

Low heritabilities, but genetic and maternal correlations between red squirrel behaviours

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Abstract

Consistent individual differences in behaviour, and behavioural correlations within and across contexts, are referred to as animal personalities. These patterns of variation have been identified in many animal taxa and are likely to have important ecological and evolutionary consequences. Despite their importance, genetic and environmental sources of variation in personalities have rarely been characterized in wild populations. We used a Bayesian animal model approach to estimate genetic parameters for aggression, activity and docility in North American red squirrels (*Tamiasciurus hudsonicus*). We found support for low heritabilities (0.08–0.12), and cohort effects (0.07–0.09), as well as low to moderate maternal effects (0.07–0.15) and permanent environmental effects (0.08–0.16). Finally, we found evidence of a substantial positive genetic correlation (0.68) and maternal effects correlation (0.58) between activity and aggression providing evidence of genetically based behavioural correlations in red squirrels. These results provide evidence for the presence of heritable variation in red squirrel behaviour, but also emphasize the role of other sources of variation, including maternal effects, in shaping patterns of variation and covariation in behavioural traits.

Introduction

Consistent individual differences in behaviour and behavioural correlations within and across contexts have been identified in many animal taxa (Sih *et al.*, 2004a; Réale *et al.*, 2010). The persistence of individual differences in behaviour has stimulated much research (Gosling, 2001; Sih *et al.*, 2004a,b; Réale *et al.*, 2007, 2010), because behaviour was previously thought to be very labile, and have been increasingly accepted as important traits with ecological and evolutionary consequences (Réale *et al.*, 2010). Animal personalities have been shown to have important fitness effects in a number of systems (Dingemanse & Réale, 2005; Smith & Blumstein, 2008) and balancing selection has been proposed as a

mechanism that could maintain these individual differences (Dingemanse *et al.*, 2004; Boon *et al.*, 2007; Wolf *et al.*, 2007). For example, Dingemanse *et al.* (2004) found that adult great tit (*Parsus major*) overwinter survival was related to exploratory behaviour and that this relationship fluctuated in direction across years. Understanding how natural selection shapes patterns of variation in animal personalities, however, requires an understanding of the underlying genetic structure of these important traits.

The genetic and environmental sources of (co)variation in specific repeatable behaviours, or personality traits, have rarely been characterized for wild populations (Dochtermann & Roff, 2010), despite the widely recognized importance of genetic variation for evolutionary processes (Roff, 1997; Lynch & Walsh, 1998). A long history of study in captive animals has established that heritable variation in behaviour is common in laboratory populations (Stirling *et al.*, 2002). However, because

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these populations have been bred in captivity for many generations and are not exposed to the degree of environmental variation found in the wild, these heritability estimates may not be representative of wild populations (Weigensberg & Roff, 1996; Hoffmann & Merilä, 1999; Hoffmann, 2000; Conner *et al.*, 2003). The number of studies that have measured heritability of personality traits in wild populations is small, but growing (Table 1), yet more estimates will be necessary before broad patterns emerge. For example, it is unclear whether certain personality traits or suites of correlated behaviours are more heritable than others, and which sources of variation in personality traits are more sensitive to changes in environmental circumstances (i.e. genotype \times environment, maternal \times environment interactions).

Correlated behaviours (sensu Sih *et al.*, 2004a,b) are an important component of animal personalities and quantifying genetic correlations between behaviours will provide important insights into the functional integration of behaviours (Cheverud, 1996) and potential constraints on behavioural evolution. A genetic behavioural corre-

lation would suggest that independent adaptation of the behavioural traits involved could be constrained (Roff & Fairbairn, 2007; but see Blows & Hoffmann, 2005). Most previous studies have quantified only phenotypic correlations between behavioural traits (Dochtermann & Roff, 2010), but the degree or sign of a phenotypic correlation may not match the underlying genetic correlation (Roff, 1997; Kruuk *et al.*, 2008). So far, there have been few estimates of genetic correlations among personality traits in wild populations but those that have estimated genetic correlations have generally found results consistent with patterns of variation observed at the phenotypic level (Bell, 2005; Réale *et al.*, 2009; Blumstein *et al.*, 2010). For example, a number of studies have found positive phenotypic correlations between aggression and boldness (Koolhaas *et al.*, 1999; Sih *et al.*, 2004a; Carere *et al.*, 2005; Boon *et al.*, 2007; Sih & Bell, 2008; Réale *et al.*, 2009) and, where examined, positive genetic correlations between boldness and aggression (Bell, 2005; