Phenological shifts in North American red squirrels: disentangling the roles of phenotypic plasticity and microevolution

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Abstract

Phenological shifts are the most widely reported ecological responses to climate change, but the requirements to distinguish their causes (i.e. phenotypic plasticity vs. microevolution) are rarely met. To do so, we analysed almost two decades of parturition data from a wild population of North American red squirrels (*Tamiasciurus hudsonicus*). Although an observed advance in parturition date during the first decade provided putative support for climate change-driven microevolution, a closer look revealed a more complex pattern. Parturition date was heritable [$h^2 = 0.14$ (0.07–0.21 (HPD interval)] and under phenotypic selection [$\beta = -0.14 \pm 0.06$ (SE)] across the full study duration. However, the early advance reversed in the second decade. Further, selection did not act on the genetic contribution to variation in parturition date, and observed changes in predicted breeding values did not exceed those expected due to genetic drift. Instead, individuals responded plastically to environmental variation, and high food [white spruce (*Picea glauca*) seed] production in the first decade appears to have produced a plastic advance. In addition, there was little evidence of climate change affecting the advance, as there was neither a significant influence of spring temperature on parturition date or evidence of a change in spring temperatures across the study duration. Heritable traits not responding to selection in accordance with quantitative genetic predictions have long presented a puzzle to evolutionary ecologists. Our results on red squirrels provide empirical support for one potential solution: phenotypic selection arising from an environmental, as opposed to genetic, covariance between the phenotypic trait and annual fitness.

Introduction

Seasonality should have historically been a strong selective force for the proper timing of energetically expensive life-history events (e.g. reproduction) in accordance with resource availabilities and abiotic conditions. Currently, climate change is rapidly altering both environmental aspects and leading to new selection pressures (Visser, 2008). The pace and magnitude of phenological adjustments are thus likely to have important consequences for both individual fitness (Ozgul *et al.*, 2010; Lane *et al.*, 2012) and population viability (Both *et al.*, 2006; Møller *et al.*, 2008; Saino *et al.*, 2011; but see Reed *et al.*, 2013a, b).

Phenological adjustments within a population can occur through two nonmutually exclusive mechanisms: phenotypic plasticity and microevolution (Boutin &

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Lane, 2014; Merilä & Hendry, 2014). Consensus climate models project extended periods of directional change (IPCC 2014) and, as phenotypic plasticity has a theoretical limit (Lande & Shannon, 1996), microevolution of phenological traits is presumed necessary to ensure the local long-term viability of populations (Visser, 2008). Although a large number of studies have attributed phenological adjustments to phenotypic plasticity and/or microevolutionary change (Parmesan, 2006; and references therein), it is exceptionally difficult to distinguish these processes. On the one hand, the necessary requirements to demonstrate microevolutionary responses to selection are rarely met. On the other, when an attempt to search for such a response is unsuccessful, phenotypic plasticity is often accepted by default (Merilä & Hendry, 2014). As a result, recent calls have been made to evaluate phenotypic plasticity and microevolution as two hypotheses, with the weight of evidence for and against being measured for each (Merilä & Hendry, 2014).

Although phenotypic plasticity is relatively straightforward to ascertain (as phenotypic variation within a genotype or individual in response to environmental variation; Pigliucci, 2001), the requirements to demonstrate a microevolutionary response to selection are more demanding. Gienapp et al. (2008) outline three prerequisites for a convincing demonstration. Studies must show that the trait in question: (i) is heritable, (ii) is under selection and, arguably the most challenging, (iii) has undergone change at a genetic level. In the current context, Merilä & Hendry (2014) add to this list the requirement of attributing climate change as the causal mechanism underlying the genetic change. The necessity of tracking fitness for a large number of individuals in pedigreed populations over multiple generations, and across a sufficient range of climatic variation, has resulted in few studies with the ability to meet this challenge. Gienapp et al. (2008) originally identified three studies that had managed to fulfill these criteria. More recently, Merilä & Hendry (2014) have expanded this list to ten species.

A population of North American red squirrels (*Tamiasciurus hudsonicus*, hereafter red squirrels) in the Yukon Territory, Canada, has previously been put forward as one that has met the requirements for a demonstration of climate change-driven microevolution (Réale et al., 2003). Red squirrels are a capital breeding species, relying on food hoarded the previous autumn to fuel current (spring) reproduction. At this location, the primary food of red squirrels is the seed contained within the cones of white spruce (*Picea glauca*; Fletcher et al., 2010). White spruce is a masting species with synchronous and highly variable (up to two orders of magnitude) annual cone production (LaMontagne & Boutin, 2007). The hoard of cones (referred to as a 'midden') forms the centre of individually defended territories. As possession of a territory, with an associated

midden, is required for overwinter survival, acquisition of a territory is a strong episode of selection (McAdam & Boutin, 2003; Fisher et al., 2017). Earlier breeding females are thus expected to realize higher fitness as their juveniles will have greater opportunity and, potentially, competitive ability to acquire available territories.

In our study population, parturition date has been shown to be both heritable (heritability (h^2) = 0.16) and under selection (selection differential (S) = −0.17; Réale et al., 2003; see also McAdam & Boutin, 2003; Fisher et al., 2017). In addition, both observed parturition dates and predicted breeding values (PBVs) for parturition date (i.e. the combined additive effects of all of an individual's genes for the trait; Lynch & Walsh, 1998) were also shown to have advanced over the course of a decade. This response was attributed to a historical increase in spring temperatures and a (albeit untested) relationship between spring temperature and annual food abundance. This response thus satisfied three of the four prerequisites for a demonstration of a microevolutionary response to selection (with the exception being an explicit demonstration of climate change as the causal factor). Following publication, however, new analytical and theoretical advances called into question the results of this study and of others studies using similar methodology (Hadfield et al., 2010).

Until relatively recently, a combination of the breeder's equation and best linear unbiased predictors (BLUPs) of PBVs had been used to, respectively, predict and estimate evolutionary responses. The breeder's equation has proved predictive under the parameters for which it was developed (i.e. plant and animal breeding), but its performance under natural conditions has been equivocal, with empirical results matching predictions in some cases (e.g. Grant & Grant, 2006), but not in others (Merilä et al., 2001). As the breeder's equation is correlative when applied to data from wild populations (Morrissey et al., 2011), observed disparities between predicted and observed responses to selection are potentially due to environmental heterogeneity having a combined influence on both individual fitness and phenotypic variation (e.g. individuals occupying higher quality territories may both breed earlier and have higher fitness, without a causal relationship between the two traits; Price et al., 1988; Rausher, 1992; Kruuk et al., 2002). For these reasons, a compelling expectation of an evolutionary response to selection requires a genetic covariance between the focal trait and fitness (Robertson, 1966; Price, 1970).

The second line of criticism concerns the use of PBVs and BLUPs (for a detailed overview of these criticisms, see Hadfield et al., 2010; Postma, 2006). True breeding values are not observable and must be predicted using quantitative genetics models. BLUPs are point estimates

of random effects and, in this context, are used to predict breeding values using phenotypic information collected from focal individuals and their relatives (Henderson, 1950, 1976). Hadfield *et al.* (2010) outline the main problems in the use of BLUPs with ecological datasets. These include the following: inappropriateness for statistical testing, genetic distributions artificially mirroring phenotypic distributions and challenges inherent in determining whether observed changes constitute microevolution vs. genetic drift.

In light of these stated challenges and potential pitfalls, our aim was to more explicitly disentangle the relative roles of phenotypic plasticity and microevolution in producing phenological variation in red squirrels. To do so, we used both the original dataset from Réale *et al.* (2003) as well as an additional decade of collected data. We first assessed whether the previously reported phenological advance continued into the subsequent decade. Next, we investigated three potential environmental determinants of phenological variation in models designed to separate within-individual phenotypic variation (i.e. phenotypic plasticity) from between-individual variation (e.g. potentially due to microevolution; van de Pol & Wright, 2009). We then estimated the strength of selection on, and the heritability of, parturition date. To enable comparison between our results and those of Réale *et al.* (2003), we estimated the standardized selection differential (*sensu* Lande & Arnold, 1983) and univariate heritability. We also investigated selection on the genetic basis to parturition date, using the genetic covariances and gradients between parturition date and annual fitness. We tested for evolutionary change in this trait using a Bayesian posterior predictive test, which benefits from being more conservative than BLUP analysis, and also in permitting the ability to statistically test whether an observed change in PBVs is greater than that which would be expected due to genetic drift (Hadfield *et al.*, 2010).

Materials and methods

Study population

We studied a wild population of red squirrels resident on one ~60 ha and two ~40 ha study grids in the south-west Yukon Territory (61°N, 138°W) from 1989 to 2008. Details of the study area and population were originally reported by Réale *et al.* (2003) (also see the Appendix S1). We monitored the two 40 ha study grids continuously from 1989 to 2008 and the 60 ha grid from 1989 to 1997 (after which we used it for food manipulation experiments and therefore we do not include the data here). Due to the masting nature of white spruce, there is a high degree of spatial synchrony in both grid-level average cone production and parturition date of red squirrels (Pearson product–moment correlations (r_P) for the average cone index (described below) and parturition date, respectively, between the 60 ha grid and the average of the two 40 ha grids: $r_{P18} = 0.90$ and $r_{P5} = 0.93$). We thus expect that the 40 ha grids provide a reliable representation of the general patterns in the area.

We followed the reproductive activity of all resident females through behavioural observation and trapping beginning in early March and continuing until the end of the breeding season. Shortly after parturition, we accessed each female's litter (typically in arboreal grass nests) to count, sex, weigh and mark all offspring with a unique ear-notch sex combination. We estimated parturition dates using a combination of visual observation, regular trapping and assessment of juvenile mass. Specifically, if the oestrous date for the female was observed, we added 35 days (i.e. the gestation period for red squirrels in this population, Lane *et al.*, 2007). If the oestrous date was missed but we captured the female as pregnant and then lactating within 10 days, we took the mid-point between these dates as the parturition date. Finally, if we missed the oestrous date and one or both of the trapping records within this window, we estimated parturition date using the mass of the juvenile(s) during handling (assuming a neonatal mass of 10 g and a growth rate of 2 g day^{-1}). We only considered the dates and offspring fates of females' first litters in analyses (females will occasionally attempt a second litter following litter loss and, rarely, following a successful litter; Boutin *et al.*, 2006; Williams *et al.*, 2014). We found most nests a second time approximately 1 month later to ear-tag the young and provide them with a unique combination of coloured discs (threaded through their ear-tags). We followed the fates of weaned juveniles through live trapping, visual observation and radiotelemetry. To facilitate comparison of our results to those in Réale *et al.* (2003), we calculated the mean parturition date of females across their lifetime and used general linear models (LM) implemented in R (ver. 3.2.2; R Core Team, 2013) to determine the relationship between year of birth and lifetime mean parturition date. However, to take advantage of the full dataset, we conducted all quantitative genetics and selection analyses below using year-specific individual parturition dates in appropriate (mixed effects) models (as opposed to lifetime averages). We report all estimates as ±1 SE or with 95% HPD intervals.

