Phenology in a wild mammal population

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Submitted for the degree of Doctor of Philosophy

University of Edinburgh

2010

Declaration

I have composed this thesis. The long-term data analysed in this thesis was collected by others. However, I have contributed to routine data collection and have a full understanding of the data collection methods. I performed all of the analyses in this thesis and composed the chapters, incorporating feedback from co-authors and supervisors. This work has not been submitted for any other degree or professional qualification.

Acknowledgements

Wow, that was an adventure. I would like to begin by thanking my supervisor, Loeske Kruuk. Loeske has been incredible throughout this whole process and I will always be grateful for her support and guidance. Loeske has managed to strike the correct balance between always being there, whilst allowing me to make my own mistakes and find my own way forward. I can only hope that some of her wisdom stays with me when the time comes for me to lead and nurture others.

The Rum red deer project is an immense data source, and I am grateful to both Fiona Guinness and Tim Clutton-Brock for the incredible amounts of time and dedication they have invested into the project. Thank you also to Steve Albon for support and to NERC and the Macaulay Institute for funding. I have been fortunate to spend considerable amounts of time on Rum, and I will always cherish my memories of fieldwork. I am grateful to Alison and Sean Morris for teaching me the fine art of calf catching and to Josephine and Loeske for funding my Home Office licence. It was truly a privilege to be able to handle wild animals and to contribute to the future of the project. I would also like to thank my second supervisor Josephine Pemberton for her support and for the many thought provoking conversations we had in the land rover, watching a hummock that may, or may not, turn out to be a deer. Additionally, Martyn Baker, Katie Stopher and Craig Walling always managed to make fieldwork more fun than sitting for 11 hours in the rain might suggest.

My analytical and reasoning skills have improved immensely whilst I have been a part of the Wild Evolution Group, led by Loeske. Special thanks are due to Jarrod Hadfield, who has helped to drag me from the quagmire of models on numerous occasions, to Dan Nussey for unfailingly smiling throughout my presentations, and to Adam Hayward for always being my left-hand man.

On a personal note, I would like to thank my family. My parents have been unwavering in their support for me, and I will always be grateful to them for providing a sense of perspective, and proper Bradford curries. My time in Edinburgh would have been so much less fun without some of the friendships that I have made in this department, and I thank those people for their companionship, and laughter over the past three and a half years.

Abstract

My thesis focuses on the phenology, or timing of annual events, in a wild red deer population on the Isle of Rum, NW Scotland. Although there has been much work on breeding time in wild populations, less is known about factors influencing variation in other phenological traits. Additionally, we know almost nothing about covariances between different phenological traits within a population, despite the fact that covariance at the genetic level could act to constrain a population's response to changing selection pressures. The long-term study on the Isle of Rum enabled me to investigate a number of phenological variables expressed in both sexes. Specifically, my thesis consists of:

i) An analysis of factors influencing variation in antler growth phenology, and implications of this variation with respect to mass of antlers grown and breeding success in the following rut. Males that both started and ended antler growth the earliest grew the biggest antlers. After correcting for age and antler mass, males that started antler growth earliest had the highest breeding success.

ii) A study of variation in gestation length. Gestation length was not associated with a female's age or previous reproductive experience, but warm temperatures in late gestation were correlated with shorter gestation lengths. I investigated, using standard and novel statistical methodology, if late conceiving females shortened gestation length to give birth at the optimal time. I found a negative covariance between conception date and gestation length but this was not found to be repeatable within females, suggesting that gestation length adjustment may be a tactic used by females only on the rare occasion they conceive outside the optimum time window. I conclude that variation in birth date is primarily a result of variation in conception date and not gestation length.

iii) An examination of variables influencing date of coat change from summer to winter coat in young and adult female deer. Coat change date became later with increasing age, and was earliest in females not bearing recent costs of lactating offspring. Coat change date was earlier in years with high average September temperatures, generating a temporal trend in coat change date, as September temperature has been increasing over time. Additionally, individuals still in summer coat on the 1st November had lower over-winter survival rates.

iv) A comparison of parameters of the distributions of seven phenology traits over time. Phenology is known to be advancing in this system, but it seems unlikely that advancement can continue indefinitely. I hypothesised that as this limit to phenological advancement was approaching, a concurrent decrease in the variance and increase in the skew and kurtosis of the distributions would be observed. I found no evidence for this, suggesting that either there is no such limit to phenological advancement, or that this limit has not yet been reached.

v) An analysis of variances and covariances of seven phenology traits, at the level of phenotype, genetic and year. Patterns of variance differed between the eight phenology traits studies. Covariances were broadly strong and positive at the level of phenotype and year. However, I detected few genetic correlations suggesting that phenology traits are free to respond to selection independently.

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Chapter 1

General Introduction

A seasonal environment imposes constraints on all living organisms, and consequently energetically-demanding life stages such as growth and reproduction occur when the weather is clement and food is plentiful (Farner, 1985). The timing of trait expression in response to seasonality, termed phenology (Sparks and Menzel, 2002), is a fundamental adaptation to life in a seasonal environment. Although there has been much work on the causes and consequences of timing of breeding in many taxa (e.g. Rathcke and Lacey, 1985, Visser et al., 2006, Réale et al., 2003b), we know less about other phenological traits and almost nothing about how phenological traits covary, both at the level of the phenotype and genotype. The aim of this PhD is to investigate the phenology of a wide range of traits in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, NW Scotland (Clutton-Brock et al., 1982). Within this thesis, each chapter is introduced independently and consequently here I focus on a brief summary of the broad topic of phenology. I then describe the study system and outline the questions addressed in this thesis.

Causes of variation in phenology

Numerous factors may generate differences in phenology, either within or between populations. I focus here on causes of variation between individuals, within a single population, and explore the multiple environmental and genetic variables that may be generating this variance in different phenological traits. Evolution, the between-generational change in the mean value of a trait due to selection, requires the

presence of heritable additive genetic variation (Lynch and Walsh, 1998). However, many other processes can contribute to phenotypic variation observed in wild systems and it is important to be able to separate these factors (Kruuk and Hadfield, 2007), as they can have differing effects on population dynamics, and ultimately on evolutionary processes. I outline below factors known to correlate with phenological variables in wild populations.

The phenology of many traits expressed repeatedly across an individual's lifetime varies with age. Prime aged individuals tend to express traits at the optimal time as younger individuals face stronger trade-offs between growth and investment in optimal timing of trait expression (Green and Rothstein, 1991). There is also evidence that learning may have a role in the phenology of breeding time in some birds (Grieco et al., 2002). Contrastingly, the weakening of selection at older ages leads to senescence and a general decrease in performance (Bonsall, 2006), although the patterns of age-related variation is not uniform across ages. For instance, the female reproductive system in mammals undergoes a rapid shutdown (Cohen, 2004), especially in humans (Armstrong, 2001), whereas offspring birth weight often shows more gradual patterns of senescence (Kirkwood and Rose, 1991).

Although there is evidence that photoperiod plays an important role in determining the expression of traits during the appropriate time period (Bradshaw and Holzapfel, 2007), environmental variation causes the optimal time of trait expression to vary. Consequently, adaptations have evolved to account for this environmental heterogeneity, termed phenotypic plasticity (Pigliucci, 2001). However, although phenotypic plasticity may reflect an adaptive condition-dependent response to a varying environment (McCleery and Perrins, 1998, Stevenson and Bryant, 2000,

Nussey et al., 2005), it may also be reflective of constraints placed on an individual due to, for example, reduced food availability (Perrins, 1970). There is also evidence that individuals differ in their response to environmental variation, termed IxE (Nussey et al., 2007).

Consistent differences between individuals, or repeatability, can be caused by factors other than due to heritable genetic variation. For instance, conditions experienced in early life can have lasting effects on a wide range of traits expressed in adults (Lindstrom, 1999). Connected to this, variation between mothers in investment in offspring can also have profound effects on an individual's phenotype; these maternal effects may themselves also be heritable (Mousseau and Fox, 1998, Rasanen and Kruuk, 2007). Interestingly, evidence has recently emerged in wild bird populations of between-male effects on the breeding time of their mates, perhaps due to genetic variation in provisioning rates and paternal care (Brommer and Rattiste, 2008, Teplitsky et al., 2010). Correctly separating each of these effects requires detailed and long-term datasets where individuals are known.

Consequences of variation in phenology

Variation in phenology is under selection in many systems. The timing of reproduction has fitness consequences acting through offspring growth and survival (e.g. Perrins and McCleery, 1989, Festa-Bianchet, 1988, Gienapp et al., 2006) and future reproductive opportunities or survival of the mother (e.g. Clutton-Brock et al., 1983, Sheldon et al., 2003). The timing of migration in passerine birds is also under selection (Brown and Brown, 2000), as is timing of moult of plumage feathers in a secondary sexual characteristic (Cockburn et al., 2008). However, directional selection on a

heritable trait does not always lead to the expected evolutionary response (Merila et al. 2001).

Phenology and climate change

Advancing phenology is one of the most striking effects of climate change (IPCC, 2007) on wild populations and has been observed in a wide range of taxa (Sparks and Menzel, 2002, Thackeray and al, 2010, Parmesan, 2006). In plants, the effects of a warming climate are most likely causal: warmer temperatures early in the season lead to faster rates of photosynthesis and thus advancing growth (Thackeray and al, 2010). In higher trophic levels, effects of a warming climate may be causal, perhaps acting through changes in energy requirements for thermoregulation; temperature may even be a direct cue for trait expression (Visser et al., 2009). However, indirect effects such as the advancement of availability of food resources will also affect phenology. There is consequently the potential for a mismatch to occur in higher trophic levels between the peak of food resources and the peak of energy requirements (Visser et al., 1998, Stenseth and Mysterud, 2002). This phenomenon has been suggested to be affecting differing rates of population decline between populations of pied flycatchers (Both et al., 2006) but its wider prevalence in wild populations is currently unknown.

There is now a pressing need for research to focus on questions other than the simply the rate of advancement of phenology. For instance, is the rate of advancement sufficient for population persistence (Visser, 2008)? Are traits expressed in the same population advancing at the same rate? Is climate change causing the selective landscape to also change (Visser et al., 1998, Both and Visser, 2001)? Will phenology continue to advance indefinitely? If phenology cannot advance indefinitely, what will happen when this limit to phenological advancement approaches? Is the observed

advancement due to phenotypic plasticity or micro-evolution or even changes in demographic parameters such as age (Réale et al., 2003b)? Will rates of micro-evolution in response to climate change be affected by genetic correlations between phenological traits? Addressing each of these questions would move us towards a more holistic understanding of the effects of climate change on wild populations and would illuminate our understanding of how wild organisms might cope in the face of climate change.

Correlations between phenological traits

A continued response to climate change will necessitate micro-evolution as the limits to phenotypic plasticity are reached (Crozier et al., 2008, Visser, 2008). Micro-evolutionary response is dependent on levels of heritable genetic variation but can also be constrained or promoted by genetic correlations between traits (Lande, 1979). However, we know almost nothing about how phenological traits in wild populations correlate at the phenotypic and genetic level (exceptions in plants are O'Neil, 1997, Kelly, 1993). An understanding of the genetic architecture underlying phenological traits is therefore essential in determining how a population might respond to climate change.

The study system

In this thesis, I will investigate the phenology of a wide range of traits in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, NW Scotland. Red deer are the archetypal example of a sexually dimorphic polygynous species, with males being up to 70% larger than females; males are also the only sex to grow antlers. Red deer are large herbivores, ranging widely across northern latitudes (Whitehead, 1972). Males (stags) play no role in offspring care. Fitness in stags is determined entirely by

performance during the annual mating season (or 'rut') between October and November (Fig 1.1), where stags use antlers grown annually during the spring in fights for access to oestrous females (Clutton-Brock et al., 1982, Clutton-Brock et al., 1997). In contrast, after conception females gestate their offspring throughout the winter following the rut, giving birth in the following spring. Birth of offspring, termed calves, is highly synchronous with over 70% of births occurring within a three-week period (Fig 1.1; Guinness et al., 1978b). The calves remain in the long grass for approximately the first two weeks of life, and the mother (hind) returns to the calf multiple times per day to feed and groom them. As the calf becomes stronger and more surefooted, it begins to spend more time with the main herd. A calf will remain in close proximity to its mother, suckling from her often until at least the autumn. If the hind is to conceive again, she will cease suckling her calf during the autumn following its birth; however lactation may continue for a further year (Clutton-Brock et al., 1982). Male offspring tend to remain with their mother until the age of two years when they will emigrate from the matrilineal group, but females are philopatric and remain in matrilineal groups, often throughout their lifetimes. Consequently the properties that constitute successful males and females are very different, skew in reproductive success is much higher in males that in females and there is even evidence for a negative cross-sex genetic correlation for fitness: genes that constitute a successful male do not necessarily perform well when expressed in a female (Foerster et al., 2007).

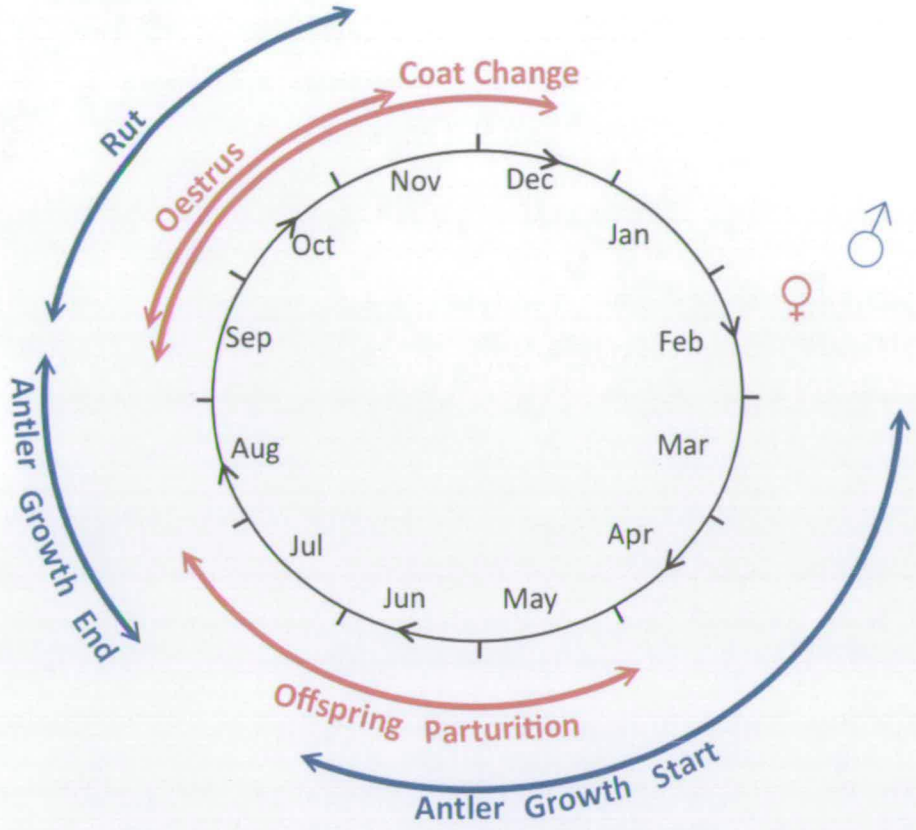


Figure 1.1 Phenological variables studied in this thesis: the arrows show the time period during which each event may occur. The inner circle (coloured red) shows traits recorded in females: oestrus date in autumn leading to parturition of the offspring in the following spring, and coat change from summer to winter coat in autumn. The outer circle (coloured blue) shows the cycle of antler growth determined by the cast date of the previous antler signaling start of growth and the cleaning of the vascular tissue surrounding the growing antler as indicating end of growth. The timing of male rutting is also shown: I analysed an individual's start, median and end date of rutting.

At 105km^2, the Isle of Rum is the largest of the 'Small Isles' lying off the West coast of Scotland. The red deer on the Isle of Rum are believed to have been introduced to the island from mainland Scotland between 1894 and 1920, following the extinction of an earlier population (Clutton-Brock et al., 1982). The habitat on the island is predominantly exposed moorland with large areas of *Culluna-Molinia-Trichophorum* vegetation and smaller patches of the more fertile *Agrostris-Festuca* grassland (Mitchell

et al., 1976). For deer management purposes the island is divided into five blocks, and prior to 1972 deer density was maintained at uniform levels across the whole island. However, in 1972 culling ceased in the North Block leading to a rapid increase in population density.

The red deer in our study system are resident in the North Block of the Isle of Rum. They have been studied intensively since 1972, although more intermittent data collection began in 1968. We aim to capture each deer within the first few days of life in order to take measurements such as body mass, to apply markings for future identification, and from 1982 to take tissue samples for genetic analyses. Although artificial markings greatly improve the identification process, natural markings and individual idiosyncrasies mean that it is possible to identify unmarked animals reliably. Continual monitoring of the study population enables the phenology of a number of traits to be known with high accuracy, and age is known for the vast majority individuals in the population (Clutton-Brock et al., 1982).

The data collected on over 4,000 red deer on the Isle of Rum has contributed to a wealth of studies investigating diverse topics such as behaviour, life history, population dynamics and evolutionary genetics. The individual-based data, often including repeated measures across an individual's lifetime, enables between and within individual causes of variance to be reliably separated. The release of the population from culling in 1972, led to a rapid increase in female population density until approximately 1981, when the population density began to stabilize, and density has since fluctuated around presumed ecological carrying capacity (Albon et al., 2000). This rapid change of density had consequences observable in a wide range of traits including offspring birth date (Nussey et al., 2005), male antler mass (Kruuk et al.,

2002), female fecundity (Kruuk et al., 1999b), and even the distribution of male mating success (Clutton-Brock et al., 1997). Weather has also been found to correlate with many traits in this system, including offspring birth date, and both juvenile and adult survival (Albon et al., 2000, Coulson et al., 2003). Although many of the effects of weather are short term, conditions in the year of birth can have effects lasting throughout an individual's lifetime (Kruuk et al., 1999b). More recently, a pedigree has been constructed for the population (Pemberton et al., 1992, Walling et al., 2010), stimulating a number of quantitative genetics studies (e.g. Kruuk et al., 2000, Coulson et al., 2003, Foerster et al., 2007). Prior to the start of my PhD, offspring birth date was the only phenological trait to have been studied intensively, and was known to vary with age, environmental and ecological conditions, and also to be heritable (Coulson et al., 2003, Nussey et al., 2005). During my PhD, I have been co-author on two papers. The first demonstrates varying patterns of senescence in a number of phenotypic characteristics, including offspring birth date and antler growth phenology (Appendix III). The second is an investigation into temporal trends in phenological variables since 1980, potentially as a direct result of climate change, finding different rates of advancement across different traits although little knock-on effects on demographic parameters (Appendix V).

The aims of this thesis was therefore to use the comprehensive and unparalleled data collected over 42 years to investigate phenology in the wild red deer population on the Isle of Rum. I begin by examining factors influencing variation in antler growth phenology, and how antler growth phenology correlates with mass of antlers grown and reproductive success (Chapter 2). Chapter 3 is an investigation into variation in gestation length, including possible correlations with conception date. In a similar vein, chapter 4 considers variation in coat change date and associations with

over-winter survival. I then move onto comparisons between phenological traits, looking first at how the mean, variance, skew and kurtosis of traits have been changing over time, potentially as a direct result of climate change (Chapter 5). Finally, Chapter 6 is a study of correlations between phenological traits expressed in this population, both at the level of phenotype and genotype.

A number of statistical techniques are presented in this thesis. The use of mixed-models (Pinheiro and Bates, 2000) allows pseudoreplication across individuals and years to be corrected for, and the parameters estimated; consequently, analyses in all chapters include mixed-models. An extension of mixed-models is the so called 'animal model' (Kruuk, 2004), which enables pedigree information to be incorporated in the random effects structure and estimates of additive genetic variance to be obtained; animal models are presented in chapter 6. Bivariate models estimate the covariance between traits at the level of the individual, year and if pedigree information is incorporated, at the genetic level (Chapters 3 and 6). Bivariate animal models therefore allow us to ask if traits expressed in different sexes are influenced by the same genes, termed cross-sex genetic correlations (Wilson et al., 2010; appendix IV, Chapter 6). Additionally I present an analysis of selection on antler growth phenology, where the analysis was carried out within a Bayesian framework (Chapter 2).

Chapter 2

Getting the timing right: antler growth phenology and sexual selection in a wild red deer population

This chapter has been published as: Michelle N. Clements, Tim H. Clutton-Brock, Steve D. Albon, Josephine M. Pemberton & Loeske E.B. Kruuk, 2010. Gettting the timing right: antler growth phenology and sexual selection in a wild deer population. *Oecologia*, **164,** 357-368.

Summary

There has been growing interest in the determinants of the annual timing of biological phenomena, or phenology, in wild populations, but research on vertebrate taxa has primarily focused on the phenology of reproduction. We present here analyses of the phenology of the annual growth of secondary sexual characteristic, antlers in red deer (*Cervus elaphus*) males. The long-term individual-based data from a wild population of red deer on the Isle of Rum, Scotland allow us to consider ecological factors influencing variation in the phenology of growth of antlers, and the implications of variation in antler growth phenology with respect to the phenotype of antler grown (antler mass) and annual breeding success.

The phenology of antler growth was influenced by local environmental conditions: higher population density delayed both the start date (during spring) and the relative end date (in late summer) of antler growth, and warmer temperatures in

the September and April prior to growth advanced start and end dates respectively. Furthermore, there was variation between individuals in this phenotypic plasticity of start date, although not in that of end date of growth. The phenology of antler growth impacted on the morphology of antlers grown, with individuals who started and ended growth earliest having the heaviest antlers.

The timing of antler growth phenology was associated with breeding success in the following mating season, independently of the mass of antlers grown: earlier start of antler growth was associated with siring a higher number of the calves born the following spring. Our results suggest that the phenology of traits that are not directly correlated with offspring survival may also regularly show correlations with fitness.

Introduction

In populations that experience a seasonal environment, the annual timing of events, or phenology, can be an important determinant of fitness (Post et al., 2008). Whilst studies of the consequences of variation in phenology have focused primarily on the timing of breeding (e.g. Charmantier et al., 2008), variation in the phenology of the growth of annually regenerating secondary sexual characteristics may also show correlations with fitness. Selection on the phenology of traits expressed annually by males might be expected in polygynous systems where there is higher variation in breeding success between males than between females, and hence the potential for strong sexual selection. Here we use data collected over 39 years from a wild red deer (*Cervus elaphus*) population on the Isle of Rum, NW Scotland (Clutton-Brock et al., 1982), to investigate factors influencing individual differences in antler growth phenology, and the implications of this variation in phenology with respect to both the antler mass grown and subsequent breeding success.

Despite much research considering selection on secondary sexual characteristics in polygynous systems, (e.g. Torbjorn von et al., 1994, Coltman et al., 2002, Kruuk et al., 2002), less attention has been paid to male phenology and its possible correlation with male reproductive success. Selection on the phenology of expression of a secondary sexual characteristic acting through female choice occurs in superb fairy-wrens, with early moulting males having highest reproductive success (Cockburn et al., 2008). However, to our knowledge, to date there has been no study examining associations between the phenology of growth of a sexually selected weaponry trait and breeding success, nor of selection on the phenology of a sexually selected trait whilst controlling for the actual phenotype of trait produced by each individual on an annual basis.

Deer antlers represent one of the most impressive examples of a sexually-selected weaponry trait in mammals and are regrown annually. In red deer, males use antlers during the rutting season in fights with other males in order to defend harems of females, and thus gain paternities. Large antlers are associated with high annual breeding success (Kruuk et al., 2002, Bartos and Bahbouh, 2006), although this association does not necessarily indicate a causal relationship (Clutton-Brock, 1982) Antler mass shows pronounced age-related variation (Kruuk et al., 2002, Mysterud et al., 2005) and sensitivity to both environmental conditions and population density (Schmidt et al., 2001, Mysterud et al., 2005, Kruuk et al., 2002) suggesting that it may be correlated with individual condition. Casting of the previous year's antlers and the immediate start of growth of the current year's antlers occurs during the spring (Li et al., 2004), when testosterone levels are very low and day length is increasing (Goss, 1983, Lincoln, 1992). Rising levels of testosterone as the autumnal rut approaches act as a brake on longitudinal growth (Goss, 1968), leading to the velvet covering, which

initially covers the antlers and is rich in vascular tissue (Goss, 1983), dying and then being cleaned from the antlers in late summer (see Fig. 1 for a representation of the deer annual cycle).

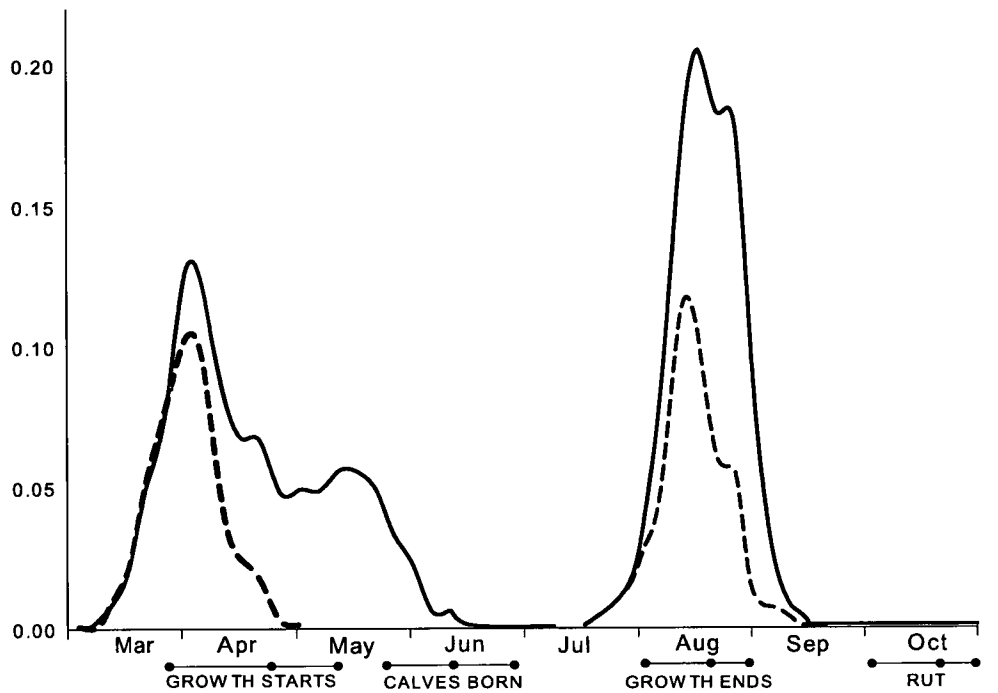


Fig. 2.1. The red deer year. Density plots of start (left) and end (right) dates of antler growth. Solid lines show all males, whereas dotted lines show only mature aged males (aged 8 to 14), where there is no age related variation in the start date of antler growth or antler mass (density adjusted to reflect proportion of mature males in population). Horizontal lines below density plot show mean date ± 1 SD of appropriate event for all individuals (rut timing was determined by taking the mean and standard deviation of the mean day each individual was observed holding a harem).

Phenotypic plasticity (Pigliucci, 2001) in red deer antler growth phenology has been observed in the start date of growth, which becomes later with increasing population density (Clutton-Brock et al., 1982) and with increasing levels of over-

winter snowfall (Watson, 1971). Recent analyses also indicate that several aspects of phenology, including the start and end dates of antler growth, have become progressively earlier in the Rum population since 1980, possibly due to effects of the warming climate and its impact on vegetation growth (Moyes et al, in review; appendix V). Antler growth phenology is age-dependent, with older males starting growth earliest (Clutton-Brock et al., 1982) and, in addition to these age effects, there may also be correlations between early start date of growth and dominance rank (Bartos, 1980). However, the implications of this variation in phenology both for the phenotype of antlers produced each year (variation in the timing and length of the growth period may generate variation in size) and ultimately for individual breeding success is not known.

The long-term data from the Isle of Rum red deer study allow us to examine the phenology of a sexually-selected trait expressed by males in a wild polygynous system and how it correlates with antler phenotype and breeding success. We ask here if relationships between environmental conditions (weather and population density) and antler mass are primarily a result of variation in antler growth phenology or if there exist independent pathways influencing both antler growth phenology and antler mass. Furthermore, we can test whether correlations between antler growth phenology and fitness are entirely due to variation in antler mass, or whether additional associations exist between phenology and breeding success independently of antler mass. Our aims in this paper are therefore to address the following three questions:

1. What are the determinants of variation between individuals in antler growth phenology, and in particular what are the impacts of age, population density and weather conditions?

2. How does antler growth phenology affect the mass of the antlers grown in a given year?
3. What are the sexual selection pressures acting on antler growth phenology?

Methods

STUDY SYSTEM

The red deer study population on the Isle of Rum has been monitored since 1968, although intensive study began in 1971 (Clutton-Brock et al., 1982). All individuals in our study system can be recognised by a combination of natural and artificial markings, enabling us to monitor them from birth, through all reproductive events, to death; age is therefore known for all individuals.

DATA COLLECTION

1. Antler and morphological data

The start and end dates of an individual's antler growth (i.e. casting and cleaning dates) were measured as the exact number of days since March 1st of each year. As not all males are sighted each day, there is often some level of uncertainty in the exact date of start or end of antler growth. Results presented here include only instances where an individual was sighted on the start or end date and also the previous day so that the relevant date was known exactly. We present here data from observations of casting and cleaning dates between 1968 and 2006.

Cast antlers were collected in the field, with the unique shape of each antler allowing the identity of the appropriate male to be determined, and then weighed. Genetic analysis of DNA extracted from antler bone indicates that recognition methods have a success rate of 93% (J. Pemberton, unpublished data) but, since the morphology

of the misidentified and correct antlers will be similar, errors are expected to have had a negligible impact on the results presented here. If both an individual's antlers were recovered, we used the average of the two measurements; if only one was found, then we used the sole measurement.

To control for variation in antler growth phenology associated with body size and for allometric effects on antler mass, we included measures of individual skeletal size in our analyses. Hind leg length, defined as the length of the metatarsus from proximal to distal metatarsal canal (mm) measured on deceased individuals, was taken as a measure of skeletal body size. As hind leg length asymptotes at three years of age (Kruuk et al., 2002), in these analyses we included only males aged three years and over during the period of antler growth.

2. Breeding success

An individual's annual breeding success (ABS) was measured as the total number of paternities amongst calves born in the spring following the rut in a given year. Paternity was inferred at 80% or greater confidence using a panel of up to 15 microsatellite loci and the parentage inference software CERVUS 3.0 (Kalinowski et al., 2007), allowing no more than one mismatch between parents and offspring. Where a genetic paternity was not assigned, we used harem holding information obtained during observations in the rut to determine likely paternities. We assigned paternity of a calf to a particular male if its mother was observed in his harem for at least 6 out of 11 days surrounding the date of probable oestrus, determined by backdating from the date of birth of the calf; this procedure assigns the correct sire in approximately 70% of cases (Pemberton et al., 1992). In order to avoid erroneously assigning zero breeding success to males who rutted in other parts of the island, in the selection analyses we

considered only males that were observed holding a harem in our study area for at least one day during the rut.

3. Environmental variables

Population density was calculated from 40 separate censuses conducted per annum, where the location of each individual observed is recorded. As with previous studies (e.g. Kruuk et al., 2002) we used the number of adult females observed in over 10% of censuses between January and May in the year of antler growth as a measure of resident population size (hereafter population density). We also tested population density in the year prior to growth but it was not found to be significant once current population density had been accounted for. The number of adult females resident in the study area increased over the study period from a minimum of 57 at the start of the study until reaching presumed ecological carrying capacity in the early 1980's, since when it has fluctuated around this level (Albon et al., 2000). The maximum number of females was 211 in 1999 and the average number was 146. We also considered the number of males and total number of adult deer in the study area as possible explanatory variables, but female density was the most predictive, possibly because it is most reflective of vegetation levels in the study area due to females remaining in their natal area (Clutton-Brock et al., 1982).

Temperature and precipitation records were taken from the Meteorological station in Kinloch on the Isle of Rum. We considered average daily temperatures (ºC) and total rainfall (mm) across one-month periods preceding the start or end dates of antler growth as possible covariates with antler growth phenology and antler mass grown. For the start date of antler growth, we considered climatic conditions between August in the year prior to growth and March in the year of growth inclusive, and for

the end date of growth and antler mass we considered all months from the August in the year prior to growth to August in the year of growth.

4. Sample sizes

The variables considered here required intensive field observations over many years; consequently observations were not available for all individuals in all years, and some years are not represented in all of the models. For instance, the model of antler weight required that the exact start and end dates of antler growth were observed in a given year and that the cast antler was collected the following year to be weighed. Sample sizes and years included in each model are quoted in the table describing the relevant model.

