s to between 30 and 40 in the last 5 years of the study ($F_{1,22} = 45.35$, $P < 0.001$, Fig. 6B).

Dispersal. On average, only 11.7% of resident adult females were located outside their natal subdivision in a given year, compared to 30.0% for adult males. The numbers and direction of males and females dispersing from their natal subdivision are shown in Fig. 7. Movement of either sex

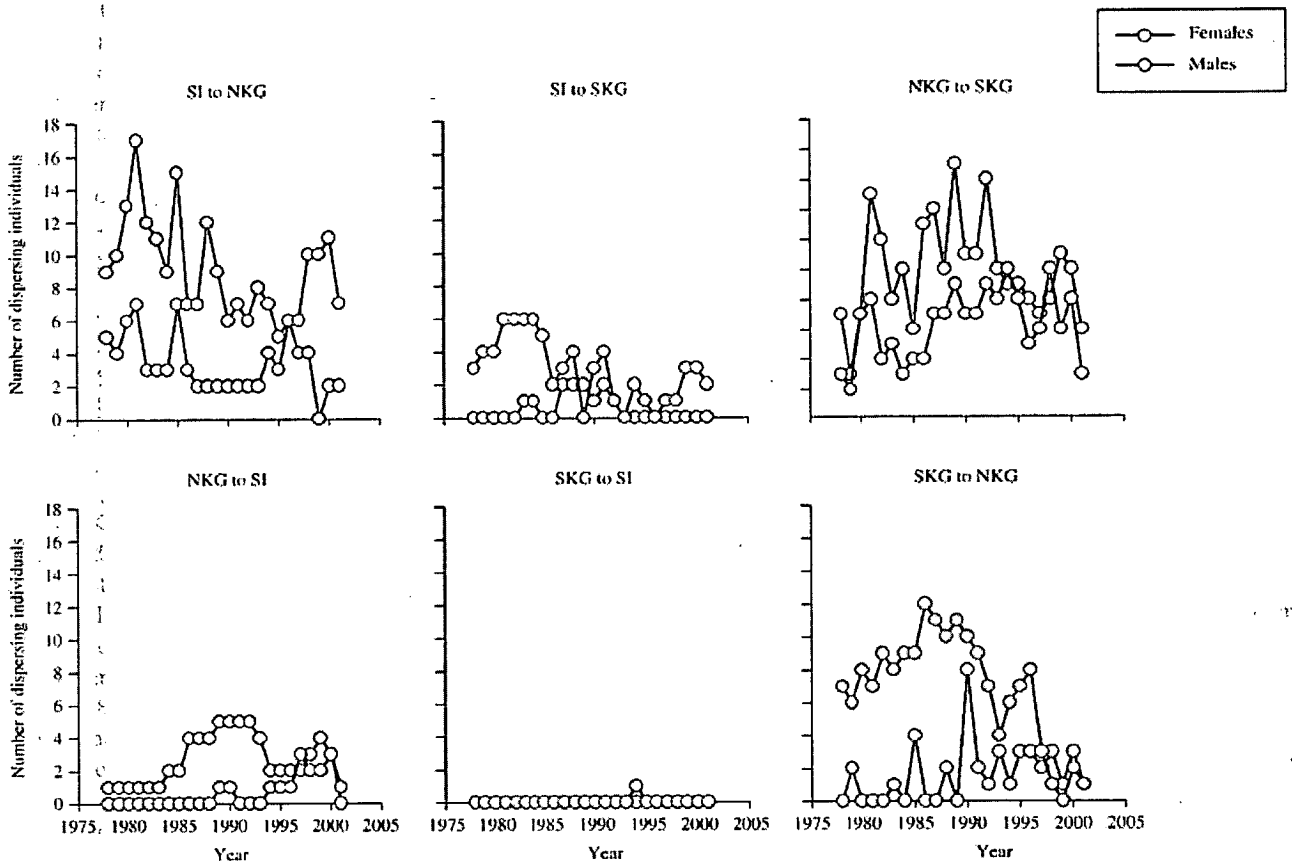

Fig. 7 Plot showing dispersal patterns in the North Block over time. *Y*-axes on each of the panels represent the number of adult deer found outside of their natal population subdivision for each of the six possible dispersal direction categories. Legends above each panel indicate dispersal direction, with natal subdivision followed by current subdivision. Numbers of dispersing females are indicated by white circles, numbers of males by grey circles. Subdivision abbreviations are as follows: SI, Shamhnan Insir; NKG, North Kilmory Glen; SKG, South Kilmory Glen.