Environmental change

We used a within-subject mean centring approach to evaluate the influence of three environmental variables (spring temperature, food abundance and population density) on parturition dates. This approach decomposes responses in the dependent variable into those associated with the average environment experienced

by an individual over its lifetime (a 'between-subject' effect) vs. deviations of the environment in a given year from the individuals' lifetime averages (the 'within-subject' plastic effect; van de Pol & Wright, 2009). We fitted individual ID and year as random effects in these models.

We collected temperature data from a nearby weather station in Burwash, YT (Burwash Environment Canada Station, http://climate.weatheroffice.ec.gc.ca; 61°N, 139°W; *ca.* 50 km from the study site) and, following Réale *et al.* (2003), we calculated spring temperature (°C) for each year as the mean temperature from 01 April to 30 June.

To assess annual food availability for red squirrels at our study site, we quantified the number of cones produced by trees each year in late summer, prior to when squirrels began harvesting them (Fletcher *et al.*, 2010). We counted all the cones visible on one side of the top 3 m of approximately 80 trees per study grid, $\ln(x + 1)$-transformed these counts and quantified annual resource availability as the average of the transformed values following previously established protocols (e.g. Boutin *et al.*, 2006). We transformed raw cone counts prior to averaging within each year so as to prevent individual trees from having disproportionate effects on annual measures of cone availability and to produce statistical models with residuals that meet the assumptions of homoscedasticity and normality of errors. We entered food abundance into models as the cone abundance in the previous year because females rely on seed cached the previous autumn to fuel spring reproduction and squirrels are known to breed earlier in the spring in response to higher cone production the previous autumn (Boutin *et al.*, 2006; Williams *et al.*, 2014).

We calculated population density, using previously established protocols (Dantzer *et al.*, 2013; Williams *et al.*, 2014), as the average number of territory occupants in the core area of each of the three (1989–1997) or two (1998–2010) study grids. The core area represented a 630×630 m (39.69 ha) area at the centre of the study grid. We unambiguously identified all territory occupants through a combination of live trapping and behavioural observation (of individuals displaying territorial behaviours such as 'rattle' calling and feeding). We entered density into the models as the number of squirrels ha^{-1}.

We used LMs to determine whether there has been a significant annual trend (year fitted as a continuous fixed effect) in spring temperature, food abundance or population density. We also used an LM to determine whether the lifetime number of cones available to females has exhibited a consistent change (for each female, by averaging the annual cone abundance over each year of her life and fitting an LM with year of birth as a continuous fixed effect).

Predicting the response to selection

We used two quantitative genetic models to predict the response to selection in parturition date. In the univariate breeder's equation

$$R = h^2 S$$

R represents the response to selection, h^2 represents the heritability of, and S represents the selection differential on, parturition date (Falconer & Mackay, 1996). We estimated h^2 using a Bayesian 'animal model' in the MCMCglmm package (Hadfield, 2010) for R. For a detailed description of animal models, see Kruuk (2004), and for our implementation, see the supplementary material. We calculated S by first estimating the standardized selection gradient (β) using a multiple regression of annual relative fitness on standardized parturition date, as well as age and age^2. We fitted the latter two variables to account for known age-related variation in both parturition date and annual fitness in this population (with a general improvement early in life, followed by a senescent decline; McAdam *et al.*, 2007; Williams *et al.*, 2014). We calculated an annual contribution to individual fitness (hereafter referred to as 'annual fitness') as the number of offspring produced in the first litter by a female that survived over the winter (overall mean = 0.55 ± 0.02). Overwinter survival (to enter the age of reproduction) is an established annual fitness metric in this study system (e.g. McAdam & Boutin, 2003) that correlates strongly with overall lifespan (McAdam *et al.*, 2007), and dispersal has been shown to not bias annual fitness calculations (Kerr *et al.*, 2007). We calculated annual relative fitness (as the difference between-individual annual fitness and population mean annual fitness) and standardized (to mean of 0 and unit variance) parturition date within each year so as to avoid confounding selection gradients with covariances between environmental conditions, trait values and annual fitness (Fisher *et al.*, 2017). Here, and below, we assume that selection based on annual fitness is consistent with lifetime selection. We then converted the selection gradient to a selection differential by multiplying it by the phenotypic variance in (standardized) parturition date. We assume that there is no selection on parturition date acting through males and therefore divided the selection differential by two before using it in the breeder's equation calculation.

