Selection on female behaviour fluctuates with offspring environment

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Abstract

Temporal variation in selection has long been proposed as a mechanism by which genetic variation could be maintained despite short-term strong directional selection and has been invoked to explain the maintenance of consistent individual differences in behaviour. We tested the hypothesis that ecological changes through time lead to fluctuating selection, which could promote the maintenance of variation in female behavioural traits in a wild population of North American red squirrels. As predicted, linear selection gradients on female aggression and activity significantly fluctuated across years depending on the level of competition among juveniles for vacant territories. This selection acted primarily through juvenile overwinter survival rather than maternal fecundity. Incorporating uncertainty in individual measures of behaviour reduced the magnitude of annual selection gradients and increased uncertainty in these estimates, but did not affect the overall pattern of temporal fluctuations in natural selection that coincided with the intensity of competition for vacant territories. These temporal fluctuations in selection might, therefore, promote the maintenance of heritable individual differences in behaviour in this wild red squirrel population.

Introduction

Fluctuating selection has long been proposed as a mechanism by which genetic variation might be maintained (Levins, 1968; Ellner & Hariston, 1994; Ellner & Sasaki, 1996) despite strong directional selection being common (Kingsolver et al., 2001). If selection fluctuates strongly in direction, then overall directional selection may be reduced, limiting any erosion of genetic variation (Bell, 2010). Fluctuating selection has been documented in a few systems (e.g. Grant & Grant, 2002), and enough longitudinal studies of selection have been undertaken that Siepielski et al. (2009) were able to conduct a meta-analysis documenting the prevalence of variation in selection. Morrissey & Hadfield (2012) sub-

sequently identified that much of this variation could be explained by sampling error, which highlights the need for comprehensive, longitudinal studies of the ecological causes of variation in natural selection.

Although behaviour was previously thought to be very labile, the persistence of individual differences in behaviour has stimulated much recent research (Gosling, 2001; Sih et al., 2004a, b; Réale et al., 2007, 2010), with persistent behavioural differences increasingly accepted as important traits with ecological and evolutionary consequences (Sih et al., 2004a, b; Réale et al., 2010). Individual differences in behaviour (established by repeatedly measuring the behaviour of individuals) have been shown to have important fitness effects in a number of systems (Dingemanse & Réale, 2005; Smith & Blumstein, 2008), and fluctuating selection has been proposed as a mechanism that might maintain these individual differences (Dingemanse et al., 2004; Boon et al., 2007; Dingemanse & Wolf, 2010).

Studies of the effects of exploration and activity on fitness in variable environments have found mixed

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results. Dingemanse *et al.* (2004) found evidence that selection on exploration in great tits (*Parsus major*) fluctuated over a 3-year period; however, Bergeron *et al.* (2013) found no evidence for fluctuations in selection on activity in eastern chipmunks (*Tamias striatus*). Bergeron *et al.* (2013) did find that selection on activity for survival was disruptive, indicating that nonlinear fitness may play important roles in maintaining variation in behavioural traits. Aggression is often used as an exemplary trait when discussing the fitness consequences of exhibiting consistent behaviour across contexts (e.g. Sih *et al.*, 2004b). It is somewhat intuitive that aggression should be beneficial in competitive environments when acquisition and/or defence of resources is valued, but detrimental in noncompetitive environments where aggression may provoke unnecessary and costly encounters with conspecifics or predators. The fitness consequences of aggression might also be nonlinearly related to competition. For example, increased aggression may be beneficial until the number of competitive encounters is so high that aggression becomes detrimental (Knell, 2009). However, to our knowledge, no one has yet measured natural selection on aggression under conditions of high competition for resources and low competition for resources.

Consistent individual differences in behaviour have been previously documented in a population of North American red squirrels (*Tamiasciurus hudsonicus*) near Kluane National Park, Canada (Boon *et al.*, 2007; Taylor *et al.*, 2012). Individual differences in red squirrel behaviour have been quantified using open-field (OF) trials, a mirror–image stimulation (MIS) test and handling tests (Boon *et al.*, 2007; Taylor *et al.*, 2012) and have been interpreted as measurements of activity, aggression and docility. Activity characterizes motor movements in response to a novel environment and is similar to measures of exploration in other systems (e.g. Walsh & Cummins, 1976; Dingemanse *et al.*, 2002; Careau *et al.*, 2008; Martin & Réale, 2008). In Kluane red squirrels, aggression and activity are positively genetically correlated (Taylor *et al.*, 2012), which is consistent with a 'proactive–reactive' behavioural syndrome (Koolhaas *et al.*, 1999). Docility is negatively phenotypically correlated with aggression and activity (Boon *et al.*, 2007; Taylor *et al.*, 2012) and is best thought of as the response to stressful confinement.

Northern populations of red squirrels experience large fluctuations in available resources due to episodic, synchronized mast seed production by white spruce (*Picea glauca*; Lamontagne & Boutin, 2007). White spruce seeds are an important winter food source for red squirrels (Fletcher *et al.*, 2013), and seed production by white spruce can vary annually across 3 orders of magnitude (Boutin *et al.*, 2006). Annual fluctuations in spruce cone production have been positively correlated with spring population density (Dantzer *et al.*, 2013), the opportunity for natural selection on red squirrels

(McAdam & Boutin, 2003a) and the strength of selection on juvenile growth rates (Dantzer *et al.*, 2013). Red squirrel females that recruit into the population (survive their first winter) have a live expectancy of 3.5 years and maximum lifespan of 8 years (McAdam *et al.*, 2007), so the vast majority experience large variation in population density and food resources over their lifetime. Furthermore, Boon *et al.* (2007) found evidence that the relationship between female red squirrel aggression and juvenile survival varied among years, but did not have data across a wide enough range of environments to test whether cone production affected selection on red squirrel behaviour.

Red squirrels defend exclusive territories and juveniles cannot dislodge adult territory owners, so they must acquire a vacant territory (and cache cones in a defendable larder hoard) to survive their first winter (Larsen & Boutin, 1994). In most cases, juveniles acquire a territory following the death of an adult, but in some cases, females bequeath part or all of their territory to one of their offspring, and in years of high cone production, juveniles can create new territories in previously unproductive areas (Price & Boutin, 1993; Berteaux *et al.* 2000). The competition juveniles face, therefore, depends on the ratio of the number of juveniles born to the number of territorial vacancies, both previously owned and newly created. Juvenile competition is both density-dependent (high population density means fewer territory vacancies) and resource-dependent; years of high cone production have reduced competition because juveniles can create new territories when resources are high (A. McAdam, S. Boutin & M. M. Humphries, unpublished data).