between SI and SKG appears to be rare, as does movement from NKG to SI. Although higher levels of dispersal are observed between NKG and SKG and, for males at least, from SI to NKG, there is little indication of increases in dispersal capable of explaining the decline in female genetic structure. The only significant positive temporal trend in dispersal was for males dispersing from NKG to SI ($F_{1,22} = 28.87$, $P < 0.001$); however, no more than three males had dispersed in this direction in any year (Fig. 7). There were significant declines over time in male dispersal from SI to NKG ($F_{1,22} = 6.88$, $P < 0.05$) and SI to SKG ($F_{1,22} = 14.36$, $P < 0.01$), and in female dispersal from SI to NKG ($F_{1,22} = 5.31$, $P < 0.05$).

Polygyny. Variance in male ABS decreased over time ($F_{1,22} = 12.14$, $P < 0.01$, Fig. 8). This decline in the level of polygyny is most likely the result of the increase in the number of males gaining at least one paternity (Fig. 6B), rather than a change in the maximum ABS, which showed no relationship with year ($F_{1,22} = 0.59$, $P > 0.05$). The removal of the four males with the largest lifetime breeding success from the data set resulted in a marginally nonsignificant decline in the variance in male ABS ($F_{1,22} = 3.14$, $P = 0.09$). The decline in polygyny was independent of the increase in the proportion of calves assigned paternities over time. In a multiple regression, the proportion of paternities assigned was not a significant predictor of the variance in male ABS ($F_{1,21} = 2.96$, $P = 0.10$), while year had a significant negative effect ($F_{1,21} = 11.33$, $P < 0.01$) on this measure of polygyny.

Discussion

Figure 3 clearly illustrates the presence of genetic structure at very fine spatial scales amongst female, but not male, red deer in the North Block study population. This was as predicted: a significant decline in genetic relatedness was observed over continuous space between pairs of the philopatric sex, and no spatial genetic structure was found between members of the dispersing sex. The presence of

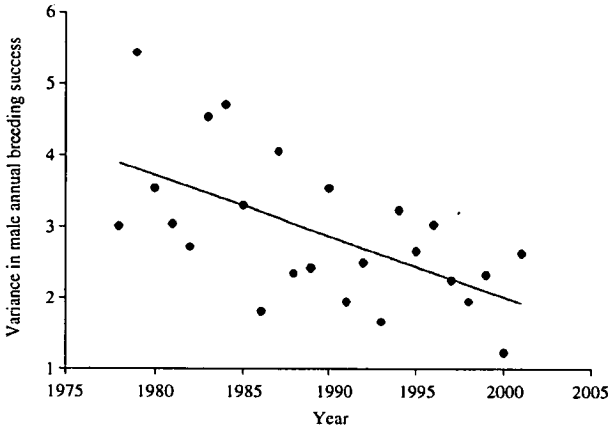

Fig. 8 Levels of polygyny have decreased over the study period. The plot shows the variance in male annual breeding success over time with a linear regression slope plotted ($b = -0.09 \pm 0.02$ SE, $P < 0.01$).

significantly positive F_{ST} and negative F_{IS} estimates across the study period imply population structure amongst females consistent with those in previous studies of mammalian population genetics (Storz 1999). In the only other study to examine genetic structure at such a fine scale in ungulates, Coltman *et al.* (2003) found similar differences between the sexes in Soay sheep on St Kilda, although the degree of spatial structure in females was lower in general and declined to zero at around 150 m. This difference between species could be explained by the fact that red deer possess a more obviously matrilocal social system (Clutton-Brock & Coulson 2002). However, it may also be the result of differences in feeding habitat and foraging behaviour: red deer on Rum have larger home ranges than Soay sheep on St Kilda.

