

Phenotypic plasticity in a maternal trait in red deer

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Summary

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

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

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

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

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

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

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

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Introduction

Phenotypic plasticity, defined as the expression of multiple phenotypic states by a single genotype under different environmental conditions (Houston & McNamara 1992),

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

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

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

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

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

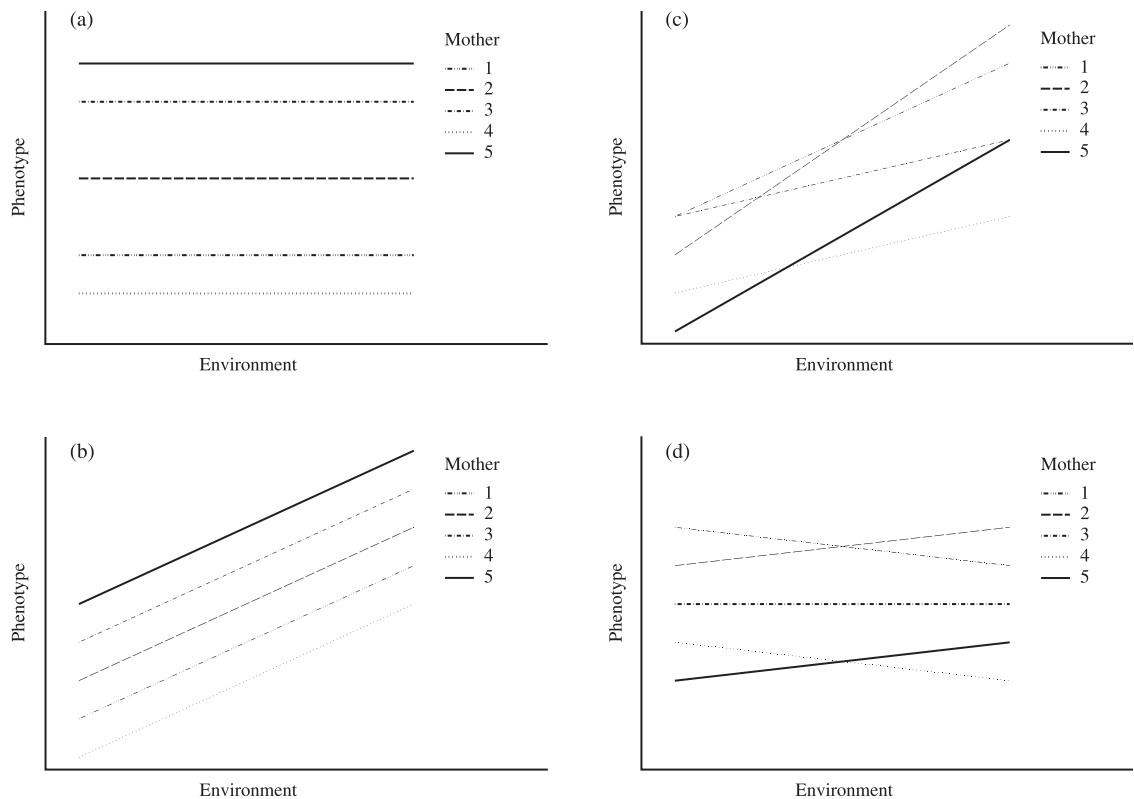


Fig. 1. Line plots of phenotypic trends across an environmental gradient for five maternal genotypes, illustrating the four main patterns of plasticity (adapted from Pigliucci 2001): (a) variation in elevation (trait means) but no average plasticity or variation in plasticity; (b) average plastic response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

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

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

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

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

There are empirical data suggesting that within population variation in plastic responses exist and can