We hypothesized that temporal fluctuations in juvenile competition for vacant territories, which are associated with annual fluctuations in spruce seed production, cause annual fluctuations in natural selection on adult female (mothers) red squirrel behaviours. We predicted that years with higher competition among juveniles for vacant territories would be associated with stronger selection for aggression, whereas years with lower competition for vacant territories would be associated with relaxed or potentially negative selection for aggression. This selection on mothers via their offspring could occur through the heritable component of behaviour or through maternal care (Taylor *et al.*, 2012). Taylor *et al.* (2012) found a positive genetic correlation between aggression and activity, so we tested whether correlational selection between aggression and activity could have contributed to this genetic correlation. Finally, following Bergeron *et al.* (2013), we also tested for disruptive selection on behavioural traits.

Materials and methods

A population of wild red squirrels has been monitored since 1989 in the southwest Yukon (61°N, 138°W), and

a detailed description of the population and general methods can be found in McAdam *et al.* (2007). Red squirrels are small (150–250 g), diurnal, semi-arboreal rodents that inhabit much of forested North America (Steele, 1998). Individuals of both sexes defend exclusive year-round territories (Smith, 1968). All squirrels in each of two study areas were uniquely marked with numbered ear-tags as nestlings or at first capture after emergence from the natal nest, and followed from birth until death. Nests of lactating females were entered immediately after parturition when young were counted and weighed, then again when young were approximately 25 days of age to tag and re-weigh the young for the estimation of growth rates (grams per day; McAdam & Boutin, 2003b). Parturition dates were calculated based on weight loss of the mother recorded during trapping or from the weight of young during the first nest entry.

Behavioural traits

We measured behavioural traits of individuals from two study areas that have been monitored continuously since 1989. Following Boon *et al.* (2007) and Taylor *et al.* (2012), we used three techniques to measure three red squirrel behavioural traits. Here, we give a brief description of the behavioural methods, but full details can be found in Boon *et al.* (2007) and Taylor *et al.* (2012). The first test, an OF trial in a portable arena, was used to measure an individual's activity in a novel environment (Walsh & Cummins, 1976; Martin & Réale, 2008). The second test was a MIS that measured aggression (Svendsen & Armitage, 1973). The testing arena for the OF and MIS tests was a 60 × 80 × 50 cm white corrugated plastic box with a clear acrylic lid through which the behaviour of the squirrel was recorded with a digital video camera. Four blind holes were placed in the floor to provide the focal squirrel with the opportunity to explore. OF and MIS trials were both performed in the same testing session (trial) with OF performance being tested first so that it would also serve as a habituation period for the MIS trial, during which a 45 × 30 cm mirror at one end of the arena was exposed. Individual squirrels were tested within 1 h of being trapped on their territory. The third test, conducted during routine handling events, measured docility as the struggle rate of individuals confined in a mesh handling bag. To measure docility, squirrels were immediately transferred from the trap into a handling bag and placed onto dry ground, and the proportion of time the squirrel spent struggling was measured over 30 s. The exclusive territoriality of red squirrels and their high trappability allowed for complete enumeration of the study population through targeted trapping and behavioural observations. Importantly, the ability to target individuals let us avoid sampling bias (Biro & Stamps, 2008; Boon *et al.*, 2008).

In 2005, 2008, 2009 and 2010, we performed 359 OF and MIS trials on 105 female red squirrels and 2266 docility tests on 141 female squirrels for which we had fitness data.

We quantified each squirrel's behaviour by scoring either the rate or the proportion of time each squirrel spent performing specific behaviours from the video-taped trials using JWatcher Video 1.0 (Blumstein & Daniel, 2007) and the same ethogram as Boon *et al.* (2007) and Taylor *et al.* (2012). We did not consider each of our measurements from the behavioural trials to be unique behaviours, and given the intercorrelation that necessarily results from mutually exclusive scores, we did not attempt to interpret them as unique behaviours. Instead, our goal was to collect many measurements that we hoped would provide a reliable overall assessment of the behaviour of squirrels under these conditions that have previously been found to have important ecological and evolutionary consequences (Boon *et al.*, 2007; Taylor *et al.*, 2012). We used the principal component loadings from Taylor *et al.* (2012) to reduce the redundancy among the measurements and calculate behavioural scores for each trial. To evaluate the appropriateness of the principal component analyses (PCA), we followed Budaev's advice (2010) and found no major violations of the assumptions of PCA. The behavioural data from both the open field and MIS tests were not multivariate normal; however, we are using the PCA to reduce dimensionality and redundancy and not as a statistical test, so the lack of normality should not be an issue. After subsampling the behavioural data to only include one sample per individual, we found nearly identical loadings as Taylor *et al.* (2012), which calculated principal component loadings using the full data set, so we used loadings from the full data set to remain consistent with Taylor *et al.* (2012).

All further analyses used the scores calculated from the first principal component loadings for each trial and will be referred to as activity (OF principal component 1) and aggression (MIS principal component 1).

Fitness

We used annual reproductive success (ARS), calculated as the number of offspring that survived overwinter to the following spring (March 1st), as our primary measure of fitness. The study areas are large relative to the dispersal distance of juvenile squirrels (Berteaux & Boutin, 2000), and previous comparisons of the survival of juveniles on the edge of our study areas compared with survival of juveniles in the core support our assumption that disappearance represents death (Kerr *et al.*, 2007; McAdam *et al.*, 2007). We used ARS as the primary measure of fitness because it combines the total production of each female (i.e. quantity) with the ability of the offspring to survive their first winter and

recruit into the population, a life-history event with a large opportunity for selection (McAdam & Boutin, 2003a; McAdam *et al.*, 2007). However, to better understand the agent of selection, we also investigated selection based on these two components of ARS: fecundity and offspring overwinter survival (OWS). Fecundity is the total number of offspring produced by each individual each year. OWS was calculated for each female as the proportion of offspring produced that survived to the following spring. Maternity was assigned during nest entries (see above).

Multiple measurements of behaviour and fitness

We measured behaviour and fitness multiple times for the majority (70% and 60%, respectively) of individuals in this study, which if unaccounted for could result in pseudoreplication in associations between traits and fitness (Hurlbert, 1984). Pseudoreplication can be accounted for in a mixed model by specifying a random effect of individual (Pinheiro & Bates, 2009) or by consolidating the multiple measures into a single estimate. In our case, accounting for the pseudoreplication via random (or grouping) effects in mixed models was not possible because current model-fitting frameworks that make use of bivariate models to measure selection do not allow for the correct error structure for multiple measures on different scales. We instead adopted a hybrid approach, consolidating the multiple measures of behaviour into a single measure and then accounting for the multiple measures of fitness per individual by fitting subsequent models of fitness with individual as a random effect. This is a common approach that has been implemented widely in evolutionary ecology research by extracting best linear unbiased predictors (BLUPs) from mixed models with the repeated-measures trait as the response and individual as a random effect. This approach has the additional benefit that fixed effects can be fitted to account for known measurement biases (e.g. effect of observer or trial order, see next section). This has the result of estimating, for each individual, one behavioural score for each trait such that each individual's behaviour was treated as a fixed trait that did not vary among years. All behavioural traits were repeatable within and across years showing that the individual differences in behaviour are persistent; therefore, this analysis concerns the consistent differences in behaviour among individuals, and not behavioural plasticity or behavioural reaction norms (Table S1).