We found significant partitioning of genetic variance between population subdivisions amongst females, but not males (Fig. 4). However, across our 24-year study period, we observed a decline in female genetic structure (Fig. 4A). Fixation indices may take many generations to reach mutation–drift equilibrium, and so it is possible that these temporal changes in genetic structure could be the product of events occurring before our study period began. F_{ST} estimates are influenced by factors such as dispersal, mating system, and effective population size. Previous work on the North Block red deer population suggests that these parameters have altered across our study period as a direct or indirect consequence of the cessation of culling (Clutton-Brock *et al.* 1982b, 1997; Albon *et al.* 1992). Increases in dispersal between population subdivision in either sex, increases in the breeding population size and decreasing polygyny levels would represent viable explanations for the observed decline in population genetic structure, although these would not represent mutually exclusive or exhaustive explanations for the observed trend.

Female population density in the North Block has increased threefold since the population's release from culling in the early 1970s. We have shown this to be concurrent with an increase in the number of females breeding in a given year (Fig. 6A). Although this increase took place mainly in the early part of our study period, the observed decline in F_{ST} and increase in F_{IS} values observed among females could be a direct result of these recent increases in effective female population size (Chesser 1991; Balloux 2004). Furthermore, as female numbers have risen, the population's female : male ratio has increased as the male bias in juvenile mortality and immigration have become more pronounced (Albon *et al.* 2000; Catchpole *et al.* 2004). As a consequence, competition for mates between males has decreased (Clutton-Brock *et al.* 1997). Our results showing a decrease in variance in male ABS, explained by an increase in the number of males obtaining at least one mating rather than an increase in maximal ABS, replicate the results for the period 1972–1990 of Clutton-Brock *et al.* (1997). The outcome of these changes has been a decline in polygyny (Fig. 8). Theoretical studies predict that reduced polygyny combined with increased numbers of reproducing females would substantially reduce co-ancestry within populations (Chesser 1991; Perrin & Mazalov 1999).

Increased dispersal of either sex from their natal population subdivision in response to rising resource competition would cause a decline in genetic structure (Slatkin 1987). While there is evidence of an overall density-driven increase in spacing between maternal relatives amongst females and in emigration amongst males in the population (Albon *et al.* 1992; Catchpole *et al.* 2004), our results do not support the hypothesis that an increase in dispersal between subdivisions is responsible for the observed breakdown in female genetic structure. Figure 7 shows little evidence of increases in dispersal in either male or female red deer within the North Block. Although dispersal patterns may not explain the decline in female global F_{ST}, the observation of relatively high levels of female NKG–SKG dispersal, compared to other possible directions, could explain the low and temporally stable pairwise F_{ST} estimates between NKG and SKG among females. This finding ties well with previous research suggesting a southward expansion of Kilmory Glen females as population density has increased to carrying capacity (Coulson *et al.* 2004). Such movement would make recent co-ancestry between females in the two subdivisions likely. Previous studies treating these two areas of the North Block as one subpopulation appear justified (Milner-Gulland *et al.* 2000).

Conclusions

Nonrandom spatial distribution of genotypes at small spatial scales can confound studies of allelic association and quantitative genetics, and may have important

evolutionary consequences such as the facilitation of kin selection and localized selection (Coltman *et al.* 2003). We found evidence of extremely fine-scale spatial structure amongst female red deer but not males, as would be expected for a typical mammalian system showing male-biased dispersal and female philopatry. Spatial structuring of genotypes amongst females declined over the course of our study period. This rapid decline in structure could be explained by both changes in female population density or levels of polygyny that are the result of the population's release from culling, but do not appear to be related to density-dependent changes in dispersal. Furthermore, we cannot be certain that the observed trends are not long-term consequences of events that took place prior to the start of our study. The analysis presented here is, to our knowledge, the first direct demonstration of a breakdown in the fine-scale genetic structure in a wild mammal population over time. The results represent an important step towards developing our understanding of the dynamic nature of population genetic structure and illustrate clearly that temporal stability in population genetic parameters cannot simply be assumed. Further research is now required to refine and expand our understanding of the interaction between the spatial distribution of genotypes, behaviour and the environment in free-living populations.