In the Robertson-Price identity

$$\Delta z = \sigma_a(z, w)$$

Δz is the expected change in mean (standardized) parturition date z between generations (generation time of red squirrels in this population is 2.7 years; McAdam & Boutin, 2003) and fitness is represented as w (analysed as annual relative fitness in our case). $\sigma_a(z, w)$

represents the additive genetic covariance between *z* and *w*. We parameterized the Robertson-Price identity by fitting a bivariate generalized linear mixed effects model in the MCMCglmm package (Hadfield, 2010) for R. In the first model, we fitted additive genetic covariance, permanent environment covariance (i.e. nongenetic individual repeatability; Kruuk & Hadfield, 2007), year covariance and residual covariance terms (as well as their univariate equivalents). We then sequentially simplified the model, by first removing the additive genetic covariance term and then removing the permanent environment covariance term. We fitted age and age^2 of reproductive females in all three models as fixed effects. To compare our phenotypic and genetic selection estimates directly, we also calculated the selection gradients by dividing the covariances by the phenotypic variance in (standardized) parturition date. Although the breeder's equation and Robertson-Price identity are both used to predict evolutionary responses, it is important to note that they will only produce quantitatively similar results if the relationship between phenotypic variance and fitness is the same as the relationship between genetic variance and fitness.

Estimating the response to selection

We used the Bayesian posterior predictive test developed by Hadfield *et al.* (2010) to estimate the genetic response to selection and to determine whether any observed change in PBVs exceeded that which would be expected due to random genetic drift. We first performed this test across the full dataset. However, evolutionary trends need not be directional across time and short-term trends can thus be masked when a single estimate over a longer period is produced (Grant & Grant, 2002). We therefore also separately evaluated a subset of the data collected during the first decade, when the phenotypic advance was most apparent. These analyses compared the slopes of breeding values predicted as part of the animal model (indicating evolutionary change) to those which have been simulated down the pedigree, using estimates of the additive genetic variance from each posterior Markov chain Monte Carlo sample of the animal model (representing random genetic drift). The proportions of iterations for which the values of the slope using the PBVs were lower than those of the simulated breeding values are the probabilities that the observed trends exceed those which would be expected due to drift.

Results

Phenotypic response and environmental trends

Lifetime measures
Our data set, collected over the 19-year study period, consisted of 1221 records from 576 females for which

we had both parturition date and annual fitness data (mean records per female = 2.1; range = 1–7). The average Julian parturition date across all years was 118.16 ± 0.71 (corresponding to 28 April in nonleap years). From 1989 to 1998, lifetime mean parturition dates of females advanced with year of birth by 1.73 ± 0.58 days year^{-1}. This trend did not continue into the second decade, however. Across the full study period, the average change in lifetime mean parturition dates was thus a nonstatistically significant advance of 0.05 ± 0.27 days year^{-1} (LM: $F_{1,17} = 0.03$, $P = 0.86$; Fig. 1a).

During the first decade, the average annual cone index experienced across the lifetime of individual females increased with year of birth at a rate of 0.18 year^{-1}. This trend did not continue into the second decade, however, and across the full study period, the trend in lifetime average cone index did not significantly differ from 0 (LM: $F_{1,17} = 3.20$, $P = 0.09$; Fig. 1b).

Annual measures
Spring ambient temperature (T_a), cone index and population density all varied annually (ranges: 3.93–7.73 °C; 0–4.85; 1.00–4.26 squirrels ha^{-1}; Fig. 2a–c). Individuals responded plastically to food abundance, indicated by a significant within-individual response (Fig. 2d; Table 1), and the between-subject effect was statistically indistinguishable from the within-subject effect (Table 1). Although there appeared to be a negative relationship between parturition date and population density (Fig. 2e), neither the within- nor the between-subject effects were statistically significant (Table 1). Similarly, neither the within- nor the between-subject effects for ambient temperature were statistically significant (Table 1). None of the environmental variables exhibited a significant temporal trend across the two decades of the study (T_a: LM: $F_{1,17} = 0.49$, $P = 0.50$; cone index: LM: $F_{1,17} = 0.18$, $P = 0.67$; density: LM: $F_{1,17} = 0.04$, $P = 0.85$; Fig. 2a–c). There has also not been a significant change in mean annual parturition dates (LM: $b_{year} = -0.62$, $F_{1,17} = 0.64$, $P = 0.43$; Fig. 3).

Evolutionary causes and consequences of phenotypic variation in parturition dates

The phenotypic variance in parturition date was comprised of a significant additive genetic variance component ($V_A = 0.14$; HPD interval: 0.07–0.19), with a corresponding heritability of 0.14 (HPD interval: 0.07–0.21). There was also selection favouring earlier breeding ($\beta = -0.14 \pm 0.06$). The breeder's equation thus predicts a microevolutionary advance of $R = 0.25$ days generation^{-1}.