However, as noted by Hadfield *et al.* (2010), using the point estimate of random effects in further analyses does not account for uncertainty around this consolidated measure and so may lead to anticonservative conclusions. In addition to performing a selection analysis using single point estimates of each individual's behaviour traits (BLUPs), we also employed a Bayesian

approach and generated a posterior distribution of BLUPs for each individual using Markov chain Monte Carlo methods (MCMC), which reflected the degree of uncertainty around each individual's behavioural value. We then used these posterior distributions, rather than single BLUPs, to carry the uncertainty around each measure of behaviour through the rest of the analyses (Hadfield *et al.*, 2010). To assess the significance of subsequent regression analyses under this approach, we fitted each regression once for each sample of the posterior distribution of random effects. This resulted in a posterior distribution of 1000 samples of each parameter from the 1000 regression models. We compared these results to our analysis that used single point estimates of each individual's behavioural traits (BLUPs; see above) to determine how these different analyses affected our inferences.

Methodological sources of variation
We controlled for a number of methodological sources of variation in the behaviour measurements prior to evaluating selection on the behavioural traits by including fixed effects in the BLUP models. To account for effects of habituation, we included fixed effect terms for lifetime and yearly trial number as covariates for the activity and aggression scores. To account for habituation to the handling test, we included lifetime and yearly handling event as covariates. We included handling events where no struggle test was performed because handling for routine data collection is very similar to the struggle rate trial. We also included a quadratic term for trial numbers to account for a nonlinear response to repeated trials. To control for effects of seasonality and study area, we included day of year as a continuous fixed effect and study area as a categorical fixed effect. Finally, we included observer as a fixed effect in the docility models. Following Wilson (2008), we only attempted to control for methodological variation (measurement error) through the inclusion of fixed effects in our models and did not attempt to account for other biological sources of variation (e.g. age, sex, birth year, reproductive status or mass) to avoid removing phenotypic variation that might be relevant to selection. Results from these models are presented and discussed further by Taylor *et al.* (2012). The behavioural traits analysed here are, therefore, equivalent to those used by Taylor *et al.* (2012) to estimate the heritabilities and genetic correlations between traits.

Natural selection

To test whether selection on behaviour fluctuated across years, we fitted a generalized linear mixed model of female ARS that included the three behavioural traits (activity, aggression and docility) and year as a fixed factor. To test for temporal variation in selection, we

included interactions between year and the behavioural traits and tested the significance of the interactions between year and the behavioural traits using a type II analysis of deviance. To account for pseudoreplication (Hurlbert, 1984), we fitted the models with study area as a fixed effect and individual as a random effect. All generalized linear mixed models for ARS and fecundity were fitted assuming a Poisson error distribution using a log link function (Bolker *et al.*, 2009). We fitted an observational level random affect to account for overdispersion (Atkins *et al.*, 2013). To estimate the effect of behaviour on OWS, we used generalized linear mixed models with a binomial error distribution weighted by fecundity.

Selection gradients

We estimated linear selection gradients (Lande & Arnold, 1983) using linear regression models of relative fitness separately for each year. Unless noted, all selection gradients are SD-standardized. Nonstandardized and mean-standardized gradients are presented in the Tables S4–S8. We calculated relative fitness and standardized traits for each study area–year combination, but combined standardized data from multiple study areas for phenotypic selection analyses within each year because of limited sample sizes within each study area–year combination. All three behavioural traits were included in the selection gradient models. The inclusion of an additional three life-history traits (offspring growth rate, litter size and parturition date) that have been shown to affect fitness in red squirrels (McAdam & Boutin, 2003b; Réale *et al.*, 2003) did not substantially affect the selection gradients for the behavioural traits, so we have reported selection gradients from the simpler models that did not include the life-history traits. We calculated summary statistics describing yearly selection gradients to facilitate comparison of our results to recent meta-analyses of variation in selection (Kingsolver & Diamond, 2011; Siepielski *et al.*, 2011; Morrissey & Hadfield, 2012). Confidence intervals for selection gradients were calculated using the sim() function in the R package 'arm' (Mitchel-Olds & Shaw, 1987; Gelman & Su, 2014).

Natural selection and juvenile competition

We assessed how selection on the three behavioural traits was affected by the amount of competition among juveniles for vacant territories. Competition was measured for each year as the total number of offspring produced in the population divided by the total number that recruited into the population. To test for an effect of juvenile competition on natural selection for these behavioural traits, we first fitted models of ARS, fecundity and OWS with the linear terms for the behavioural traits and interactions between competition and the behavioural traits. Then, to investigate our *a priori* predictions about nonlinear selection, we fitted models of

ARS, fecundity and OWS, to linear and quadratic behavioural terms and the interaction between aggression and activity. We also included interaction terms between competition and the linear and quadratic behavioural terms and the three-way interaction between competition, aggression and activity, which assessed whether correlational selection on aggression and activity depended on juvenile competition. Removing nonlinear and interaction terms from the model had no effect on linear selection, so we report results from the complete model.

All analyses were performed using R 3.0.3 (R Core Team, 2014). Generalized linear mixed models were fitted using the lme4 package (Version 1.0-4; Bates *et al.*, 2014) and the MCMCglmm package (Version 2.19, Hadfield, 2010), and figures were plotted using the ggplot2 package (Version 0.9.3.1, Wickham, 2009).

Results

Temporal variation and annual selection

Aggression and activity each interacted significantly with year (aggression: $\chi^2 = 22.84$, d.f. = 7, $P < 0.005$; activity: $\chi^2 = 22.92$, d.f. = 7, $P = 0.005$), showing that selection for these traits was not consistent across the 8 years that we studied (Table S3), but we did not find a significant interaction between year and docility ($\chi^2 = 10.33$, d.f. = 7, $P = 0.17$). We, therefore, estimated linear selection gradients separately for each year.

Standardized selection gradients for female behavioural traits based on single BLUP realizations (Table 1 and Fig. 1a) fluctuated in sign across years and were significantly positive for aggression in 2006 ($\beta' = 0.66$, CI = 0.13–1.19), 2008 ($\beta' = 0.78$, CI = 0.20–1.54) and 2009 ($\beta' = 0.56$, CI = 0.05–1.20), but negative in 2005 ($\beta' = -0.41$, CI = −0.71 to −0.04). Selection for activity was significantly negative in 2009 ($\beta' = -1.01$, CI = −1.84 to −0.47) and, selection for docility was significantly negative in 2009 ($\beta' = -0.56$, CI = −1.07 to −0.06). Selection on activity and selection on docility occurred in the same direction in each of the 8 years, but was not significantly correlated (correlation among years, $r = 0.50$, $n = 8$ years, CI = −0.31 to 0.89, Fig. 2), but selection on activity was in the opposite direction of aggression for seven of those years (correlation between activity and aggression for all years, $r = -0.80$, $n = 8$ years, CI = −0.96 to −0.21) and selection on aggression and docility was opposite in 7 of 8 years and negatively correlated (correlation between aggression and docility for all years, $r = -0.72$, $n = 8$ years, CI = −0.94 to −0.03).