Acknowledgements

The authors are extremely grateful to the Scottish Natural Heritage and their staff for permission to work on Rum and for local support, as well as Fiona Guinness and many other project field workers. Thanks must also go to Jon Slate, Kevin Connaghan, Kate Byrne, and Sarah Lewis for genotyping work, and to Dany Garant, Silvia Pérez-Espona, Felicity Jones, Francois Balloux and two anonymous referees for constructive comments and discussion regarding earlier drafts of the manuscript. Both the Rum research project and DHN's studentship were funded by NERC.

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This forms part of Dan Nussey's PhD thesis in population and quantitative genetics of red deer on Rum, supervised by Loeske Kruuk. The analysis is the brainchild of Dave Coltman and Tim Coulson, and Dave undertook much of the genetic analysis. Genotyping was conducted in Josephine Pemberton's lab in Edinburgh. The red deer project is run and maintained by Tim Clutton-Brock in Cambridge, with Ali Donald and Sean Morris – the current and most recent Kilmory field researchers – collecting much of the data presented here.

Science Reprint

Selection on Heritable Phenotypic Plasticity in a Wild Bird Population

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14 October 2005, Volume 310, pp. 304-306

Selection on Heritable Phenotypic Plasticity in a Wild Bird Population

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Theoretical and laboratory research suggests that phenotypic plasticity can evolve under selection. However, evidence for its evolutionary potential from the wild is lacking. We present evidence from a Dutch population of great tits (*Parus major*) for variation in individual plasticity in the timing of reproduction, and we show that this variation is heritable. Selection favoring highly plastic individuals has intensified over a 32-year period. This temporal trend is concurrent with climate change causing a mismatch between the breeding times of the birds and their caterpillar prey. Continued selection on plasticity can act to alleviate this mismatch.

Phenotypic plasticity—defined as the ability of a single genotype to alter its phenotype in response to environmental conditions—is an important mechanism by which populations can respond rapidly to changes in ecological conditions (*1–3*). Plasticity in life history traits is ubiquitous in animal populations (*1*), with traits often varying within the lifetimes of individuals depending on the conditions they experience (*4*, *5*). It is typically conceptualized and measured using reaction norms: linear functions describing the change in a trait across an environmental gradient (*3*, *6*). Laboratory research has shown that genetic variation for plasticity exists (*7*, *8*) and that heritable plasticity can respond to artificial selection (*2*, *9*).

Given that many species are currently experiencing long-term anthropogenically driven environmental change (*10*, *11*), a better understanding of how natural selection acts on plasticity under altered levels of environmental variation in the wild is imperative. Detailed analyses of within-population variation in life history plasticity are rarely undertaken in naturally occurring populations, because such analyses require data from large numbers of individuals breeding repeatedly across their lifetimes. Recent research using mixed-effects linear models has shown that individuals in two wild vertebrate populations vary in their levels of life history plasticity (*4*, *12*). At present, little is known about the consequences of environmental change for the action of natural selection on plasticity and, ultimately, the ability of populations to continue to respond adaptively to environmental variation. Here we present data from a wild bird population showing temporal trends in natural selection on heritable phenotypic plasticity in the timing of reproduction, which are concurrent with changes in climate and the timing of food availability.

After a warm spring, female passerines often breed earlier than they do after a cold spring (*13*, *14*). This is a result of phenotypic plasticity (*14*, *15*), an individual-level response to temperature. Such a response is considered adaptive because it synchronizes the birds' phenology with the temperature-dependent hatching times and growth rates of the caterpillars they rely on to feed their nestlings (*16*, *17*).

A long-term study of great tits (*Parus major*) in the Hoge Veluwe, one of the Netherlands' largest national parks, has revealed that after recent warming of spring temperatures in

Table 1. Linear mixed-effects model of 2195 laying date observations from 833 female great tits that bred in more than 1 year during the period 1973 to 2004. Estimated covariance (female, female × spring temperature) = 1.16 ± 0.31 (SE).

	Random effects		
Term	Variance	SE	LRT
Year of breeding	9.54	2.55	558.33***
Female	8.05	0.76	226.73***
Female × spring temperature	1.05	0.31	27.07***
Residual	14.97	0.64	
	Fixed effects		
Term	Wald statistic	df	Wald/df
Spring temperature	45.47	1	45.47***
Age	116.91	1	116.91***
Age × spring temperature	7.26	1	7.26**

**$P < 0.01$. ***$P < 0.001$.

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the region, the timing of growth of their caterpillar prey has advanced while the phenology of the birds has not (*17*). As a result, over the past three decades, the laying dates of female great tits have moved closer to the peak in the caterpillar biomass, so that the peak in demand for food for their offspring no longer coincides with the peak in prey availability (*14*, *17*). Selection on the heritable component of great tits' plastic responses to spring temperatures could act to reduce this phenological mismatch (*14*).

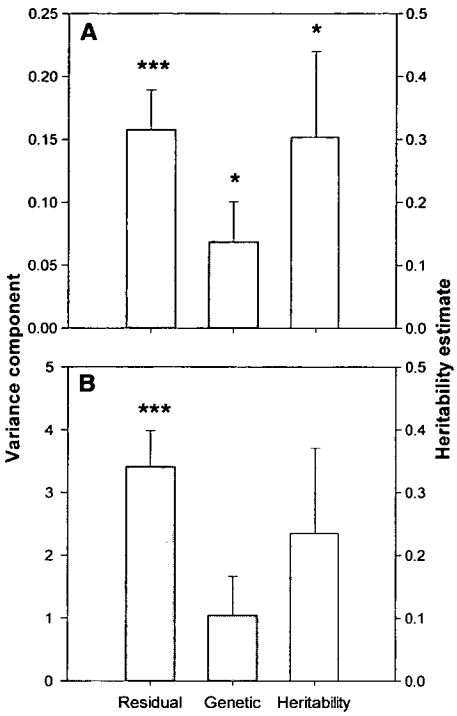

Fig. 1. Significant genetic variation exists for laying date plasticity. The bar plots show "animal model" estimates of residual and additive genetic variance (gray) and heritability (white) with SE bars for (**A**) laying date–spring temperature slope and (**B**) laying date elevation. The left *y* axis shows variance component values for the gray bars; the right *y* axis shows predicted heritabilities for the white bars. Asterisks above bars indicate an estimate that is significantly greater than 0 (*$P < 0.05$, ***$P < 0.001$).

We used information on laying dates for 833 females breeding in more than 1 year between 1973 and 2004 to examine variation among females in their laying date reaction norms. A random-coefficients model of laying dates (*18*) showed that after a warm spring, on average, females began laying earlier than they did after a cold spring (Table 1). We found significant variation between females in both their estimated laying date at the average spring temperature [likelihood ratio test (LRT) = 226.73, df = 1, $P < 0.001$] and the magnitude of their response to spring temperature (LRT = 27.07, df = 2, $P < 0.001$). Females in this population lay early after a warm spring, but the magnitude of this plastic response varies between females (*19*). There was a significant, positive correlation between elevation and slope ($r = 0.40$, LRT = 15.41, df = 1, $P < 0.001$): Females that lay early in the average environment are also the most plastic females.