In the bivariate animal model, the level of additive genetic variance in parturition date was similar to that estimated in the univariate model ($V_A = 0.14$; HPD
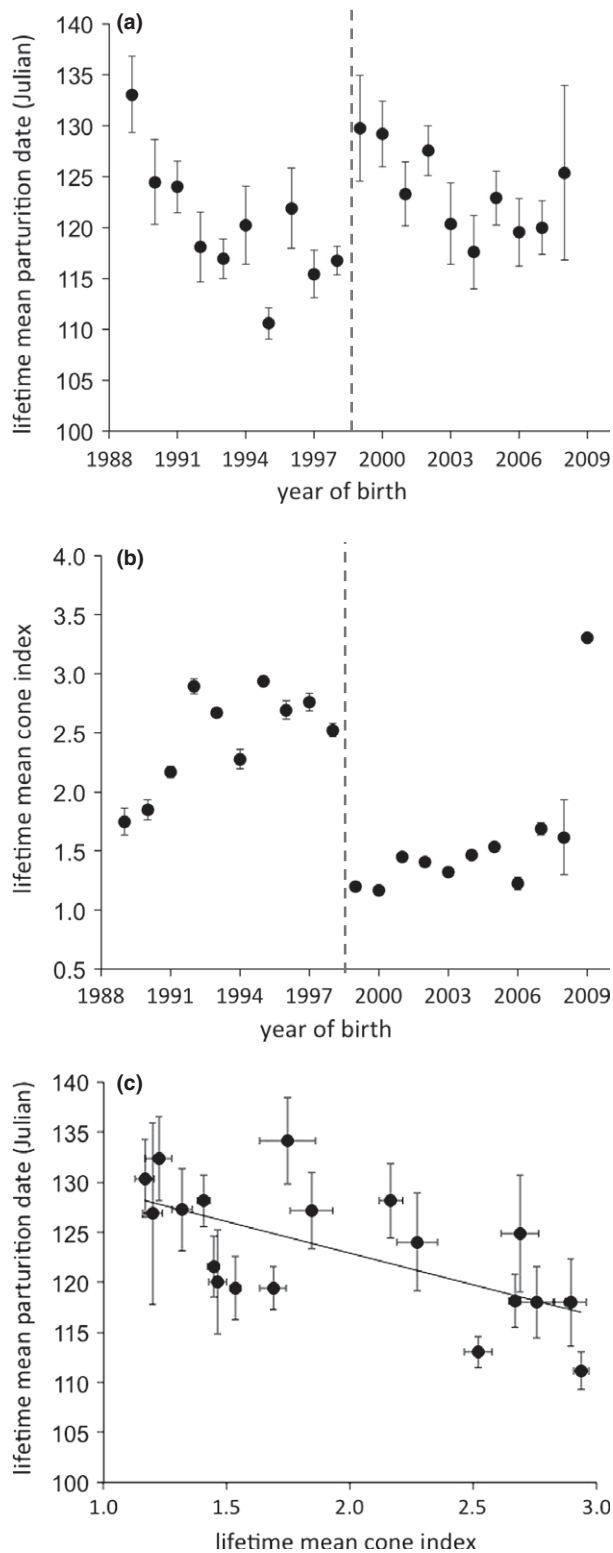
Fig. 1 Temporal trends in, and relationships between, mean parturition date of female North American red squirrels and average food abundance across their lives. (a) Variation in mean lifetime parturition date (Julian date $\pm$ SE) of females representing different cohorts from 1989 to 2007. Although an advance is apparent during the first decade (demarcated by a vertical dashed line), there is not a significant trend across the full study period. (b) Lifetime mean cone index (average cone index of females across all years of life) of females representing different cohorts from 1989 to 2007. Whereas an increase in lifetime mean cone index is apparent in the first decade (demarcated by a vertical dashed line), corresponding to the advance in average parturition date, there is not a significant trend across the full study period. (c) The relationship between lifetime mean cone index and lifetime mean parturition date.

1.02×10^{-7} to 0.13). The additive genetic covariance ($COV_A = -3.75 \times 10^{-5}$; HPD interval: -0.01 to 0.02; Table 2, full model) and permanent environment covariance ($COV_{PE} = -4.84 \times 10^{-4}$; HPD interval: -0.12 to 0.03; Table 2, model 2) between these two traits, as well, were both negligible. The observed phenotypic selection on parturition date was therefore due to a negative residual covariance between parturition date and annual relative fitness ($COV_R = -0.15$; HPD interval: -0.22 to -0.01; Table 2, model 3).

There was no evidence of a trend in breeding values across either the first decade ($R = -2.0 \times 10^{-3}$ days $year^{-1}$) or the full study duration ($R = 2.0 \times 10^{-3}$ days $year^{-1}$; Fig. 4). The Bayesian probabilities that the magnitude of these trends was less than 0 were 0.58 (first decade) and 0.56 (full duration) and the probabilities that they were less than what would be expected by random genetic drift were 0.55 (first decade) and 0.54 (full duration).

Discussion

Microevolution and plasticity can both contribute to phenotypic responses to climate change, but rarely are their roles explicitly distinguished (Gienapp *et al.*, 2008). On the surface, a trend in a heritable phenotypic trait in a direction concordant with natural selection provides putative support for microevolution. Our results reveal, however, the complexities that can underlie such patterns. In red squirrels, the association between parturition date and annual fitness was not due to a genetic or a permanent environment covariance, but rather to a residual covariance. As there was no genetic covariance between parturition date and annual fitness, PBVs had unsurprisingly not advanced over the two decades. Moreover, phenotypic variation in parturition date is adequately explained by a plastic response to variation in food abundance. In contrast, there is little evidence of a role of abiotic climate change because there has not been a significant change in temperature, during the specific time period we