After accounting for uncertainty in our BLUP measures, standardized selection gradients for docility were significantly negative in 2006 ($\beta' = -0.25$,

Table 1 Selection gradients for aggression and activity significantly fluctuated in sign over an 8-year period. Standardized linear selection gradients (β') ± standard errors for female behavioural traits through annual reproductive success (ARS). Selection gradients were estimated from single realized best linear unbiased predictors (BLUPs) of behavioural traits. 95% confidence intervals were generated using the sim() function in the R package arm. Sample sizes for the number of adult females each year (N females), the number of offspring produced by the females (N offspring) and the number of offspring that survived overwinter to the next spring (N ARS) are given for each year. Bold highlights confidence intervals that do not overlap zero.

Year	Aggression	Activity	Docility	N females	N offspring	N ARS
2003	−0.17 (−1.25 to 0.57)	0.86 (−0.20 to 1.96)	−0.40 (−1.22 to 0.61)	13	36	11
2004	−0.62 (−1.17 to 0.22)	0.49 (−0.37 to 1.29)	0.26 (−0.29 to 1.01)	21	59	17
2005	**−0.41 (−0.71 to −0.04)**	0.34 (−0.10 to 0.76)	0.19 (−0.11 to 0.54)	41	184	62
2006	**0.66 (0.13 to 1.19)**	−0.23 (−0.96 to 0.35)	−0.41 (−0.92 to 0.17)	48	149	20
2007	−0.07 (−0.57 to 0.48)	0.15 (−0.44 to 0.63)	0.03 (−0.47 to 0.52)	34	127	35
2008	**0.78 (0.20 to 1.54)**	−0.44 (−1.64 to 0.39)	−0.17 (−0.81 to 0.45)	37	127	12
2009	**0.56 (0.05 to 1.20)**	**−1.01 (−1.84 to −0.47)**	**−0.56 (−1.07 to −0.06)**	33	115	22
2010	−0.02 (−0.43 to 0.56)	0.10 (−0.34 to 0.50)	−0.21 (−0.47 to 0.16)	18	125	36

$CI_{0.95} = -0.58$ to -0.03), 2009 ($\beta' = -0.30$, $CI_{0.95} = -0.53$ to -0.05) and 2010 ($\beta' = -0.23$, $CI_{0.95} = -0.35$ to -0.10; Fig. 1b and Table S5). 95% credible intervals of selection gradients for female aggression and activity overlapped zero in all years.

When comparing selection gradients from analyses based on single BLUP realizations vs. the MCMC approach (Fig. 3 and Table 4), we found that selection gradients estimated from the two approaches were highly correlated (Aggression = 0.96, $CI_{0.95} = 0.79$–0.99; Activity = 0.89, $CI_{0.95} = 0.51$–0.98; Docility = 0.95, $CI_{0.95} = 0.74$–0.99), but selection gradients based on single BLUP realizations were higher (Mean absolute difference: Aggression 0.23, Activity 0.24, Docility 0.09).

We calculated summary statistics for the standardized selection gradients in order to frame our results in terms of recent meta-analyses of fluctuating selection (Table 2). A high ratio of 'mean of absolute values' to the 'absolute value of the mean' of selection gradients indicates that fluctuations in selection reduced overall directional selection (*sensu* Kingsolver & Diamond, 2011). These ratios were high for aggression (4.5 : 1) and activity (15 : 1), but lower for docility (1.75 : 1). We found larger standard deviations among selection gradients relative to the mean of their standard errors for aggression (0.52–0.30) and activity (0.58–0.36), which indicate that variation in selection was not due to sampling error (*sensu* Morrissey & Hadfield, 2012). In contrast, the standard deviation of selection gradients for docility was low and similar to the mean standard error (0.30–0.28).

Natural selection and juvenile competition for vacant territories

We tested whether competition among juvenile squirrels for vacant territories explained the observed variation in temporal selection by (i) testing for correlations between selection gradients and juvenile competition and (ii) fitting generalized linear mixed models.
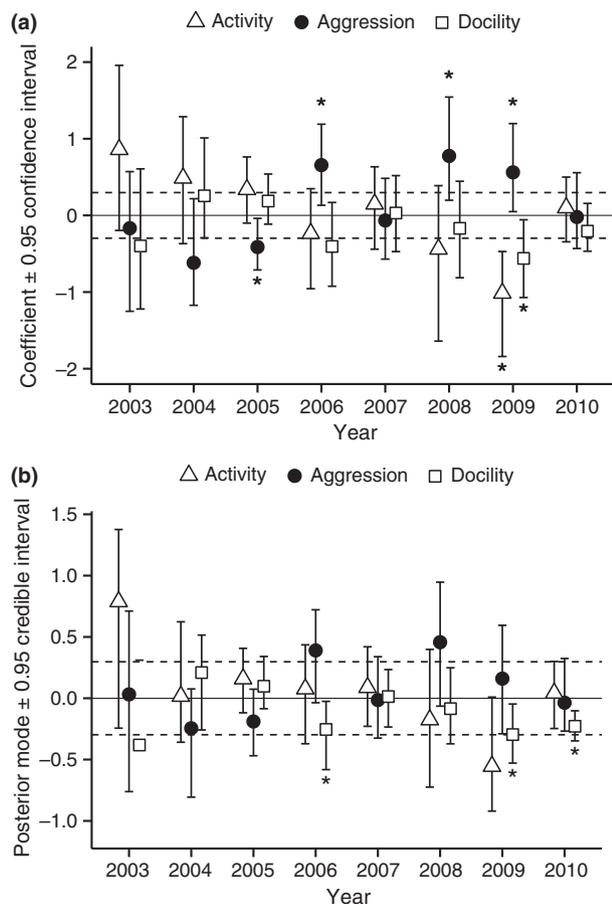
Selection gradients
Aggression selection gradients (β') for ARS were positively correlated with competition (Fig. 4, r = 0.87, CI = 0.43–0.98, $N = 8$ years). Docility selection gradients were negatively correlated, but the relationship was not significant ($r = -0.65$, CI = −0.93 to 0.01). Activity selection gradients were also negatively, but not significantly correlated with competition ($r = -0.48$, CI = −0.89 to 0.34). After accounting for uncertainty in the behavioural measurements, we found very similar correlations between selection gradients and juvenile competition. Selection gradients (β') for female aggression, through ARS, were positively correlated with competition ($r = 0.95$, $CI_{0.95} = 0.76$–0.99), while selection gradients for activity ($r = -0.14$, $CI_{0.95} = -0.77$ to 0.63) and docility ($r = -0.52$, $CI_{0.95} = -0.90$ to 0.29) were negatively, but not significantly correlated.