Significant genetic variation in a trait must exist for there to be any response to selection (*20*). We generated predictors for the two components of each female's reaction norm: her laying date in the average environment (elevation), and her change in laying date in response to temperature (plasticity or slope) (*3*). We used an "animal model" (*21*) to estimate the genetic component of phenotypic variance in predictors of female elevation and slope (*18*). We found that significant genetic variation for laying date plasticity exists in the Hoge Veluwe great tit population and that laying date plasticity was significantly heritable [$h^2 = 0.30 \pm 0.14$ (SE), $z_{(<0)} = 2.21$, $P < 0.05$ (Fig. 1A)]. Genetic variation and heritability estimates for laying date elevation were relatively high but were not significantly greater than 0 [$h^2 = 0.24 \pm 0.14$, $z_{(<0)} = 1.73$, $P > 0.05$ (Fig. 1B)]. However, the genetic correlation between slope and elevation was highly positive and not significantly different from 1 ($r_A = 0.77 \pm 0.18$, $z_{(<1)} = 1.28$, $P > 0.05$).

To investigate selection on laying date plasticity across the study period, we measured the relationship between a female's lifetime reproductive success (LRS) and predictors of her laying date elevation and slope (*18*). There was evidence for directional selection on both reaction norm components, and there was no evidence of stabilizing or correlated selection (table S1). Females that laid earlier in the average environment (low elevation) and responded more strongly to temperature (more negative slope) had significantly more of their offspring recruit into the population as breeding adults (standardized selection gradients for elevation and slope: –0.094 ± 0.039 and –0.085 ± 0.039, respectively).

Over the study period, selection favoring females that advance their laying dates strongly in response to warm spring temperatures increased (Fig. 2; slope × cohort interaction: $F_{1,804} = 7.22$, $P < 0.01$). It is clear from both the fitted interaction (Fig. 2A) and the data themselves (Fig. 2, B to D) that selection has been strongest in the last two decades of the study, during which time the phenological mismatch with the peak in caterpillar biomass first emerged and then increased (*14*). The same pattern of changing selection over time is observed on estimates of females' laying date elevation (table S2) (*18*).

It appears that the strong correlation between females' elevation and slope renders these two components of their reaction norms indistinguishable. Selection favored those highly plastic females that also lay early on average. How can we explain the correlation between elevation and slope? Both plasticity