interval: 0.07–0.20). Annual relative fitness, however, displayed negligible additive genetic variance, with wide HPD intervals ($V_A = 2.50 \times 10^{-4}$; HPD interval:
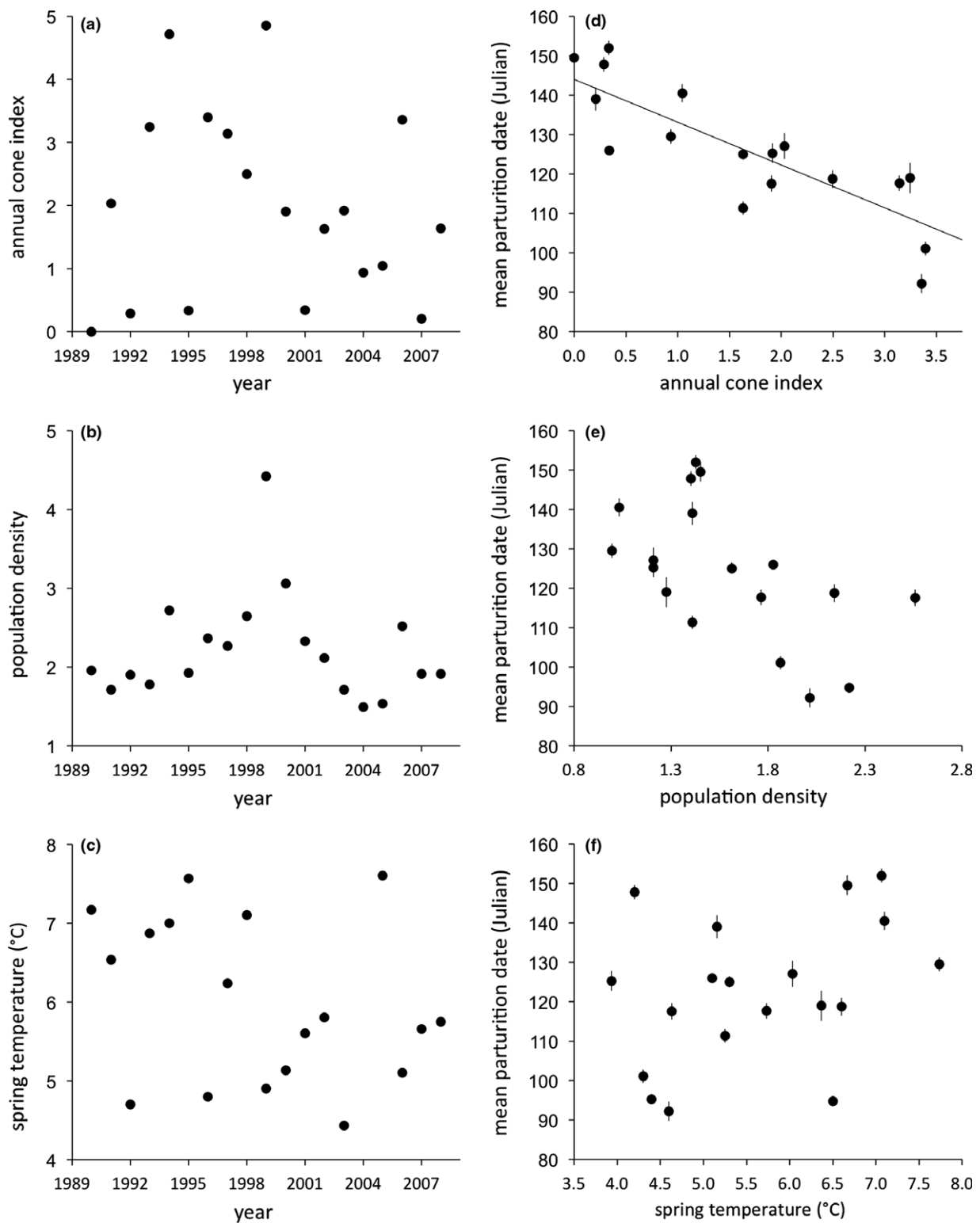
Fig. 2 Annual variation in, and the relationship to mean female red squirrel parturition date, for three environmental variables across two decades: (a) annual cone index of white spruce trees; (b) annual population density; (c) mean spring temperature; (d) relationship between annual cone index and mean parturition date; (e) relationship between annual population density and mean parturition date; and (f) relationship between mean spring temperature and mean parturition date.

Table 1 Model coefficients (fixed effects) and estimated variances (random effects) with 95% highest posterior density intervals of a within-subject mean centred Bayesian mixed effects model used to investigate influences on parturition date of female North American red squirrels.