Generalized linear mixed models
For ARS, we found a significant positive interaction between maternal aggression and the degree of competition among juveniles for vacant territories (Table 3, $b = 3.40 \pm 0.92$, $Z = 3.68$, $P < 0.001$), and negative interactions between activity and competition ($b = -2.45 \pm 0.84$, $Z = -2.90$, $P = 0.004$), and docility and competition ($b = -1.41 \pm 0.69$, $Z = -2.04$, $P = 0.041$). We also found a significant interaction between the effects of aggression and activity on ARS ($b = 1.01 \pm 0.43$, $Z = 2.35$, $P = 0.019$), and a three-way interaction between the effects of aggression, activity and competition on ARS was significant ($b = 2.62 \pm 1.32$, $Z = 1.99$, $P = 0.046$; after removing the three-way interaction from the model, the two-way interaction between aggression and activity was not significant). Finally, there were no significant interactions between competition and quadratic terms for the behavioural traits on ARS.

(a)

(b)

Fig. 1 Directional selection gradients (β') $\pm$ credible or confidence intervals (0.95) of annual reproductive success for female aggression, activity and docility. Dashed lines represent 75th percentiles for directional selection gradient magnitudes measured in the wild as compiled by Kingsolver *et al.* (2001). Stars indicate 95% credible or confidence intervals that excluded zero. The selection gradient coefficients in plot (a) were calculated from single realized best linear unbiased predictors (BLUPs) of each individual's behavioural traits, while the posterior mode of selection gradients in plot (b) incorporated uncertainty around the behavioural measures.

Results for OWS were similar to those for ARS. Aggression interacted positively with competition (Table 3, $b = 4.75 \pm 1.28$, $Z = 3.70$, $P < 0.001$), while activity interacted negatively ($b = -2.89 \pm 1.20$, $Z = -2.40$, $P = 0.016$. However, docility did not interact significantly with competition on OWS ($b = -0.78 \pm 0.90$, $Z = -0.86$, $P = 0.39$). There was a significant interaction between aggression[2] and competition ($b = -3.11 \pm 1.36$, $Z = -2.29$, $P = 0.022$) that resulted in stabilizing selection on aggression in high-competition environments, but no nonlinear selection in low-competition environments (Fig. S1). Finally, the three-way interaction between aggression, activity and competition was significant ($b = 3.95 \pm 2.13$, $Z = 2.13$, $P = 0.034$), indicating that correlational selection

between aggression and activity was strong when juvenile competition was high but weak when competition was low (Figs S1 and S2).
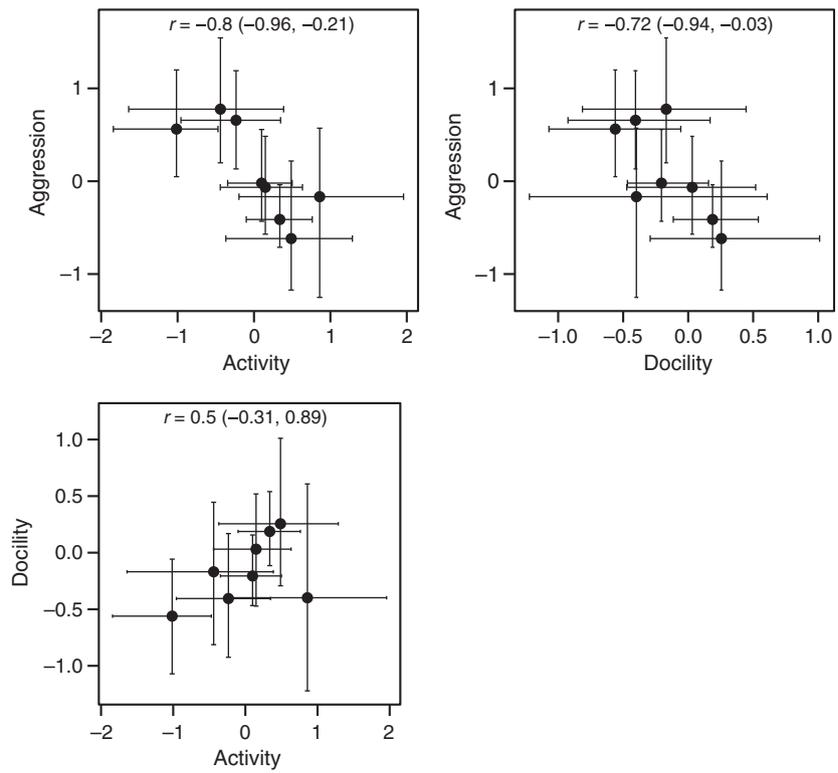
We did not find any significant effects of the behavioural traits on fecundity (Tables S9 and S10).

After accounting for uncertainty in the behavioural measurements, only the interactions between aggression and competition (Table S9; $b = 1.71$, $CI_{0.95} = 0.48$–2.66), and docility and competition ($b = -0.70$, $CI_{0.95} = -1.55$ to -0.26) on ARS remained significant, as assessed by overlap of 0.95 credible intervals with zero. For selection through OWS, only the interaction between competition and aggression was significant ($b = 2.16$, $CI_{0.95} = 0.55$–3.53).
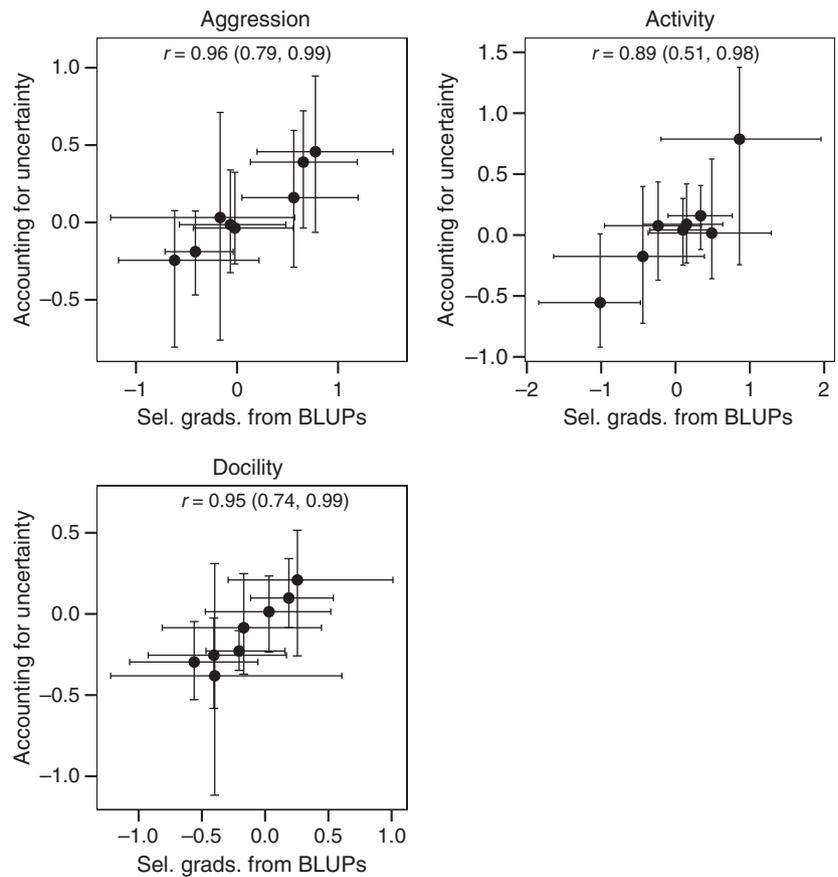
Discussion