STATISTICAL ANALYSIS

1 & 2. Variation in antler growth phenology and antler mass

Linear mixed effects models (Pinheiro and Bates, 2000) with a Normal error distribution were used to investigate factors influencing the phenology of antler growth and antler mass. The variables included in these models as fixed effects were: age as a multi-level factor (3-15 years inclusive), hind leg length as a measure of skeletal size, population density in the year of growth and its quadratic, and climatic variables. All continuous explanatory variables were mean-centred. In the model of end date, we corrected for start date, allowing us to investigate factors determining the end date relative to start date. The model of antler mass included the start and end dates of antler growth as fixed effect covariates. Random effects of individual identity (ID) and year of growth, as a factor, were included in all models.

We created maximal models including all explanatory variables (except hind leg length) for the start and end dates of antler growth and antler mass, which were then dropped from the model in a sequential fashion if not significant. Significance of fixed effects was assessed using a chi-squared test on the ratio of log-likelihoods of compared models, obtained using maximum likelihood (ML) methodologies (Pinheiro and Bates, 2000), until only variables significant at the 5% level, and a maximum of one climatic variable due to the high correlations between weather variables, remained.

We fitted age as a factor, rather than a continuous covariate, as graphs of antler growth phenology and antler mass as a function of age (Fig 2.2) did not appear to follow a distribution that could be readily described using a linear or quadratic term. Antler growth phenology became earlier and antler mass became greater with increasing age, until approximately eight years old, after which point there was a marked flattening in the graph. We began by fitting age in years as a factor, with each age added independently. We then grouped consecutive ages with similar parameter estimates as one factor and tested, using log-likelihood ratio tests, if models with and without the selected age grouping were significantly different. We accepted the largest age range for which there was no significant difference between models where age was grouped and ungrouped and then ensured that all other fixed effects remained significant at the 5% level.

As with fixed effects, significance of random effects were assessed using chi-squared tests on log-likelihood ratios, with the distinction that likelihoods were obtained using restricted maximum likelihood methodologies (REML, Pinheiro and Bates, 2000). In the models of start and end dates of antler growth we tested for individual variation in responses to climatic conditions (i.e. individual variation in

phenotypic plasticity, or "IxE" interactions; Nussey et al., 2007) by fitting random regression models (a form of linear mixed effect model) where an interaction term between individual identity and the climatic variable most influencing start or end date was added to the random effect structure (Nussey et al., 2007).

Once the most appropriate model had been determined, we then added hind leg length to the fixed effect structure. Inclusion of this variable caused a large decrease in sample sizes and consequently we used this model to determine the significance of hind leg length only. To ensure that we were accounting for all allometric effects in the model of antler mass, we took the natural logarithms of antler mass and hind leg length prior to inclusion in this step of the model (Kerkhoff and Enquist, 2009).

3. Selection on antler growth phenology: associations with annual breeding success

Analysis of annual breeding success (ABS) was carried out in a Bayesian framework (Sorensen and Gianola, 2002) using the MCMCglmm package in R (Hadfield, 2010). Fitting likelihood-based mixed models can be problematic where the error structure is Poisson distributed because the likelihood has to be approximated rather than obtained analytically (Breslow and Clayton, 1993, Breslow and Lin, 1995). This approximation can perform poorly, especially when there are low numbers of replicates per random effect (Breslow, 2003). The use of Markov Chain Monte Carlo (MCMC) techniques allowed us to sample from a series of more analytically manageable conditional distributions, which converge on the joint posterior distribution (Sorensen and Gianola, 2002).

A linear model of ABS was fitted that predicted the Poisson rate parameter on the logarithmic link scale. The Poisson rate parameter is unobserved and is treated as

an augmented latent variable updated using Metropolis-Hastings updates. These logged rate parameters are assumed to follow a Normal distribution with mean determined by the fixed effects structure, and covariance determined by the random effects and an additional residual term to account for over-dispersion (see Sorensen and Gianola, 2002 for more details). Fixed effects, random effects and variance components were Gibbs sampled using the Gibbs sampling method of Garcia-Cortes and Sorensen (2001). Weakly informative priors for the variance components were obtained by determining between-individual and between-year variances for those males who participated in the rut but for whom we do not have data on the timing of antler growth. Each chain was run for 400,000 iterations with a burn-in of 20,000 and thinning interval of 380.

Significance of fixed effects in the models were tested for by examining 95% confidence intervals of parameter estimates obtained from the model; where the estimate did not range over zero, the parameter was considered to be significant. Maximal models were created and non-significant fixed effects were sequentially removed from the model until only those significant at a 95% confidence level remained. Results are reported as mean parameter estimate ± 1 posterior standard deviation (PSD), which is equivalent to standard error in likelihood based models, and the associated 95% confidence interval. Individual ID and year were used as independent random effects throughout. We approached selection analysis in two ways:

i. Total selection on antler phenology: To determine how the observed timing of antler growth correlated with ABS, we first constructed models where the start and

end dates of growth were the only covariates with ABS. Significance of interactions and second order terms were also tested for.

ii. Direct selection: We define direct selection as the association between antler growth phenology and ABS whilst also correcting for other known correlates of ABS (Kruuk et al., 2002), specifically age and its quadratic, antler mass, population density and a measure of skeletal size (hind leg length).

All models were run in R v2.6.2 (R Development Core Team, 2008). Models of antler growth phenology and antler mass used the package lme4 (Bates, 2007); http://lme4.r-forge.r-project.org/), whereas the Bayesian models of ABS were ran in MCMCglmm (Hadfield, 2010; http://cran.r-project.org/web/packages/MCMCglmm/index.html).

Results

1. Variation in antler growth phenology

(i) Summary statistics

Across our study population, the mean start date of antler growth was the 19th April (day 50, SD=21 days; Fig. 2.1), and mean end date was the 16th August (day 169, SD=9 days; Fig. 2.1). The within-year correlation between an individual's start and end dates of antler growth was 0.59 (n=629, p<0.001).

(ii) Age-related variation and the influence of start date on the end date of growth

The average start date of antler growth showed large amounts of age-related variation up until 8 years of age. For instance, the average start date for males aged 8 was almost 50 days earlier than that for 3-year-olds (Table 2.1, Fig. 2.2). However no

age-related variation was observed in start date for males of 8 years and above (comparing models with and without age groupings: $\chi^2_{(d.f.=7)}=13.84$, P=0.054). In the model of end date, the parameter estimate for start date (b=0.22±0.01SE; Table 2.1) showed that an advance in start date of approximately 5 days was correlated with average end date advancing by one day. After correcting for start date, end date varied by less than 2 days between 3 and 10 years of age (Table 2.1), although it became later in individuals aged 11 and over (Table 2.1). Consequently, those individuals who started growth early grew antlers for a longer period (Table 2.1), resulting in an increased growth period with increasing age until the age of 8 (Fig. 2.2). For example, the average growth period for 8-year-old males was 144 days, whereas that of 3-year-old males was 105 days, a difference of 39 days.

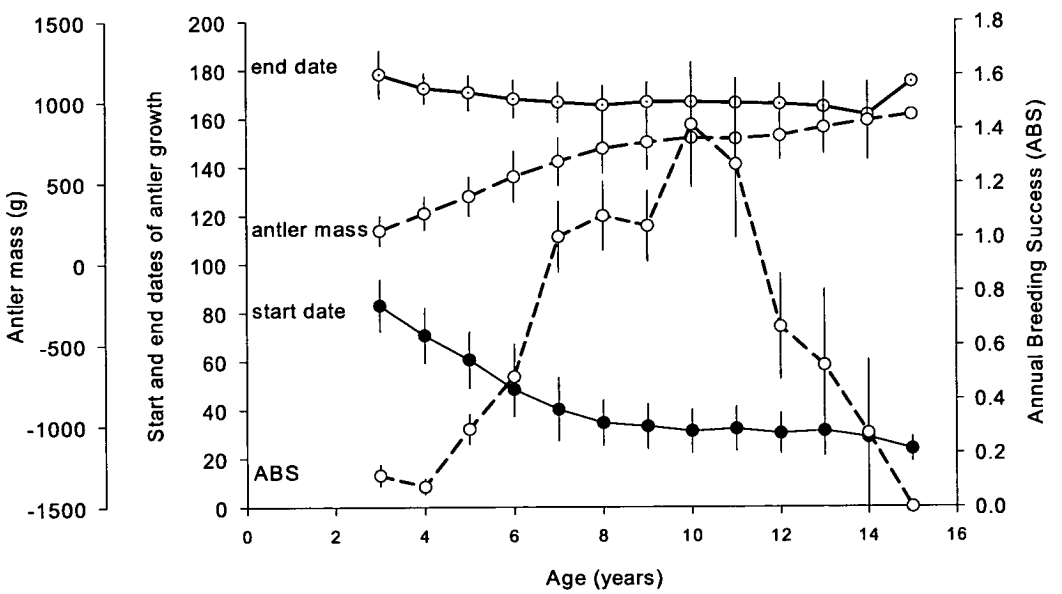


Fig. 2.2. Age related variation in antler growth phenology, antler mass and annual breeding success among males. Error bars show mean value ±1SD for start date of antler growth, end date of antler growth and antler mass and mean value ±1SE for annual breeding success as a function of male age (years).

(iii) Current environmental conditions

Higher population density was associated with later start and end dates of antler growth (Table 2.1). An increase in density of 100 females delayed the average start date of growth by approximately 9 days and delayed the average end of growth by approximately 7 days, once the effect of start date on end date had been accounted for (end date=5+0.22*9; Table 2.1), leading to a decreased growth period at high population density (Table 2.1). Warmer temperatures in the September prior to the start date of antler growth led to an earlier average start date (average September temperature = 12.1°C; ±0.8 SD; range=10.3°C-13.6°C; Table 2.1), and end date responded in a similar manner to the temperature in April in the year of growth (average April temperature = 7.3°C; ±1.0 SD; range=5.4°C-9.1°C; Table 2.1).

(iv) Associations with body size

Adding body size, measured as hind leg length, to the models of start and end dates reduced sample sizes to n=471 and n=217 observations respectively. There was no evidence that skeletal body size was associated with either start date ($\chi^2_{(1)}$=0.707, P=0.400) or end date ($\chi^2_{(1)}$=0.188, P=0.665) of antler growth.

(v) Components of variance (random effects)

Individual identity and year of antler growth were significant variance components in the models of start and end date, indicating consistent differences between individuals and years over and above those corrected for by the fixed effects described above (Table 2.1). Individual identity explained 42% of the variation remaining in start date, and 58% of the variation in end date of antler growth. Unexplained differences between years accounted for 9% of the remaining variance in start date, and 16% in end date.

Individuals varied in their phenotypic plasticity in response to variation in climatic conditions influencing start date (Table 2.1), as seen by the significance of a random effect interaction (IxE) between individual identity and the climatic variable (September temperature) influencing start date of antler growth. However there was no evidence that individuals varied in their response to those climatic conditions influencing end date (April Temperature: $\chi^2_{(2)}=2.130$, P=0.345).

Table 2.1. Estimates of fixed and random effects from linear mixed effect models of (a) start and (b) end dates of antler growth. All continuous explanatory variables were mean-centred prior to inclusion in the model. In the model of antler growth start date, the significant random regression IxE term is shown. Significance of fixed and random effects was assessed by log-likelihood ratio tests of models with and without the appropriate parameter.

Fixed effect	Parameter Estimate (SE)	df	Chi-sq.	p-value
a) Start Date of Growth				
Intercept	81.92 (1.02)			
Age = 3	0.00	5	2705.8	<0.001
Age = 4	-10.95 (0.75)			
Age = 5	-20.75 (0.76)			
Age = 6	-33.30 (0.77)			
Age = 7	-41.42 (0.77)			
Age 8 - 15	-49.40 (0.67)			
Population Density	0.09 (0.01)		28.49	<0.001
September Temp	-1.96 (0.70)		7.53	0.007
Random effects	**Variance component (SD)**			
Individual	40.76 (6.38)	1	442.92	<0.001
Individual x Sep Temp	9.10 (3.02)	2	19.70	<0.001
Year of Antler Growth	8.51 (2.92)	1	103.66	<0.001
Residual	43.97 (6.63)			
	n = 1,559; 412 individuals; 39 years (1968-2006)			
b) End Date of Growth				
Intercept	169.03 (0.96)			
Start Date of Growth	0.22 (0.01)	1	195.91	<0.001
Age = 3	0.00	4	63.22	<0.001
Age 4 - 7	-1.20 (0.59)			
Age 8 - 9	0.35 (0.85)			
Age = 10	1.77 (1.05)			
Age 11 - 15	3.71 (0.98)			
Population Density	0.05 (0.01)	1	11.19	0.001
April Temp	-1.20 (0.53)	1	4.55	0.033
Random effects	**Variance component (SD)**			
Individual	23.10 (4.81)	1	285.17	<0.001
Year	6.48 (2.54)	1	118.59	<0.001
Residual	10.04 (25.34)			
	n = 629; 249 individuals; 31 years (1971 – 2006 excluding 1973, 1976, 1977, 2002 & 2004)			

2. The effect of phenology on antler mass

Antler growth start and end dates were significant predictors of antler mass grown: individuals that started and ended growth early also had the heaviest antlers (Table 2.2, Fig. 2.3). The significant interaction between these dates showed that those individuals who both started and ended growth early had heavier antlers than would be predicted from the additive effects alone (Table 2.2, Fig. 2.3c). There was no evidence of a quadratic relationship of either start or end date with antler mass (start: $\chi^2_{(1)}=0.38$, P=0.536; end: $\chi^2_{(1)}=2.37$, P=0.124).

Average antler mass increased with age (Fig. 2.2; Table 2.2). As with start date of growth, we observed no significant age-related variation within mature males (those aged eight years and over; $\chi^2_{(6)}=10.26$, P=0.114).

Environmental factors influenced antler mass, even after correcting for start and end dates. Increasing population density was linked with lighter antlers, although a significant quadratic effect indicated a higher impact at low density (Table 2.2). Warm temperatures in March of the year of growth led to a heavier average antler mass (Table 2.2; average March temperature = 5.7°C; SD=1.1; range=3.4°C-7.8°C).

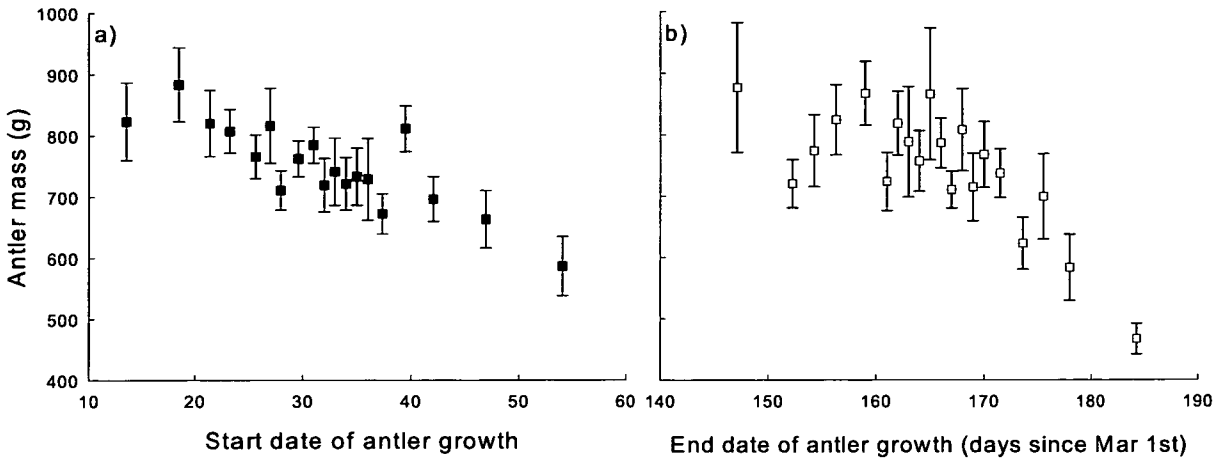
Individual identity was a highly significant component in the model of antler mass, accounting for 82% of the remaining variance (Table 2.2). In contrast, year of antler growth was not a significant component in this model, consisting of just 0.7% of the variance once the fixed effects described above had been entered into the model.

When examining the relationship between body size and antler mass, we fitted a separate model including all of the fixed effects described above and an additional

term of the natural logarithm of hind leg length (doing so reduced the sample size to 113 observations from 50 individuals across 21 years). We also log-transformed the response variable antler mass in this analysis. There was a significant positive association between antler mass and body size ($b=2.25\pm0.09$, $\chi^2_{(1)}=5.20$, $P=0.023$) which on the untransformed scale of antler mass corresponds to antler mass increasing at a rate of hind leg length to the power of 2.25.

Table 2.2. Analysis of antler mass (g). Estimates of fixed and random effects from linear mixed effects models with Normal error distributions. Significance of effects was assessed using log-likelihood ratio tests between models with and without the appropriate effect. All continuous fixed effects were mean centered. Start and end dates refer to the timing of antler growth as measured annually in each individual.

Fixed effect	Parameter Estimate (SE)	df	Chi-sq.	p-value
Antler Mass				
Intercept	187.68 (19.23)			
Age = 3	0.00	5	99.87	<0.001
Age = 4	100.10 (17.23)			
Age = 5	185.36 (16.69)			
Age = 6	248.01 (16.19)			
Age = 7	327.27 (16.37)			
Age 8 - 15	389.15 (15.63)			
Start Date	-2.95 (0.71)	1	8.12	0.004
End Date	-4.10 (1.06)	1	19.36	<0.001
Start:End Dates	0.09 (0.04)	1	6.09	0.014
Population Density	-0.97 (0.24)	1	9.34	0.002
Population Density^2	0.007 (0.003)	1	4.20	0.040
March Temp	20.21 (5.94)	1	12.70	<0.001
Random effects	**Variance Component (SD)**			
Individual	16290.87 (127.64)	1	132.52	<0.001
Year	133.45 (11.55)	1	0.64	0.42
Residual	3545.54 (59.54)			
	n = 293 ; 155 individuals; 26 years (1971-2006 excluding 1972, 1973, 1976, 1977, 1995, 1999, 2000, 2002, 2004 & 2005)			



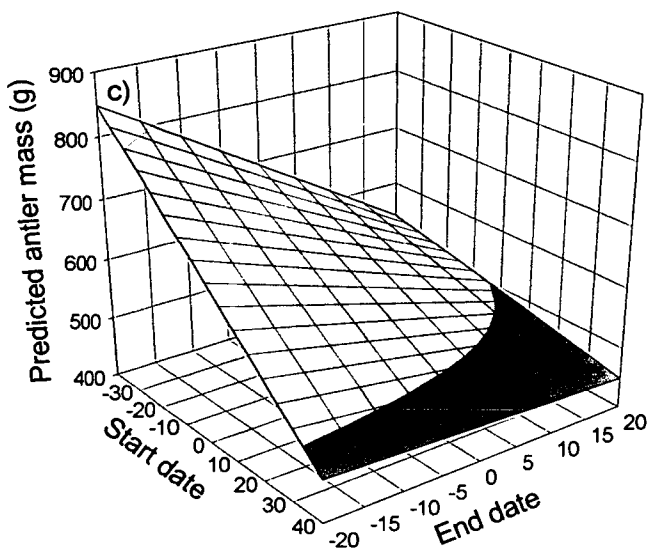


Fig. 2.3. Antler mass as a function of the (a) start date and (b) end date of antler growth, measured as the number of days since March 1st. Each point shows mean value (±1SE) of 5% of mature males (aged 8 to 14) ordered by appropriate date.

c) Predicted antler mass from linear mixed effect model described in Table 2.2 for males aged 8 in average environmental conditions. The start date and end dates of antler growth were mean centered prior to inclusion in the models.

3. Selection on antler growth phenology

i. Total selection

Including only the start date of antler growth as a fixed effect in a model of annual breeding success (ABS) showed that males who started growth earliest had the highest ABS in the following rut (Table 2.3i). Similarly, individuals that ended growth earliest had higher ABS (Table 2.3i). When both start and end dates of antler growth were entered into the same model, start date was the only significant predictor of ABS (Table 2.3i) and there was no evidence of a significant interaction between start date and end date (parameter estimate = -0.00026 (±0.00096PSD), 95% CI=(-0.00210,0.00165)).

ii. Direct selection

ABS was age dependent, showing a quadratic relationship with age (Table 2.3ii; Fig. 2.2). There was also a significant association between antler mass and ABS: males with heaviest antlers gained more paternities in the following rut (Table 2.3ii). When hind leg length was added to the model in Table 2.3ii, reducing the sample size to 154 observations (55 individuals), it was not found to be a significant predictor of ABS (parameter estimate = 0.0154 (±0.024PSD), 95% CI=(-0.0304,0.0595)).

Antler growth phenology was directly associated with ABS independently of the other variables known to influence breeding success (Table 2.3ii). The start date of antler growth was negatively correlated with ABS: individuals who started growth early were more successful in the following rut (Table 2.3ii; Fig. 2.4). However there was little evidence of an association between end date and ABS (parameter estimate=-0.040 (±0.021PSD), 95% CI =(-0.082,0.005)) nor any evidence of any non-linear selection on either start date or end date (quadratic start date term: 95% CI= (-

0.001,0.001); quadratic end date term: 95% CI=(-0.005,0.001)). We considered the interaction of start date of antler growth with the climatic variable found to most influence start date (September temperature) but did not find any significant effects (95% CI=(-0.010,0.003)). There was also no evidence of any interaction between antler growth phenology and age on ABS (start date: 95% CI=(-0.016,0.009); end date 95% CI=(-0.020,0.015)).

Individual identity accounted for 62% of the remaining variance in ABS once fixed effects had been accounted for, whereas year accounted for 7% of the remaining variance (Table 2.3ii).

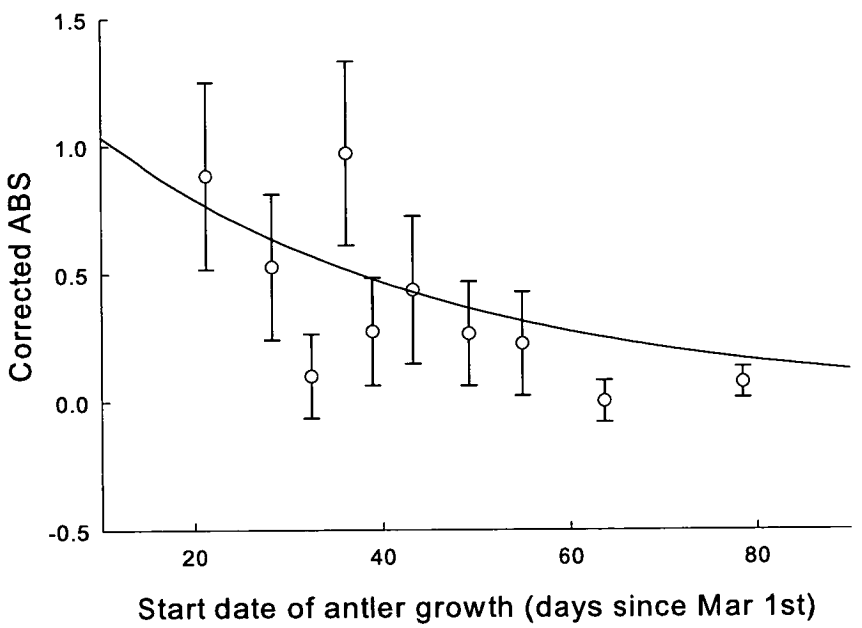


Fig. 2.4. Annual breeding success (ABS) and antler phenology. ABS corrected for age, population density and antler mass as a function of start date of antler growth. Each point shows mean value (±1SE) of groups each containing 10% of males ordered by start date. The line shows the log-transformed parameter estimate from Table 3ii.

Table 2.3. Analysis of Annual Breeding Success (ABS). i) total selection on the dates of start and end of antler growth and ii) direct selection, accounting for other significant predictors of ABS. Results are from Markov Chain Monte Carlo simulations with a Poisson logarithmic link function. Individual identity and year were fitted as random effects and appropriate variance components are shown. Significance of fixed effects was assessed by examining 95% confidence intervals of parameter estimates – where these do not include 0 the appropriate effect is taken to be significant. *n* shows the number of observations and individuals included in the analysis.

Fixed Effect	Parameter Estimate (Posterior SD)	95% Confidence Interval
i) Total Selection		
Start Date	-0.043 (0.005)	-0.053 -0.034
Random Effects		
Individual	1.063 (0.148)	0.618 1.631
Year	0.137 (0.074)	0.046 0.308
Residual	0.614 (0.148)	0.368 0.943
n = 730 ; 243 individuals; 35 years (1971 – 2006 excluding 1973)		
End Date	-0.076 (0.013)	-0.103 -0.051
Random Effects		
Individual	1.109 (0.309)	0.639 1.791
Year	0.239 (0.134)	0.075 0.593
Residual	0.734 (0.217)	0.370 1.200
n = 511 ;238 indivs; 33 years (1971-2006 excl 1973, 1976 & 2002)		
Start Date	-0.034 (0.010)	-0.053 -0.016
End Date	-0.040 (0.022)	-0.082 0.001
Random Effects		
Individual	0.814 (0.308)	0.362 1.544
Year	0.295 (0.188)	0.076 0.756
Residual	0.836 (0.311)	0.355 1.569
n = 315;159 indivs; 1 yrs (1971-2006 excl. 1973,1976,1977, 2002, 2004)		
ii) Direct Selection		
Age	0.627 (0.471)	-0.257 1.575
Age^2	-0.053 (0.026)	-0.106 -0.003
Population Density	0.012 (0.005)	0.004 0.022
Antler Mass	0.004 (0.001)	0.002 0.006
Start Date	-0.027 (0.014)	-0.054 -0.002
Random Effects		
Individual	1.052 (0.171)	0.555 1.768
Year	0.108 (0.060)	0.037 0.259
Residual	0.542 (0.317)	0.277 0.928
n = 345 ;143 indivs; 32 years (1971-2006 excl 1972, 1973, 2002 & 2005)		

Discussion

Our results have shown that the phenology of growth of a secondary sexual trait was highly dependent on an individual's age and on environmental conditions, and that there was also a substantial component of between-individual variation representing consistent differences across individuals' lifespans, presumably attributable to either heritable genetic or permanent environment effects. Differences in phenology were associated with differences in the morphology of antlers grown, and, even after correcting for this, with differences in subsequent breeding success: earlier individuals were more successful.

The phenology of antler growth showed sensitivity to population density and the local climate, indicating condition-dependent expression of these traits. The critical time periods for which climatic variables influenced antler growth start and end dates coincided with the primary and secondary peaks of plant productivity in this system respectively (Albon et al., 1992, Langvatn et al., 1996). The significant IxE variance component in the model of start date showed that individuals varied in how they responded to changes in September temperature, indicating different condition-dependent responses to environmental variation (Nussey et al., 2007). In contrast, males all appeared to respond similarly to variation in April temperature with respect to end date of antler growth.

Why might early antler growth phenology be associated with heavier antlers? Males starting growth earliest both grew for the longest period and produced the heaviest antlers. It is not possible with correlational data such as these to determine whether there are direct causal effects of timing of the start of growth on antler size, or

whether the association is driven by joint relationships with individual condition, but a strong role for the latter seems likely. The start date of antler growth was correlated with temperature in the previous autumn, which may be linked to vegetation availability when males finish rutting and feed intensely to regain condition prior to winter (Clutton-Brock et al., 1982). This suggests some condition-dependent relationship between antler growth start date and antler mass: those individuals who started growth early were in good condition and consequently also grew bigger antlers. Such condition-dependence could also generate variation between individuals within a year. Furthermore, within a group of males starting growth at a similar time, males who finished earliest would, on average, have produced the largest antlers, implying that heavy antlers were not simply a consequence of a longer growth period but were also associated with a higher growth rate.

Annual breeding success (ABS) was influenced by an individual's age, population density, antler mass and start date of antler growth, and also showed permanent differences between individuals. The associations between ABS and phenology over and above the latter's effect on antler mass are probably mediated by individual condition, with early start date reflecting good condition (Verhulst et al., 1995) and presumably hence strength and fighting ability, rather than due to any direct causal relationship between the timing of start date of antler growth in spring and rutting success in the subsequent autumn. Such arguments would indicate that, were we able to include an explicit measure of condition in our models of 'direct' selection, we would no longer detect any association between ABS and antler growth phenology. If this unmeasured aspect of condition is largely environmentally-determined, antler growth phenology will not be genetically correlated with ABS (Rausher, 1992). This mechanism has been proposed to explain the lack evolution of laying dates in birds,

despite apparent directional selection (Price et al., 1988) and is supported by empirical data from wild populations (Cargnelli and Neff, 2006, Bety et al., 2003). An environmentally-based covariance via some non-genetic component of condition is a possible reason for the observed phenotypic association between antler mass and fitness (Kruuk et al., 2002). It may be that the same mechanism is driving the observed correlations between antler growth phenology and ABS; testing this hypothesis will require the calculation of the genetic correlation between the two.

We found no association between skeletal body size and ABS or antler growth phenology in our analyses. In the Rum red deer, lifetime (rather than annual) breeding success is correlated with body size (Kruuk et al., 2002) suggesting that this variable may influence fitness through longevity rather than success in any particular year, although the significant positive relationship between body size and antler mass may be suggesting that body size increased ABS by acting through the antler mass.

Our results showed antler growth phenology to be under directional selection after correcting for the morphology of antler grown. These results are therefore similar to those of studies of female breeding time, in which there is evidence of negative directional selection favouring earlier breeders in a range of taxa (e.g. Perrins, 1970, Green and Rothstein, 1993, Coulson et al., 2003). The physiological or evolutionary constraints which prevent males casting the previous year's antlers earlier, and initiating growth of the new set, remain to be explored. There are also striking differences with other cervid systems, specifically reindeer, in which males cast antlers much earlier in the year, immediately following the rut at the end of December (Bubenik et al., 1997). Our results suggest that an understanding of the evolutionary pressures determining the annual timing of events within a population

needs to consider both the natural and the sexual selection pressures acting on both males and females.

Chapter 3

Variation in wild ungulate gestation length

Summary

In species inhabiting a seasonal environment, timing of birth may be highly correlated with fitness. However, we know little about the relative effects of conception date and gestation length in determining birth date in wild mammalian populations. Here, we analysed gestation length in a wild ungulate population (red deer). Higher spring temperatures during gestation were associated with shorter average gestation length, but we found no significant correlations with female age, recent reproductive history or offspring sex.

We also tested whether late conception was associated with shorter gestation lengths, to enable late conceiving females to give birth closer to the optimum time. Simply fitting conception date as a fixed effect in a model of gestation length is not statistically sound as errors in determining conception date could be driving an observed negative covariance; consequently we also approached this problem using a novel technique of bivariate models. Late conception was correlated with shorter gestation lengths although this was not found to be repeatable within females, indicating that gestation length adjustment may only be apparent on the rare occasions that females conceive outside the optimum time window.

Although there were no consistent differences between females in gestation length, there were differences between males in the gestation lengths of females they mated with. This is possibly a local environment effect, grouping females who were primarily in the same area during conception and gestation.

We conclude that observed variation in birth date is predominantly due to systematic variation in conception date as opposed to gestation length.

Introduction

The timing of reproduction in a seasonal environment has important fitness consequences, with regards to both offspring growth and survival (e.g. Perrins and McCleery, 1989, Festa-Bianchet, 1988, Gienapp et al., 2006) and future reproductive opportunities or survival of the mother (Clutton-Brock et al., 1983, Sheldon et al., 2003). In many taxa the majority of offspring births occur within a short time window that coincides with periods of maximum food availability (Visser et al., 1998, Arlettaz et al., 2001, Ryan et al., 2007), and synchronization of offspring births may even be adaptive in some systems (Sinclair et al., 2000, Reed et al., 2006). In mammals, variation in birth date could be driven by either variation in conception date, or by variation in gestation length. However, due to the inherent difficulty of measuring conception date (Berger, 1992), much less is known about the relative effects of conception date and gestation length in determining birth date in wild mammal populations. We present here an analysis of variation in gestation length in a wild population of red deer on the Isle of Rum, Scotland.

Previous studies of the Isle of Rum red deer population found longer gestation length in male than female offspring (Clutton-Brock et al., 1982), but no effect of a female's previous reproductive experience on gestation length (Guinness et al., 1978a). Gestation was also longer in wild than supplementary fed females from the same population, suggesting effects of nutrition on gestation length (Guinness et al., 1978a). With reference to these studies, Clutton-Brock et al 1982 concluded (page 62) that 'although gestation length may be influenced by environmental variables, such effects

are not pronounced and represent a small percentage of the total length'. Unfortunately, these conclusions are sometimes interpreted as an assertion that gestation length is constant within species (e.g. citations in Asher et al., 2005b, Garcia et al., 2006, Scott et al., 2008, Mysterud et al., 2009), and an implication that there is little potential for a plastic response to local environmental conditions during gestation, nor for any adjustment in gestation length if conception is late. However, the finding by Kiltie (1982) that intraspecific variation in gestation length in mammals increases with average body size, and the study by Berger (1992) demonstrating shorter gestation lengths in some late conceiving wild bison, provoked a number of studies to consider whether varying gestation length is a reproductive tactic (Mysterud et al., 2009) dependent on factors such as age and body condition. Table 3.1 summarises previous studies of gestation length in deer, whether captive or wild, and in all other non-captive ungulate populations. We split the populations into three types reflecting increasing amounts of supplementary feeding and decreasing levels of environmental heterogeneity: wild unmanaged, wild managed and captive. Across the different studies, no clear pattern emerges of factors influencing variation in gestation length in ungulates. However, due to the vastly differing levels of nutritional provision between populations, the different species studied and the small sample sizes used in many of the studies, which complicate the interpretation of non-significant results, it is difficult to draw any firm conclusions. Gestation length has been shown to vary with female age or reproductive status in wild bison (Berger, 1992) and managed reindeer (Mysterud et al., 2009) but no effects have been detected in captive or unmanaged red deer (Table 3.1). There is some evidence that high levels of nutrition are related to a shorter gestation length (e.g. Verme, 1965, Asher et al., 2005a; Table 3.1) but, perhaps because of the different variables used as a proxy for condition, the picture is not entirely clear (e.g. Rowell and Shipka, 2009; Table 3.1). Effects of offspring sex were found in 3 out of

the 11 studies that tested for them, with sons having longer gestation lengths on average (Clutton-Brock et al., 1982, Holand et al., 2006, Mysterud et al., 2009).