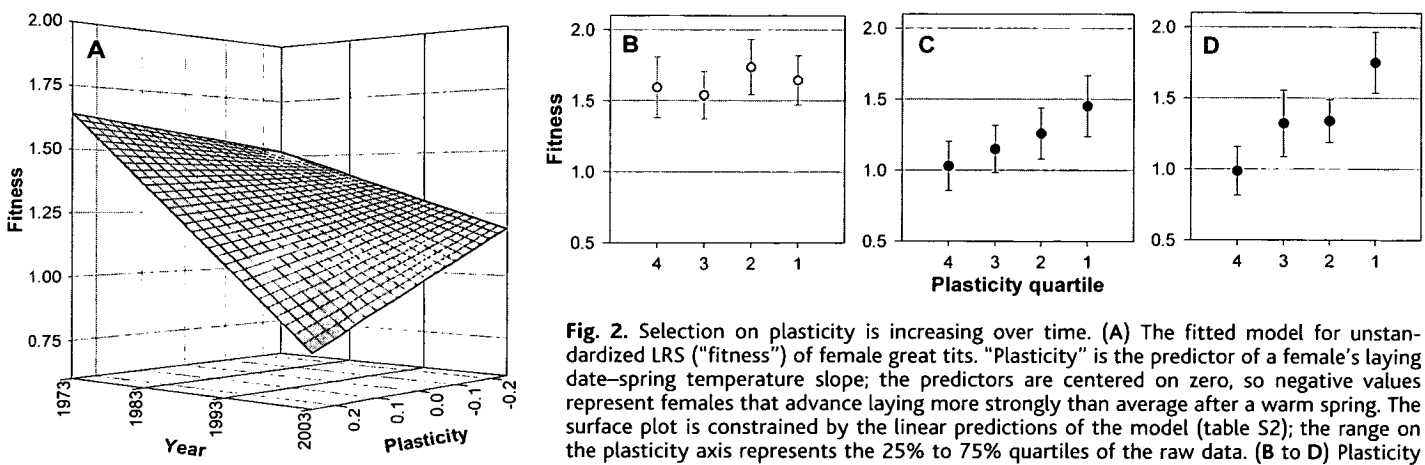

Fig. 2. Selection on plasticity is increasing over time. (**A**) The fitted model for unstandardized LRS ("fitness") of female great tits. "Plasticity" is the predictor of a female's laying date–spring temperature slope; the predictors are centered on zero, so negative values represent females that advance laying more strongly than average after a warm spring. The surface plot is constrained by the linear predictions of the model (table S2); the range on the plasticity axis represents the 25% to 75% quartiles of the raw data. (**B** to **D**) Plasticity predictor quartiles were estimated across the entire study period; mean LRS values for each quartile (with SE bars) are shown for females first breeding during the periods 1973 to 1982 (B), 1983 to 1992 (C), and 1993 to 2002 (D). Quartile 1 contains the most plastic females.

and elevation in laying date may be correlated to some unmeasured aspect of individual quality or condition (*4*, *12*, *22*). If birds differ in their ability to lay early in the year because of variation in some aspect of individual quality—for example, because of differences in their ability to gather resources—high-quality birds will lay early in warm years and later in cold years and will have steeper slopes and lower elevations, whereas poor-quality birds will usually lay later regardless of temperature and will therefore have shallower slopes. The early/plastic birds would be expected to match their reproductive timing better with the peak in caterpillar biomass, especially as spring temperatures become increasingly warm.

To substantiate the relationship between the great tits' reaction norms and the mismatch with the peak in food availability, we estimated each female's "lifetime synchrony" (*18*). Improved lifetime synchrony was associated with increased laying date plasticity ($F_{1,806} = 189.4$, $P < 0.001$) and earlier breeding in the average environment ($F_{1,806} = 582.4$, $P < 0.001$). Furthermore, the synchrony of highly plastic females increased over time relative to less plastic individuals (slope × cohort interaction: $F_{1,804} = 55.8$, $P < 0.001$). The observed changes in selection on plasticity, as the phenological mismatch has increased over time, appear to be driven by the fact that highly plastic females breed in closer synchrony with the peak in food availability and hence have more resources available for provisioning their young.

Female LRS has decreased across the study period (Fig. 2; cohort main effect: $F_{1,804} = 9.92$, $P < 0.01$). If such a decline persists alongside increased mismatching of phenologies between birds and caterpillars, the population's viability may ultimately be threatened. A phenotypic response to recent selection on laying date reaction norms cannot yet be demonstrated in this population (*23*), although the presence of additive genetic variance for plasticity means that a response to selection is predicted (*20*, *21*). However, a microevolutionary response to selection on the laying date reaction norms toward lower elevations and stronger plasticity would be expected to result in closer synchrony between the great tits' laying dates and the peak in food availability, and ultimately could alleviate the trophic mismatch.

We have shown that selection affects life history plasticity and that it can change with prevailing ecological conditions to potentially alter reaction norms in a wild population. This finding has wider implications because climate change has the potential to induce mismatches in the timing of breeding between trophic levels across a wide variety of ecosystems (*17*, *24*, *25*). The capacity for evolutionary change in phenological reaction norms shown here represents a potential means for natural selection to alleviate such mismatches and their ultimately negative consequences for population viability and ecosystem function (*11*, *26*). However, it remains to be seen whether microevolutionary change in reaction norm shape can occur fast enough to keep up with the rapid rate of change in ecological conditions.

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27. We thank J. H. van Balen and J. Visser for managing the long-term study for many years, the board of De Hoge Veluwe for kindly allowing us to work on their property, and L. Kruuk, J. Bommer, K. Lessells, and four anonymous referees for comments on earlier drafts of the manuscript. Supported by British Ecological Society small ecological project grant 2429 (D.H.N.).

Supporting Online Material

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6 July 2005; accepted 7 September 2005
10.1126/science.1117004