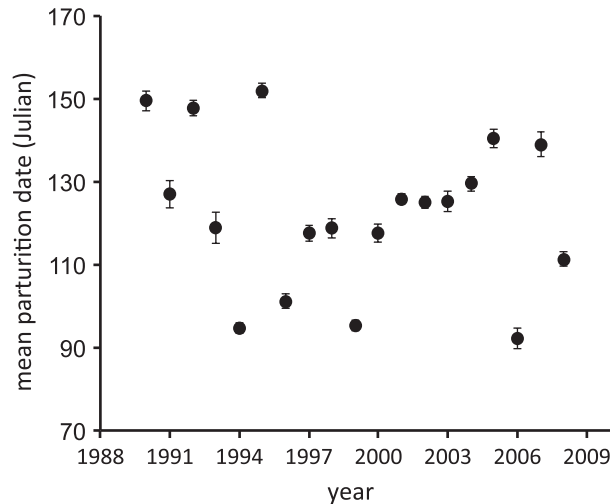| Effects	Model term	Estimate	95% Highest posterior density interval
Fixed effects	Intercept	140.71	135.08 to 145.81
	Age	−13.41	−15.96 to −11.46
	Age^2	1.42	1.19 to 1.86
	Between-subject effect (cone abundance)	−10.25	−13.62 to −5.55
	Within-subject effect (cone abundance)	−12.36	−15.27 to −7.28
	Between-subject effect (spring temperature)	2.79	−1.11 to 8.02
	Within-subject effect (spring temperature)	2.23	−1.69 to 7.47
	Between-subject effect (density)	−3.10	−11.23 to 6.51
	Within-subject effect (density)	4.16	−5.00 to 12.63
Random effects	ID	36.82	20.39 to 54.81
	Year	64.17	33.39 to 167.49
	Residual	211.76	191.56 to 237.31

Fig. 3 Variation in annual mean parturition date (Julian date ± SE) of female North American red squirrels in the study population over two decades.

analysed (01 April to 30 June) at our study site over the course of our study.

In some cases, the breeder's equation has proven predictive under natural conditions (Grant & Grant, 2006). Yet, in others it has been shown to have little predictive ability (e.g. Merilä *et al.*, 2001; and references

therein). As a result, the general utility of this tool for inferring microevolutionary change under natural conditions has been called into question (Morrissey *et al.*, 2011). While a heritable phenotypic trait not responding to selection at first seems puzzling, such stasis is understandable if the phenotypic association between trait variation and annual fitness is generated entirely by environmentally induced covariance (Price *et al.*, 1988; Rausher, 1992; Kruuk *et al.*, 2002), which was the case in our study of red squirrels. In this population, although heritability of parturition date is not exceptionally high (the mean heritability of life-history traits in a review of wild vertebrate populations was 0.33 ± 0.04; Postma, 2014), the breeder's equation did predict an advance in parturition dates of 0.25 days generation^{-1}. However, our multivariate analyses identified that the phenotypic association between parturition dates and annual fitness was due to a residual covariance as opposed to genetic or permanent environment covariance.

Statistically, a residual covariance indicates that a latent factor (i.e. an unmeasured variable) affects the two modelled traits (Brommer *et al.*, 2014). Ecologically, if such a trait (e.g. territory quality) independently influences parturition date and annual fitness, individuals on higher quality territories may both breed earlier and realize higher annual fitness, without a causal relationship between the two traits (Price *et al.*, 1988). The presence of a residual environmental covariance, but not a permanent environmental covariance in our dataset indicates that such environmentally induced covariance must be temporary for red squirrels. Occupancy of a territory and an associated food cache is a prerequisite for survival in this population, and most individuals, as a consequence, remain on a single territory across many years, if not their entire lives (Berteaux & Boutin, 2000). If specific territories were consistently of higher/lower quality, we would expect a repeatable, nongenetic, association between parturition date and annual fitness (i.e. a significant permanent environment covariance). Our estimates of a negligible permanent environment covariance, but a significant residual covariance suggests instead that variation in territory quality must be transient (i.e. a given territory may produce higher relative numbers of cones in some years, but lower than average numbers in others; LaMontagne *et al.*, 2013). A negative residual covariance and the low level of genetic variance in annual fitness (see also McFarlane *et al.*, 2014) are also consistent with previous work showing that, although selection on parturition date is typically negative (e.g. Fisher *et al.*, 2017), there are temporal fluctuations in the strength, and occasionally direction, of selection (McAdam & Boutin, 2003; Williams *et al.*, 2014).

Phenological shifts are the most commonly reported ecological responses to anthropogenic climate change

Table 2 Estimates of (co)variance components of and between annual parturition date (PD) and annual fitness (AF) for female North American red squirrels.

Model	Variance components (PD)				Variance components (AF)			
	Year Variance (V_{YEAR})	Additive genetic variance (V_A)	Permanent environment variance (V_{PE})	Residual variance (V_R)	Year variance (V_{YEAR})	Additive genetic variance (V_A)	Permanent environment variance (V_{PE})	Residual variance (V_R)
1) Full	6.85×10^{-5} (2.15×10^{-8} to 0.02)	0.14 (0.07 to 0.20)	3.56×10^{-4} (5.26×10^{-11} to 0.04)	0.74 (0.66 to 0.80)	8.20×10^{-5} (6.73×10^{-8} to 0.02)	2.50×10^{-4} (1.02×10^{-7} to 0.13)	1.81×10^{-3} (1.33×10^{-7} to 0.20)	4.20 (3.77 to 4.47)
2) V_A and COV_A omitted	1.56×10^{-4} (1.09×10^{-9} 0.02)	n.f.	0.12 (0.06 to 0.18)	0.74 (0.68 to 0.83)	1.94×10^{-8} (1.20×10^{-8} to 0.02)	n.f.	1.36×10^{-3} (3.87×10^{-8} 0.23)	4.14 (3.83 to 4.55)
3) V_A, COV_A, V_{PE} and COV_{PE} omitted	7.39×10^{-5} (1.30×10^{-7} to 0.02)	n.f.	n.f.	0.87 (0.80 to 0.94)	1.26×10^{-4} (7.01×10^{-9} to 0.02)	n.f.	n.f.	4.26 (3.89 to 4.57)