In addition to testing for effects of individual characteristics, previous studies of gestation length have often found a negative covariance between the length of gestation and conception date in both wild and captive ungulates: it appears that females conceiving late may be able to adjust gestation so that birth is closer to the optimum time window (Table 3.1), although this adjustment has not yet been shown to be adaptive. However in testing whether late conceptions are associated with shorter gestation time, it is important to consider the potential for spurious correlations to arise merely due to measurement error. Gestation length is the difference between two observed time-points of conception date and offspring birth date. The use of a parameter that is defined as the difference between two variables can be problematic when testing for statistical associations between the difference and one or other of these variables, and the problems is amplified when one or both variables are not known with high certainty (Kelly and Price, 2005). Consequently even if there is no biological relationship between conception date and gestation length, errors in determining conception date can result in a negative covariance between the two. To illustrate this imagine a hypothetical population where all individuals conceive on the same date, gestate for the same period and give birth on the same date, but that observational difficulties mean that conception date is not always determined accurately. If conception date is estimated as two days earlier than the true date this will have the effect of lengthening estimated gestation length by two days, and vice versa, generating a negative covariance between conception date and gestation length. One way of addressing this issue is to use bivariate analyses to look for an association between conception date and gestation length at the level of the female, on the

assumption that observational errors in conception date are distributed randomly across females and so contribute to residual variance but not differences between females. This then allows us to ask the question: do females who consistently conceive late have consistently short gestation lengths, and vice versa?

We also consider whether, more generally, there are consistent differences between individuals in gestation length. In labile traits that are expressed repeatedly across an individual's lifetime, selection acts on such differences (or repeatability); if a component of the variation is heritable, then a trait can evolve (Lynch and Walsh, 1998). Many studies have examined both repeatability and heritability in wild populations, most often at the level of the individual expressing the trait (Kruuk et al., 2008). However an individual's phenotype may be influenced by the phenotype of others, the most obvious example being maternal effects where differences in maternal phenotypes translate into differences in the phenotype of the offspring (Rasanen and Kruuk, 2007). Recent studies in birds have examined breeding time as both a maternal and paternal trait and found significant differences between males in the lay dates of females with which they mate (Brommer and Rattiste, 2008, Teplitsky et al., 2010). This phenomenon has been ascribed to differences between males in levels of provisioning to the female and paternal care. Clearly these are not traits attributable to males in the polygynous mating system of ungulates, but there are a number of findings that suggest that there may exist differences between males in the gestation lengths of females they mate with. In red deer, there is a strong social component to oestrus synchrony (Iason and Guinness, 1985) and the presence and roaring of males is known to bring females into oestrus (Shelton, 1960, McComb, 1987). If there is variation between males in the ability to bring females into oestrus, then it is possible that there may also be between-male effects on gestation length, as gestation length may be

correlated with oestrus date. Variation between males may also be environmentally generated if there are local environment effects acting on gestation length, as males rut in very localized areas (Clutton-Brock et al., 1982). Additionally, paternal effects on gestation length could occur through variation between males in the quality of females they mate with, or direct genetic effects on the growing offspring. Direct paternal genetic effects on gestation length have been observed in crosses between different subspecies of red deer (Asher et al., 2005b), but it is not known if this result extends to genetic variation within a population of males of the same species. Consequently in this paper we test for between individual variation both at the level of the female and the male.

In this paper we therefore use long-term data from a wild red deer population to address the following questions:

1. How much phenotypic variation is there in gestation length and is the observed variation a function of female age, reproductive status or ecological conditions?
2. Is there any evidence of gestation length adjustment in response to conception date, and could this relationship be generated through errors in determining conception date?
3. Is the observed variation a function of between-individual variation, resulting from heritable genetic or permanent environmental effects at the level of the individual female and male?

Table 3.1 (overleaf). Summary of published individual based studies of gestation length in all deer populations and other wild ungulate populations, grouped by population type then species. N: number of observations. Years: number of years. Oestrus: occurring naturally or artificially induced using hormones. Population type: captive, wild managed (i.e. supplementary fed), wild unmanaged (i.e. not supplementary fed). Calf sex/female age/female (reproductive) status/conception date: whether these parameters were tested for associations with gestation length and if significant effects found, the direction of the effect. Effect of conception date: estimate of the conception date regression coefficient in a model of gestation length, where this was significant. *Cervus elaphus,* red deer or wapiti; *Bison bison,* bison; *Elaphurus davidianus,* Pere David's deer; *Antilocapra americana,* Pronghorn; *Ovis canadensis,* bighorn sheep; *Rangifer tarandus,* reindeer; *Cervus nippon,* Sika deer; *Alces alces,* Alaskan moose; *Odocoileus virginianus,* white-tailed deer. The multiple regression coefficients in Scott 2008 is due to two populations with natural oestrus being analysed separately and an additional population where oestrus was artificially synchronized

Study	Species	N/yrs	Oestrus	Average gestation (days)	Offspring sex	Female age	Female Status	Effect of conception date?	Magnitude of conception date effect	Other significant variables	Other non-significant variables
Wild unmanaged populations											
Berger, 1992	*B. bison*	261/4	Natural		No		Primiparous > multiparous	Yes	good condition & late conception=>shorter		Female mass
Guinness et al., 1978a	*C. elaphus*	47/5	Natural	236.6			No			Supplementary fed females => shorter	Year
Clutton-Brock et al., 1982	*C. elaphus*	70/-	Natural	Males: 236.1±4.8 Females: 234.2±5.0	Sons longer						
Wild managed populations											
Byers and Hogg, 1995	*A. americana*	82/5	Natural	250±8.78 95%CI		No				Dry previous summer => longer	
Guinness et al., 1971	*C. elaphus*	13/1	Natural	231±4.5SD							Year; Birth date
Guinness et al., 1978a	*C. elaphus*	8/5	Natural	231.5			No				Year; Birth date; Individual;
Byers and Hogg, 1995	*O. canadensis*	75/10	Natural	173 ± 3.31 95%CI	No	No					Summer rainfall
Holand et al., 2006	*R. tarandus*	100/1	Natural		Sons longer			Yes	-0.41 ±0.062SE		Female mass (autumn)
Mysterud et al., 2009	*R. tarandus*	88/10	Natural	221 (211-229)	Sons longer	Older > younger		Yes	-0.35 ±0.21 95%CI		Individual
Captive populations											
Schwartz and Hundertmark, 1993	*A. alces*	22/7	Natural	231±5.4SD			No				Litter size; Year
Haigh, 2001	*C. elaphus*	26/3	Artificial	247±5SD							
Asher et al., 2005a	*C. elaphus*	51/3	Artificial	235						High nutrition levels in late pregnancy =>shorter	Female mass (autumn); Change in body condition score
Asher et al., 2005b	*C. elaphus*	18/1	Artificial	range 227-245						Female condition. C.e.roosevelti sire longer than C.e.scoticus sire	Nutrition levels in late pregnancy; Female mass
Garcia et al., 2006	*C. elaphus*	36/1	Artificial	234.2	No	No		Yes	-0.36 ±0.10SE	Female mass; Twins longer	Subspecies
Scott et al., 2008	*C. elaphus*	393/1	Natural		No			Yes	-0.49 ±0.036SE & -0.36 ±0.066SE	Female subspecies – C.e.scoticus x C.e.hippelaphus crosses shorter than pure lines	Male subspecies; Calf mass
Scott et al., 2008	*C. elaphus*	91/1	Artificial		No			Yes	-0.19 ±0.040SE		Male subspecies; Calf mass
Matsuura et al., 2004	*C. nippon*	42/2	Natural	230.1±11.3	No	No	No	No		High female mass winter & spring=>shorter (bad years only)	Female mass (good years)
Brinklow and Loudon, 1993	*E. davidianusde*	11/1	Artificial	284d				No			
Verme, 1965	*O. virginianus*	91/7	Natural							Nutrition levels (higher=>shorter)	
Rowell and Shipka, 2009	*R. tarandus*	11/2	Artificial	212-229	No			Yes	-0.31±0.02SE		Female mass; Calf mass
Rowell and Shipka, 2009	*R. tarandus*	70/21	Natural	203-229	No	No		Yes	-0.37±0.04SE		

Methods

We used data collected over 34 years from an individual-based study of a wild population of red deer on the Isle of Rum, Scotland (Clutton-Brock et al., 1982). The population has been monitored intensively since the cessation of culling in the study area since 1972. Individuals are followed throughout their lifetimes and genetic relationships are known through observations in the field (for maternity) and genetic analyses (for paternity; Walling et al., 2010).

Conception date is determined by daily monitoring of the study population from 15th September to 15th November (covering the mating season, or rut) each year. We aim to observe each female each day and record whether she is in a 'harem' and, if so, the male owner of that harem. Females are also watched for signs of oestrus such as being mounted, and intense attention from males (Guinness et al., 1971). Less than 10% of females each year were recorded as being in oestrous multiple times, and less than 4% of females had multiple oestrouses that differed by more than 10 days. If more than one oestrus was observed for a given female in one season, we consider the last observed instance to be the conception date. Conception date is measured as the number of days from 14th September in the year of conception.

Regular monitoring during the calving season enabled birth date to be known with high accuracy. The majority of births occur within the space of just a few weeks in late May and early June (Guinness et al., 1978b) although births have been recorded as late as September. So that extreme outliers did not bias results, we restricted birth dates to offspring born between May and July of each year, which represent 97.3% of

all recorded birth dates. Birth date is measured as the number of days from 15th September in the year of conception, i.e. the previous year.

Gestation length is the period between conception and birth. To ensure that we were not assigning extraordinarily long gestation lengths to individuals who had not conceived during the observed oestrus, but had instead cycled and conceived during a following unobserved oestrus (approximately 18 days later, Guinness et al., 1971), we confined results to gestation length less than 235+17 (252) days (Fig. 3.1; average taken from Clutton-Brock et al., 1982). There is also some evidence in the literature of oestrus occurring during pregnancy and consequently we also removed estimated gestation lengths shorter than 235-17 (218) days (Guinness et al., 1971), on the assumption that these were based on observations of a later oestrus that did not lead to pregnancy. These restrictions reduced the number of observations from 542 to 465, a reduction of 11% (Fig. 3.1). Studies from farmed red deer, where conception date is known with higher certainty, have found gestation length to range between 227 and 245 days (Asher et al., 2005b), showing that the restrictions used here are reasonable. Asher (2007) found higher variation in gestation length in naïve females (range 193-263) than females that had reproduced previously. However the percentage of naïve hinds in our excluded data (19.4%) was less than in the observations analysed (21.7%) suggesting that naïve females did not have unusually variable gestation lengths. It is also possible that by excluding long gestation lengths we removed females who conceived early and extended gestation length to be closer to the optimum time period of birth. However the average conception date of gestation lengths greater than 252 days that were excluded from the analyses was the same as the average of observations included in analyses, suggesting that these long gestation lengths were most likely due to a later unobserved repeat oestrus cycle.

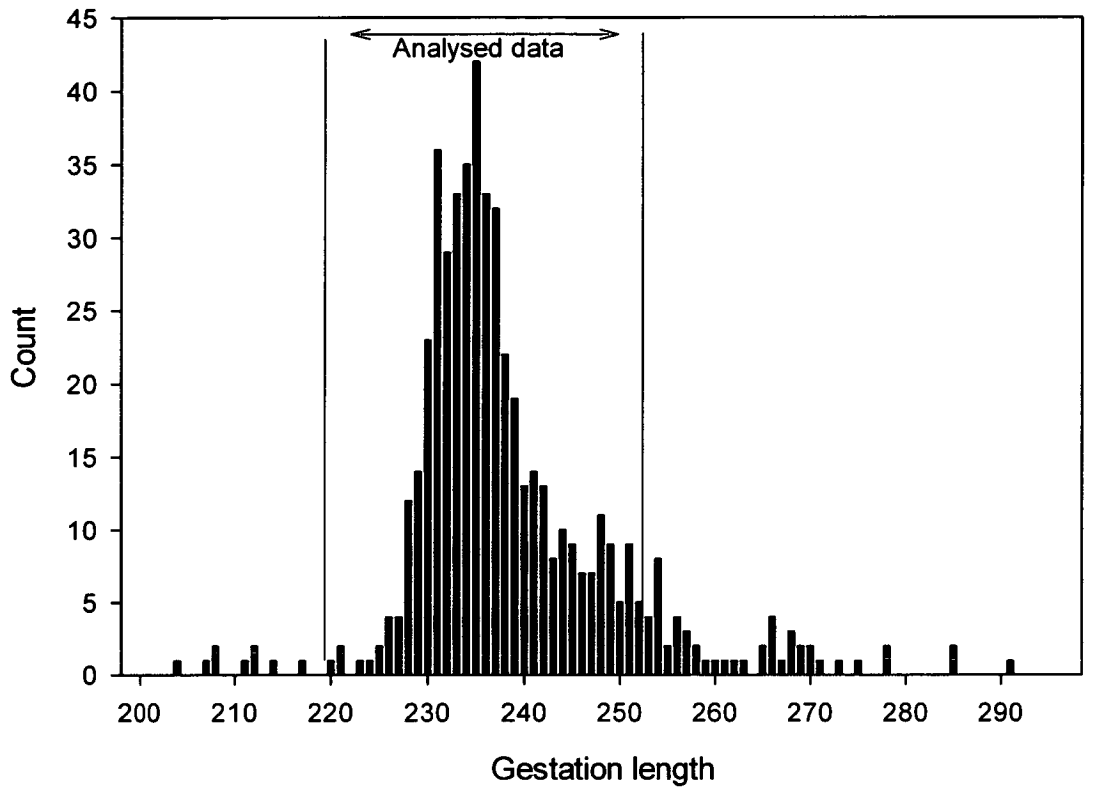


Figure 3.1. Distribution of observed gestation length in our study population of wild red deer. Female red deer have an oestrus cycle every 18 days and we restricted analyses to gestation length in the range of 218-252 days as shown.

We investigated if parameters known to influence other phenology variables in this system (Coulson et al., 2003, Nussey et al., 2005, Clements et al., in press, chapter 2) were also correlated with gestation length. Variables used in these analyses were:

Female age: the age of the mother in years at the time of conception, as a covariate, plus its quadratic.

Female reproductive status: A female was classed as Naïve (N) if she has never bred before; True Yeld (TY) if she has bred before but did not reproduce in the previous breeding season; Summer Yeld (SY) if she reproduced in the previous breeding season but the offspring died before 15th September of that year; Winter Yeld (WY) if she

reproduced in the previous breeding season but the offspring died between 15th September and 15th May in the year following birth; and Milk (M) if she reproduced in the previous season and the offspring was still alive on 15th May in the following year.

Population density: the number of females aged 1 year or more resident in the study area. Population censuses are carried out between January and May each year and a female is considered to be resident in the study area if she was observed in more than 10% of the censuses. Analyses presented here consider density in the year of conception; we also considered density in the year of offspring birth but it was not found to be significant.

Weather variables: Daily weather records are taken from the meteorological station in the village of Kinloch on the Isle of Rum. We considered total monthly rainfall and average daily temperature from the June in the year prior to offspring birth to the May in the year of offspring birth as possible covariates with gestation length.

Offspring sex: Whether the offspring was male or female, determined either at capture in the days following birth or, less often, through observation of the growing offspring. If the offspring died before the sex was known then it was excluded from analyses.

Female identity: the mother of the offspring, determined by observation.

Male identity: the genetic father of the offspring (see pedigree information).

Male identity-year: A factor grouping all of the offspring fathered by a single male in a particular year.

Pedigree information: A pedigree is available for the study population and has been described in full elsewhere (Walling et al., 2010). Briefly, maternal links are known through observation of the study population, and paternal links are found using microsatellite genotypes and a combination of two parentage inference packages - MasterBayes (Hadfield et al., 2006) and COLONY 2 (Wang and Santure, 2009). MasterBayes uses behavioural and genetic information to assign the most probable sire

which we accepted if individual-level certainty was greater than 0.8. We then used COLONY 2 to group full and half sibs and accepted any additional paternities found by COLONY 2 that were assigned with an individual-level certainty of at least 0.8. COLONY 2 can group half sibs even when the father has not been genotyped. When this occurred we used behavioural information to assign the most probable sire or, when this was not possible, a dummy sire was created to preserve the half-sib groupings within the pedigree. The pedigree consisted of 3,931 individuals, 3,330 of whom had known mothers and 1,840 had known sires. There were 571 individuals in the pedigree where neither parent was known.

Sample sizes

All analyses were performed on data where both the conception date and birth date were known. The dataset consisted of 465 births from 299 females across 34 years (1971 - 2007, excluding 1972, 1973, 2000). When male identity was included in the model, sample sizes dropped to 322 births from 229 females, 140 males and 33 years (1971 - 2006, excluding 1972, 1973, 2000).

Statistical analyses

We used linear mixed models (Pinheiro and Bates, 2000), run in ASReml-R (Gilmour et al., 2009), with female identity and year as random effects in order to determine the factors influencing gestation length. Fixed effects were female age and its quadratic, female status, population density and weather variables. To avoid issues of collinearity between weather variables, we fitted all other fixed effects before finding the single month weather variable that was most significant in the model described above. Significance of fixed effects was assessed using Wald statistics whereas the significance of random effects was determined by log-likelihood ratio tests on models

with and without the respective random effect. So that the intercept measured the gestation length at the beginning of the season in an average environment all continuous explanatory variables except conception date were mean-centred prior to inclusion in the model. We compared models with and without conception date as a fixed effect due to the methodological difficulties in fitting conception date in the model of gestation length.

We used two approaches to investigate the relationship between conception date and gestation length. To allow us to make comparisons with other studies of gestation length, we first fitted conception date as a fixed effect in the model of gestation length described above. We then approached the problem using a bivariate mixed model with conception date and *birth* date as dependent variables (so as to avoid both variables being affected by measurement error in conception date), and random effects of female identity and year. Fixed effects in the bivariate model were female age and its quadratic, female reproductive status and calf sex. We omitted population density and weather from the fixed effects structure so as to allow the effects to fall into a general measure of between-year variance. The estimates of variance and covariance in conception date and birth date at each random effect level can then be used to derive the equivalent parameters for gestation length. Thus, for example:

$$var_{fem}(\text{gestation length}) = var_{fem}(\text{conception}) + var_{fem}(\text{birth}) - 2*cov_{fem}(\text{conception, birth}) \quad (1)$$

$$cov_{fem}(\text{conception, gestation length}) = cov_{fem}(\text{conception, birth}) - var_{fem}(\text{conception}) \quad (2)$$

where var_{fem} is the variance between females in a given trait, and cov_{fem} is their covariance for two traits. Using this methodology, errors in assigning conception date will fall into the residual covariance and will not affect estimates at the individual or year level. We calculated the standard errors associated with these estimates in a similar manner and tested their significance using t-tests. We reasoned that a significant (negative) covariance between conception date and gestation length at the level of the individual female would indicate that some females repeatedly conceive later than others and late conception is repeatedly associated with shorter gestation length. Similarly, a significant negative covariance between conception date and gestation length at the year level would indicate that in some years average conception date is relatively late, and average gestation lengths are subsequently shorter.

We then explored between-individual variation in gestation length by fitting additional parameters to the random effect structure and in cases where the random effects were linked to a pedigree structure, tested for heritable genetic variation in gestation length using an animal model (Kruuk, 2004). Female identity and year were included as random effects in all models, as were fixed effects of female age and its quadratic, female status and offspring sex. To allow for direct comparisons with the bivariate model described above we did not include weather or population density as fixed effects in these models. We considered firstly addition of a single random effect term of male identity. Where we found significant effects of female or male identity, we used an animal model to partition this variation into heritable genetic and permanent environment effects. We found significant effects of male identity, which could be due either to intrinsic differences between males or to differences between males in the females that they mated with. We reasoned that if the effect was due to intrinsic differences between males, perhaps acting through genetic effects on the growing

offspring or differences in bringing females into oestrus, then the male effect would be repeatable across years. To test this we added an additional effect of male identity-year, where a different random effect was fitted for each male in each year, to the model and compared likelihoods of the different models.

Results

Variation in gestation length

The average gestation length across the study period was 236.5 days with an associated standard deviation of 6.2 days; 95% of gestations lay between 228 and 249 days in length (Fig. 3.1). The average conception date was 14th October ±9.1 SD days and the average birth date was 7th June ±12.3 SD days.

Is gestation length a function of female characteristics?

We found no significant effects of female age, female reproductive status, offspring sex or population density on gestation length, both before (Table 3.2i) and after (Table 3.2ii) correcting for conception date. There was however a significant effect of March temperature during gestation, with high temperatures associated with a shorter average gestation length (Table 3.2; Fig. 3.2). After correcting for the effect of March temperature on gestation length, between-year variance was not significant (Table 3.2). The results from model 3.2i did not qualitatively change when all observations (i.e. gestation length not restricted to 218-252 days; Fig. 3.1) were included in the analysis.

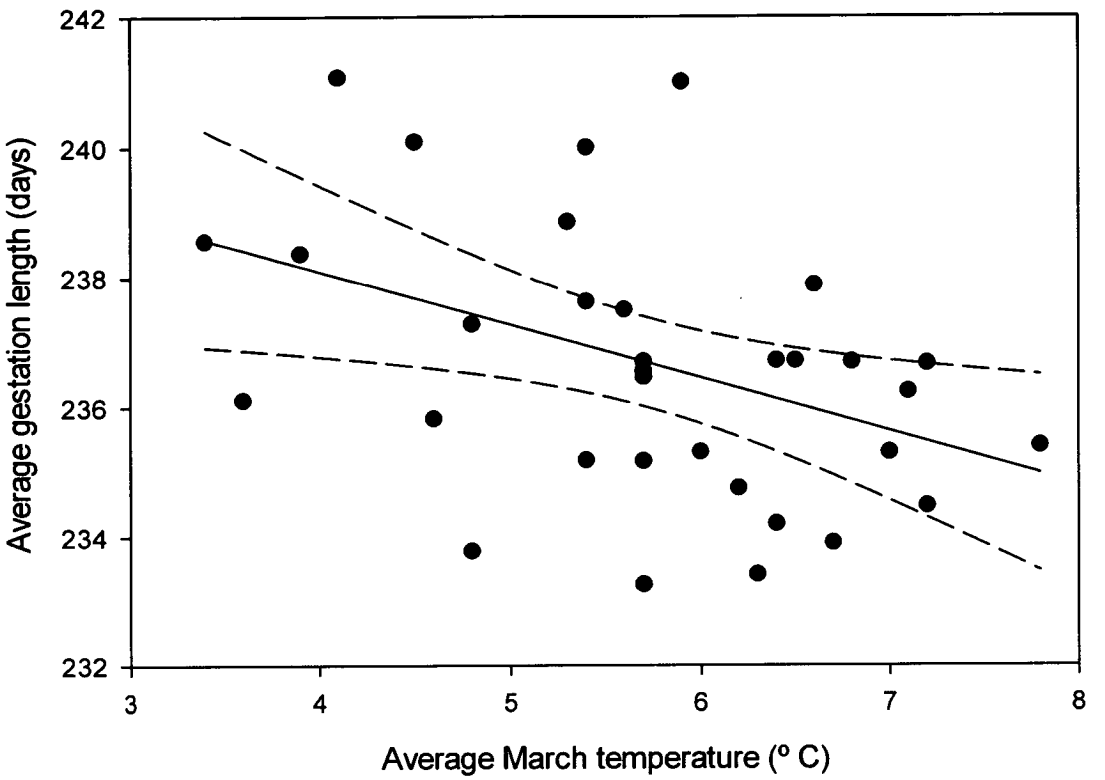


Figure 3.2. Average gestation length was shorter in years with high March temperature. Plot showing annual mean gestation length as a function of average March temperature. Trend line and 95% confidence intervals are calculated from the data points shown.

Table 3.2. Analysis of gestation length both before (i) and after (ii) correcting for conception date. Results are from linear-mixed models with a Normal error distribution. Population density and March temperature were mean-centred prior to inclusion in the model. Significance of fixed effects was assessed using Wald statistics whereas significance of random effects was assessed using log-likelihood ratio tests. Significant effects are shown in bold.

i)	Parameter Estimate (SE)	Df	W-stat	p-value
Intercept	238.99 (2.75)	1	23520	<0.001
Age	-0.58 (0.67)	1	0.74	0.393
Age^2	0.05 (0.04)	1	1.25	0.266
Female Status= M	0	4	1.35	0.254
Female Status= N	-1.24 (1.39)			
Female Status= TY	-1.13 (0.86)			
Female Status= SY	-1.54 (1.02)			
Female Status= WY	1.03 (1.33)			
Offspring sex = M	0	1	0.08	0.779
Offspring sex = F	-0.17 (0.60)			
Population density	-0.01 (0.01)	1	0.51	0.482
March Temp	**-0.66 (0.30)**	**1**	**4.85**	**0.04**

Random effects	Variance Component (SE)	Df	chi-sq	p-value
Female Identity	1.33 (2.86)	1	0.295	0.587
Year	0.64 (0.92)	1	0.364	0.547
Residual	34.87 (2.75)			
	n = 465 ; 299 individuals; 34 years			

ii)	Parameter Estimate (SE)	df	W-stat	p-value
Intercept	242.67 (3.13)	1	14570	<0.001
Age	-0.79 (0.67)	1	1.39	0.24
Age^2	0.06 (0.04)	1	1.98	0.161
Female Status= M	0	4	2.02	0.094
Female Status= N	-1.66 (1.38)			
Female Status= TY	-1.52 (0.87)			
Female Status= SY	-2.00 (1.03)			
Female Status= WY	1.10 (1.33)			
Offspring sex = M	0	1	0.12	0.736
Offspring sex = F	-0.20 (0.60)			
Population density	-0.004 (0.012)	1	0.1	0.757
March Temp	**-0.77 (0.32)**	**1**	**5.68**	**0.026**
Conception Date	**-0.08 (0.04)**		**5.52**	**0.02**

Random effects	Variance Component (SE)	df	chi-sq	p-value
Female Identity	1.92 (2.88)	1	0.416	0.519
Year	1.10 (1.06)	1	1.657	0.198
Residual	33.61 (3.68)			
	n = 465 ; 299 individuals; 34 years			

Does gestation length change with conception date?

Conception date as a fixed effect: Conception date was significantly negatively associated with gestation length: a delay in conception of 18 days (one oestrus cycle) led to average gestation length being approximately 1.4 days shorter (Table 3.2ii; Fig. 3.3). There was no significant relationship between gestation length and the quadratic of conception date ($W\text{-}stat_{(d.f.=1)}=0.051$, $p=0.822$).

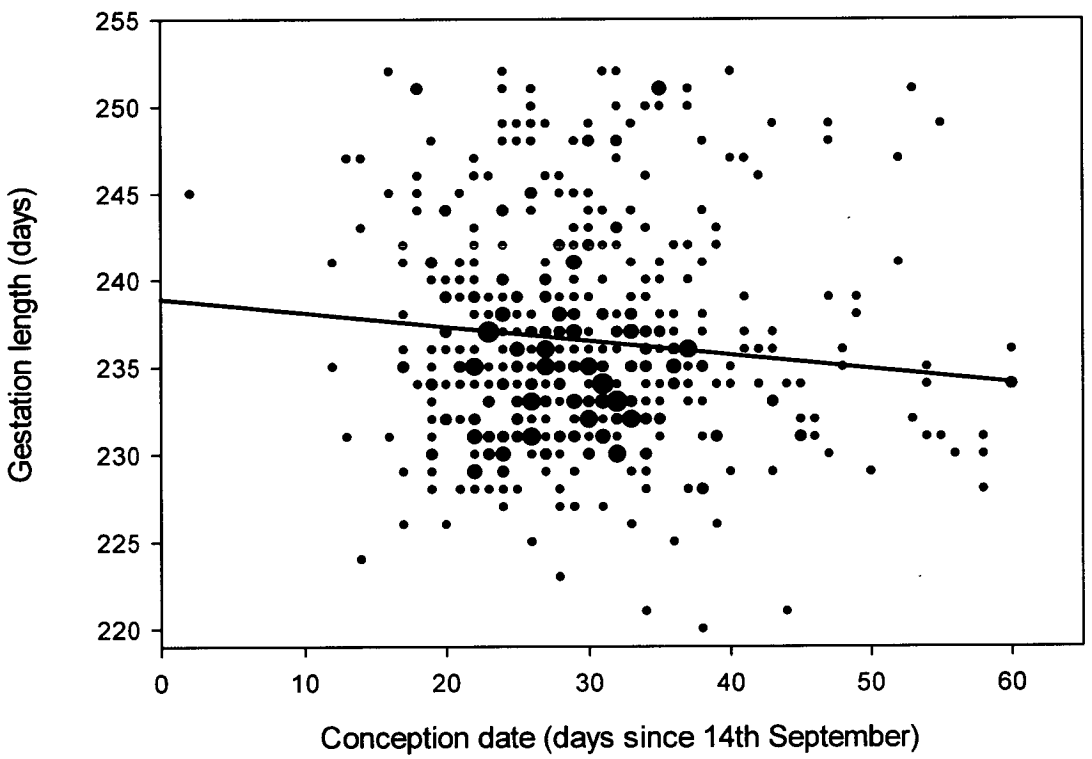


Figure 3.3. Gestation length decreased with advancing conception date. Scatter plot of phenotypic values, size of dots represents number of observations. Line shows slope estimated in Table 2ii: b=-0.08 ±0.04SE.

Bivariate model of conception and birth dates: Table 3.3 shows the output from a bivariate model of conception date and birth date; parameters including gestation length were inferred from the bivariate model. Between-female variances in conception

and birth dates were high and strongly correlated (ρ=1.0). In years where average conception date was late, birth date was also late on average (ρ=0.97). Inferred parameter estimates indicated that there was no significant covariance between conception date and gestation length at the level of the individual female: females with consistently late conception dates did not have consistent differences in gestation length in comparison to those females with consistently early conception dates (Table 3.3). There was a significant covariance between conception date and gestation length at the year level: in years where the average conception date was late, average gestation length was longer (Table 3.3). However there was a significant negative covariance between conception date and gestation length found in the residuals (Table 3.3).

Table 3.3. Observed and inferred variance-covariance matrix of conception date, birth date and gestation length where female identity and year were included as random effects. Variance components are shown on the diagonal, covariances are underneath the diagonal with the associated correlation above the diagonal. Bold indicates outputs from a bivariate model of conception date and birth date, where female age and its quadratic, female status and offspring sex were included as fixed effects; otherwise components are inferred from this bivariate model (see eqns 1 and 2 in Methods). Significant covariances are highlighted with a *. N= 465, females=299, years=34.

	Conception date	Birth date	Gestation length
FEMALE IDENTITY			
Conception date	**11.99 (4.87)**	**1.02***	1.94
Birth date	**15.67 (5.36)***	**19.63 (7.28)**	
Gestation length	3.67 (2.58)		0.29 (2.77)
YEAR			
Conception date	**14.68 (4.85)**	**0.96***	0.49*
Birth date	**17.14 (5.60)***	**21.30 (7.00)**	
Gestation length	2.46 (1.11)*		1.70 (5.09)
RESIDUALS			
Conception date	**47.67 (5.18)**	**0.68***	-0.23*
Birth date	**37.94 (5.37)***	**65.01 (7.39)**	
Gestation length	-9.71 (3.15)*		36.65 (3.70)

Is there between-individual variation in gestation length?

There was mixed evidence of significant between-female variance in gestation length. Female identity was not significant in the model of gestation length (Table 3.2i) and accounted for only 3.61% of the variation remaining once the fixed effects of age, reproductive status, population density and March temperature had been corrected for (Table 3.2i). When conception date was added to the model, the between-female variance remained non-significant and represented 5.24% of the remaining variance (Table 3.2ii). Similarly, when between-female variance in gestation length was calculated from a bivariate model of conception date and oestrus date, it was not significant and represented only 0.75% of remaining variance (Table 3.3). Table 3.4 contains a summary of models of gestation length with environmental variables (such as population density and March temperature) excluded from the fixed effects and hence allowed to fall into a general term of between-year variance; different random effects structures are compared. In a model with just female identity and year, between-female variance was non-significant and accounted for just 2.11% of the variance (Table 3.4i). However, when model 2i was rerun using all available data points (i.e. gestation length not restricted to 218-252 days) there was a marginally significant level of between-female variance in gestation length accounting for 12% of the remaining variance ($\chi^2_{(d.f.=1)}=3.94$, $p=0.047$).