We found temporal fluctuations in selection on female aggression and activity that confirm Boon *et al.*'s (2007) suggestion that selection for activity and aggression in red squirrels fluctuates through time. Moreover, the magnitude of linear selection gradients for dam aggression and activity repeatedly exceeded the 75th percentile of selection gradients assembled by Kingsolver *et al.* (2001). In a meta-analysis of a large data set, Siepielski *et al.* (2009) found that selection commonly varies from year to year, but acknowledged that measurement error may contribute substantially to this variation. Morrissey & Hadfield (2012) re-analysed a portion of Siepielski *et al.*'s data set to show that the majority of variation in selection could be explained by measurement error, which led to their conclusion that selection was actually 'remarkably consistent in time' (Morrissey & Hadfield, 2012). Nevertheless, fluctuating selection clearly plays a strong role in a number of natural systems (e.g. Darwin's finches, Grant & Grant, 2002; side-blotched lizards, Svensson & Sinervo, 2004; see also Bell, 2010) and remains an intuitive and plausible mechanism for the maintenance of genetic variation. We calculated summary statistics for the 8 years of selection gradients in our study (Table 2) and found evidence that fluctuations in aggression and activity were unlikely to be due to sampling error, additionally generalized linear mixed models supported an interaction between year and the behavioural traits on fitness.

Evidence of fluctuating selection on behaviour in other systems is mixed. In a 3-year study of exploration and selection in great tits (*P. major*), Dingemanse *et al.* (2004) found evidence that the direction of selection on exploration fluctuated across years, but in a 5-year study of eastern chipmunks (*T. striatus*), Bergeron *et al.* (2013) found no evidence of fluctuating selection on exploration. Our results, along with a previous study showing that red squirrel behavioural traits are heritable (Taylor *et al.*, 2012), suggest that temporally fluctuating selection could play a role in maintaining variation in red squirrel behaviour despite associations

Fig. 2 Pairwise correlations of selection gradients for the three behavioural traits. Selection gradients were estimated from single realized best linear unbiased predictors (BLUPs) of behavioural traits.

Fig. 3 Selection gradients estimated after accounting for uncertainty (*y*-axis) were highly correlated with gradients estimated from single realized best linear unbiased predictors (BLUPs) (*x*-axis); however, they were generally lower magnitude.

Table 2 Summary statistics for standardized linear selection gradients (β') provide evidence for fluctuations in selection on aggression and activity. A high ratio of 'mean of absolute values' to the 'absolute value of the mean' of selection gradients indicates that fluctuations in selection reduced overall directional selection (*sensu* Kingsolver & Diamond, 2011). Large standard deviations among selection gradients relative to the mean of their standard errors indicate that variation in selection was not due to sampling error (*sensu* Morrissey & Hadfield, 2012). The frequency of sign changes was calculated as the number of changes in direction between successive years relative to $n-1$, where n is the total number of years (*sensu* Siepielski *et al.*, 2011). Standard errors were calculated as the credible interval/3.92.

	BLUPs analyses			Accounting for uncertainty		
	Aggression	Activity	Docility	Aggression	Activity	Docility
Mean $|\beta'|$	0.41	0.45	0.28	0.25	0.26	0.11
$|$Mean $(\beta')|$	0.09	0.03	0.16	0.09	0.12	0.03
SD of β'	0.52	0.58	0.30	0.30	0.33	0.15
Mean SE of β'	0.30	0.36	0.28	0.22	0.22	0.14
Frequency of β' sign changes	0.57	0.57	0.57	0.29	0.29	0.71

with fitness, a result that is consistent with the oligogenic rather than infinitesimal model of adaptation (Bell, 2010).

Juvenile competition as an agent of selection

We found strong associations between the direction of linear selection on maternal aggression and our hypothesized agent of selection – juvenile competition for vacant territories. Intraspecific aggression has long been hypothesized to have a close relationship with intraspecific competition (Stamps, 1977), and more recently, this relationship continues to be invoked as an intuitive example of how behaviour may be maladaptive when the environmental context varies (e.g. Sih *et al.*, 2004b; Bergmüller 2010; Bergmüller & Taborsky; Bell & Sih, 2007; Bell 2007; Dingemanse *et al.*, 2010). Aggression is thought to be favoured only when the benefits of winning intraspecific conflicts outweighs the potential costs of either unnecessary intraspecific encounters or carry-over into other contexts such as predation. Therefore, selection for aggression should be strong when intraspecific competition is high, but reversed when competition is low. Our evidence that selection for aggression varies positively with the intensity of intraspecific competition represents what we believe is the first empirical support for this commonly held expectation.
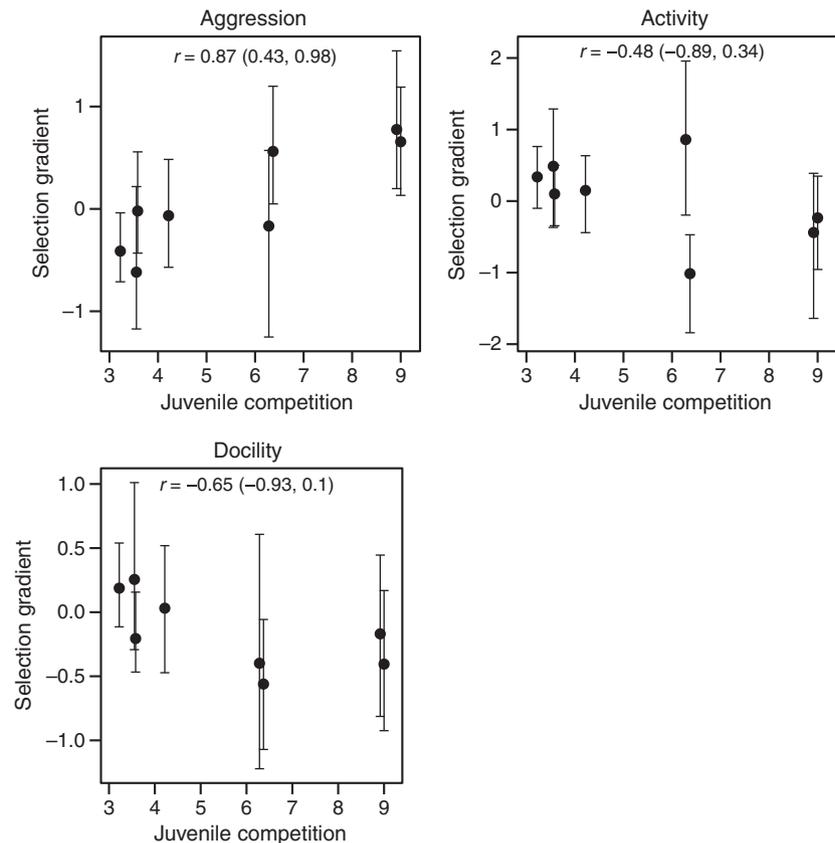
Linear selection for maternal docility was negatively correlated with juvenile competition. Positing explanations why selection on female docility varies with juvenile competition is not straightforward because we