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

PHENOLOGICAL PLASTICITY IN RED DEER

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

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

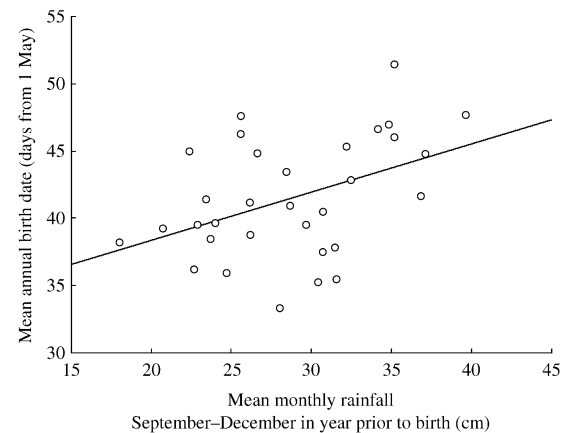


Fig. 2. Scatter-plot showing the mean monthly rainfall between September and December in year prior to birth and annual average birth date (days after 1 May), with regression line ($b = 0.36 \pm 0.10$ se).

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

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

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

Materials and methods

STUDY POPULATION

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

After the cessation of culling, the density of resident adult females in the study area increased steadily to

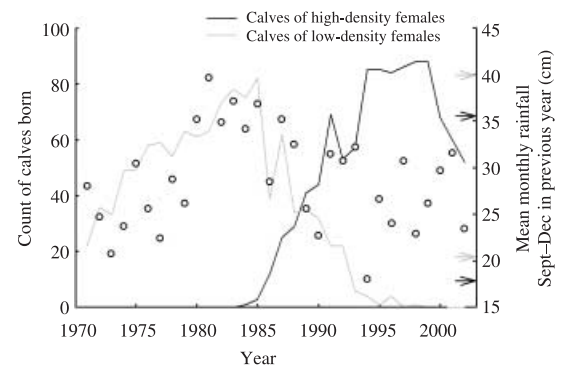


Fig. 3. Line and scatter-plot showing the number of calves born per year of the study period to low (born up to and including 1980, grey line) and high (born after 1980, black line) density females, and mean monthly rainfall between September and December of the previous year ('autumn rainfall', open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

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

MONITORING AND MEASUREMENT

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

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

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

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

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

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

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

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

Statistical analysis

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

All data analysis was conducted using GENSTAT version 6.1 (VSN International).

MIXED-EFFECTS LINEAR MODELS OF CALVING DATE

We used the same fixed-effects terms as Coulson *et al.* (2003) to generate a maximal model for calving date, but with an expanded data set (including 4 more years of data). Climate covariates were estimated as comparisons between years, and so should be tested against unexplained year-to-year variation (Milner, Elston & Albon 1999). Consequently, a random effect for offspring's year of birth was added to this initial maximal model, which was used to screen covariates before moving onto the more advanced models. The significance of fixed-effects terms was assessed by referring

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

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

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

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

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

NATURAL SELECTION ON PHENOTYPIC PLASTICITY

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

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

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

Results

PATTERNS OF PLASTICITY FOR CALVING DATE

Tests comparing LMM deviances revealed that the random effect for female identity explained a significant

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

In the model with all random effects, the fixed-effect estimate of autumn rainfall indicated that calving dates were delayed by an average of 0.41 (± 0.15 SE) days cm^{-1} of rain: there was an average within-female response significantly greater than zero. It appears that the trend in calving date with autumn rainfall observed at the population level is largely explained by within-individual variation at the maternal level.

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

As predicted, females experiencing high population densities showed a reduced response to autumn rainfall. Low density females responded by 0.36 days cm^{-1} of rain more than high density females, a marginally non-significant difference (female density group * autumn rainfall interaction: $t = 1.44$, $P = 0.08$, one-sided t -test). LMMs were run for each of the two female density groups separately. Table 2 shows that variation in individual calving date–autumn rainfall slopes was very similar in the high- and low-density groups of females (0.56 ± 0.32 SE and 0.52 ± 0.21 SE, respectively), while females in the high-density group varied more in their calving date elevations than those at low density (35.13 ± 9.97 SE compared with 23.80 ± 7.87 SE). Correlations between