We also found significant between-male effects on gestation length (Tables 3.4ii & 3.4iii). The log-likelihood of the model containing male identity-year (Table 3.4iii) was higher than that containing male identity (Table 3.4ii), indicating that the random effect of male identity-year best explained the variance in the data. Fitting male identity-year into the model caused the parameter estimate of female identity to become negative but highly non-significant (Tables 3.4iii).

Attempting to split male identity into permanent environment and additive genetic effects resulted in convergence problems. We also did not attempt to fit female additive genetic effects as female identity was not a significant parameter in any model (Table 3.4).

The random effect of year was not significant when male identity-year was included in the model (Table 3.4iii) but was significant otherwise (Table 3.4i & 3.4ii). The models in table 3.4 differ from table 3.2 as the environmental effects of population density and March temperature were not included as fixed effects in the models in table 3.4.

Table 3.4. Estimates of variance components from models of gestation length where female age and its quadratic, female status and offspring sex were included as fixed effects. Male identity-year fitted a different random effect for each male in separate years, grouping offspring born to the same male in the same year, and allowed us to determine if male identity effects were repeatable across years. Each column shows parameter estimate, associated standard error, chi-squared statistic from models with and without the appropriate random effect and associated p-value (degrees of freedom equals one in all cases). The log likelihood ("loglik") of each model is shown. Models 4i: N= 465, females=299, years=34; 4ii,4iii : N births = 322, females=229, males=140, years=33. * indicates p<0.05

	Variance components					
	Female identity	Male identity	Male identity-year	year	residual	loglik
i	0.72 (3.17; 0.06; 0.801)			2.01 (3.17; 5.10; 0.024)*	31.40 (4.08)	-730.1
ii	0.58 (2.98; 0.04; 0.844)	5.50 (2.63; 7.10; 0.008)*		2.31 (1.50; 4.94; 0.026)*	26.40 (4.11)	-726.6
iii	-0.27 (2.83; 0.01; 0.923)		11.32 (3.75; 9.75; 0.002)*	1.02 (1.33; 0.78; 0.378)	22.51 (4.21)	-725.2

Discussion

Using a large and long-term dataset from a wild ungulate population we investigated sources of systematic variance in gestation length and found evidence only for warmer temperatures during gestation being associated with shorter gestation lengths and between-individual variation at the level of the male. Many studies have investigated whether gestation length is negatively correlated with conception date to promote synchronicity of births (Table 3.1). We detected a negative covariance between conception date and gestation length but found no evidence for gestation length adjustment being a tactic used repeatedly by females across their lifetimes. We conclude that observed variation in birth date is primarily a result of variation in conception date but there is some flexibility in gestation length in response to environmental conditions.

We estimated an average gestation length of 236.5 days, which is strikingly similar to that found from a much smaller sample of the same unmanaged study population on Rum in 1978 (236.6 days; Guinness et al., 1978a), and slightly longer than the average found in a managed population on Rum (231 days, Guinness et al., 1978a) and in captive red deer populations (235 days, Asher et al. 2005a; 234.2 days, Garcia et al. 2006). Measures of variation have been reported less often than average gestation length in studies of red deer, but our measure of a standard deviation of 6.2 days was slightly greater than that found previously in this study system (4.8 days for female calves and 5.0 days for male calves) and in a managed population on Rum (4.5 days, Guinness 1978a). These differences are presumably due to higher levels of environmental variation both in our study system compared to captive populations and over time within our population.

High spring temperatures were linked to shorter average gestation lengths. Previous work in captive red deer has shown that increased nutrition in the last trimester of pregnancy decreases gestation length (Asher et al., 2005a), and it is possible that increased temperature in March is driving this effect through increased vegetation growth. Oestrus date has been advancing at a faster rate than birth date in our study system since 1980 (Moyes et al., in review; appendix V) possibly due to increasing March temperature over time, although a higher proportion of females conceiving during the first oestrus of the season could also produce the same trends. In common with previous results from this study system, we did not find any effects of female reproductive status on gestation length (Guinness et al., 1978a). However, we also did not find any evidence of a longer gestation length in male than female offspring, as was found previously in this population (Clutton-Brock et al., 1982). This difference may be related to the high population density experienced in this population since 1982 (Albon et al., 2000), which is believed to have led to changes in the offspring sex ratio over time (Kruuk et al., 1999a); the same processes may also have affected between-sex differences in gestation length. A recent study of wild managed reindeer (Mysterud et al., 2009) found increased gestation length in older females, but, in common with all previous studies of red deer (Table 3.1), we found no such effects. It is possible that there are intrinsic differences in the natural histories of red deer and reindeer that are driving these results.

Shortening of gestation length by late conceiving females would enable offspring birth date to be closer to an optimum window, with the consequence that the highest period of nutritional demand by the offspring is better matched to the period of highest food resources for the mother (Loudon et al., 1984). This could be expected to

have fitness consequences both for the offspring, acting through milk quality influencing growth and overwinter survival (Landete-Castillejos et al., 2000), and for the mother, acting through condition, as lactation is the most energetically demanding component of raising offspring (Clutton-Brock et al., 1989). Conception date was a significant fixed effect in the model of gestation length. However, our parameter estimate of -0.08 days was less than those found previously in red deer, which are in the region of -0.2 to -0.5 (Table 3.1). Although it is possible that errors in determining conception date were responsible for some of the association between conception date and gestation length in our analyses, negative associations between conception date and gestation length have also been reported in captive populations where conception date is known with higher accuracy (e.g Scott et al, 2008; Asher et al., 2005a). This suggests that gestation length adjustment may be a true biological phenomenon and not a statistical artifact, although the true parameter estimate may be lower than that estimated. It must also be acknowledged that a negative covariance between conception date and gestation length does not necessarily imply that gestation length adjustment is adaptive: late conceiving females may simply experience better environmental conditions during late gestation than early conceiving females, and consequently have shorter gestation lengths.

Although gestation length was negatively associated with conception date, gestation length adjustment was not a tactic used by females repeatedly across their lifetimes. We used bivariate models to ask if females that consistently conceived late had correspondingly short gestation lengths, but we found no evidence for this. An assumption of the bivariate technique is that females are consistent in conception dates throughout their lifetimes: if this adjustment of gestation length is only apparent on rare occasions when females conceive outside the optimum time window, we would

not expect to see a negative covariance at the level. Oestrus date was repeatable within females (Chapter 6) although female identity accounted for just 9% of the residual variance, suggesting the potential for gestation length adjustment to be a true biological phenomenon but apparent only on rare occasions when females conceive outside the optimum time window. Additional evidence for gestation length adjustment not being a prominent force in this system can be found by examining phenotypic variances: the variance of birth date was greater than that of conception date, which would not be expected if females were adjusting gestation length in order to synchronize birth date. Further, when conception date was fitted as the only fixed effect in a model of birth date without random effects, conception date explained 64% of the variance in birth date (b=0.97±0.03 SE), compared to the 31% accounted for when gestation length was included as the only fixed effect (b=0.93±0.06 SE; analysis not shown).

We tested for between-individual level variation in gestation length at the level of both the female and the male sire of her offspring, as recent studies in wild bird populations have found male effects on lay date (Brommer and Rattiste, 2008, Teplitsky et al., 2010). We found mixed evidence of between-individual variation at the level of the female, with female identity accounting for no more than 12% of the remaining variance after correction for fixed effects. Previous work in both our study population (Guinness et al., 1978a) and in an unmanaged population of reindeer (Mysterud et al., 2009) have found no evidence of between-female variation in gestation length, although the number of repeated measures across females in these studies was very low. Power may be limiting our ability to detect between-female variation in gestation length but, as we found significant levels of between-female variation in both conception date and birth date with the same sample size (Table 3.3),

we can conclude that the true magnitude of between-female variation in gestation length is likely to be low.

We found high levels of between-male variation in gestation length. Due to the polygynous mating system of red deer, these effects cannot be ascribed to differences between males in levels of provisioning to females, as is believed to occur in birds (Brommer and Rattiste, 2008, Teplitsky et al., 2010). The male term can be thought of as a harem effect, grouping females who primarily reside in the same area and, as individual males do not rut throughout the season (Clutton-Brock et al., 1982), who conceived around the same time and are therefore likely to be in similar condition. Our results suggest that between-male effects were not consistent in the same male across different years. Consequently it is probable that this result was not being driven by intrinsic differences between males and was perhaps more likely to be driven by local environmental effects; testing this hypothesis would require modeling the spatial correlations between individuals in the random effects structure. These results are perhaps highlighting the importance of local environment, both physical and social, on gestation length. Caution would therefore be advised in extrapolating results from captive studies, where females are in broadly benign and uniform environments, to wild populations, where there are much higher levels of environmental variation in time and space.

The analyses presented here found very little systematic variation in gestation length, a result that is out of step with the majority of recent analyses, but that is unlikely to be due simply to lack of statistical power as our sample sizes are substantially larger than almost all of the other studies listed in Table 3.1. However, there was a significant effect of spring conditions, with warm March temperatures

associated with shorter average gestation lengths, and significant between-male variation perhaps reflecting micro-environmental conditions. In this study population there was a negative covariance between gestation length and conception date, but gestation length adjustment was not a tactic used repeatedly by females throughout their lifetimes. It appears that systematic variation in gestation length is predominantly driven by variation in conception date in wild red deer populations with some capacity for adjustment in gestation length in response to environmental conditions, and consequently birth date should be considered as a trait predominantly under maternal control in wild red deer.

Chapter 4

Coat change date

Summary

Phenology, the timing of annual events, is important in a seasonal environment with short growing seasons. Despite many studies on the causes and consequences of variation in phenology of traits associated with breeding, much less is known about traits expressed when organisms are preparing for winter. Here we analysed the date of coat change from summer to winter coat over 21 years in a wild population of red deer on the Isle of Rum, Scotland. Coat change date became earlier from the first year of life until aged 3, when it started becoming later. This is in contrast to other phenological traits studied in this population, which have shown advancement with age over a much larger proportion of the lifespan (until approximately aged 9), followed by senescence. In common with other phenological traits in many taxa, annual average date of coat change in the population has become progressively earlier over the past 21 years: increased September temperatures explained much of this trend in adult females. Coat change date has advanced faster than any other phenological trait in this study system, becoming earlier by approximately 1 day per year. Coat change is also a good indicator of individual condition, as individuals who have changed into winter coat by the 1st November showed higher over-winter survival, especially in immature deer.

Introduction

Organisms living in seasonal environments have to be able to withstand large fluctuations in environmental conditions, but the regularity of seasons has enabled the evolution of a large suite of adaptations to this heterogeneity (Farner, 1985). For instance, at northern latitudes spring is a season of growth and reproduction, whereas the harsher conditions of winter have given rise to adaptations focused on survival, such as dormancy of trees (Perry, 1971) and hibernation of some small mammals (Lyman and Chatfield, 1955). The correct timing, or phenology, of these adaptations is therefore fundamental for organisms to be able to cope with a seasonal environment. Studies on phenology have focused predominantly on timing of reproduction during the spring such as germination in plants (Rathcke and Lacey, 1985), breeding time in birds (Visser et al., 2006) and birth date in mammals (Réale et al., 2003b) . However much less is known about factors influencing traits expressed during the autumn when organisms are preparing for winter. Here we use data collected over 21 years from a wild population of red deer on the Isle of Rum, Scotland (Clutton-Brock et al., 1982), to consider factors influencing the date of change from summer to winter coat during autumn and consequences of this variation with respect to over-winter survival.

To gain a true understanding of factors influencing phenological variables in wild populations, intensive long-term individual-based studies are necessary to allow different effects to be properly partitioned. The phenology of many traits expressed repeatedly across an individual's lifetime varies with age. The timing of trait expression is generally optimal in prime-aged individuals as younger individuals are still investing in growth (Green and Rothstein, 1991), and there is also evidence that breeding time in some birds may be learnt (Grieco et al., 2002). Contrastingly, older individuals

experience senescence due to the weakening of selection at older ages leading to a general decrease in performance (Bonsall, 2006). Additionally, recent reproductive experience can affect the phenology, indicating a condition-dependent response (Nussey et al., 2005).

Advancing phenology is one of the most striking effects of climate change in wild mammal populations and has been observed in a wide range of taxa (Parmesan and Yohe, 2003, Sparks and Menzel, 2002). In the UK, a recent meta-analysis has shown phenology to be advancing at a faster rate than previously recognised (Thackeray and al, 2010). Advancing phenology may be due to direct effects such as changes in thermoregulation energy requirements affecting condition or temperature acting as a cue to trait expression (Visser et al., 2010). Indirect effects, of as increased vegetation growth can also affect phenology through condition. In our study population, a wide range of phenological variables have advanced over the past 30 years (Moyes et al., in review; appendix V), believed to be a direct result of a longer growing season (Barnett et al., 2006) due to climate change.

Red deer (*Cervus elaphus*) are large ungulates adapted to a seasonal environment. Their natural range covers most Northern temperate regions (Whitehead, 1972) and consequently they have developed a range of adaptations to cold winters. Appetite levels of red deer fall during winter (Heydon et al., 1993), even in captivity when food is plentiful (Pollock, 1975), indicating a physiological adaptation to periods of low food availability in the wild. Additionally, coat change in red deer from summer to winter coat occurs in the autumn where a woolly undercoat is grown to provide insulation from the cold winters (Ryder, 1976, Ryder and Kay, 1973) and

lowers the energy required in order to maintain a comfortable core body temperature. This winter coat is then shed in the spring as temperatures rise.

Relatively little is known about the phenology of coat change in red deer. Day length is an important factor in controlling the timing of coat change: when individuals are held in artificial light with a shortened annual cycle of 6 months (i.e. 'dawn' occurs twice every 24 hours) individuals change into winter coat twice per calendar year (Kay and Ryder, 1978). Despite the influence of daylength, there is evidence that coat change date is correlated with an individual's condition as the proportion of females in winter coat by November 1st is highest at low population densities and in females not bearing the cost of lactation (Clutton-Brock et al., 1982; pages 263 & 74). There is also evidence of between-individual variation in coat change date in captive populations (Ryder and Kay, 1973), perhaps reflecting consistent differences between individuals that are either genetically or environmentally induced (Lynch and Walsh, 1998).

Here we use data from a long-term study of a wild red deer population in order to investigate factors influencing the timing of coat change from summer to winter coat in immature deer of both sexes and adult females (insufficient data are available to consider adult males). We consider first correlations of coat change date with age and female reproductive status, an individual's sex in immature deer, and environmental factors of population density and weather. Second, we determine if average coat change date has been advancing in this population over time, possibly as a direct result of climate change. Third, we investigate correlations between coat state on the 1st of November and over-winter survival.

Methods

We analysed data from the long-term study of red deer (*Cervus elaphus*) in the North Block of the Isle of Rum, NW Scotland (Clutton-Brock et al., 1982). The population is unmanaged and population density has been naturally regulated since culling ceased in the study area in 1972. The date of female coat change from summer to winter coat has been collected in the study population since 1987. Coat change is a gradual process (Ryder and Kay, 1973) and as some individuals often have small clumps of hair remaining for quite some time after the remainder of the coat has changed, we define coat change date to be the date when an individual is in 90% of winter coat. The winter coat of red deer is much thicker and of a duller colour than the summer coat and consequently the growth of winter coat is obvious to see. Consistency in observations over time was ensured by the same person (Fiona Guinness) collecting the majority of the data (approximately 65%); where additional observers collected data they were carefully trained by FEG and observations frequently calibrated. FEG was not present during periods of coat change from winter to summer coat and consequently these observations could not be calibrated between observers; here we analyse only coat change date from summer to winter coat. Observations were only included in this analysis when the animal was clearly visible to the observer and was seen on a regular basis so the exact date of coat change could be determined. Analyses on deer aged 2 or more years were restricted to females as males range too widely for reliable daily observations. As immature individuals of both sexes tend to remain with their mothers, we also analysed data from both males and females aged 0 and 1 years. A total of 2,178 coat change observations from 21 years (1987-2007) on a total of 860 individuals were recorded. Additionally, a specific census took place on the 1st

November each year, where the 'coat state' of every individual was scored as being summer coat (S), half winter coat (H), or full winter coat (W).

The vast majority of individuals in this study population are born in the study area so their age is known exactly; we included only individuals with known birth year in analyses. Age was included in models as a continuous covariate with its associated quadratic term. The youngest individuals were just a few months old (0 year) and the oldest female was aged 19 years.

We investigated if coat change date was correlated with an individual's immediate reproductive history, or 'reproductive status'. As with previous studies in this system e.g. (Coulson et al., 2003, Nussey et al., 2005), a hind was classed as Naïve (N) if she had never bred before; True Yeld (TY) if she had bred before but did not calve in the previous calving season; Summer Yeld (SY) if she calved in the previous calving season but the calf died before 15th September of that year; Winter Yeld (WY) if the calved in the previous breeding season but the calf died between the 15th September and the 15th May of the following year; and Milk (M) if she calved in the previous season and the calf was still alive on 15th May of the following year.

In this system, numerous phenology traits are correlated with population density e.g. (Nussey et al., 2005, Clements et al., in press; chapter 2) and consequently we looked for associations between population density and coat change date. Population density was taken to be the number of females aged 1+ years resident in more than 10% of censuses conducted between January and May in the year of coat change (e.g. Coulson et al., 2003, Nussey et al., 2005).

Weather variables were taken from the meteorological station on the Isle of Rum and consisted of monthly measurements of average temperature and total rainfall. For the purposes of this analysis, we determined the most significant single one-month weather variable over the 12 months prior to the autumn coat change.

Statistical analyses

Factors influencing coat change date

Factors influencing coat change date were determined using mixed models (Pinheiro and Bates, 2000). As all individuals aged 2 years and under had not yet reproduced, we classified them as 'immature' and analysed coat change date separately to 'mature' females aged 3 years and above. We first created a base model including as fixed effects age and its quadratic and population density; we also included reproductive status in the model of mature deer and an individual's sex in the model of immature deer. Individual identity, mother's identity and year (as a factor) were included as random effects. Significance of all fixed effects was assessed using Wald statistics. We then found the most significant one-month weather variable when added to the base model above. Significance of all variables was then assessed and significance of random effects determined by performing log-likelihood ratio tests on models with and without the appropriate effect (Pinheiro and Bates, 2000). We then tested for significant interactions between main effects.

Temporal trends in coat change date

Phenology across a wide range of traits has been advancing in this system since 1980 (Moyes et al., in review; appendix V), believed to be as a direct result of climate change. Consequently, we tested if the average date of coat change each year has also advanced over time. To do this, we first fitted year as a continuous covariate in the

fixed effects structure of the base model described above and tested its significance using a Wald test. To determine if observed temporal trends could be explained by trends in the weather variable found to be the most significant predictor of coat change date, we fitted both this weather variable and year as a continuous covariate in the base model. If year was no longer significant in the model with weather, we concluded that systematic changes in the weather variable were driving the temporal trend. Again, we modelled immature and mature deer separately.

Is coat change phenology associated with over-winter survival?

Assessing whether coat change date in a given year was associated with over-winter survival was problematic as coat change date occurred annually but an individual could die just once. Therefore, if we were to use a glmm with a binary response variable of survival, the response variables would not be independent. Instead, we used Chi-squared tests to determine if there was an association between coat state and over-winter survival. Splitting the data by age enabled us to check that repeated measures across individuals were not driving observed results. However, it must be noted that analysis at each age - by definition -cannot include data from individuals who died before attaining that age, a concept known as the missing fraction (Hadfield, 2008). Although Chi-squared tests are a comparatively crude method of analysis, and do not account for variation in coat state due to reproductive status or environmental variables, their shortcomings are at least obvious to see.

We tested if coat state on the 1st of November was associated with over-winter survival as many more observations of coat state than exact coat change date were recorded. To do this we categorised all individuals by coat state and determined the number in each category that were still alive on May 15th of the following year. We then

used a Chi-squared test to determine if there was a statistical association between coat state and over-winter survival.

To determine in which groups coat change date was most predictive of over-winter survival, we split all observations firstly into immature and mature deer and repeated the analyses. We then split further by age, reproductive status and sex with caveat that at least 100 observations were present in each test. If less than 5 expected values were present in each category, a continuity adjusted-Chi-squared test was used.

All analyses were performed in ASReml-R (Gilmour et al., 2009).

Results

The average date of coat change of immature deer (aged 0-2 years) of both sexes in this population was 24th October and the standard deviation was 16.6 days (Figure 4.1). In mature female deer (aged 3+ years), the average coat change date was 21st October ± 18.3 SD (Figure 4.1).

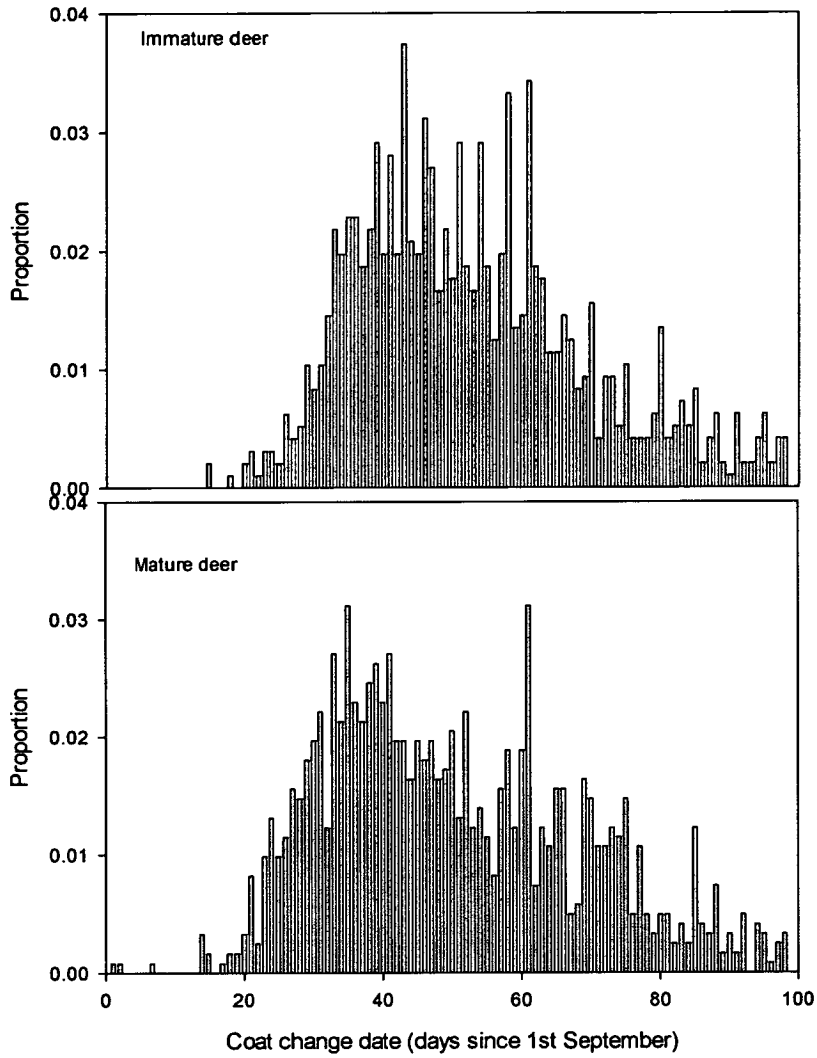


Figure 4.1. Coat change date distribution in immature (both sexes, top) and mature (females, bottom) red deer. Distributions show all observations across all years.

Coat change date was influenced by age. In the model of immature deer, 4 year olds changed coat earliest (Table 4.1; Figure 4.2). In mature females, 3 year-olds changed coat earliest (Table 4.2; Figure 4.2). Coat change date then became later with increasing age, and the significant quadratic term indicated that the difference between ages increased with age (Table 4.2; Figure 4.2). The average coat change date of

individuals 16 years and over was almost 1 month later than that of 3 and 4 year olds (Table 4.2; Figure 4.2).

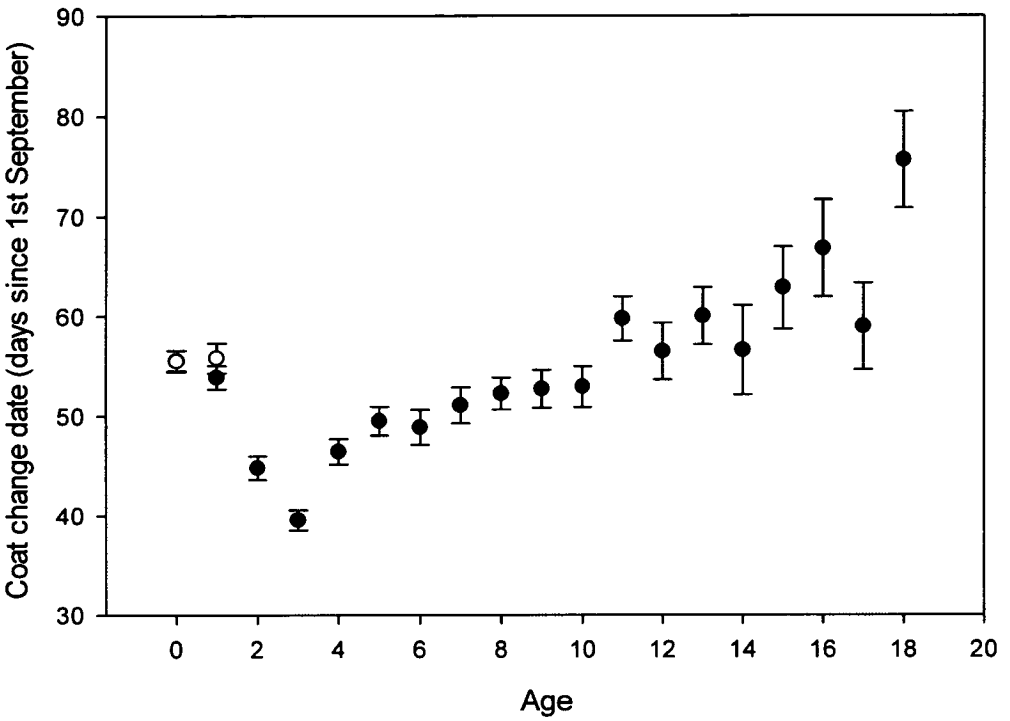


Figure 4.2. Coat change date in females became earlier with age until the age of 3, when it started becoming later (filled circles). Average coat change date by age ±1SE. Coat change date was recorded in both sexes until the age of 1 year; average coat change date of males aged 0 and 1 years is shown as unfilled circles. The average coat change date of males and females aged 0 was so similar that the estimate for females cannot be seen behind the estimate for males on this graph.

A female's reproductive status was a significant predictor of her coat change date, with females who have never reproduced (Naïve) expressing the trait the earliest and hinds with young calves (Milk and Winter Yeld) the latest (Table 4.2; Figure 4.3).

Environmental conditions influenced coat change date. Warm temperatures in the September prior to coat change were associated with earlier average coat change

date in immature (Table 4.1) and mature (Table 4.2) deer. There was a significant interaction between female reproductive status and September temperature (Table 4.2; Table 4.3; Figure 4.3) suggesting that Milk and Winter Yeld females were most affected by changes in temperature. However, there was no evidence of an association between coat change date and population density (Table 4.1; Table 4.2).

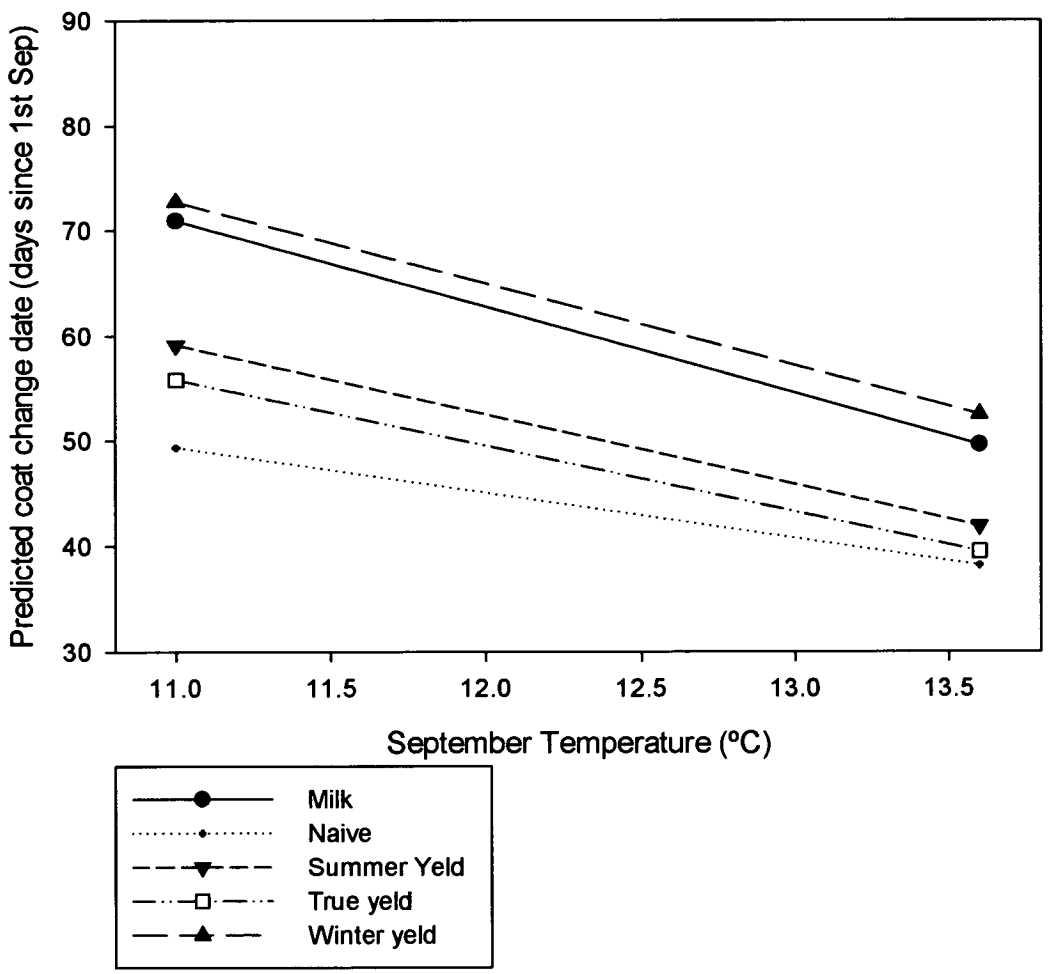


Figure 4.3. Females bearing the coat of lactation advanced coat change date most in response to increased September temperatures. Predicted coat change dates from model described in Table 4.2.

In the model of immature deer, sex was not a significant predictor of coat change date (Table 4.2). Additionally, there were no significant interactions between

any of the variables in either model of coat change date (Table 4.3), except the interaction between female reproductive status and September temperature (Figure 4.3).

The random effects structure showed that there were high levels of repeatability in coat change date, with individual identity accounting for 33% of the variation remaining after correction for fixed effects in immature deer (Table 4.1) and 40% in mature deer (Table 4.2). There was no significant effect of maternal identity in any model (Table 4.1; Table 4.2). After correction for September temperature and population density, year accounted for 10% of the remaining variation in immature deer (Table 4.1) and 13% in mature deer (Table 4.2).

Table 4.1. Factors influencing coat change date in immature red deer (aged 0-2 years). Outputs from a mixed-model with Normal errors and random effects of individual, maternal identity and year. Significance of fixed effects was assessed using Wald statistics whereas significance of random effects was tested by comparing log-likelihoods of models with and without the appropriate random effect.

Fixed effects	Estimate	SE	df	W-stat	p-value
Intercept	56.94	1.66	1	1348	
Age	5.72	1.95	1	8.65	0.004
Age^2	-5.20	0.97			
Sex= M	0.50	1.19	1	0.179	0.674
Sex= F					
Pop dens	0.17	0.09	1	3.681	0.074
Pop $dens^2$	-0.0019	0.0025	1	0.585	0.456
Sep Temp	-8.13	1.80	1	20.31	<0.001
Random	**Estimate**	**SE**	**df**	**Chi-sq**	**p-value**
Individual	78.42	14.71	1	31.48	<0.001
Maternal ID	9.44	8.00	1	1.52	0.218
Year	24.97	10.55	1	54.01	<0.001
Residual	127.82	11.43			
	N=963, code=701, mumcode=290, year=21 (see below)				

Table 4.2. Factors influencing coat change date in adult female red deer (aged 3-19 years) on the Isle of Rum. Outputs from a mixed-model with Normal errors and random effects of individual, maternal identity and year. Significance of fixed effects was assessed using Wald statistics whereas significance of random effects was tested by comparing log-likelihoods of models with and without the appropriate random effect. Status = reproductive status, with N = Naïve, M=milk, TY=true yeld, SY=summer yeld, WY= winter yeld.