have little insight into how docility might relate to agents of selection. Docility is the response to being trapped and handled and not simply the opposite of aggression (though it is weakly negatively correlated with aggression; Taylor *et al.*, 2012). Docility is also correlated with activity and may include a response to novel situations. It certainly captures the behavioural stress response of an individual, but whether the stress response of an individual to handling is correlated with its response to natural stress is unknown.

We also found that linear selection for maternal activity was negatively correlated with juvenile competition. Exploring possible mechanisms behind this relationship requires an understanding of the ecological dynamics behind variation in juvenile competition. Years with weak competition among juveniles for vacant territories are typically years of mast seed production during which a satiating level of spruce cones are produced (Fletcher *et al.*, 2010). In these years of super-abundant food, juveniles do not necessarily need to acquire a traditional territory following the death of a previous territory owner, but can instead establish a new territory. This requires the hoarding of sufficient spruce cones to be able to survive the winter, which might favour more active offspring. Activity has been found to be associated with food intake in a number of systems (reviewed in Biro & Stamps, 2008), but further work will be necessary to elucidate the relationships between hoarding ability and activity in red squirrels. In our discussion of the mechanisms by which maternal behaviour might affect the number of offspring recruited, we have implicitly assumed similarity in behaviour between mothers and offspring. We have previously found these behavioural traits to be heritable (Taylor *et al.*, 2012), but discuss this assumption further below.

Nonlinear selection

Selection for optimal combinations of traits can build up linkage disequilibrium between those traits, contributing to a genetic correlation. The correlational selection we identified between aggression and activity might, therefore, contribute to the genetic correlation that we have previously identified between these traits (Taylor *et al.*, 2012). However, recombination acts to break apart those linkages and will erode the linkage disequilibrium unless correlational selection is strong and persistent (Lynch & Walsh, 1998; Sinervo & Svensson, 2002). The correlational selection we identified is modulated by juvenile competition, such that it is strongest in high-competition years, but reduced or absent in low-competition years, so it seems unlikely that correlational selection alone maintains the observed genetic correlation. There might, therefore, be other mechanisms, such as physical linkage or genes with pleiotropic effects that contribute to the observed genetic correlation.

Fig. 4 Selection gradients (β') for female aggression through annual reproductive success were correlated with juvenile competition. Selection gradients were calculated from single point estimates of individual behaviour (BLUPs, best linear unbiased predictors) and were estimated for yearly data combined across study areas and plotted $\pm$ 0.95 confidence intervals.

The genetic correlation between aggression and activity also has implications for any expected response to linear selection. The overall response to selection is predicted to result from direct selection on the focal trait as well as an indirect response to selection on a genetically correlated trait (Lande & Arnold, 1983). We have previously found evidence of a positive genetic correlation between aggression and activity (Taylor *et al.*, 2012), but here found that directional selection on activity and aggression were consistently opposite in sign to one another (Fig. 4). This means that an evolutionary response to selection on aggression will be opposed by the indirect response to selection on activity, and vice versa, which will limit the degree to which directional selection can cause an evolutionary response in either of these behavioural traits. Opposing directional selection on these two traits is also expected to reduce the genetic correlation between aggression and activity through linkage disequilibrium (Lande & Arnold, 1983).

Fitness components

Our measure of ARS was comprised of a fecundity component and an offspring survival component. The separate analysis of these two components of ARS revealed that selection on female behavioural traits through ARS acted almost entirely through OWS (Table 4); we did not find any significant effect of female behaviour on fecundity (correlations between female-selection gradients for behaviour through ARS and OWS ranged from 0.94 to 0.99 all $P < 0.001$). Female behaviour could directly affect OWS if females intervened in offspring competition for vacant territories or preparation for winter, but these behaviours have not been observed, so if they occur they would be exceedingly rare. Females occasionally bequeath their territory to a juvenile (Price & Boutin, 1993; Berteaux & Boutin, 2000), and this could be considered a direct reflection of the mother's behaviour, but Boon *et al.* (2007) found no relationship between bequeathal and female aggression, activity or docility. In this population, it is more likely that parental behaviour influences OWS indirectly either through correlated parental care (e.g. maternal effects) or through parent–offspring phenotypic resemblance in behaviour given that behaviour is heritable and subject to maternal effects in this population (Taylor *et al.*, 2012). Juvenile squirrels, tested with the same behavioural trials shortly after emergence from their nest, exhibit the same OF and MIS behaviours as adult squirrels (Kelley, 2014). Maternal behaviour could also affect some other offspring trait, or traits, that causally affect offspring survival. Interestingly, Dantzer *et al.* (2013) showed that red squirrel

Table 3 Nonlinear selection on female aggression and activity interacted with juvenile offspring competition. A positive interaction between aggression and activity (positive correlational selection) was stronger in high-competition years. Also, a negative quadratic term for aggression (stabilizing selection) was stronger in high-competition years. See Figs S1 and S2 for visualizations of nonlinear interactions. The generalized linear mixed model for ARS assumed a Poisson error distribution, while the model for offspring overwinter survival (OWS) assumed a binomial error distribution weighted by fecundity. Random effects for identity and grid year were included in both models to account for pseudoreplication. An observation level random effect was included to account for overdispersion. Bold highlights coefficients where $P < 0.05$.