Model	Covariance between PD and AF				Selection gradient between PD and AF			
	Year covariance (COV_{YEAR})	Additive genetic covariance (COV_A)	Permanent environment covariance (COV_{PE})	Residual covariance (COV_R)	Year gradient	Additive genetic gradient	Permanent environment gradient	Residual gradient
1) Full	-4.54×10^{-5} (-8.42×10^{-3} to 8.38×10^{-3})	-3.75×10^{-5} (-0.01 to 0.02)	-1.26×10^{-4} (-0.04 to 0.02)	-0.07 (-0.18 to 0.03)	-0.15 (-3.61 to 3.60)	-1.85×10^{-3} (-0.83 to 0.13)	3.63 (-15.04 to 12.75)	-0.10 (-0.25 to 0.05)
2) V_A and COV_A omitted	-4.50×10^{-5} (-8.16×10^{-3} to 7.42×10^{-3})	n.f.	-4.84×10^{-4} (-0.12 to 0.03)	-0.08 (-0.19 to 0.03)	0.49 (-3.83 to 3.07)	n.f.	3.66×10^{-4} (-1.13 to 0.22)	-0.10 (-0.26 0.04)
3) V_A, COV_A, V_{PE} and COV_{PE} omitted	1.68×10^{-5} (-5.84×10^{-3} to 8.37×10^{-3})	n.f.	n.f.	-0.15 (-0.22 to -0.01)	0.19 (-2.94 to 4.44)	n.f.	n.f.	-0.16 (-0.25 to -0.01)

Estimates are provided for three models: (1) the full model includes the year, additive genetic, permanent environment and residual (co)variance estimates; in (2), the additive genetic (co)variance terms are omitted; and in (3), the permanent environment (co)variance terms are omitted. Values are reported with 95% HPD intervals in parentheses. 'n.f.' indicates 'not fitted'.
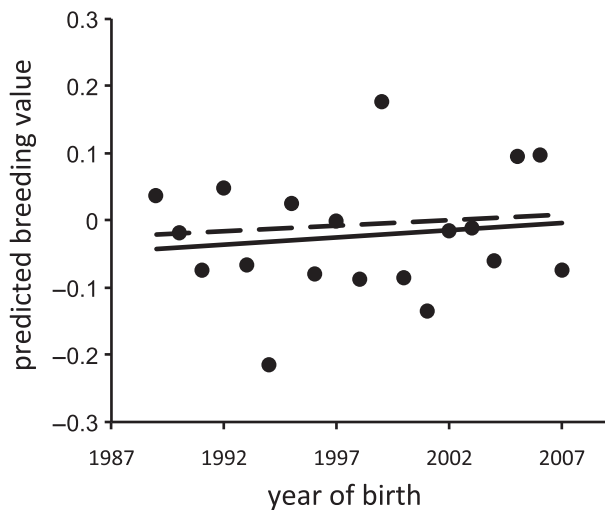
Fig. 4 Predicted breeding values for parturition date in female North American red squirrels during 1989–2007 (filled circles and solid line). The expected change due to random genetic drift (dashed line) is also represented.

(Parmesan, 2006). However, climate change is occurring in concert with other aspects of environmental change (anthropogenic or otherwise; Millenium Ecosystem Assessment, 2005). There is thus a risk of incorrectly assigning climate change as the cause of an observed phenological shift. There has not been a trend in spring temperatures at our study location during the specific period we investigated (01 April to 30 June) in either of the two decades we assessed. Instead, food abundance strongly influences parturition dates (Figs 1c and 2d; Williams *et al.*, 2014), with earlier breeding occurring in years following mast events (i.e. the synchronous production of very large seed crops; LaMontagne & Boutin, 2009). During the first decade, there was an increase in the number of lifetime available cones (Fig. 1b) that, in turn, was driven by two mast events (in 1993 and 1998; Fletcher *et al.*, 2010). Across the full study period, however, there has been neither a significant linear trend in cone abundance or parturition date. Thus, while the initially reported advance resembled that which has been reported across myriad taxa in response to climate change, it is now apparent that it resulted from a plastic response to natural environmental variation (above average food production) rather than climate change.

Globally, the effects of climate change are expected to be particularly pronounced at high latitudes (IPCC 2014). In the Kluane region, although we did not detect a trend in spring temperatures during the two decades we investigated, there has been a general warming in overall annual temperatures in recent decades (Krebs, C.J., unpub. data). There is thus the potential for life histories within this population to be affected by climate change, but this effect is likely to occur indirectly. The size of the white spruce cone crop in a given year is correlated to climate conditions one and 2 years prior (Krebs *et al.*, 2012). Shifts in parturition dates of red squirrels, as a result, could occur, and these shifts are most likely to be due to indirect influences operating through changes in their food resources.

To date, the majority of phenological research on vertebrates has tended to focus on income breeding species, such as insectivorous passerines (e.g. Both *et al.*, 2006). In these systems, selection favours synchronization of breeding with seasonal variation in food abundance. By storing food or fat in anticipation of reproduction, capital breeding species are freed from this constraint. We expect that phenological responses to food abundance (as opposed to temporal availability) may thus be common in these species and encourage further research into influences on phenology across a range of breeding strategies.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1 Supplementary materials and methods.