Fixed effects	Estimate	SE	df	W-stat	p-value
Intercept	54.11	3.06	1	198.6	
Age	0.20	0.61	1	0.104	0.748
Age^2	0.08	0.03	1	5.76	0.017
Repro Status= M	0		4	81.9	<0.001
Repro Status= N	-16.11	1.38			
Repro Status= TY	-12.43	0.97			
Repro Status= SY	-9.63	1.26			
Repro Status= WY	2.37	1.29			
Population density	0.12	0.10	1	1.58	0.227
Population $density^2$	-0.001	0.003	1	0.223	0.643
September Temp	-8.20	2.10	1	10.92	0.004
Status= M:Sep temp	0		4	2.54	0.042
Status= N:Sep temp	3.90	1.32			
Status= TY:Sep temp	1.95	1.17			
Status= SY:Sep temp	1.59	1.48			
Status= WY:Sep temp	0.42	1.53			

Random effects	Estimate	SE	df	Chi-sq	p-value
Female Identity	101.73	15.43	1	202.89	<0.001
Maternal identity	14.40	12.32	1	1.52	0.218
Year	31.83	12.25	1	130.86	<0.001
Residual	106.33	5.39			

N=1215, N individuals=375, N mothers=230, N years=21

Table 4.3. Significance of interaction terms in i) immature and ii) mature deer. Results show the significance of adding the appropriate interaction to the models without interactions, described in tables 1 and 2.

i) Immature	Df	W-stat	P-value
Sex:Sep-temp	1	1.578	0.210
age:Sep-temp	1	0.4336	0.513
age:Sex	1	0.3896	0.535
Sex:dens	1	0.2994	0.587
dens:Sep temp	1	0.0266	0.873
age:dens	1	0.0180	0.894

ii) Mature	Df	W-stat	P-value
status:Sep temp	4	2.54	0.042
status:dens	4	1.467	0.213
age:Sep temp	1	1.053	0.305
age:dens	1	0.6121	0.438
age:status	4	0.8689	0.490
dens:Sep temp	1	0.00452	0.947

Temporal trends in coat change date

Coat change date became earlier over the study period in both immature and mature deer (Figure 4.4). Coat change date advanced by 1.14±0.19SE days per year in immature deer, and 0.93±0.21SE days per year in mature deer; this represents an advancement of almost 23 days in immature deer and 19 days in mature deer over the course of the study period Although coat change date in immature deer appeared to be advancing at a faster rate than in mature deer, this difference was not statistically significant (unpaired t-test of parameter estimates, p=0.47).

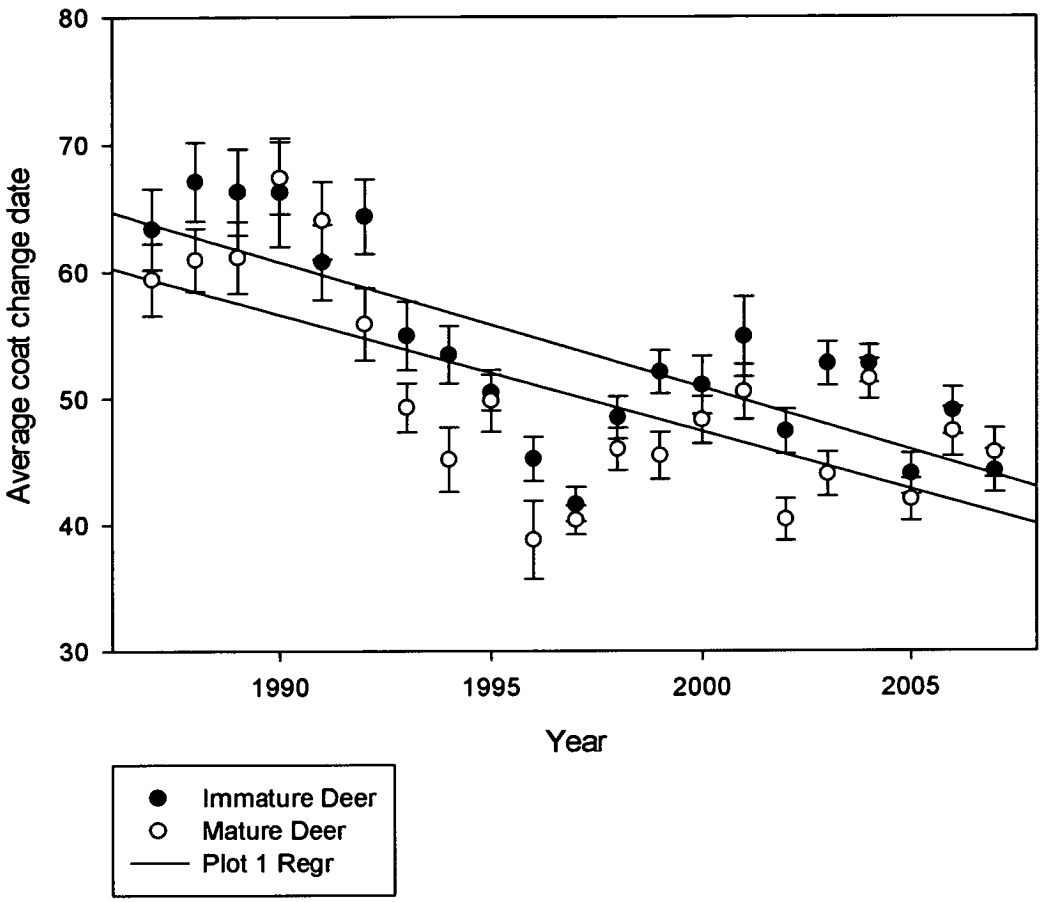


Figure 4.4. Average coat change date advanced over the study period in both immature (filled circles, top line) and mature deer (open circles, bottom line). Trend lines were calculated from the data points shown.

We next included both year as a covariate and September temperature in models to determine if increase in September temperature over time (Figure 4.5) was driving temporal trends in coat change date. Inclusion of September temperature in the model of immature deer caused the estimate of temporal change to decrease by 29%, although the trend remained significant (b=-0.81±0.30SE, p=0.017). In contrast, September temperature appeared to explain much of the temporal trend in coat change

date in mature deer as year became non-significant and the parameter estimate dropped by 25% when September was added to the model (b=-0.70±0.35, p=0.060).

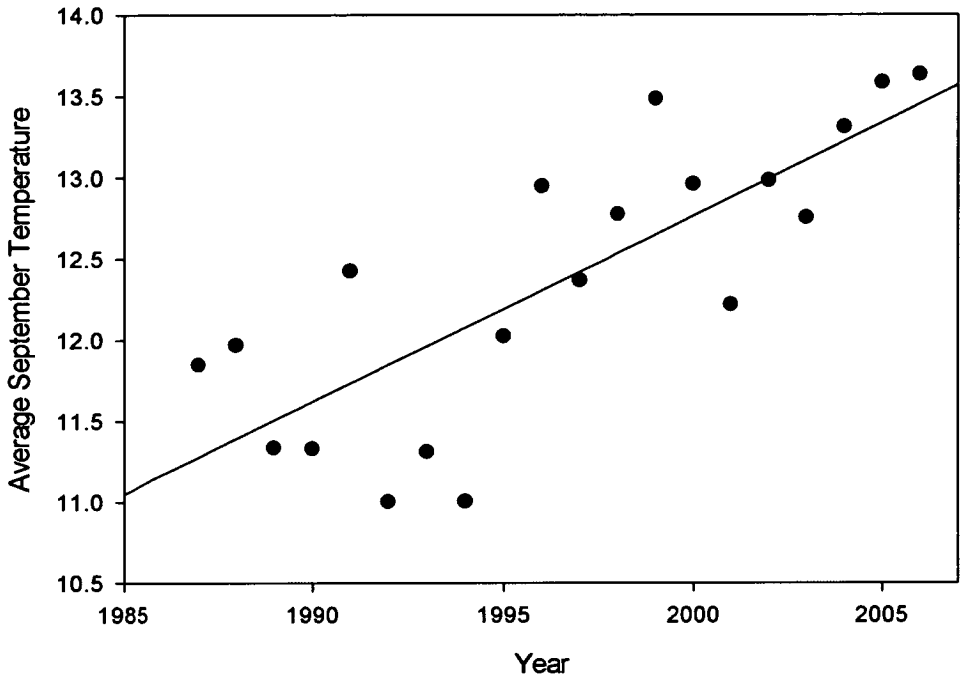


Figure 4.5. Average September temperatures (°C) increased over the study period (b=0.11±0.02). Trend line was calculated from data points shown.

Is coat change phenology associated with over-winter survival?

Across all deer, coat change state on November 1st was a significant predictor of over-winter survival (Table 4.4; Figure 4.6). We split the data further to determine where coat change state was most predictive of over-winter survival. Females whose calves would die over the upcoming winter (winter yeld) had the only significant relationship between coat change date and over-winter survival when split by reproductive status (Table 4.4). When split by age, a significant relationship between coat change state and over-winter survival was only evident in individuals aged 0, 1 and 5 years (Table 4.4).

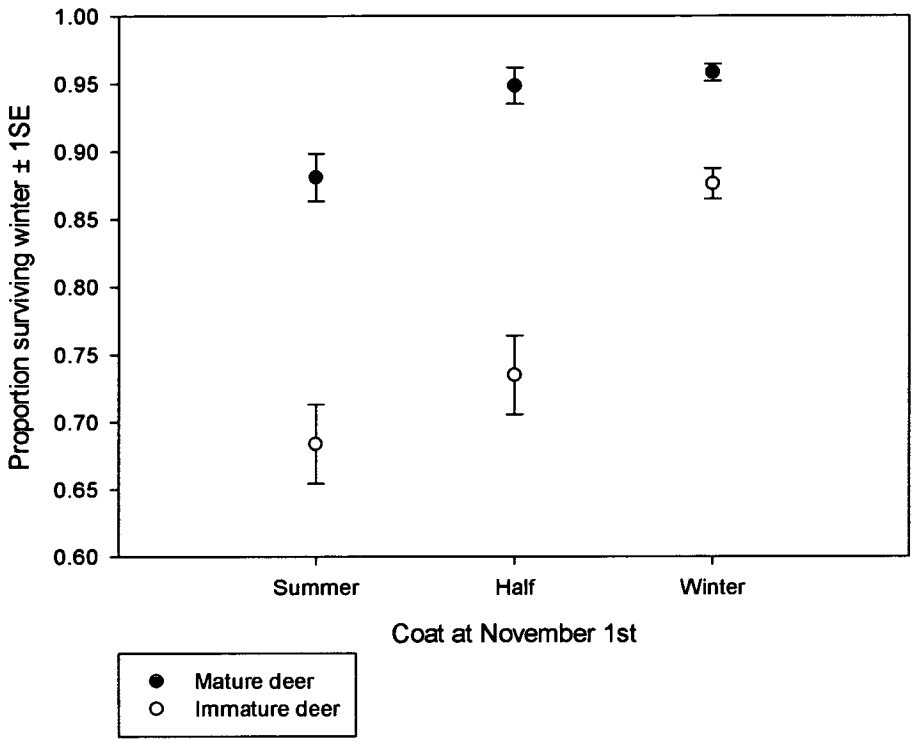


Figure 4.6. Coat change state on the 1st November was correlated with over-winter survival in both immature (open circles) and mature deer (filled circles). Individuals were classified according to coat state on 1st November; the percentage surviving winter and associated standard error was then calculated. Differences in survival were significant when tested using Chi-squared tests (Table 4).

Table 4.4 (overleaf). Coat state on 1st November was a significant predictor of over winter survival. We used chi-squared tested to determine if coat state on the 1st of November (Summer, Half or Winter) was predictive of over-winter survival. N shows the number of individual's categorised in each coat state, mean and stderr describes the average survival rates and associated standard error. df, chi-squared stat and p-value related to the chi-squared test assessing differences in survival rates between coat states.

state	N	mean	stderr	df	chisqstat	p
All deer						
S	597	0.80	0.02			
H	501	0.85	0.02			
W	1869	0.92	0.01	2	74.9324	**<.0001**
Mature/immature						
Mature deer						
S	344	0.88	0.02			
H	271	0.95	0.01			
W	1029	0.96	0.01	2	27.9164	**<.0001**
Immature deer						
S	253	0.68	0.03			
H	230	0.73	0.03			
W	840	0.88	0.01	2	59.5164	**<.0001**
Sex						
Immature female deer						
S	159	0.74	0.04			
H	131	0.82	0.03			
W	567	0.91	0.01	2	36.9601	**<.0001**
Immature male deer						
S	94	0.60	0.05			
H	99	0.63	0.05			
W	273	0.80	0.02	2	19.9083	**<.0001**
Female reproductive status						
Mature milk females						
S	131	0.93	0.02			
H	95	0.98	0.01			
W	288	0.97	0.01	2	4.3712	0.112
Mature winter yeld females						
S	92	0.74	0.05			
H	45	0.84	0.05			
W	91	0.93	0.03	2	12.8064	**0.002**
Mature summer yeld females						
S	32	0.94	0.04			
H	39	0.90	0.05			
W	113	0.95	0.02	2	1.1684	0.558
Mature true yeld females						
S	81	0.93	0.03			
H	70	0.99	0.01			
W	284	0.95	0.01	2	2.94	0.230
Mature naïve females						
S	8	1.00	0.00			
H	22	1.00	0.00			
W	253	0.97	0.01	2	0.9762	0.614

state	N	mean	stderr	df	chisqstat	p
Age						
Immature age=0						
S	153	0.59	0.04			
H	132	0.64	0.04			
3W	406	W	0.02	2	29.2066	**<.0001**
Immature age=1						
S	73	0.81	0.05			
H	66	0.82	0.05			
W	237	0.94	0.02	2	13.8481	**0.001**
Immature age=2						
S	27	0.89	0.06			
H	32	0.97	0.03			
W	197	0.97	0.01	2	4.1746	0.124
Mature age=3						
S	8	0.88	0.13			
H	19	0.95	0.05			
W	208	0.97	0.01	2	1.8606	0.394
Mature age=4						
S	31	0.94	0.04			
H	28	0.96	0.04			
W	156	0.97	0.01	2	0.7628	0.683
Mature age=5						
S	29	0.90	0.06			
H	33	1.00	0.00			
W	124	0.98	0.01	2	7.9588	**0.019**
Mature age=6						
S	29	0.90	0.06			
H	26	1.00	0.00			
W	106	0.92	0.03	2	2.5539	0.279
Mature age=7						
S	34	0.88	0.06			
H	25	1.00	0.00			
W	94	0.96	0.02	2	4.4901	0.106
Mature age=8						
S	39	1.00	0.00			
H	25	1.00	0.00			
W	81	0.96	0.02	2	2.4204	0.298
Mature age=9						
S	37	0.92	0.05			
H	24	0.96	0.04			
W	77	0.94	0.03	2	0.3712	0.831
Mature age=10						
S	28	0.86	0.07			
H	23	1.00	0.00			
W	61	0.92	0.04	2	3.4919	0.175
Mature age=11						
S	31	0.90	0.05			
H	24	0.88	0.07			
W	41	1.00	0.00	2	4.9548	0.084
Mature age=12						
S	20	0.80	0.09			
H	17	0.94	0.06			
W	31	0.90	0.05	2	2.0034	0.367
Mature age=13						
S	17	0.82	0.10			
H	13	0.77	0.12			
W	24	0.96	0.04	2	3.1551	0.207

Discussion

The aim of this study was to investigate factors influencing variation in coat change date, and implications of this variation with respect to over-winter survival. Patterns of age related variation differed from those found previously in other traits in this system (see below) and average coat change date advanced faster that any other phenological trait in this system over time. Additionally we showed coat change date to be correlated with over-winter survival.

Patterns of age-related variation in coat change date differed substantially from those observed in other female traits in this population (Nussey et al., 2009); appendix III). Female fecundity and offspring birth weight, birth date and first year survival have been shown to improve from 2 years old to approximately 9 years old, followed by senescence from 9 years (Nussey et al., 2009; appendix III). However, patterns of senescence differ between traits: fecundity and offspring birth date show only slight senescence from 9 years followed by a pronounced decline at around 14 years, whereas offspring birth weight and survival both show marked declines from 9 years. Female fecundity and offspring birth date are believed to be primarily a function of oocyte number, which decreases sharply with age in many different taxa (Gosden and Faddy, 1998, Cohen, 2004), including humans (Armstrong, 2001) and farmed red deer (Fisher et al., 2000). In contrast, offspring birth weight and over-winter survival are related to investment of resources by the female during gestation and lactation, and may therefore decline with somatic deterioration (Kirkwood and Rose, 1991, Cohen, 2004). Coat change was earliest in 3 year olds and became later with increasing age. Until the age of 8 years, the difference between consecutive years was less than 1.5 days and this difference accelerated with age until the predicted difference between

females aged 18 to 19 years was over 3 days. This unprecedented pattern in this system may indicate different physiological processes underlying the phenology of coat change date and other female traits studied previously in this population. If so, there may be very little correlation, either phenotypic or genetic, between coat change date and other traits in this population. This hypothesis is confirmed explicitly in chapter 6.

Further evidence of different physiological processes underlying coat change date and other phenological variables comes from experiments on farmed deer. Implanting melatonin, mimicking the effects of shortened days, in adult female red deer caused very differing patterns of advancement in oestrus date and coat change date (Fisher et al., 1990). Indeed, the pattern of age-related variation found the analyses presented here resembles that found in kidney fat index in this population, which is highest in young adult females (Clutton-Brock et al 1982), suggesting that coat change date may be reflective of individual condition. Finally note that this age-related pattern is also unlikely to be due to non-random disappearance of individuals, as results remain qualitatively the same when age of first and last reproduction are included in the model (van de Pol and Verhulst, 2006; analysis not shown).

Female reproductive status was significantly associated with coat change date. Hinds who had never bred before (Naïve) and hinds without a calf (Yeld or Summer Yeld) i.e. those in best condition (Mitchell and Lincoln, 1973), had the earliest average coat change date. Together with the patterns of age-related variation, these results suggest that those individuals in the best physiological condition change into winter coat the earliest.

Although we found evidence of condition-dependence acting through female status, we did not find any significant effect of population density. This was unexpected as a previous study in this system prior to 1980 found that the proportion of females in winter coat by the 1st of November decreased with increasing density (Clutton-Brock et al 1982). Female population density in the study area increased after the cessation of culling in 1972 before reaching apparent carrying capacity approximately 10 years later (Albon et al., 2000). The analysis presented here includes only data from 1987 and consequently does not cover the period of large-scale density changes observed in the early part of the study, which may be why the effect of population density was non-significant in this analysis, but significant previously. The trend was for later average coat change date at high density, which is consistent with the trend for females in good condition change to winter coat earliest.

Average coat change date was earliest in years with high September temperatures. A similar relationship has also been found between September temperature and early average start of antler growth in adult males during the following spring (Clements et al., in press; chapter 2). As early antler growth is under selection (Clements et al., in press; chapter 2), warm September temperatures are likely to be associated with improved vegetation growth and increased individual condition. There was a significant interaction between September temperature and reproductive status indicating that females bearing the recent cost of reproduction and were consequently in the worst condition advanced coat change date most in response to high September temperatures.

Although coat change date appears to be linked to condition, coat change date was highly repeatable indicating consistent differences between

individuals throughout their lifetimes. Interestingly, maternal identity was not a significant random effect in the model of immature individuals suggesting that consistent differences between individuals at this early age were not due differences consistent differences between mothers in maternal provisioning (Rasanen and Kruuk, 2007). The high levels of repeatability were therefore presumably due to heritable genetic variation or environmentally induced effects other than maternal effects (Lynch and Walsh, 1998). Indeed, coat change date is shown in chapter 6 to be significantly heritable. These differences between individuals may be reflective of an individual's 'quality' (Wilson and Nussey, 2010); determining such an association would require calculation of the selection gradients acting on the average trait value.

Increases in September temperature over the study period were correlated with advancement in average coat change date, although September temperature alone did not explain all of the advancement in immature deer, and year as a fixed effect was only marginally non-significant in the model of mature deer ($p=0.060$). Advancement over time has been observed in a wild range of taxa in the UK (Thackeray and al, 2010) and is believed to be a direct result of increased average temperatures (Barnett et al., 2006) due to climate change (IPCC, 2007). Coat change date advanced by approximately 1 day per year across the study period, by far the biggest advance in any phenological trait in this study system (Moyes et al., in review; appendix V), perhaps again reflecting the high condition-dependence of coat change date.

Consistent with coat change date being reflective of an individual's condition, individuals not in winter coat by the 1st November had a reduced probability of over-winter survival. This effect was most pronounced in immature deer, perhaps because survival rates were lowest in this group, and thus enabled effective discrimination

between coat change states with the technique of a chi-squared analysis. In correlational analyses such as these, it is impossible to separate the ultimate and proximate relationships between coat change date and over-winter survival: individuals in winter coat earliest may have to expend less energy to maintain body temperature (Arnold et al., 2004) and consequently there may be a causal relationship between coat change date and over-winter survival. Alternatively, early individuals may also growth the thickest coat as they are in the best condition, or coat change date may more generally reflect an individual's condition, leading to non-causal correlations between coat change date and over-winter survival.

In a seasonal environment, expression of an adaptive trait at an appropriate time can have important fitness consequences, acting through either individual survival or reproduction, or even through offspring survival. In red deer the growth of the winter coat enables individuals to expend less energy in order to maintain their body temperature (Arnold et al., 2004), increasing the probability of over-winter survival. However, we found very different patterns of age-related variation and degree of advancement over time in comparison to other traits in this system. These results emphasise the importance of understanding the biology of a species: if we had conducted climate change trends analyses on coat change date alone, assuming it to representative of all phenological traits in the system, advancement over time would appear much greater than it truly is. There is therefore a need to approach analysis from a more holistic viewpoint (Post et al., 2008), perhaps using multivariate techniques to study the whole system and how different factors integrate.

Chapter 5

Distribution trends

Summary

It is widely acknowledged that global climate change is causing advancement in the timing, or phenology, of a wide range of biological phenomenon. However, it seems unlikely that advancement can continue indefinitely. We hypothesise that as this limit to phenological advancement is approached, there will be a concurrent decrease in the variance and possibly a related increase in the levels of skew and kurtosis recorded over time. Changes in these parameters may therefore hold important, and inexpensive to obtain, clues about how a population is responding to climate change. We tested this hypothesis using data from a wild population of red deer in NW Scotland, where the mean timing of annual events is known to have been advancing over the past 30 years. However, we found very little evidence of systematic changes in the higher moments of the trait distributions, perhaps suggesting that there is opportunity for phenology to continue advancing in this population. Testing for trends in variance, skew and kurtosis is a trivial matter but despite the vast number of studies of trends in mean values in wild populations, we know of none that have considered the higher moments of the distribution.

Introduction

There is now substantial evidence that global climate change is affecting wild animal and plant populations, and projections of further increases in temperature indicate that populations will continue to come under increasing stress (IPCC, 2007). One of the most immediately striking effects of climate change can be seen in the

phenology, or timing, of annually occurring events, which are predominantly becoming earlier (Walther et al., 2002, Parmesan and Yohe, 2003, Thackeray and al, 2010). Although there is widespread evidence that the mean of many distributions of phenology traits is advancing, we know little about how other parameters that characterise a distribution – namely the variance, skew and kurtosis – are changing over time; these parameters could hold important clues to how a population is responding to climate change. Additionally we know little about how the parameters of the distribution differ between phenology traits within a population. Here we use long-term data from a wild population of red deer on the Isle of Rum, NW Scotland, to compare the moments of the distribution of seven phenology variables and test for temporal trends in these moments.

A recent meta-analysis found phenology across a wide range of taxa in the United Kingdom to be advancing at a faster rate than previously realised (Thackeray and al, 2010). At northern latitudes, temperature is often a limiting resource and it is likely that increasing temperatures are linked to better ecological conditions and advancing phenology in the UK (Thackeray and al, 2010). For instance, plants are experiencing the fastest rate of phenological advancement in the UK, most probably reflecting the strong causal relationship between temperature and growth in this taxa (Thackeray and al, 2010). However it seems unfeasible that phenology will continue to advance indefinitely, and therefore probable that that there exists a time point beyond which phenology traits cannot advance. In plants, for example, very high increases in temperature may result in a delay in phenology as plants become heat stressed in the summer months (Sherry et al., 2007) or do not experience sufficiently low temperatures during winter for optimal growth in spring (Thompson and Clark, 2008). Over time we would therefore expect to observe greater numbers of the population expressing a trait very close to this limit of phenological advancement: concurrent with this will be

associated changes in the parameters of the distribution. Monitoring the variance, skew and kurtosis of phenology variables over time would be a relatively simple and potentially very illuminating exercise but it is unclear what might be expected to happen to all of these parameters as the limit to phenological advancement approaches. Below we outline our expectations of changes in these parameters over time and then test our hypotheses on seven different phenology traits collected over 30 years from a wild red deer population in Scotland.

It seems difficult to predict what might happen to the variance of phenology traits with a slight improvement in ecological conditions: more individuals may be able to express the trait at the optimum time which would lead to a decrease in variance but it is also feasible that an increase in ecological conditions could lead to differences between individuals being emphasised which may act to increase variance (as has been observed in crop yields in the USA, Chen et al., 2004). Recent individual-based studies have found evidence that the magnitude of response to environmental change varies between individuals (termed IxE interactions, e.g. Brommer et al., 2005, Nussey et al., 2005)). A consequence of IxE interactions is that the phenotypic variation in a trait changes with the environmental variable under consideration (Nussey et al., 2007), implying that changing climate may lead to not only shifts in mean values recorded but also to changes in the total variance over time; but to our knowledge, there have been no explicit tests for systematic trends in the variance of traits in wild populations. As the population approaches the limit to phenological advancement we predict that the total variance would decrease as the earliest individuals begin to reach the point beyond which they cannot advance.

The skew of phenology variables measures whether the majority of the population express a trait before or after the mean date; if the majority of the population

are expressing a trait before the mean date there must also be a small number of individuals expressing the trait very late, and vice versa. Slight increases in ecological conditions have been found to decrease skew in yields of crops (Day, 1965), but a simulation study predicted skew to increase with improved ecological conditions (Park and Sinclair, 1993). These results are not in complete disagreement as the simulation study incorporated the concept of a maximum yield and simulated changes in parameters when average yields were close to this point. If the yields of crops in the empirical study were not close to the maximum yield, then trends in the higher moments of the distribution may be different to those predicted in the simulation study. As the concept of maximum yield appears to be similar to our concept of a limit to phenological advancement, we predict that if a population is approaching this limit, the expected increasing numbers of individuals expressing the trait at a similarly early time-point would cause an increase in skew. Skew may also increase further if some especially poor quality individuals cannot advance phenology as fast as the rest of the population, creating a longer tail in the distribution.

The kurtosis of a distribution is a parameter that receives relatively little attention in ecology, perhaps because few people are entirely sure how to interpret it. High kurtosis indicates that many individuals are expressing traits close to the mean value and is expected in canalized characters (Waddington, 1957) where there are high costs, and thus selection against, deviating from the mean (Fraser, 1977). As with skew, an empirical study in crop systems has found kurtosis to decrease with improving environmental conditions (Day, 1965), but a simulation study has predicted kurtosis to increase (Park and Sinclair, 1993). As the simulation study included maximum yield, we follow Park and Sinclair (1993) in predicted increased kurtosis as the population approaches the limit to phenological advancement.

Here we use data from a wild ungulate population to explore how the distribution of a variety of phenological traits have changed over time in response to climate change. The red deer (*Cervus elaphus)* population on the Isle of Rum has been studied intensively since 1972 and continuous monitoring enables the timing of expression of a number of traits on an individual basis to be determined (Clutton-Brock et al., 1982; Figure 2). The phenology of traits in this population has been advancing since 1980 (Moyes et al., in review; appendix V), believed to be as a result of increased temperatures leading to advancement in the vegetation growing season in Scotland (Barnett et al., 2006) . Early parturition (Coulson et al., 2003) and antler growth start dates (Clements et al., in press; chapter 2) are under selection in this study system suggesting that the advancement in phenology is linked to higher average individual condition. In this population significant levels of IxE are present in parturition dates in relation to autumn rainfall (Nussey et al., 2005) and antler growth start dates in relation to autumn temperature respectively (Clements et al., in press; chapter 2), suggesting that these variables may show changes in variance over time, if the relevant climate variables are also changing over time. However, there is no evidence of IxE in antler growth end date in relation to spring temperature (Clements et al., in press; chapter 2) where consequently variance may be less likely to change with time. Here we use the same phenology variables as Moyes et al, including the additional trait of coat change date (see Figure 5.2 for a schematic of the deer year), to investigate trends in the higher moments of the distributions of the phenology traits over the past 30 years.

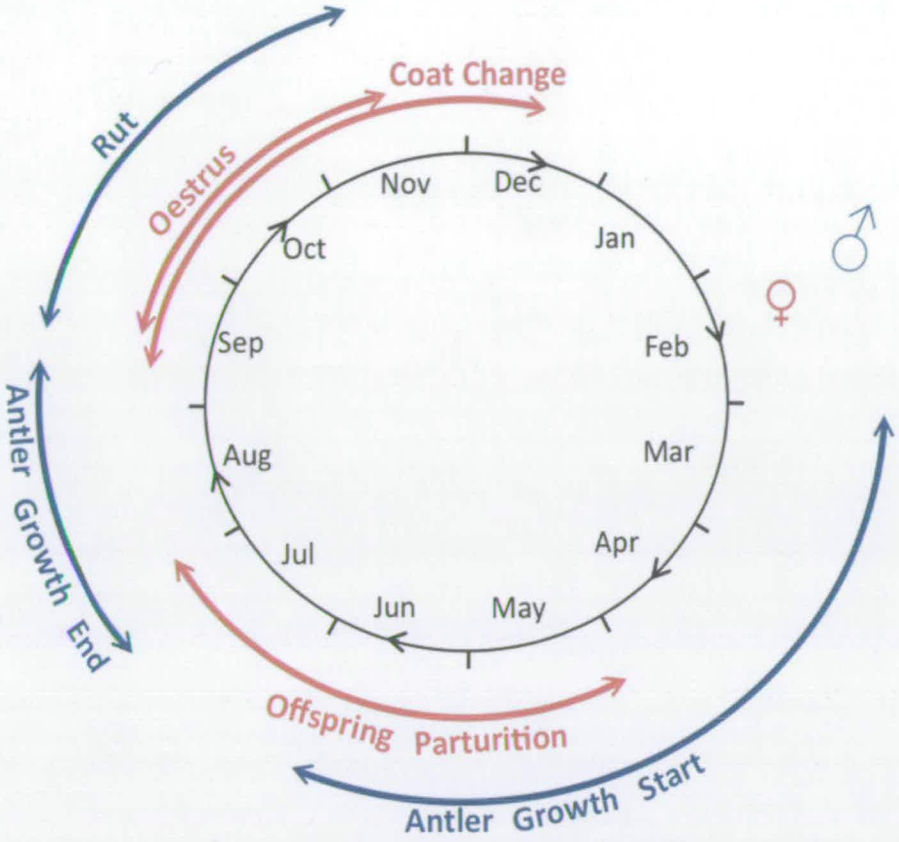


Figure 5.2. Annual cycle of deer phenology: the arrows show the time period during which each event may occur. The inner circle (coloured red) shows traits recorded in females: oestrus date in autumn leading to parturition of the offspring in the following spring, and coat change from summer to winter coat in autumn. The outer circle (coloured blue) shows the cycle of antler growth determined by the cast date of the previous antler signalling start of growth and the cleaning of the vascular tissue surrounding the growing antler as indicating end of growth. The timing of male rutting is also shown: we analysed an individual's start, and end date of rutting.

Methods

The wild red deer population in the North block of the Isle of Rum, Scotland has been studied intensively since 1972, since when it has also been free from culling (Clutton-Brock et al., 1982). Individuals are identified through both artificial tags and natural markings and followed throughout their lives, consequently the age of each individual was known. The date of trait expression for each individual was determined through regular censuses of the study population, undertaken during the same period

each year. In accordance with (Moyes et al., in review; appendix V), we used data from 1980 onwards as large-scale population density increases occurred before this point (Albon et al., 2000) that may act to mask systematic environmentally-induced changes in this system. If the timing of trait expression was recorded in less than five individuals in a given year, we removed all observations in that year from the analyses of that trait; this enabled sufficient degrees of freedom for the mean, variance, skew and kurtosis to be calculated for each trait in each year.

We considered the following phenology variables:

Parturition date: the date on which a female gave birth to her offspring was determined through daily censuses of the study population between May and July each year. We included only births in May, June and July of each year so that extremely late births did not bias results, which reduced sample sizes by less than 3%. We analysed 2,379 observations from 594 individuals and 30 years (1980-2009).