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

Model	Random variables included in mixed-effect model			Deviance of model	d.f.	Test	Log-likelihood test statistic
	Offspring year of birth	Maternal variables					
		Identity	Slope for autumn rainfall				
Across study period (1971–2002)							
1	√			14385.85			
2	√	√		14344.87	1	1 vs. 2	40.98**
3	√	√	√	14329.91	2	2 vs. 3	14.96**
Offspring of females born at low population density (up to and including 1980)							
4	√	√		7457.19			
5	√	√	√	7446.65	2	4 vs. 5	10.54**
Offspring of females born at high population density (1981–2002)							
6	√	√		6885.81			
7	√	√	√	6879.55	2	6 vs. 7	6.26*

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

Random effects	(a) Up to and including 1980		(b) 1981–2002	
	Variance component	SE	Variance component	SE
Offspring year of birth	18.0	7.8	9.4	5.6
Female elevation	23.80	7.87	35.13	9.97
Female slope on autumn rainfall	0.52	0.21	0.56	0.32
Residual	248.3	12.3	246.5	13.5
Fixed effects	Estimate	SE	Estimate	SE
Reproductive status				
Milk	44.70	1.38	46.37	1.47
Naïve	40.19	2.16	44.08	1.96
Summer yield	36.73	1.62	34.55	1.70
Winter yield	46.95	2.06	44.94	1.91
Yield	40.14	1.41	37.84	1.38
Female's age	-2.92	0.97	-2.26	1.21
Female's age ²	0.18	0.05	0.15	0.06
Autumn rainfall	0.52	0.20	0.18	0.21

elevations and slopes in the two groups were also different: both were negative, but females born at high densities showed a closer relationship between elevation and slope ($r = -0.35$ and -0.10). Females born at high density that showed early calving dates in the average environment were more likely to also show a strong positive response to autumn rainfall.

Females born up to and including 1980 (low density) showed an average positive plastic response to autumn rainfall of $+0.52 \pm 0.20$ SE days cm^{-1} of rain per month (see Table 2). These females are giving birth earlier in response to dry autumn conditions, and vary significantly in their responses ($\chi^2 = 10.54$, d.f. = 2, $P < 0.05$, Table 1). This pattern of plasticity is illustrated by Fig. 1c.

Among females born at high density, there was a substantially reduced average response of calving date to autumn rainfall ($+0.18 \pm 0.21$ SE days cm^{-1} of rain per month, Table 2), which was not significantly different from zero. However, there was still variation between females in their plastic responses to autumn rainfall ($\chi^2 = 6.26$, d.f. = 2, $P < 0.05$, Table 1). This situation is described visually in Fig. 1d.

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

NATURAL SELECTION ON PHENOTYPIC PLASTICITY

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

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

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

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

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

A possible factor influencing the shift in selection on females' plastic responses between these two groups could be differences in their experiences of autumn rainfall. There was no evidence of a linear temporal trend in autumn rainfall across the study period ($b = -0.03 \pm 0.10$ SE cm per year, d.f. = 32, $t = 0.27$, $P > 0.05$), or any difference in the mean autumn-rainfall conditions experienced by the two groups (t -test comparing mean autumn rainfall in years in which 10 or more females from either low- or high-density groups bred (see Fig. 3): $t = 0.89$, d.f. = 32, $P > 0.05$). While it does appear that the high-density group of females experienced slightly lower variation in autumn rainfall, it is clear that there was still considerable variation in rainfall across their lifetimes (Fig. 3).

Discussion

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

The observed variation between females in mean calving date is not surprising and can be ascribed to differences in genetic or non-genetic components of individual quality (Clutton-Brock, Guinness & Albon 1983). However, to date few studies have examined or discussed variation in responses to the environment

between reproductive females in naturally occurring populations (although see Brommer *et al.* 2003). Across the study period we observed variation in females' response of calving date to autumn rainfall. If we assume the plastic response of a female's calving dates to autumn rainfall to be the result of physiological condition-dependent decision making, then we can see that the optimal response to rainfall may vary depending on a female's physiological condition at the start of her reproductive cycle (i.e. August or September).

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

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

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

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

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

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

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

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

Conclusions

This study illustrates how mixed models can provide a valuable and readily available means of analysing

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

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