Component of fitness	ARS			OWS		
	Coefficient	Z value	P value	Coefficient	Z value	P value
Intercept	**−0.68 ± 0.31**	**−2.23**	**0.025**	**−1.92 ± 0.39**	**−4.87**	**< 0.001**
Competition	**−2.24 ± 0.91**	**−2.46**	**0.014**	**−2.48 ± 1.18**	**−2.11**	**0.035**
Aggression	**0.96 ± 0.31**	**3.06**	**0.002**	**1.33 ± 0.42**	**3.17**	**0.001**
Activity	**−0.66 ± 0.28**	**−2.35**	**0.019**	**−0.82 ± 0.38**	**−2.14**	**0.032**
Docility	**−0.51 ± 0.23**	**−2.18**	**0.029**	−0.49 ± 0.29	−1.70	0.088
Aggression^2	**−0.65 ± 0.32**	**−2.07**	**0.039**	**−1.02 ± 0.43**	**−2.36**	**0.018**
Activity^2	−0.30 ± 0.26	−1.16	0.247	−0.37 ± 0.36	−1.03	0.304
Docility^2	−0.18 ± 0.12	−1.48	0.139	−0.18 ± 0.15	−1.17	0.242
Competition × Aggression	**3.40 ± 0.92**	**3.68**	**< 0.001**	**4.75 ± 1.28**	**3.70**	**< 0.001**
Competition × Activity	**−2.45 ± 0.84**	**−2.90**	**0.004**	**−2.89 ± 1.20**	**−2.40**	**0.016**
Competition × Docility	**−1.41 ± 0.69**	**−2.04**	**0.041**	−0.78 ± 0.90	−0.86	0.388
Aggression × Activity	**1.01 ± 0.43**	**2.35**	**0.019**	**1.40 ± 0.60**	**2.35**	**0.019**
Competition × Aggression^2	−1.86 ± 0.96	−1.93	0.053	**−3.11 ± 1.36**	**−2.29**	**0.022**
Competition × Activity^2	−0.81 ± 0.77	−1.05	0.295	−1.17 ± 1.10	−1.06	0.291
Competition × Docility^2	−0.63 ± 0.39	−1.61	0.107	−0.50 ± 0.50	−0.98	0.325
Agg. × Act. × Competition	**2.62 ± 1.32**	**1.99**	**0.046**	**3.95 ± 1.86**	**2.13**	**0.034**
Random effect variance	ARS					OWS
Observation	0.000005					0.96
Individual identity	0.05					0.0001
Grid year	0.02					0.0006

mothers in this population respond plastically to cues from their environment to increase offspring survival, but our results here imply that mothers are unable to entirely adjust their behaviour to match conditions and therefore produce offspring with high survival only in years when environmental conditions match their behavioural phenotype. An analysis of the importance of behavioural plasticity and of consistent individual differences in plasticity (behavioural reaction norms; Dingemanse *et al.*, 2010) would be very interesting in this system.

Because male red squirrels do not provide parental care, an analysis of selection on male behavioural traits through OWS could provide evidence that selection is acting through heritable components of fitness. Alternatively, behaviour could be measured directly on juveniles to determine how this affects their survival under conditions of high and low competition. An analysis of juvenile overwinter survival (Kelley, 2014) did not find an effect of juvenile activity or aggression on overwinter survival; however, this study was limited to 1 year for which there was intermediate competition for vacant territories. Studies of juvenile squirrels are difficult because trappability of juveniles is low immediately after emergence, and juvenile death prior to measure-

ment could result in a bias (e.g. 'the invisible fraction'; Grafen, 1988; Hadfield, 2008). The relative importance of parental behaviour and offspring behaviour to offspring survival could be assessed directly in future studies by measuring juvenile behaviour before emergence or by looking for evidence of a genetic correlation between personality and survival using the analysis of a multigenerational pedigree (Hadfield, 2008; Sinervo & McAdam, 2008) even if phenotypic behavioural measurements are not available for offspring.

Uncertainty in behavioural measurements

In addition to the primary selection analyses that relied on single point estimates of behaviour (BLUPs), we also performed an analysis that accounted for uncertainty around the behavioural measurements. Although the point estimates from both analyses were highly correlated ($r = 0.89$–0.96), the MCMC analysis resulted in selection gradients and model coefficients that were lower in magnitude (approximately 50% mean reduction in magnitude), which resulted in more conservative estimates of selection. As a result, some of the higher-order effects that we saw based on single point estimates were not supported in our models that incor-

Table 4 Comparison of selection gradients accounting for behavioural measurement uncertainty (MCMC) with selection gradients calculated from single realized best linear unbiased predictors (BLUPs).

	Aggression	Activity	Docility
Correlation	0.96 (0.79, 0.99)	0.92 (0.61, 0.99)	0.95 (0.74, 0.99)
|Mean| MCMC	0.20	0.24	0.20
|Mean| BLUPs	0.41	0.45	0.28
Mean |Difference|	0.23	0.24	0.09
n years |BLUPs| > |MCMC|	8	7	7

porated uncertainty in our behavioural scores (e.g. effects of competition on nonlinear selection). However, our main findings that selection on female behaviour varies temporally, acts through OWS and coincides with competition among juveniles were robust to the incorporation of uncertainty in individual behavioural measures in our selection analysis. The potentially anticonservative use of BLUPs (Hadfield *et al.*, 2010) remains widespread in the behavioural literature, and our results represent a cautionary example of the hidden uncertainty in selection gradients that can arise when variable traits are statistically analysed as though they are known with certainty.

Conclusion

We tested the hypothesis that ecological changes through time lead to fluctuating selection, which might maintain variation in behavioural traits. Our results show that linear selection on female red squirrel aggression and activity is sometimes very strong, but fluctuates in sign across years depending on the level of competition among juveniles for vacant territories. Interestingly, selection on female aggression and activity was typically opposite in sign, but these two behavioural traits are positively genetically correlated, which means that evolutionary responses to selection would be reduced by selection acting on the other correlated trait. Our decomposition of ARS into its fitness components showed that selection on female red squirrel behaviour acted primarily through juvenile overwinter survival rather than fecundity.

These results, combined with previous work showing that these behavioural traits are heritable (Taylor *et al.*, 2012), suggest that variation in selection across years could help to maintain consistent individual differences in behaviour in this population. Furthermore, our results suggest that multivariate selection acting on multiple behavioural traits and the genetic associations between those traits are likely to have important consequences for our understanding of the evolution of animal personality.

Data accessibility

Data and code used in the analyses are available on git-hub (https://github.com/rwtaylor/2014-female-selection) and archived on Zenodo (http://dx.doi.org/10.5281/zenodo.11568)

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Repeatability within and across years.

Table S2 Variation in competition across years and study areas.

Table S3 Generalized linear mixed model results showing the interactions between year and the behavioural traits (BLUPs).

Table S4 Non-standardized linear selection gradients () for female behavioural traits through annual reproductive success after accounting for behavioural measurement uncertainty (see methods for details).

Table S5 SD-standardized linear selection gradients () for female behavioural traits through annual reproductive success after accounting for behavioural measurement uncertainty (see methods for details).

Table S6 Mean-standardized linear selection gradients () for female docility through annual reproductive success after accounting for behavioural measurement uncertainty (see methods for details).

Table S7 Non-standardized linear selection gradients () ± standard errors for female behavioural traits through annual reproductive success.

Table S8 Mean-standardized linear selection gradients () ± standard errors for female docility.

Table S9 Selection on female aggression and docility, through annual reproductive success, interacted significantly with juvenile offspring competition for vacant territories after accounting for behavioural measurement uncertainty.

Table S10 There was no evidence for selection on female behaviour through fecundity.

Figure S1 High activity females (black lines) were favoured when they were also aggressive in high competition environments (left panel).

Figure S2 The predicted relative fitness surface, as a function of female aggression and activity, was saddle shaped when competition among juveniles for vacant territories was high.