Oestrus date: the rutting season occurs in the autumn and intensive monitoring of the population occurs during between 15th September and 15th November each year. A female in oestrus shows characteristic behaviours and receives increased attention from males (Guinness et al., 1971). If more than one oestrus was observed for a particular female in a given season, we considered the first oestrus in results presented here. Analyses consisted of 691 observations from 373 individuals and 29 years (1980-2009, excluding 2000 for which no observations were available).

Coat change date: the date on which a female was observed in 90% of winter coat, determined during censuses from 15th September until 8th December of each year. Although coat change is a trait expressed by both sexes, the movement of males from the study area following rutting led to low sample sizes, which were potentially biased by

non-random movements of males. Consequently we considered here only data from females, who predominantly remain in the same area throughout the year. Coat change date was only available from 1987 and consequently we analysed 1,619 observations from 474 individuals and 23 years (1987-2009).

Antler growth start date: the date when antlers fall off the male (are cast) and immediate growth of the new antlers begins (Li et al., 2004). We analysed 960 observations from 299 individuals and 29 years (1980 – 2009, excluding 2002; see Clements et al., in press for further details).

Antler growth end date: the end of antler growth is evident when the rich vascular tissue that surrounds the growing antler dies and is cleaned from the antler (Goss, 1983). We analysed 691 observations from 249 individuals and 25 years (1980 – 2009, excluding 2002, 2007, 2009, 2006, 2008).

Rut start and end dates: the first and last dates that an individual was observed holding a harem. To ensure that we were considering only adult males who gain the majority of paternities, and as with (Moyes et al., in review), analysis was restricted to individuals who were observed holding a harem for at least 5 days in a given year. Analyses consisted of 1,787 observations from 541 individuals and 30 years (1980-2009).

If the phenology of trait expression is correlated with age, changes in the parameters of the distribution over time could simply be due to changes in the age-structure of the population, as opposed to a true trend in phenology driven by changes in environment. Consequently we analysed the mean, variance, skew and kurtosis, and their associated trends over time, both before and after correction for age, and also for other non-climatic factors known to be associated with phenology variables in this system

(Coulson et al., 2003, Nussey et al., 2005, Clements et al., in press). We therefore corrected for:

Individual's age: included as a factor as patterns of age-related variation are known to differ remarkably between phenology variables in this system (Nussey et al., 2009; appendix III). To ensure that at least 10 individuals were represented for each variable in each age class (Bolker et al., 2009), we restricted data to males aged 3 to 14 and females aged 2 to 15 inclusive.

Population density: the number of females resident in the study population each winter, measured as the number of females recorded in more than 10% of censuses between January and May (Coulson et al., 2003). In all analyses presented here, we used population density in the same calendar year as the relevant trait was observed in.

Female reproductive status: describes a female's previous breeding experience and is classified as: *Naïve*, a female who has never bred before; *True yeld*, a female who has bred before but didn't give birth during the last breeding season; *Summer yeld*, a female who gave birth in the previous breeding season and the calf died before 15th September of that year; *Winter yeld*, a female who gave birth in the previous breeding season and the calf died between 15th September of that year and 1st May of the following year; *Milk*, a female who gave birth in the previous breeding season and the calf was still alive on 1st May of the following year.

STATISTICAL METHODS

We performed all analyses both before and after correction for the above parameters. Modeling on residuals from a model with fixed effects of age, population density and female reproductive status and random effects of individual and year allowed us to remove variance attributable to these factors and consequently examine

trends driven by environmental change only. To do this we ran linear mixed effects models (Pinheiro and Bates, 2000) with fixed effects of age, population density and female reproductive status; individual identity and year were used as random effects in each of the seven models, to control for pseudoreplication across these factors (Pinheiro and Bates, 2000).

Dates before correction: refers to the exact date of trait expression without any correction for age, population density or female reproductive status.
Dates after correction: refers to the date of trait expression after correction for the effects of age, population density and female reproductive status, individual identity and between year variance, i.e. the residuals from the models described above.

Summary statistics were then calculated for each variable both before and after correction. We considered unbiased estimators of the mean, variance, skew and kurtosis for each variable (Joanes and Gill, 1998). Calculations are as below:

$$\text{Variance} = \frac{1}{(n-1)}\sum_{i=1}^{n}(x_i-\bar{x})^2$$

$$\text{Skew} = \frac{\sqrt{n(n-1)}}{(n-2)}\frac{\frac{1}{n}\sum_{i=1}^{n}(x_i-\bar{x})^3}{\left(\frac{1}{n}\sum_{i=1}^{n}(x_i-\bar{x})^3\right)^{\frac{3}{2}}}$$

$$\text{Kurtosis} = \frac{(n+1)n(n-1)}{(n-2)(n-3)}\frac{\sum_{i=1}^{n}(x_i-\bar{x})^4}{\left(\sum_{i=1}^{n}(x_i-\bar{x})^2\right)^2} - 3\frac{(n-1)^2}{(n-2)(n-3)}$$

Where n is the number of observations in the sample ($x_1, \ldots x_n$) and $\bar{x}$ is the mean value of the sample.

Skew is a measure of the symmetry about the mean of a distribution. A symmetrical distribution, such as the Normal distribution, has a skew of zero. On a

graph of a distribution with negative skew (or left skew) the left tail is longer and the mass of the distribution is concentrated on the right. Alternatively, a positive skew (or right skew) indicates that the right tail of the distribution is longer and the mass of the distribution is concentrated on the left.

Kurtosis measures how concentrated the data is about the mean relative to the Normal distribution where kurtosis equals zero. A negative value of kurtosis indicates that the data is less concentrated about the mean leading to a flatter peak in the distribution that is said to be platykurtic. Alternatively a positive value of kurtosis shows that the data is more concentrated about the mean than would be expected under the Normal distribution causing a higher steeper peak in the distribution and is termed leptokurtic.

We used D'Agostino's K-squared test (D'Agostino et al., 1990) to determine if the population level measures of skew and kurtosis differed significantly from those expected under a Normal distribution. Here a transformation is applied to the skew and kurtosis to obtain a test statistic for each variable that follows Chi-squared distribution if the skew or kurtosis does not significantly differ from that expected under a Normal distribution. We then corrected for multiple testing (14 tests) using sequential Bonferroni tests (Rice, 1989). Sequential Bonferroni testing was preformed separately for parameters obtained before and after correcting for fixed effects.

To determine if the distributions were changing over time we calculated the summary statistics described above for each variable in each separate year. We then fitted a linear model to each of these summary statistics where year as a continuous covariate was the only fixed effect and tested its significance using an F test. To ensure that the observed results were not being driven by a small number of outliers, we

examined the graphs of the summary statistics over time to identify any outliers and then re-ran the analysis (including calculation of the residuals) without data from the year(s) where the outlier(s) occurred. We then corrected for the multiple significance tests (28 tests) using a sequential Bonferroni test, and as above, tests were performed separately for trends obtained before and after correction for fixed effects.

Results

Phenology distributions

We analysed a total of seven traits, three of which were expressed by females and four by males. The average timing of expression of traits ranged from late April for antler growth start date to the end of October for rut end date (Table 5.1; Figure 5.2).

The highest level of variance before correction was in antler growth start date whereas the lowest was in antler growth end date (Table 5.1i). The high levels of age-related variation in antler growth start date led to it showing very low levels of variance after correction, greater only than antler growth end date. The highest level of variance after correction was in coat change date (Table 5.1ii, Figure 5.3).

All distributions except antler growth end date and rut end date showed significant positive skew before correction, implying a longer right tail (Table 5.1i). After correction only parturition, oestrus, and coat change and antler growth start dates had distributions with significantly positive skew (Table 5.1ii; Figure 5.3). Traits expressed in males showed either significant negative skew after correction (antler growth start date and rut end date) or no significant skew after correction (antler growth end date and rut start date; (Table 5.1ii; Figure 5.3). All significant parameters remained significant when a sequential Bonferroni test was applied (Table 5.1).

Kurtosis before correction was significantly negative in all traits (implying a flatter peak about the mean relative to the Normal distribution; Table 5.1i) except parturition and oestrus dates which showed significantly positive kurtosis (implying a steeper peak; Table 5.1i). Contrastingly, kurtosis after correction was significantly positive for all variables implying a steeper peak about the mean relative to the Normal distribution (Table 5.1ii; Figure 5.3). All significant parameters remained significant when a sequential Bonferroni test was applied (Table 5.1).

Table 5.1. Distribution of phenology variables expressed in red deer on the Isle of Rum, combining data from all years since 1980 both (i) before and (ii) after correcting for effects of age, population density and female reproductive status, i.e. residuals from a model including the above parameters as fixed effects and individual identity and year as random effects. Measures of skew and kurtosis were tested for significant deviance from that expected under the Normal distribution using a D'Agostino's K-squared test; * indicates $p<0.05$; ** $p<0.01$; *** $p<0.001$. $^{\$}$ indicates that the parameter remained significant after correction for multiple testing using a sequential Bonferroni test.

	Mean	Variance	Skew	Kurtosis
i) Before correction				
Parturition	8th June	150.01	1.04***$^{\$}$	1.76***$^{\$}$
Oestrus	14th October	93.64	0.62***$^{\$}$	0.29***$^{\$}$
Coat change	20th October	337.13	0.53***$^{\$}$	-0.30***$^{\$}$
Antler growth start	22nd April	469.39	0.42***$^{\$}$	-0.97***$^{\$}$
Antler growth end	17th August	70.61	-0.05***$^{\$}$	-0.27***$^{\$}$
Rut start	7th October	231.19	0.33***$^{\$}$	-0.56***$^{\$}$
Rut end	30th October	191.86	-0.30***$^{\$}$	-0.46***$^{\$}$
ii) After correction				
Parturition	-	1.31	1.43***$^{\$}$	3.52***$^{\$}$
Oestrus	-	54.88	0.82***$^{\$}$	0.92***$^{\$}$
Coat change	-	101.68	0.48***$^{\$}$	0.47***$^{\$}$
Antler growth start	-	28.52	-0.60***$^{\$}$	2.41***$^{\$}$
Antler growth end	-	7.92	0.11	1.04***$^{\$}$
Rut start	-	94.25	-0.11	0.55***$^{\$}$
Rut end	-	67.05	-0.42***$^{\$}$	1.22***$^{\$}$

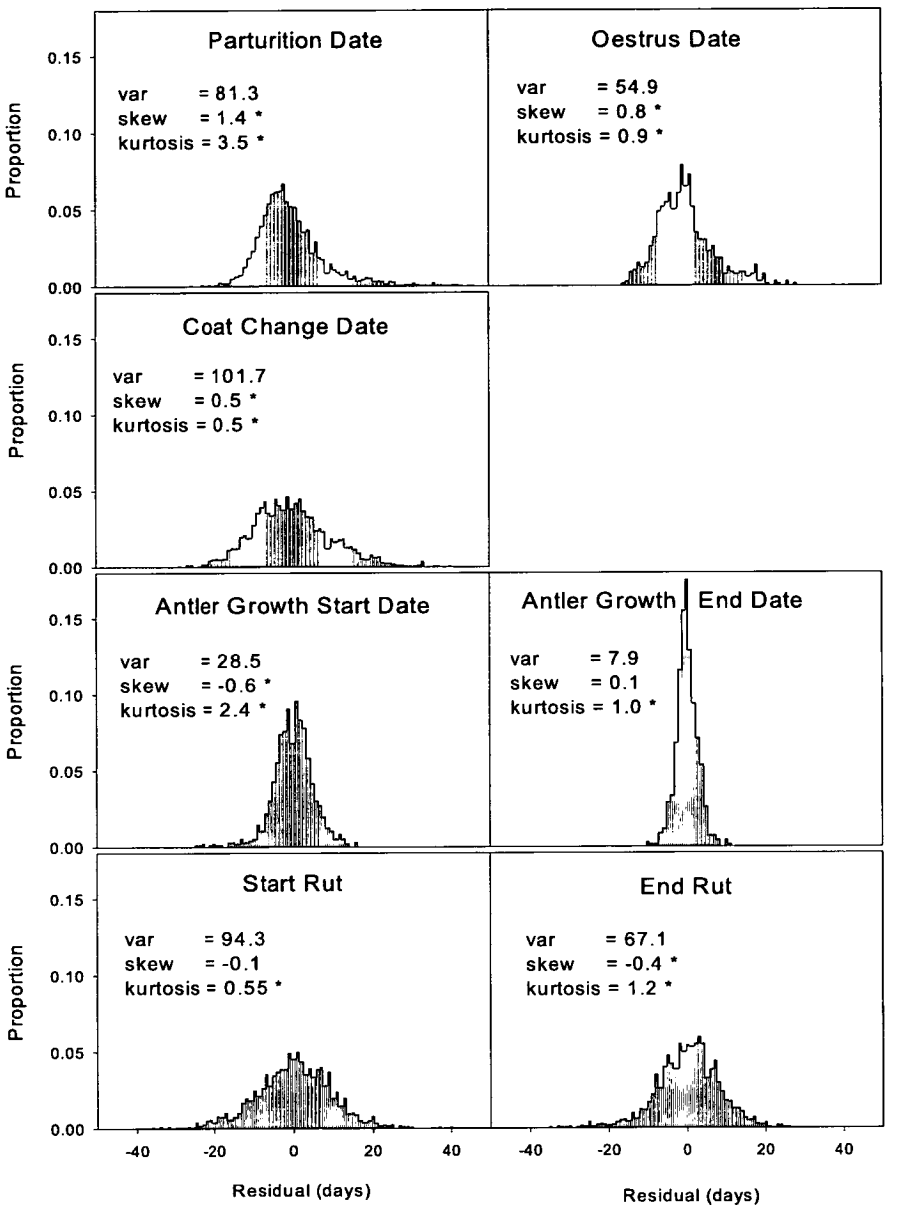


Figure 5.3: Distribution of phenology variables after correction for age, population density and female reproductive status since 1980. Graph show distribution of residuals from a linear-mixed model, correcting for the above factors, rounded to the nearest day. Measures of variance, skew and kurtosis (from Table 1ii) are shown. Where the skew and kurtosis deviated significantly from the Normal distribution, this is marked with a *. Note that the scales on each graph are identical for comparison.

Changes in distributions over time

The mean value of all traits before correction became significantly earlier over the study period (Table 5.2i), except antler growth start and end dates which became earlier, but not significantly so. The advancement in coat change date became non-significant when a sequential Bonferroni test was applied (Table 5.2i). After correction for age, density and female reproductive status, the mean value of all traits became significantly earlier over time (Table 5.2ii, Fig 5.4). However, sequential Bonferroni testing caused only the trends in parturition and rut end dates to remain significant (Table 5.2ii). We visually inspected the trends in Figure 5.4 to determine years that appeared to be outliers; we then removed all observations from outlying years and re-ran analyses to determine if the outliers were driving observed results (Table 5.3). The trends in mean values after correction of all variables remained significant when outliers were removed (Table 5.3).

The variance of most traits before correction showed no significant trends over time, except coat change date which increased in variance over the study period, and rut end date where the variance deceased significantly (Table 5.2i); neither of these trends were significant after application of a sequential Bonferroni test (Table 5.2i). There were also broadly no significant changes in variances of phenology variables after correction (Table 5.2ii). However, the variance of antler growth start date after correction decreased significantly over the study period (Table 5.2ii; Fig 5.4); the trend remained significant when outlying years of 1981 and 1982 (Fig 5.4) were removed from the analyses (Table 5.3) and remained significant after application of a sequential Bonferroni test (Table 5.2ii). Additionally, removal of a single outlier caused the variance trend in coat change date after correction to become significantly negative (Table 5.3) and the variance trend in rut end date after correction to become

significantly positive (Table 5.3); corresponding with the observed trends in these variables before correction (Table 5.2i).

Rut end date was the only variable before correction to show significant changes in skew over time, becoming greater over the study period (i.e. the left tail decreased over time; Table 5.2i); this result remained significant after application of a sequential Bonferroni test (Table 5.2i). In contrast, the skew of rut start date was the only variable after correction that changed significantly over the study period, where the skew became less over time (Table 5.2ii), but this result was not significant under a sequential Bonferroni test (Table 5.2ii).

Kurtosis of traits before correction significantly increased over time in antler growth start and end dates but significantly decreased over time in oestrus date and rut end date (Table 5.2i); none of these trends remained significant after sequential Bonferroni testing. There were no significant changes detected the kurtosis of any phenology variables after correction over time (Table 5.2ii). However, the removal of the outlier in rut start date after correction in 1998 (Fig 5.4) caused the trend in kurtosis to become significantly positive (Table 5.3).

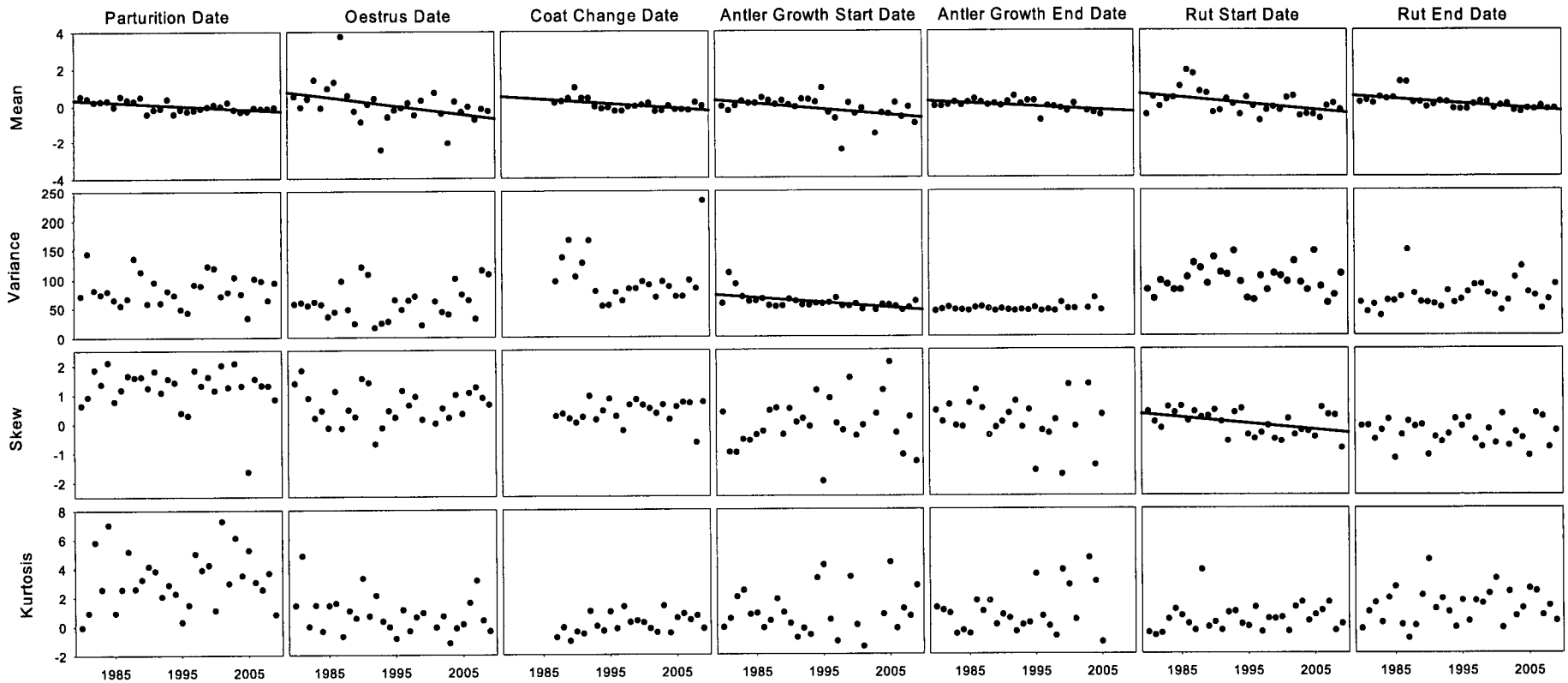


Figure 5.4: Trends in mean, variance, skew and kurtosis over time in phenology variables, in units of days. Graphs show parameter estimates after correction for age, population density and female reproductive status. Where a trend was significant (see Table 2ii), the slope estimate was added to the graph. For comparison, the scales of all graphs for each parameter are identical

Table 5.2. Trends in mean, variance, skew and kurtosis over time in seven different phenology variables both i) before and ii) after correcting for effects of age, population density and female reproductive status i.e. residuals from a model including the above parameters as fixed effects and individual identity and year as random effects. Estimate of change in parameter over time and, in brackets, associated standard error and p-value from an F-test testing significance of the slope. * indicates p<0.05; ** p<0.01; *** p<0.001. $^{\$}$ indicates that the parameter remained significant after correction for multiple testing using a sequential Bonferroni test.

	Mean	Variance	Skew	Kurtosis
i) Before correction				
Parturition	-0.359 (0.057; <0.001)*** $^{\$}$	-0.350 (0.846; 0.683)	-0.003 (0.011; 0.783)	0.049 (0.032; 0.134)
Oestrus	-0.287 (0.080; 0.001)** $^{\$}$	0.362 (0.735; 0.627)	-0.013 (0.013; 0.325)	-0.070 (0.023; 0.005)**
Coat change	-0.668 (0.213; 0.005)**	-9.947 (4.152; 0.026)*	0.007 (0.019; 0.710)	0.069 (0.037; 0.075)
Antler growth start	-0.375 (0.185; 0.052)	-0.091 (4.372; 0.984)	0.024 (0.017; 0.168)	0.081 (0.036; 0.032)*
Antler growth end	-0.179 (0.131; 0.186)	1.493 (1.292; 0.260)	0.026 (0.026; 0.325)	0.096 (0.035; 0.011)*
Rut start	-0.239 (0.062; 0.001)** $^{\$}$	-0.126 (0.980; 0.899)	-0.010 (0.004; 0.018)	-0.004 (0.006; 0.506)
Rut end	-0.447 (0.061; <0.001)*** $^{\$}$	2.251 (1.031; 0.038)*	0.039 (0.009; <0.001)*** $^{\$}$	-0.070 (0.027; 0.017)*
ii) After correction				
Parturition	-0.020 (0.005; <0.001)*** $^{\$}$	-0.111 (0.571; 0.848)	-0.012 (0.015; 0.431)	0.025 (0.041; 0.541)
Oestrus	-0.046 (0.022; 0.042)*	0.678 (0.628; 0.290)	-0.003 (0.013; 0.844)	-0.040 (0.028; 0.154)
Coat change	-0.025 (0.008; 0.007)**	-0.460 (1.354; 0.737)	0.006 (0.012; 0.600)	0.039 (0.020; 0.064)
Antler growth start	-0.030 (0.013; 0.026)*	-1.002 (0.280; 0.001)** $^{\$}$	0.017 (0.019; 0.374)	0.001 (0.040; 0.976)
Antler growth end	-0.019 (0.007; 0.009)**	0.244 (0.155; 0.128)	-0.022 (0.021; 0.307)	0.061 (0.041; 0.143)
Rut start	-0.034 (0.012; 0.011)*	0.044 (0.510; 0.932)	-0.021 (0.008; 0.015)*	0.017 (0.019; 0.383)
Rut end	-0.026 (0.006; <0.001)*** $^{\$}$	0.693 (0.485; 0.164)	-0.002 (0.009; 0.797)	0.020 (0.025; 0.438)

variable	Year removed	N/average	Parameter estimated	Estimate	Std err	p-value	Significance changed?
Parturition	2005	62/79	Skew	0.006	0.009	0.512	No
Oestrus	1987 1993 2003	35/72	Mean	-0.245	0.060	<0.001	No
Coat change	2009	115/70	Variance	-15.525	2.871	<0.001	Yes
Antler start	1998	4/33	Mean	-0.030	0.009	0.004	No
Antler start	1981 1982	134/66	Variance	-0.367	0.124	0.007	No
Antler start	1995	8/33	Skew	0.017	0.017	0.316	No
Antler end	1996	5/28	Mean	-0.018	0.005	0.003	No
Start rut	1988	64/59	Kurtosis	0.030	0.013	0.034	Yes
End rut	1986 1987	100/118	Mean	-0.039	0.009	<0.001	No
End rut	1987	56/59	Variance	1.012	0.351	0.008	Yes
End rut	1990	65/59	Kurtosis	0.027	0.022	0.232	No

Table 5.3. Significance of trends over time after correction for effects of age, population density and female reproductive status after removal of outlier(s) determined by visual examination of Figure 4. N/average shows the number of observations in the outlier year compared to the number of observations that would be expected if observations were distributed evenly across all years. 'Significance changed?' indicates if the removal of the outlier(s) identified in 'year removed' caused a qualitative change from results recorded in table 2.

Discussion

We have shown here that, consistent with an earlier individual-based analysis of the same population (Moyes et al, in review; appendix V), the mean timing of expression of traits in this population is predominantly becoming earlier over time, potentially as a direct result of climate change. However, contrary to our expectations, very few changes were observed in the higher moments of the distributions.

The distribution of all variables, after correction for age, population density and female reproductive status, differed between phenology traits. The skew of all traits expressed by females was positive whereas for males the skew was both significantly negative and not significantly different from zero. As antler growth start and rut start dates are under selection, with the earliest males siring the most offspring (Clements et al., in press, Moyes et al., in review; chapter 2 & appendix V respectively), the significant negative skew in both of these traits indicates that there were a small number of males who expressed these traits earliest and were perhaps the most successful. It is not clear why females would show different patterns of skew to males, but it is possible that there is some balancing selection acting on parturition and oestrus dates preventing very early trait expression as birth timing has to match vegetation availability for the female to be able to provide high quality milk to the growing offspring (Loudon et al., 1984). Consistent with the idea of kurtosis reflecting past selection on a small phenotypic range (Fraser, 1977), kurtosis of all traits after correction was positive, perhaps indicating high costs of deviating from the mean and reflecting past selection on the optimum timing of expression in a highly seasonal environment with a short growing season (Fraser, 1977).

We investigated the trends in the mean, variance, skew and kurtosis of seven different phenology traits over 30 years. We first corrected for effects of age, population density and female reproductive status so that observed trends reflected changes in climate and were not confounded by changes these other variables over time. As with (Moyes et al., in review; appendix V) and in common with many other organisms in the UK (Thackeray and al, 2010), we found the mean phenology of all traits to be advancing over time, although the parameter estimates differed to that found in the previous study. Moyes et al found in an individual-based analysis that rut end date is advancing almost twice as fast as rut start date leading to a reduction in rut duration over time, but we found no evidence for this in our analysis of annual means. However, the trend in rut start date but not rut end date became non-significant when a sequential Bonferroni test was applied, perhaps suggesting a decrease in average rut duration over time in our study. Although both studies detected advancement of phenology over time, the discrepancies serve to highlight the importance of individual-based studies in understanding patterns of population change in response to climate change. We analysed trends in population-level annual estimates as it is impossible to test for trends in variance, skew and kurtosis over time within an individual-based framework, but we advocate the use of individual-based models wherever possible.

After correction for effects of age, population density and female reproductive status, the variance of just three traits showed evidence of systematic changes over time: the variance in antler growth start date decreased over the study period and, in both cases after the removal of a single outlier, the variance in coat change date significantly decreased and in rut end date significantly increased over the 30 years of the study. Recent ecological studies have focused on individual by environment interactions (IxE; Nussey et al., 2007), where the magnitude of response to environmental conditions differs between individuals. Consequently population level

variance should also correlate with the environmental variable under consideration. If the environmental variable is changing over time an associated temporal trend in variance would be expected, although this has never been explicitly tested. Globally, climate change is leading to systematic changes in mean temperature but the effect on rainfall is less pronounced (IPCC, 2007). In Scotland (Barnett et al., 2006), and specifically on the Isle of Rum (Moyes et al., in review; appendix V), average temperatures have increased significantly over the past 30 years; in Scotland significant changes in rainfall have only been detected in winter (Barnett et al., 2006). Consequently we predict significant changes in variance over time only in those variables where IxE has been detected in response to temperature and winter rainfall. In our study system IxE interactions have previously been tested for in three phenology variables. Significant IxE occurs in antler growth start date in response to autumn temperatures (Clements et al., in press; chapter 2) and consistent with expectations variance decreased in this variable over the past 30 years. No IxE is detectable in antler growth end date in response to spring temperatures (Clements et al., in press; chapter 2) and correspondingly we did not detect any significant trends in this variable over time. In parturition date, significant IxE is present in relation to autumn rainfall (Nussey et al., 2005) where we found no significant trends in variance over time. Consequently, these results are the first indication that IxE interactions detectable in individual-based models may translate into population level changes in variance over time, if the ecological variable under consideration is also changing over time.

There were broadly very few changes in the measured levels of variance, skew and kurtosis over time. We hypothesised that the mean data of expression of phenology traits could not continue to advance indefinitely, and as this limit to phenological advancement was approaching we would observe a concurrent decrease in variance and increase in skew and kurtosis. Our results are therefore implying that either there is not

a limit to phenological advancement or that it has not yet been reached. In red deer the limit to phenological advancement could be caused by vegetation growth no longer advancing, or perhaps hormonal cycles acting as a brake to advancement as photoperiod is known to be an important determinant of timing of phenology traits in this system (e.g. Pollock, 1975, Webster and Barrell, 1985), and so it seems implausible that phenology will continue to advance in this system indefinitely. More likely is the possibility that the limit to phenological advancement has not yet been reached. Scottish red deer are especially small due to the poor ecological conditions experienced (Suttie et al., 1983) and consequently the effects of climate change experienced thus far may be beneficial. If this analysis was repeated on an organism residing in a previously optimal environment and with low stress tolerance, the trends observed may be very different. There is therefore a clear requirement for more studies to report trends in variance, skew and kurtosis over time so that patterns observed can be better understood through comparative analyses.

We predicted that as a population average timing of trait expression became closer to the limit of phenological advancement, we would observe a concurrent decrease in variance and increase in skew and kurtosis, evidence for which was weak, indicating that perhaps advancement of the population mean may continue for some further years. Ours is the first study examining trends in parameters of the distribution other than the mean, which seems surprising given the number of studies examining changes in mean across a wide range of taxa (Menzel and al, 2006, Parmesan, 2007, Thackeray and al, 2010).

When calculating the change in mean values over time, as is so often done in studies linking phenology and climate change, it is a relatively trivial matter to also calculate how the higher moments of a distribution are changing; these higher moments

may hold important, but inexpensive to obtain, clues to how a population is responding to climate change.

Chapter 6

The internal clock goes tick-tock? Variance and covariances of phenological traits in a wild mammal population

This chapter is in press as: Michelle N. Clements, Tim H. Clutton-Brock, Fiona E. Guinness, Josephine M. Pemberton & Loeske E.B. Kruuk. Variances and covariances of phenological traits in a wild mammal population. *Evolution*.

Summary

In a seasonal environment there are multiple aspects of timing, or phenology, that contribute to an individual's fitness. Several studies have shown a genetic basis to variation between individuals in breeding time, but we know little about the heritability of other phenological traits in wild populations. Furthermore, the presence of genetic correlations between phenological variables could act to constrain or promote any response to selection, but less is known of the multivariate genetic relationships underlying phenological traits in the wild. Here we use data from a wild population of red deer on the Isle of Rum, Scotland, to investigate covariances between eight phenological traits. Variation was characterised at the level of the phenotype, genotype and year, and traits measured in different sexes enabled us to test for cross-sex genetic correlations. Phenotypic correlations were broadly strong and positive, as were correlations between traits expressed in the same year. We found evidence of significant additive genetic variation in five of the eight phenological traits studied.

However there was little evidence of genetic correlations between traits, implying that much of the observed phenotypic correlation was environmentally induced. Our results suggest that different phenological traits may be free to move along independent evolutionary trajectories.

Introduction

A seasonal environment imposes selection pressures on the timing, or phenology, of annual events in wild populations. There have been many studies examining causes and consequences of the timing of breeding in wild mammal and bird populations (e.g. Sheldon et al., 2003, Reed et al., 2009, Réale et al., 2003a), but the timing of expression of other traits also has important fitness consequences. For instance, the timing of migration in birds has been shown to be under selection (Brown and Brown, 2000), as has the timing of expression of secondary sexual characteristics in both birds and mammals (Cockburn et al., 2008, Clements et al., in press; chapter 2). Different phenological traits are often correlated both within individuals and within years, although less is known about the extent of genetic correlations between them: whilst there is some evidence of genetic correlations underlying phenological traits in plants (Kelly, 1993, O'Neil, 1997), to our knowledge, genetic correlations between phenological traits in wild animal populations have not been estimated. Here we use data collected from a wild population of red deer (*Cervus elaphus*) across 42 years to investigate factors influencing the observed variation in, and the covariances between, a number of phenological variables expressed in both sexes.

Genetic variation is central to evolutionary dynamics. Evolution proceeds most freely along lines of high genetic variance (Falconer and Mackay, 1996 , Lynch and Walsh, 1998, Schluter, 1996) and consequently a population's ability to respond to

changing selection pressures is dependent of levels of genetic variation. In phenological traits, significant genetic variation has been found in taxa as diverse as fungi (Lehman and Oudemans, 2000), plants (Geber and Griffen, 2003), insects (van Asch et al., 2007), birds (McCleery et al., 2004) and mammals (Réale et al., 2003b). However, most studies of heritability in phenological traits in wild animal populations have focused on a single trait (although see Svensson, 1997 for a study of two traits) and we know little about how patterns of variance differ between multiple phenological traits within a single population, nor how these traits co-vary. Genetic correlations occur when the same genes, or genes linked on the same chromosome, influence different phenotypic traits. Consequently selection cannot act independently on these traits, as selection on one trait will produce a correlated response in the other (Lande, 1979). If traits that are negatively genetically correlated are under unidirectional selection, or traits that are positively genetically correlated are under antagonistic selection (i.e. genetic variance-selection concordance), then evolutionary response will be faster than if the traits were genetically independent. However, if the opposite occurs (genetic variance-selection antagonism), any evolutionary response will be constrained and may be in a very different direction to selection. An understanding of the genetic variances and covariances between phenological traits, i.e. the genetic architecture underlying traits, is therefore essential to predict how a population may respond to changing selection pressures.

The study of genetic correlations requires multivariate analyses to construct a **G** matrix describing the genetic covariance between multiple traits; estimation of the **G** matrix can provide insights into genetic architecture undetectable with univariate analyses (Blows, 2007, Walsh and Blows, 2009). Multivariate analyses can also be used to detect cross-sex genetic correlations, which requires knowledge of the genetic

relationships between individuals within the study population. The detection of genetic correlations in wild populations often requires detailed and long-term datasets and consequently our understanding of multivariate genetic architecture in this area is poor. To our knowledge, the only previous studies of genetic correlations between phenological traits are in plants. Significant genetic correlations have been found between the flowering start and end dates in purple loosestrife (O'Neil, 1997), but there was no evidence of genetic correlations between the dates of flowering and fruit maturation in, an annual legume, *Chamaecrista fasciculata* (Kelly, 1993). In wild animal populations, genetic correlations between phenological and other phenotypic traits have been found in passerine birds where lay date and clutch size are often negatively genetically correlated (e.g. Sheldon et al., 2003, Garant et al., 2008, Husby et al., In press), and in the male barn swallow where there is a negative genetic correlation between arrival date following migration and tail length, a sexually selected trait (Møller, 2001).

An understanding of the genetic architecture underlying phenological traits is especially pertinent as advancing phenology is one of the most immediately striking effects of climate change on natural populations and has been observed in a wide range of wild organisms (reviewed in Menzel and al, 2006, Parmesan and Yohe, 2003, Thackeray and al, 2010). Although some of the advancement thus far may be primarily due to phenotypic plasticity, if environmental conditions continue to change as predicted (IPCC, 2007), limits to plasticity may be reached and microevolution may be required for a continued response (Visser, 2008, Crozier et al., 2008); the genetic structure of phenological traits will therefore influence a population's response to environmental change.

Here, we investigate patterns of genetic variance and covariance in phenology traits in a wild population of red deer. Red deer in Scotland live in a highly seasonal environment where vegetation growth is the predominant limiting resource (Suttie et al., 1983). The mating system is polygynous with males taking no part in offspring care; consequently the selection pressures acting on males and females are strikingly different (Clutton-Brock et al., 1982). There is even evidence of a negative genetic correlation for fitness between males and females - genes that make a good male do not necessarily perform well when expressed in a female (Foerster et al., 2007) - although it is not known whether this result extends to traits other than fitness. Due in part to these differences between males and females, the sequence of events to produce one offspring takes over 12 months, represented schematically in figure 6.1. Males cast their antlers in the spring, followed by the immediate growth of a new set (Li et al., 2004). When antler growth has ceased in late summer the rich vascular tissue surrounding the growing antler dies and is cleaned from the hard antler (Goss, 1983). Females come into oestrus from mid September and during this time males use their antlers in fights with other males to hold harems of females and gain matings with oestrous females, termed rutting. Male reproductive success is dependent on males both holding harems at the optimum time when many females are in oestrus, and male ability to fight and defend those harems from other males (Mysterud et al., 2008). From early October all individuals change into winter coat, which provides additional insulation (Ryder, 1976); summer coat is regrown in spring. Gestation of the offspring by the female occurs over winter and birth of the offspring is between May and July of the following year (Guinness et al., 1978b). During the period of offspring birth, males grow the next set of antlers and feed to regain condition for the upcoming rutting season (Lincoln, 1992). Females suckle their calves throughout the summer. If a female is to conceive again in the next rutting season, lactation ceases before oestrus;

otherwise a female may continue to suckle her calf throughout the following winter (Clutton-Brock et al., 1982).

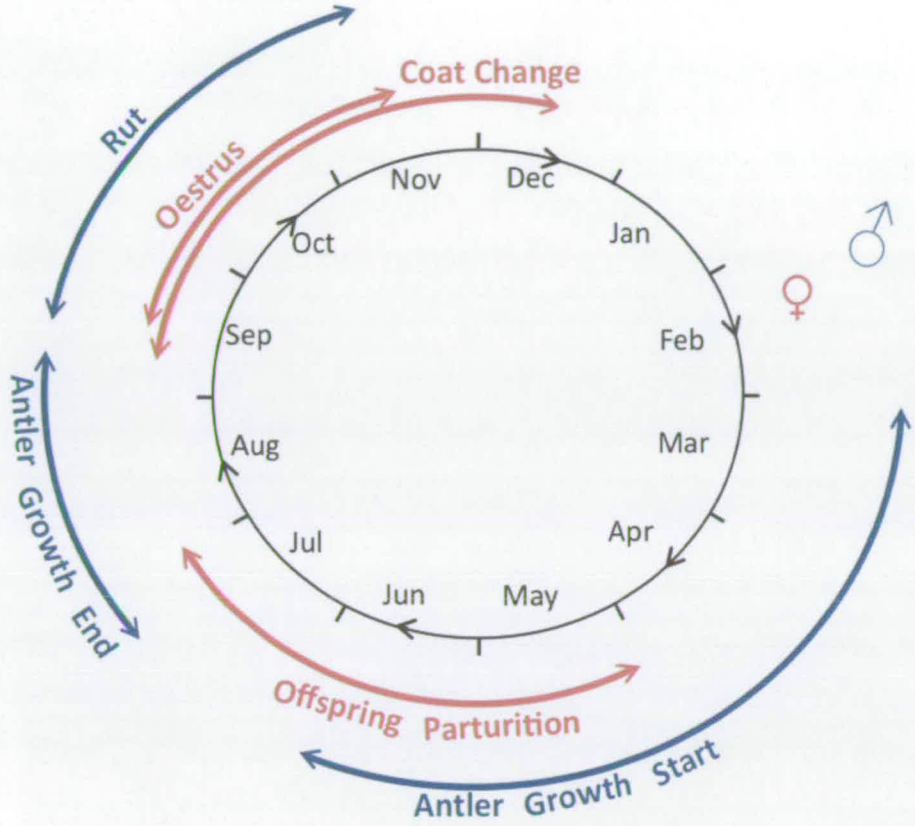


Figure 6.1. Annual cycle of deer phenology: the arrows show the time period during which each event may occur. The inner circle (coloured red) shows traits recorded in females: oestrus date in autumn leading to parturition of the offspring in the following spring, and coat change from summer to winter coat in autumn. The outer circle (coloured blue) shows the cycle of antler growth determined by the cast date of the previous antler signaling start of growth and the cleaning of the vascular tissue surrounding the growing antler as indicating end of growth. The timing of male rutting is also shown: we analysed an individual's start, median and end date of rutting.

The study population of red deer on the Isle of Rum is an ideal system in which to study genetic correlations between phenological traits in a wild population. Individuals are followed throughout their lives, resulting in the longitudinal data necessary to separate variation into between and within individual sources (Clutton-

Brock et al., 1982). A pedigree has been constructed for the population (Walling et al., 2010), which allows us to separate additive genetic and environmental effects, and to calculate genetic correlations between traits. Phenological traits have been advancing in this system since 1980, believed to be as a direct result of an extended growing season due to a changing climate (Moyes et al., in review; appendix V), although the rate of advancement has differed between traits. In males, antler growth start and end dates, rut start date and antler weight are highly correlated within individuals in a given year (Clements et al., in press, Moyes et al., in review; chapter 2 and appendix V respectively), suggesting the same underlying hormonal mechanism, and thus that these traits may potentially show significant levels of genetic covariance. Although selection on phenological traits in this system seems to be unidirectional across traits, with early expression associated with high fitness, the magnitude of selective pressure differs between traits. Selection for early parturition by females acts through both offspring survival (Coulson et al., 2003) and the probability of the female reproducing in the following breeding season (Clutton-Brock et al., 1983). In males, individuals with the biggest antlers both start and end antler growth and rutting the earliest, and have the highest breeding success (Clements et al., in press, Kruuk et al., 2002, Moyes et al., in review). However after correcting for antler mass, only the start date of antler growth is independently under selection (Clements et al., in press, Moyes et al., in review).

In this paper we use data collected from the Rum red deer study population to investigate patterns of phenotypic and genetic variance and covariance in eight phenological traits. First, we ask how much of the observed phenotypic variance in the different traits is determined by differences in age and reproductive status, by variance between years due to local ecological conditions affecting all individuals, and by

consistent differences between individuals due to permanent environment and heritable genetic effects. Second, we calculate phenotypic correlations between variables expressed in the same sex. Finally, we investigate genetic and within-year correlations expressed both within and between the sexes.

Methods

Study system

The wild population of red deer in the North Block of the Isle of Rum, Scotland (57°0'N, 6°20'W) has been studied continuously since the cessation of culling in the study area in 1972, although more intermittent data collection began in 1968 (Clutton-Brock et al., 1982). The deer are individually recognisable through natural and artificial markings and are followed throughout their lives. Intensive monitoring during the birth (calving) season enables the majority of offspring to be caught during the first few days of life to determine sex and weight, to apply artificial markings and to sample for genotyping.

Phenological variables

Dates are measured as the number of days since 1st March of each calendar year, with the exception of parturition date, which is measured from 1st March in the previous calendar year, so that it matches the relevant oestrus date.

Female traits

Oestrus date: Intensive censusing of the rutting season occurred between 15th September and 15th November of each year. We recorded the location of each female and, if she was in a harem, the harem male. Females were defined as being in oestrus if they were seen to be mounted or continuously followed for extended periods of time by a male (Guinness et al., 1971). Multiple oestrouses per season were recorded for less

than 10% of all females observed in oestrus. In the majority of these cases, oestrus dates differed by just a few days, and less than 4% of all females had oestrouses that differed by more than 10 days. As failure to conceive and repeated cycling may in part be due to males in the population, we took the first observed oestrus so that traits were observed independently across the sexes. Oestrus dates were available for 839 observations across 37 years, from 1971 to 2009, excluding 1973 and 2000 as no data were available for these years.

Coat Change Date: The date a female was judged to be in 90% of winter coat, determined during censuses until the 8th December of each year. Although males also change into winter coat, due to their movements during the rutting period we have much less detailed information on male coat change date and so here we only analysed females. Summer coat is regrown in spring but as this variable is observed less frequently than winter coat change, it was not included in results presented here. Data were collected only in the later period of the study and consequently here we analysed 1,619 observations over 23 years from 1987 to 2009 inclusive.

Parturition date: The date of offspring birth, determined through daily censuses between May and July of each year. So that extremely late births did not bias results, we included only births in May, June and July of each year, which represent 97.3% of all known parturition dates. We analysed 2,858 parturition dates collected over 42 years, from 1968 to 2009 inclusive.

Male traits

Antler growth start and end dates: The exact date of antler casting and cleaning. Cast dates were collected over 42 years from 1968 to 2009 (1,536 observations) and clean dates (908 observations) were available for 33 years from 1971 to 2008 excluding 1973, 1976, 1977, 2002 and 2007, as no cleaning dates were recorded in these years.

Rut start/median/end dates: During the rut censuses, any male holders of harems are recorded daily. As rutting timing is dependent on both the distribution of female oestrus date and intensity of competition between males, we analysed the start, median and end rutting dates to determine how patterns of variance differed between them. As with Moyes et al (in review), we included only males that held harems for at least 5 days in a given year. The first, last and median day of rutting was then determined and was available for 2,002 observations from 38 years from 1971 to 2009 excluding 1973.

Other phenotypic variables

To correct for variation due to factors intrinsic to each individual, and which have previously been found to correlate with phenological variables in this system (Nussey et al., 2009, Coulson et al., 2003); appendix III), we included the following variables in all analyses:

Age: The age of the individual in years. As patterns of age-related variation are known to differ between phenological traits in this population (Nussey et al., 2009; appendix III) we fitted age in years as a multi-level factor. To ensure that at least 10 individuals were present for each variable in each age class (Bolker et al., 2009), we restricted data to males aged 3 to 14 and females aged 2 to 15 inclusive.

Female reproductive status: Females were classified according to recent reproductive history. A female was classed as "naïve" if she had never bred before; "true yeld" if she had bred before but not in the previous reproductive season; "summer yeld" if she had bred in the previous reproductive season but the offspring had died before 15th September in the year of birth; "winter yeld" if she had bred in the previous reproductive season and the offspring had died between 15th September in the year of birth and 15th May in the following year; and "milk " if she had bred in the previous reproductive season and the offspring was still alive on 15th May of the following year.

Pedigree

We used pedigree information to determine the genetic architecture of traits. The methodology of pedigree determination has been described in full elsewhere (Walling et al., 2010), consequently we include only a brief description here. An individual's mother is known with certainty through close observation of the study system, whereas the father is determined by genetic analyses based on information on up to 15 microsatellite markers. The majority of paternal links in the pedigree were determined using the paternity inference program MasterBayes (Hadfield et al., 2006) that incorporates genetic and behavioural information to assign the most likely sire; we accepted paternities that were assigned with individual confidence levels of 80% or above. When MasterBayes did not assign a sire we used the program COLONY2 (Wang and Santure, 2009) to add additional links to the pedigree. COLONY2 incorporates genetic information from full and half siblings to increase the power of genetic analyses. We accepted any links found in COLONY2 but not in MasterBayes that were assigned with an individual confidence of at least 80%. Additionally COLONY2 can group paternal half-sibs even when the sire has not been genotyped. If an ungenotyped male was observed holding the female in his harem in at least one of the 11 days surrounding probable oestrus date (determined by backdating 235 days from offspring birth date, (Clutton-Brock et al., 1997)), then he was considered as a candidate sire. If the same ungenotyped male was a candidate sire for at least 50% of the offspring grouped as paternal half sibs, then the male was assigned as the sire for all offspring in the half-sib grouping. If the sire could not be identified, the sire was coded as a dummy variable to preserve the half-sib grouping within the pedigree.

Statistical analyses

Univariate analyses

We investigated sources of variance in each variable using a univariate animal model (Kruuk, 2004, Wilson et al., 2010; appendix IV). Age, and female reproductive status where appropriate, were included as fixed effects in all models. Random effects in all models were additive genetic effects (V_A) incorporating pedigree information, individual identity to account for repeated measures across individuals (permanent environment effects, or V_{PE}), year (V_{YR}) and unexplained residual effects (V_R). The phenotypic variance was therefore partitioned as below:

$$V_P = V_A + V_{PE} + V_{YR} + V_R \quad (1)$$

Significance of random effects was determined using log-likelihood ratio tests to compare models with and without the appropriate random effect (Pinheiro and Bates, 2000). Environmental effects such as density and weather are known to affect phenological traits in this system (Clutton-Brock et al., 1987, Moyes et al., in review). However, as we were primarily interested in how effects covary, we elected not to model these variables explicitly but to allow their effects to fall into a general measure of between-year variance. We then calculated heritability as $h^2=V_A / V_P$ where V_P was the total variance remaining after correction for fixed effects (i.e. the sum of all variance components from the random effect structure). Heritability was taken to be significant if the associated additive genetic variance component was also significant. Repeatability was estimated as $(V_A + V_{PE})/ V_P$ and measures the proportion of variance

attributable to consistent differences between individuals, whether genetically or environmentally induced.

We calculated the percentage of total phenotypic variance attributable to fixed effects of age and female reproductive status using $(V_T - V_P)/ V_T$ where V_T is the total phenotypic variance calculated from the raw data.

Although we controlled for repeated measures across individuals and years, other sources of variance may cause estimates of variance components to be artificially inflated or deflated (Kruuk and Hadfield, 2007). A primary example of such sources of variance are maternal effects (Rasanen and Kruuk, 2007), caused largely by differences between mothers in provisioning to offspring. However, maternal identity was not significant in any univariate model of phenology traits. Additionally, as the inclusion of maternal identity in a model caused a reduction in sample sizes, and the parameter estimate was often so small as to be inestimable, we did not include maternal identity in any models presented here. Recent studies have found male effects on female lay date in birds (Brommer and Rattiste, 2008, Teplitsky et al., 2010) and in this system there are differences between males in the gestation lengths of females they mate with (Chapter 3). Consequently we attempted to fit sire identity in models of oestrus and parturition dates.

Overall phenotypic correlations

Phenotypic correlations were calculated in traits expressed by the same individual, i.e. calculation of cross-sex phenotypic correlations were not possible. As phenological traits were recorded repeatedly across an individual's lifetime, we approached the calculation of phenotypic correlations in two ways. We first

determined the overall phenotypic correlations between traits expressed by the same individual in a given year by calculating the Pearson correlation coefficient between all observations and testing if it was significantly different from zero. However, the overall phenotypic correlation did not correct for variation due to age, female reproductive status or between-year variation and thus was not directly comparable to estimates of genetic correlations. Consequently, we also tested for individual-level phenotypic correlations using bivariate models.

Individual-level phenotypic, genetic and within-year correlations

We next used bivariate models to determine the covariances between traits. Briefly, bivariate models incorporate two response variables, which are linked through a covariance term in the random effects structure to determine the covariance between the traits at different levels of individual or year, and ultimately genotype. For a more detailed introduction to bivariate animal models see (Wilson et al., 2010; appendix IV). Significance of all covariance terms was tested by performing log-likelihood ratio tests to compare the full model with one where the appropriate covariance term was set to zero. Associated correlations were found by dividing the covariance component by the square root of the product of the variance components, and were taken to be significant if the associated covariance component was significant.

Firstly we determined the individual-level phenotypic correlations between phenology traits expressed in the same sex. Random effects in this model were individual, year and a residual error term, and included all associated covariance terms. Fixed effects were age and female reproductive status. Significance of individual-level phenotypic covariance would indicate that individuals that were on average early (or

late) at expressing one trait also expressed the other trait early (or late) on average, after correction for fixed effects.

We then incorporated pedigree information to determine levels of additive genetic covariance between traits. As this analysis was at the level of the genotype, both within and between sex comparisons were possible. In models of traits expressed in the same sex, random effects were additive genetic variance, individual (or permanent environment effect), year and residual error, and all associated covariances. Where the traits were expressed in different sexes we could not model the covariance term at the level of the individual or residual. Significance of the genetic covariance parameter indicated that genetic variation associated with early (or late) expression of one trait was also associated with early (or late) expression of the other trait.

From the bivariate models incorporating genetic information we also extracted between-year covariances. Significance of between-year terms indicated that in years where the average timing of expression of one trait was early (or late), the average timing of expression of the other trait was also early (or late), after correction for effects of age and female reproductive status.

The inclusion of sire effects in bivariate models including oestrus and conception dates caused both a substantial reduction in sample sizes and convergence problems; consequently we did not include sire identity as a random effect in bivariate models presented here.

All analyses were performed in ASReml v3 (Gilmour et al., 2009).

Results

Univariate analyses

The percentage of total phenotypic variance attributable to the intrinsic factors of age and female reproductive status was not constant between phenological variables (Table 6.1). Age accounted for 77% of the phenotypic variance observed in antler growth start date but age and reproductive status accounted for only 6% of the variance observed in female parturition date (Table 6.1). Phenological traits became earlier with increasing age until prime age but the patterns of age-related variation differed between phenological traits (Figure 6.2).

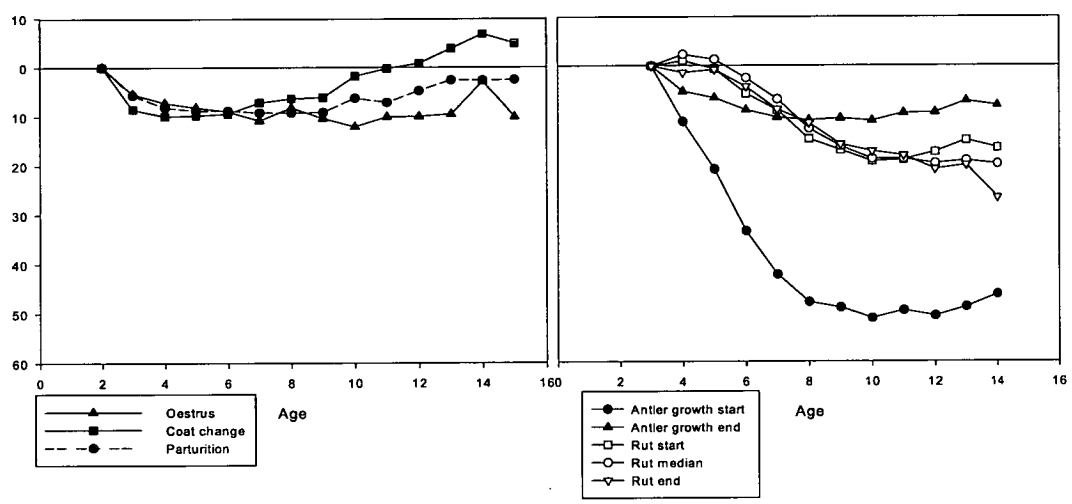


Figure 6.2. The timing of expression of phenological traits became earlier with increasing age, followed by senescence, but patterns of age-related variation differed between traits. Females (left) and males (right). The graph shows the predicted difference in timing of expression of each trait in days relative to the predicted value at age two in females and three in males from univariate models of each phenological trait.

We detected significant levels of additive genetic variance - and consequently also significant heritability - in five of the eight phenological traits studied (Table 6.1): coat change and parturition dates in females and rut start, median and end dates in males (Table 6.1). We found no significant additive genetic variance in oestrus date in females and antler growth start and end dates in males (Table 6.1), although the p-value of the additive genetic component of antler growth start date was 0.067 (Table 6.1). There was a strong and positive correlation between estimates of additive genetic variance and residual variance (ρ=0.74; p=0.035; Figure 6.3). The conclusions did not qualitatively change when we corrected for multiple testing of eight significance tests using sequential Bonferroni methodology (Rice, 1989).

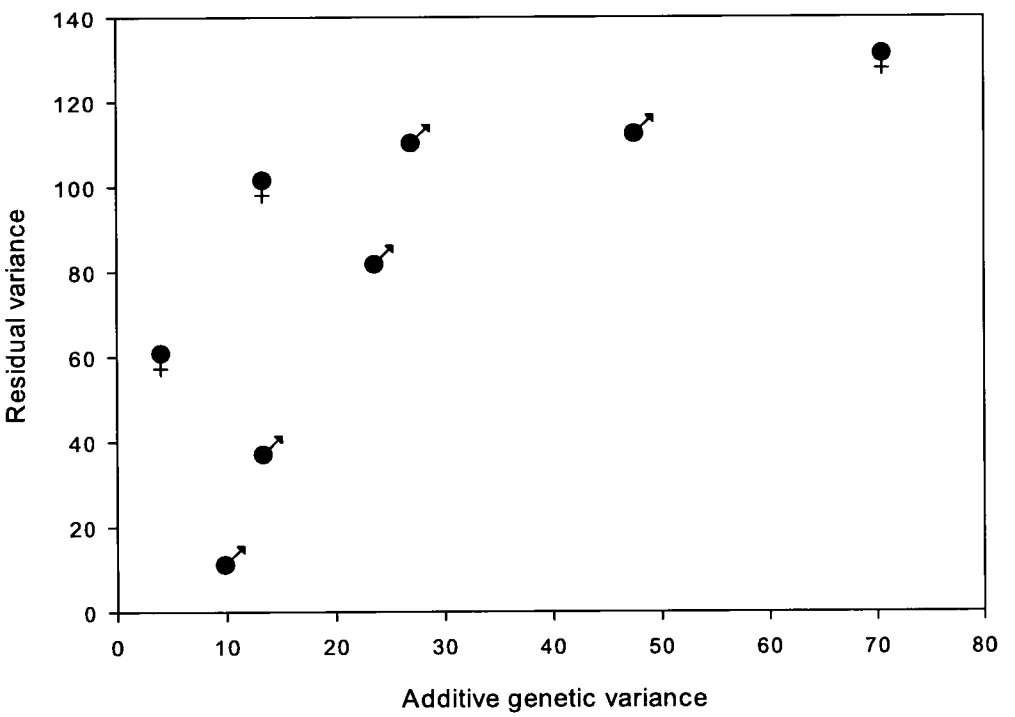


Figure 6.3. Residual variance estimates were positively correlated with estimates of additive genetic variance in males and females (ρ=0.74; p=0.035). Traits expressed by males or females are indicated by the symbols.

Estimates of heritability ranged from 0.05 (±0.04SE) for oestrus date to 0.26 (±0.06SE) for coat change and rut start dates (Table 6.1, Figure 6.4). Heritability was highly positively correlated with additive genetic variance (ρ=0.90; $p<0.001$).

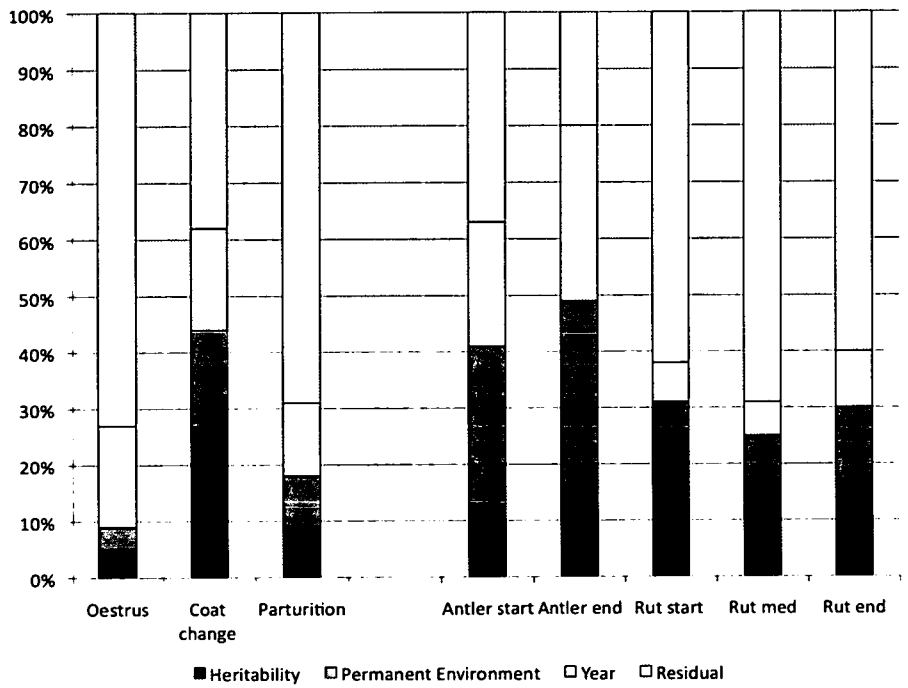


Figure 6.4. Patterns of variance differed between phenological traits. The graph shows the partitioning of variance after correction for effects of age and reproductive status into constituent parts of heritability; permanent environment effects; year and residual deviance

In models of oestrus and parturition dates there was a significant random effect of sire identity, although inclusion of this factor caused a dramatic reduction in sample sizes (down to 355 observations of oestrus date and 1,724 of parturition date). Inclusion of sire effects caused instability in the models' ability to discriminate female additive genetic and permanent environment effects, and consequently we fitted only female identity in these models. The sire effect was significant in both traits, accounting for 12% of the remaining variance after correction for fixed effects in oestrus date and 30% in parturition date. However, there was no evidence of heritable genetic variance

underpinning differences between sires (oestrus date: estimate=1.59±8.15 SE, h^2=2.5%, p=0.89; parturition date: parameter inestimable), suggesting that the sire effects are presumably driven by local environmental effects as males rut in very localized areas (see discussion; (Clutton-Brock et al., 1982).

Overall phenotypic correlations

There were broadly strong and significant overall phenotypic correlations between phenological traits within an individual in any given year (Figure 6.5). All covariance estimates were positive (Figure 6.5), suggesting that individuals that express a trait early in a given year also express other phenological traits early in that year. Within females there was no significant correlation between coat change date and oestrus date (ρ=0.047; n=254; p=0.455; Figure 6.5).

Individual-level phenotypic correlations

We also examined phenotypic correlations using a bivariate model correcting for effects of age, female reproductive status and within-year effects to ask if individuals that on average express a trait early also express other traits early on average. As with the overall phenotypic correlation, correlations were broadly strong and positive (Table 6.2). However, the correlations between coat change and both oestrus and parturition dates were non-significant, as were correlations between rut start date and both antler growth start and end dates (Table 6.2; Figure 6.5).

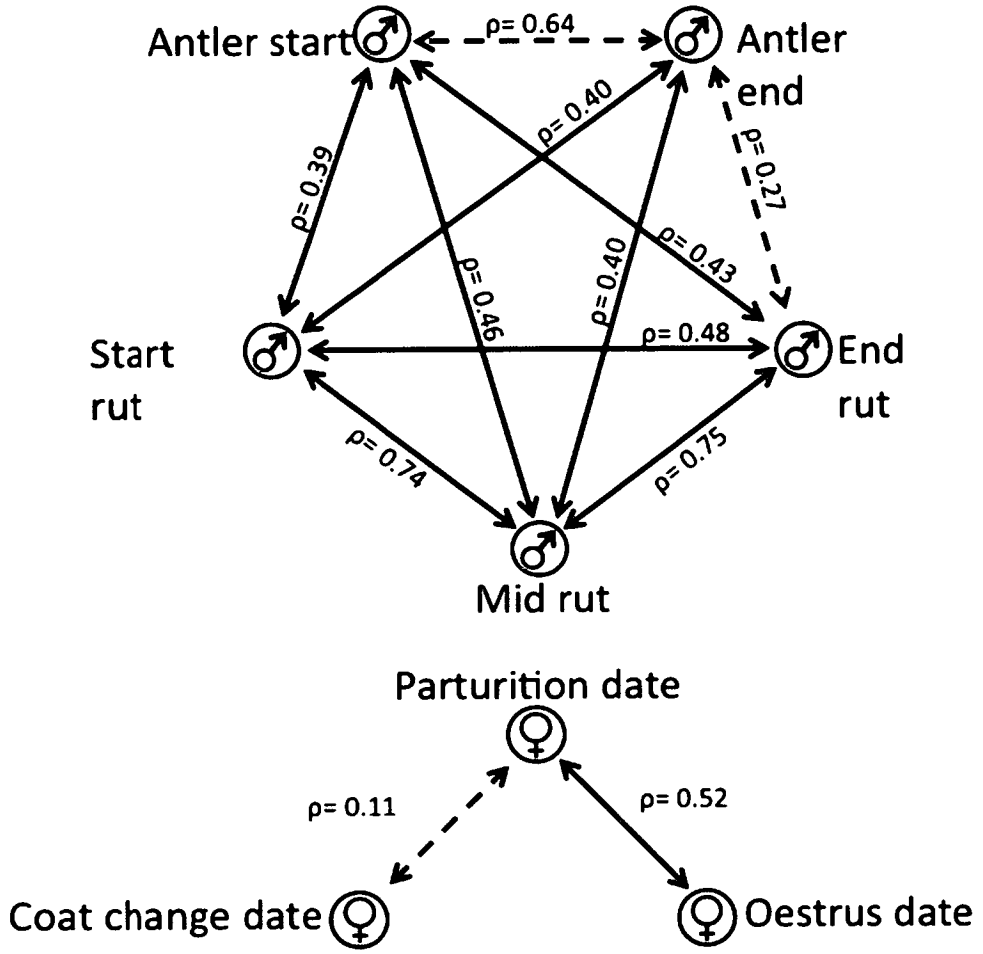


Figure 6.5. Phenotypic correlations between phenological variables were broadly strong and positive. Solid lines link traits that were significantly correlated both before (overall correlation) and after (individual-level correlation; Table 2) correction for effects of age and female reproductive status. Dashed lines link traits where the overall correlation was significant but the individual-level correlation was not. Diagram shows estimates of all significant overall phenotypic correlations. There was no significant overall phenotypic correlation between coat change date and oestrus date (ρ=0.047; n=254; p=0.455).

Genetic correlations

We detected fewer significant genetic correlations between phenological traits (Table 6.3; Figure 6.6). There was a significant genetic correlation between oestrus date and both parturition date and rut start date (Table 6.3; Figure 6.6). However,

caution is advised when interpreting these results as there was no significant additive genetic variance in the univariate model of oestrus date (Table 6.1). Rut median date was significantly genetically correlated with rut start date and rut end date (Table 6.3; Figure 6.6), but there was no significant genetic correlation between rut start and rut end dates. We detected significant genetic correlations only in variables with the highest individual-level phenotypic correlations (Figure 6.7), but the correlation between estimates of phenotypic and genotypic correlations was not significant (ρ=0.50; p=0.083; Figure 6.7). Results did not qualitatively change when we corrected for the 28 tests using sequential Bonferroni testing.

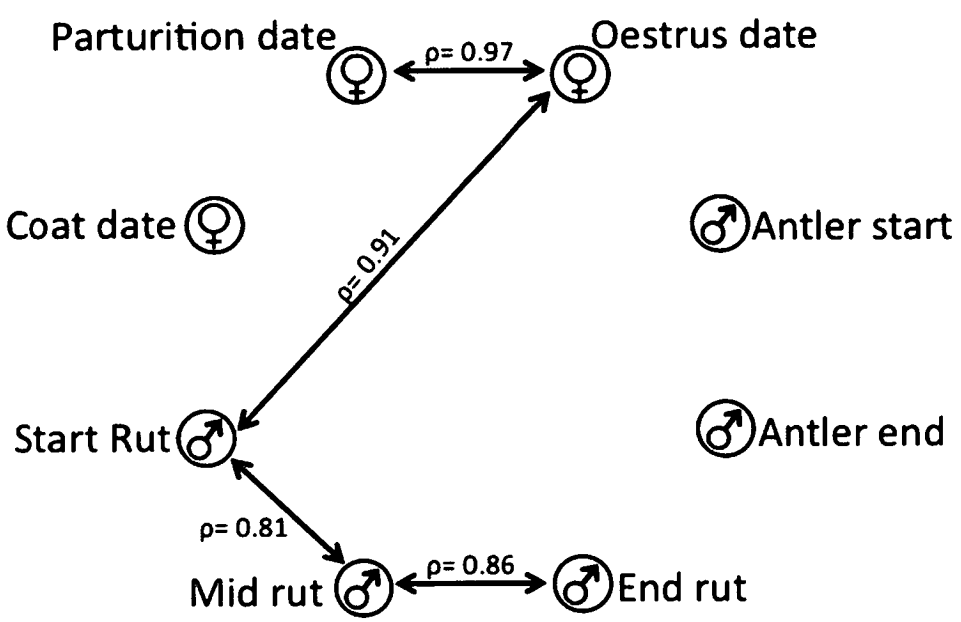


Figure 6.6. There was little evidence for the presence of strong genetic correlations between phenological traits. Black lines denote significant genetic correlations of phenological traits obtained from Table 3.

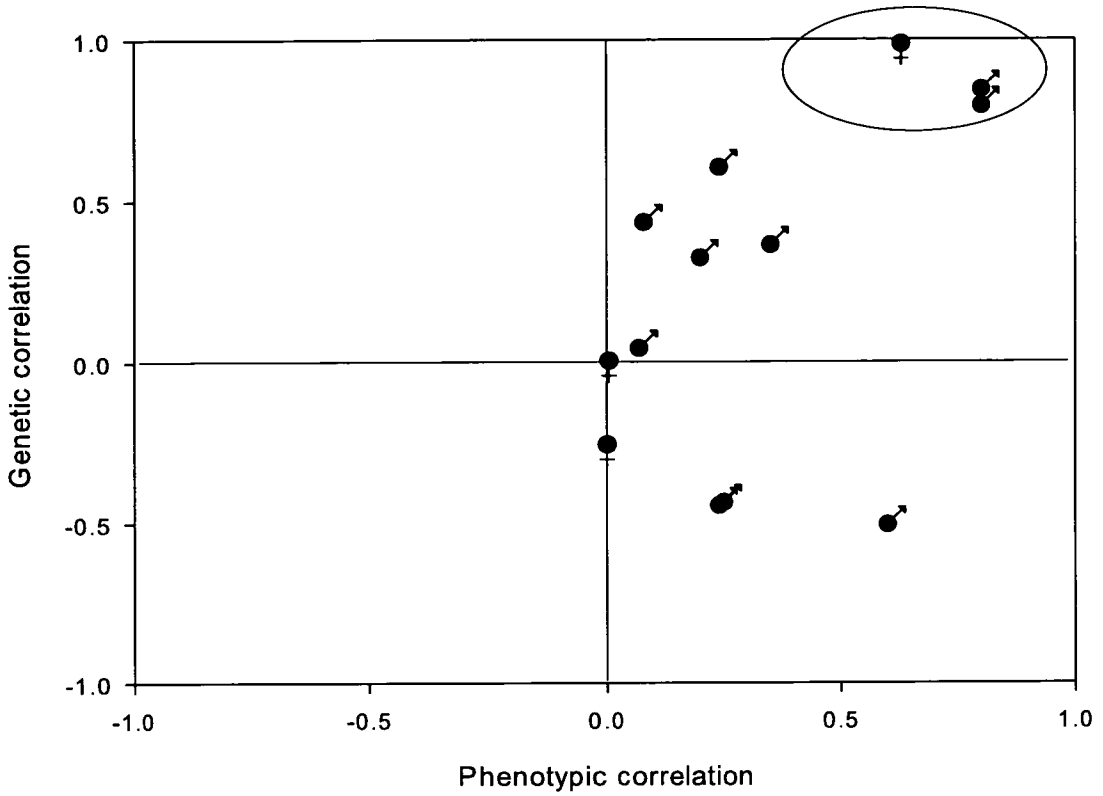


Figure 6.7. Within-sex individual-level phenotypic correlations were uncorrelated with genotypic correlations (ρ=0.40; p=0.171). Symbols denote males and females. We detected significant genetic correlations only where there were also large phenotypic correlations. The ellipse contains the three significant genetic correlations from table 2.

Within-year correlations

Within-year correlations were always positive and mostly significant (Table 4; Figure 6.8), suggesting that environmental conditions affect traits in similar ways. The clear exception to this was coat change date, for which there were no significant within-year correlations with oestrus, parturition, rut end, or rut median dates (Table 6.4; Figure 6.8). When we corrected for the 28 significance tests using sequential Bonferroni testing, three within-year covariances became non-significant: coat change

date and antler growth end dates; antler growth end date and rut end date; and coat change date and rut start date.

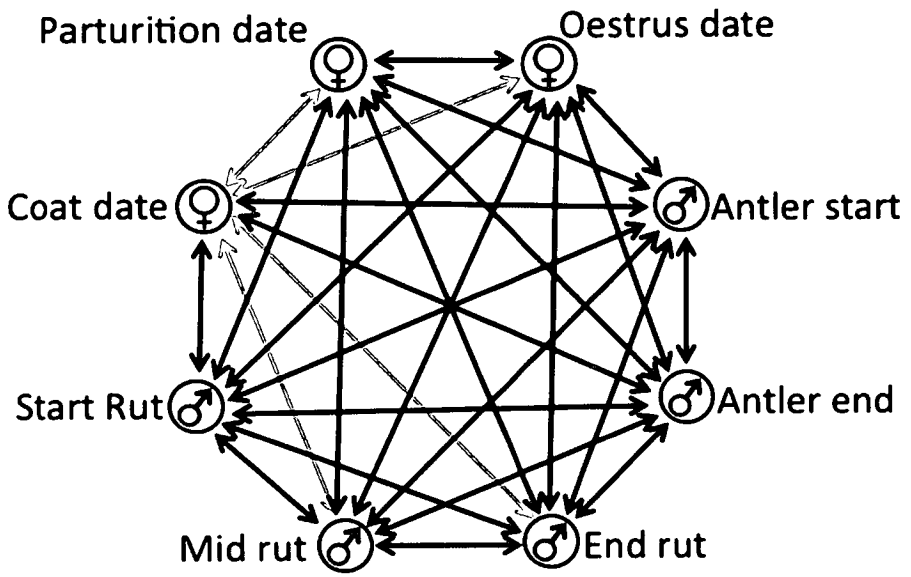


Figure 6.8. Within year correlations between phenological traits were broadly strong and positive. The black lines denote significant correlations and the grey non-significant correlations.

Permanent environment and residual correlations

The genetic and within-year correlations were taken from the same bivariate model for each pair of traits. Where the trait was expressed in the same individuals (i.e. same sex), we also calculated covariances at the level of individual (permanent environment covariances) and residual. Permanent environment correlations were broadly positive, indicating that consistent differences between individuals over and above genetic effects had similar effects across all traits. However, the only significant correlations were between antler growth start date and antler growth end, rut start and rut median dates. For completeness, these covariances are included in appendix I.

Table 6.1. Sample sizes, summary statistics and outputs from univariate animal models correcting for age and female reproductive status. Obs. = number of observations; indiv = number of individuals; yrs = number of years. Additive genetic, permanent environment, year and residual: Variance components from univariate model reported ±1SE. Significance was tested using log-likelihood ratio tests. * indicates p<0.05; ** p<0.01; *** p<0.001. "Age & status" is the proportion of total variance attributable to intrinsic factors. All other summary statistics are reported relative to variance after correcting for fixed effects. Repeat is the proportion of variance attributable to individuals ($V_A + V_{PE}$)/ V_P; h^2, and year show the percentage of variation attributable to additive genetic effects (i.e. heritability, reported ±1SE) and between-year variance respectively. P-value denotes the significance of the additive genetic (and consequently the heritability) term.

	Sample sizes			Summary statistics		Variance components				Proportion of variance				
Variable	obs	indiv	yrs	mean	var	Additive genetic	Permanent Environ	Year	Residual	Age & status	Repeat	h^2	p-value	year
Oestrus	811	423	36	15th Oct	92	4.0 (3.8)	3.0 (4.6)	14.6 (4.4)***	59.6 (4.2)	12%	9%	5% (4%)	0.282	18%
Coat change	1619	474	23	20th Oct	339	70.5 (17.6)***	40.9 (13.6)***	45.5 (14.8)***	130.1 (5.5)	15%	44%	26% (6%)	<0.001	18%
Parturition	2858	634	42	7th Jun	154	13.3 (4.3)***	12.7 (4.0)***	18.6 (4.7)***	100.3 (3.0)	6%	18%	9% (3%)	<0.001	13%
Antler start	1539	417	42	20th Apr	445	13.8 (8.2)	29.4 (8.2)***	21.3 (5.4)***	38.2 (1.6)	77%	41%	13% (8%)	0.067	22%
Antler end	908	333	33	17th Aug	79	10.31(6.3)	21.4 (6.0)***	22.9 (6.5)***	12.3 (0.8)	15%	49%	15% (9%)	0.108	31%
Rut start	1930	573	37	7th Oct	231	48.0 (11.3)***	9.1 (8.8)	13.1 (4.1)***	113.6 (4.4)	20%	31%	26% (6%)	<0.001	7%
Rut med	1930	573	37	20th Oct	228	27.4 (8.7)***	12.5 (7.4)	9.8 (3.1)***	111.4 (4.2)	29%	25%	17% (5%)	<0.001	6%
Rut end	1930	573	37	31st Oct	190	24.0 (8.1)***	17.3 (7.1)**	14.0 (4.0)***	82.9 (3.2)	27%	30%	17% (6%)	<0.001	10%

Table 6.2. Within-individual level phenotypic correlations were broadly strong and positive in both (a) female and (b) males. Output from a bivariate model correcting for effects of age, female reproductive status and between-year environmental effects. Covariances are shown below the diagonal with the associated correlation above the diagonal, both are shown with associated standard error. Significance of covariances was tested using log-likelihood ratio tests and correlations were considered significant if the appropriate covariance component was also significant. * indicates p<0.05; ** p<0.01; *** p<0.001

a)	Oestrus	Coat change	Parturition
Oestrus		0.00 (1.23)	0.63 (0.16)***
Coat change	0.004 (4.941)		0.005 (0.05)
Parturition	9.15 (2.19)***	0.27 (4.60)	

b)	Antler start	Antler end	Rut start	Rut median	Rut end
Antler start		0.61 (0.06)***	0.25 (0.08)**	0.26 (0.09)**	0.08 (0.12)
Antler end	21.09 (3.43)***		0.21 (0.09)*	0.25 (0.09)**	0.09 (0.11)
Rut start	12.38 (4.49)**	8.91 (3.78)*		0.81 (0.10)***	0.36 (0.08)***
Rut median	10.99 (4.04)**	9.06 (3.35)**	38.43 (4.81)***		0.81 (0.10)***
Rut end	3.47 (4.06)	3.10 (3.24)	17.55 (3.96)***	33.10 (4.12)***	

Table 6.3. Genetic covariances and correlations from bivariate animal models. Covariances are shown below the diagonal with the associated correlation above the diagonal, both are shown with associated standard error. Significance of covariances was tested with log-likelihood ratio tests and correlations were considered significant if the appropriate covariance component was also significant. * indicates p<0.05; ** p<0.01; *** p<0.001

	Oestrus	Coat change	Parturition	Antler start	Antler end	Rut start	Rut median	Rut end
Oestrus		-0.27 (0.28)	0.97 (0.33)**	0.07 (0.34)	0.2 (0.50)	0.91 (0.36)	0.72 (0.39)	0.09 (0.37)
Coat change	-5.87 (6.22)		-0.01 (0.12)	0.28 (0.33)	-0.32 (0.33)	-0.05 (0.21)	-0.07 (0.20)	-0.04 (0.17)
Parturition	8.31 (2.90) **	-0.44 (6.59)		0.53 (0.32)	0.33 (0.32)	0.01 (2.58)	-0.07 (0.25)	-0.2 (0.24)
Antler start	0.68 (4.27)	8.89 (10.25)	7.16 (4.30)		-0.49 (0.76)	-0.43 (0.36)	-0.42 (0.38)	0.06 (0.45)
Antler end	1.63 (3.75)	-8.43 (8.34)	3.94 (3.74)	-3.48 (5.18)		0.34 (1.02)	0.62 (0.36)	0.45 (0.38)
Rut start	11.98 (4.81)*	-3.19 (11.45)	0.15 (5.38)	-8.34 (7.29)	2.28 (6.71)		0.81 (0.24)***	0.38 (0.22)
Rut median	7.67 (4.15)	-3.09 (9.72)	-1.29 (4.54)	-6.60 (6.19)	9.42 (5.49)	29.61 (8.88)***		0.86 (0.28)***
Rut end	0.89 (4.21)	-1.78 (9.37)	-3.57 (4.37)	1.14 (6.33)	6.43 (5.31)	12.87 (7.47)	23.17 (7.64)***	

Table 6.4. Within year covariances and correlations between phenological traits from bivariate animal models. Covariances are shown below the diagonal with the associated correlation above the diagonal, both are shown with associated standard error. Significance of covariances were tested with log-likelihood ratio tests and correlations were considered significant if the appropriate covariance component was also significant. . * indicates p<0.05; ** p<0.01; *** p<0.001

	Oestrus	Coat change	Parturition date	Antler start	Antler end	Rut start	Rut median	Rut end
Oestrus		0.45 (0.31)	0.84 (0.20)***	0.79 (0.18)***	0.67 (0.31)***	0.83 (0.24)***	0.85 (0.27)***	0.82 (0.21)***
Coat change	11.64 (6.80)		0.52 (0.31)	0.78 (0.28)**	0.68 (0.21)**	0.55 (0.19)*	0.46 (0.44)	0.34 (0.56)
Parturition	13.79 (3.91)***	16.10 (7.96)		0.62 (0.23)***	0.65 (0.25)***	0.72 (0.27)***	0.72 (0.23)***	0.64 (0.27)***
Antler start	14.29 (4.21)***	27.92 (8.99)**	12.47 (4.09)***		0.83 (0.19)***	0.88 (0.31)***	0.88 (0.28)***	0.69 (0.32)***
Antler end	11.36 (3.90)***	22.64 (8.59)**	12.86 (4.25)***	19.11 (4.93)***		0.85 (0.25)***	0.78 (0.24)***	0.49 (0.24)*
Rut start	12.53 (3.78)***	14.28 (6.91)*	11.66 (3.74)***	17.68 (4.75)***	14.74 (4.17)***		0.96 (0.32)***	0.87 (0.27)***
Rut median	11.38 (3.42)***	10.20 (5.55)	10.21 (3.31)***	15.34 (4.19)***	11.96 (3.66)***	10.40 (3.25)***		0.94 (0.24)***
Rut end	12.23 (3.68)***	8.64 (6.17)	10.56 (3.58)***	12.01 (3.89)***	8.21 (3.63)*	10.87 (3.30)***	10.70 (3.21)***	

Discussion

The aim of this study was to characterise the genetic architecture influencing variance in a number of phenological traits, and covariances between them at the level of the phenotype, genotype and year. Most traits showed significant levels of heritability and significant phenotypic and year correlations, but we did not find strong evidence for the presence of genetic correlations between traits. We discuss below possible reasons and implications for these results.

We first investigated patterns of variance using univariate animal models to partition phenotypic variance into its constituent parts of additive genetic, permanent environment, year and unexplained residual variation. Theory predicts depletion of additive genetic variance in traits closely related to fitness, as directional selection on fitness itself will drive beneficial alleles to fixation (Fisher, 1958): traits under stronger selection should therefore have lower levels of V_A than those under weaker selection. When traits have been measured on different scales, comparison of their relative levels of V_A requires use of a standardized measure such as the heritability or the coefficient of additive genetic variance (Houle, 1992; for examples, see Merila and Sheldon, 1999, Kruuk et al., 2000). However both measures may generate artefactual associations with selection independent of any differences in V_A (see discussion in Kruuk et al., 2000). Here, all traits were measured on the same scale (in days) and so direct comparison of variance components is possible. The highest estimate of additive genetic variance in our study was in coat change date, whereas the lowest was found in oestrus date. Parturition date is known to be under directional selection in this system (Coulson et al., 2003) and, as gestation length is thought to be relatively constant (Guinness et al., 1978a, chapter 3), it is likely that oestrus date is also under similar selection. We know

little about the selection pressures acting on coat change date, but as it is the only phenological trait we studied that is not related to timing of breeding or parturition, or growth of a secondary sexual characteristic, it is likely that coat change date is under the weakest selection of all the phenological variables presented here; these results may therefore provide support within a group of phenological traits for the suggestion that traits under stronger selection may have relatively less genetic variance.

In this study, we found no evidence of significant heritability in antler growth start and end dates, or in oestrus date. Drawing robust conclusions from non-significant results is problematic as the result could be simply due to a lack of statistical power rather than reflecting true biological non-existence (Lynch, 1999); in analyses of variance and covariance, it may be unlikely that the true estimate is exactly zero, but proving that estimates are significantly different from zero may be more difficult in analyses of data from natural populations (Kruuk and Hill, 2008). Both the sample sizes and parameter estimates of additive genetic variance were relatively low in the phenological variables where we did not find significant heritability, suggesting that even if larger sample sizes were to produce significant effects the magnitude would remain small. Indeed the parameter estimate of additive genetic variance in antler growth start date was similar to that of parturition date, but the parturition date model incorporated almost twice the number of observations than the model of antler growth start date and hence had a correspondingly smaller standard error.

We found significant effects of sire identity, indicating consistent differences between males in the oestrus and parturition dates of the females with which they mate. However, there was no evidence of heritable genetic variance underlying these effects and, as inclusion of sire identity caused substantial difficulties in model fit, we

did not include sire effects in bivariate analyses. Heritable genetic variation between males in the lay date of females they mate with has been detected in wild bird systems (Brommer and Rattiste, 2008, Teplitsky et al., 2010), and has been attributed to between male differences in levels of provisioning to the female. However, in a polygynous, harem-based system such as the red deer, provisioning cannot be responsible for such differences between males. We believe that the sire effects most likely represent differences between the environments experienced by females mating with different males, as males rut in very localized areas (Clutton-Brock et al., 1982). Sire identity will therefore group together females living in a particular area and experiencing the same local environmental conditions. In addition, in red deer, there is a strong social component to oestrus synchrony (Iason and Guinness, 1985) and the presence and roaring of males is known to bring females into oestrus (Shelton, 1960, McComb, 1987). Although significant male effects on traits expressed by females is an exciting area of future research, our results also demonstrate the potentially substantial contributions of common environment effects in wild populations (Kruuk and Hadfield, 2007).

Covariances between traits were strong and significant at the level of the phenotype and year but broadly not significant at the genetic level, implying that many of the observed phenotypic correlations between traits were environmentally induced and consequently condition-dependent plastic responses (Pigliucci, 2001, Nussey et al., 2007). Our results demonstrate that phenotypic correlations are not necessarily accurate surrogates for genotypic correlations, as is often assumed (reviewed in Kruuk et al., 2008 and debunked in Hadfield et al., 2007). This 'phenotypic gambit' (Grafen, 1984) is likely to hold in traits with high heritability, as the environmental component of the phenotypic correlation will be lower than in traits with less heritability

(Cheverud, 1984). The assumption that phenotypic correlations reflect genotypic correlations is perhaps erroneous for phenological variables due to the strong influence of environmental variation on an individual's phenotype. Our results therefore suggest caution in extrapolating from phenotypic studies of phenological traits to the likely impact on evolutionary processes.

Maintenance of genetic diversity poses a problem to evolutionary biology – why would less fit genotypes persist in a population in the face of selection against low fitness? Studies on wild populations (e.g. Brommer et al., 2007, and in this study system Foerster et al., 2007), have suggested that a negative genetic covariance in fitness between the sexes may maintain genetic variance, as there is no optimal genotype within the population (Lynch and Walsh, 1998). The presence of negative cross-sex genetic correlations within phenological traits could be envisaged if, for instance, genes that when expressed in a female lead to early breeding could perform badly in the testosterone-rich environment of a male, perhaps leading to later rutting and reduced breeding success. However we found no evidence for sexually antagonistic gene expression, suggesting that the negative cross-sex genetic covariance detected by Foerster et al. (2007) was not underpinned by negative genetic covariances between phenological traits. It must be noted that our analyses have not investigated the genetic correlations between phenological and either morphological or life-history traits in this system – if significant genetic correlations are present, our interpretation of the genetic landscape of phenological traits may alter radically. However, recent work in this study system suggesting that genetic effects on males and females are concordant for juvenile and adult survival traits, but discordant only for reproductive traits (Walling et al., in prep) implies that significant genetic variance-selection antagonism is not a prevalent

feature in our study system. Additionally we have not investigated how covariances between traits vary with environment or with an individual's age (Kruuk et al., 2008).

We found a large and significant cross-sex genetic correlation (0.91±0.36SE) between female oestrus date and male rut start date. However, this result may be spurious for a number of reasons. Firstly the additive genetic component of oestrus date was very low and non-significant, and consequently it is difficult to draw conclusions from a covariance without any clear evidence of underlying variance in one or more variable. Secondly oestrus date and parturition date were very highly genetically correlated, suggesting that there should also be a genetic correlation between rut start date and parturition date, for which there was both significant levels of genetic variance and high sample sizes, but we found no evidence for this. Finally, note that although the correlation was significantly greater than zero, its standard error was sufficiently high as to generate a very broad confidence interval.

What do the results presented here imply regarding the population's ability to adapt to a novel environment, particularly in the light of the widespread evidence for effects of recent climate change on phenology in wild populations (Menzel and al, 2006, Thackeray and al, 2010)? The absence of strong genetic correlations between phenological variables suggests that response to future selection will not be constrained by genetic correlations between traits (Lynch, 1999). However, it remains possible that genetic correlations between phenological and other traits may act to constrain or facilitate adaptation. To our knowledge this is the first time that the genetic correlations between phenological traits have been characterised in a wild population and it is consequently unknown how these results will be representative of other systems. It remains to be seen if it is a general phenomenon that high phenotypic

correlations between phenological traits are not accompanied by a tight coupling at the genetic level in wild populations.

Chapter 7

General Discussion

The aim of this thesis was to investigate phenology in a wild population of red deer. I discuss here the main findings presented in this thesis and suggest areas of possible future research.

Causes of variation in phenology

In this thesis, I investigated factors influencing phenology in eight different traits. In all traits studied, patterns of age-related variation differed between traits suggesting differing physiological processes underlying these traits. Further support for differing physiological processes underlying phenological traits in this system was found in chapter 6 where I detected few significant genetic correlations between phenological traits.

Phenotypic plasticity, in the form of significant associations between phenology and prevailing environmental conditions, (Pigliucci, 2001) was found in all traits where I tested for it. However, the critical time periods found to affect traits differed between traits: early average antler growth start date and coat change date were associated with warm September temperatures, whilst shorter average gestation lengths and early average antler growth end dates were correlated with warm spring temperatures (during March and April respectively). Additionally, the time period between the most significant weather period and the average timing of trait expression differed between traits (Table 7.1). Where the time-lag is very small, such as in coat change date,

gestation length and antler growth start date, it is possible that increased vegetation during these periods was having a direct effect on trait timing. For instance, increased food resources may be directly channeled into energy for the growing calf or antler, leading to a decrease in growth period. This is supported by the interaction between antler growth start and end dates in the model of antler mass showing that the males with the heaviest antlers did not simply grow for the longest period. However, in traits such as antler growth start date, a role for condition seems likely as the intervening period between the critical climatic variable and the average timing of trait expression includes winter. These results suggest that the effects of environmental variation on a phenotype can be complex and different for each aspect of phenology.

Table 7.1 Time-lag between most significant 1 month weather variable and average time of trait expression in the phenological traits investigated in this thesis. As gestation length is the time period between conception and birth dates, the average of each of these traits is reported.

Chapter	Phenological trait	Average date	Significant weather month	Time-lag (months)
2	Antler growth start	19th April	September	7
2	Antler growth end	16th August	April	4
3	Gestation length	14th Oct – 7th Jun	March	0-3
4	Coat change	22nd October	September	1

I have documented the first, to my knowledge, evidence in mammals of between-male differences in the breeding time of females they mate with. Two previous studies have found evidence of this phenomenon in wild bird populations, which they attributed to between-male differences in levels of provisioning to the female and paternal care (Brommer and Rattiste, 2008, Teplitsky et al., 2010). Additionally, both studies found evidence of heritable genetic variation in males, suggesting the potential for between-male effects to influence evolutionary processes (Lynch and Walsh, 1998). Clearly, in the polygynous mating system of red deer,

paternal care cannot be responsible for these between-male differences, and analyses of gestation length suggested that these effects may be environmentally induced. Consequently, a fruitful area of research may be to incorporate information of spatial home ranges into the random effects structure to model common-environmental effects explicitly (Kruuk and Hadfield, 2007). Common-environmental effects may be affecting estimates of quantitative genetic parameters in many systems and a comparative study of the magnitude of these effects across a range of traits and study systems would undoubtedly be of use.

Although there has been growing interest in correlations between conception date and gestation length in ungulates (e.g. Berger, 1992, Asher, 2007, Mysterud et al., 2009), no study has yet recognised the potential for such a relationship to be a statistical artifact due to errors in determining conception date (Kelly and Price, 2005). I approached this problem using novel methodology of bivariate models to show that gestation length adjustment was not a tactic used repeatedly by females across their lifetimes. My work raises questions around the prevalence of gestation length adjustment in wild systems, and suggests that parturition date in wild ungulates is predominantly a function of conception date rather then gestation length.

Consequences of variation in phenology

Early antler growth phenology was associated with higher breeding success in the following rut, and early coat change date was correlated with over-winter survival. Although selection is an intuitive concept, correctly testing for selection is more difficult that one might imagine. Firstly, how does one define fitness? Studies such as these on wild populations must often choose a proxy for fitness such as survival or number of offspring, both of which measure very different processes (Kingsolver et al.,

2001). Secondly, the measurement of direct selection is complicated as there may be an unmeasured variable such as condition that affects both the trait under focus and fitness. Consequently, apparent selection will not lead to changes in gene frequency between generations. This mechanism has been proposed for the apparent lack of evolution in breeding time in birds (Price et al., 1988) and antler mass in red deer (Kruuk et al., 2002), suggesting that environmentally-induced correlations may be a common feature in studies of wild populations (Merila et al., 2001). Thirdly, selection may be estimated from the genetic covariance between a trait and fitness, implying that it is a relatively simple matter to test for this association in a bivariate framework (Walsh and Blows, 2009). However, genetic variation in fitness itself is predicted to be low (Fisher, 1958) as it is by definition under directional selection, which will act to erode genetic variance. Consequently, the analytical difficulties in testing for a covariance between a trait and fitness at the genetic level may be insurmountable. Finally, the death of individuals prior to trait expression in wild systems may lead to biased estimates of selection gradients if the trait under selection is correlated with survival (Hadfield, 2008).

Despite the numerous difficulties in testing for selection in wild systems, it is still an important area of future research. Evidence of changing selective pressures as a result of climate change (Visser et al., 1998, Both and Visser, 2001) imply that it is more important than ever that we gain a better understanding of how selection acts in natural populations. Selection analyses coupled with findings from experimental systems may be the most fruitful way forward with this problem (Verhulst and Nilsson, 2008, Visser et al., 2009)

Phenology and climate change

It is now undisputed that phenology is advancing across a wide range of taxa (Sparks and Menzel, 2002, Parmesan, 2006, Thackeray and al, 2010), including this study system (Moyes et al., in review; appendix V), most likely as a direct result of climate change (IPCC, 2007). Consequently, future research must move away from simply documenting rates of advancement towards an understanding of what the effects of climate change on wild populations might be (Visser, 2008). I attempted to address this question by asking how the variance, skew and kurtosis of phenological traits have been changing over time in this study system. I hypothesized that phenology cannot continue to advance indefinitely and as this limit to phenological advancement approaches, change in the higher moments of the distribution may be evident, but I found no evidence for this. My results therefore suggest that there is either no limit to phenological advancement, or that it has not been reached. As meta-analyses of phenology over time calculate the mean trait value in each year, the required data to calculate the variance, skew and kurtosis must also be available. Consequently, a meta-analysis of trends in the higher moments of the distribution of a number of different phenological traits would be an exciting, and relatively inexpensive, area of future research.

Correlations between phenological traits

I present in this thesis the first analysis of genetic correlations between phenological traits in a wild animal population. I found correlations to be broadly strong and significant at the level of the individual and year, but not significant at the genetic level. These results therefore imply that phenological variables are free to respond to varying selection pressures independently (Lande, 1979). However it is

unknown whether these results generalize to other systems. A better understanding of the genetic architecture underlying phenological traits in a wide range of systems, and their relationship with other non-phenological traits, is therefore imperative to determine how populations might respond to climate change.

Final thoughts

This thesis represents a rare attempt to systematically evaluate causes and consequences of variation in phenology in a wild mammal population. I found the phenological traits expressed in this population to show differing patterns of age related variation, sensitively to environmental conditions and to even differ in their genetic architecture. None of this work would have been possible without the detailed and comprehensive datasets gathered from years of painstaking work in the field. These results emphasise that all phenological traits are not equal and it is therefore imperative to choose the trait under study carefully and with consideration of the underlying biological processes and questions.

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Appendices

Appendix I: Chapter 6 supplementary material

Table S1. Permanent environment covariances and correlations between phenological traits from bivariate animal models in (a) females and (b) males. Covariances are shown below the diagonal with the associated correlation above the diagonal, both are shown with associated standard error. Significance of covariances were tested with log-likelihood ratio tests and correlations were considered significant if the appropriate covariance component was also significant. . * indicates $p<0.05$; ** $p<0.01$; *** $p<0.001$.

a)	Oestrus	Coat change	Parturition
Oestrus		1.02(1.05)	0.13(0.56)
Coat change	5.88(6.02)		0.06(0.24)
Parturition	0.65(2.91)	1.45(5.71)	

b)	Antler start	Antler end	Rut start	Rut median	Rut end
Antler start		0.82(0.18)***	0.88(0.23)***	0.75(0.26)**	0.10(0.25)
Antler end	24.40(5.47)***		0.65(0.49)	0.018(0.28)	-0.16(0.24)
Rut start	17.07(4.61)***	7.17(5.38)		0.92(0.67)	0.46(0.44)
Rut median	16.97(5.99)**	0.22(5.08)	9.88(7.18)		0.73(0.44)
Rut end	2.30(5.60)	-3.38(5.02)	6.37(6.20)	10.75(6.47)	

Table S2. Residual covariances and correlations between phenological traits from bivariate animal models in (a) females and (b) males. Covariances are shown below the diagonal with the associated correlation above the diagonal, both are shown with associated standard error. Significance of covariances were tested with log-likelihood ratio tests and correlations were considered significant if the appropriate covariance component was also significant. . * indicates $p<0.05$; ** $p<0.01$; *** $p<0.001$.

a)	Oestrus	Coat change	Parturition
Oestrus		0.029(0.07)	0.36(0.04)***
Coat change	2.54(6.81)		0.003(0.048)
Parturition	27.8(3.42)***	0.3089(5.0679)	

b)	Antler start	Antler end	Rut start	Rut median	Rut end
Antler start		0.35(0.04)***	-0.01(0.04)	0.08 (0.05)	0.02(0.05)
Antler end	7.69(1.07)***		0.21(0.06)**	0.24(0.06)***	0.07(0.06)
Rut start	-0.67(3.08)	7.88(2.21)**		0.57(0.03)***	0.23(0.02)***
Rut median	5.43(3.08)	8.88(2.22)***	64.2(3.49)***		0.571(0.03)***
Rut end	0.93(2.81)	2.14(2.04)	22.27(2.70)***	54.66(2.98)***	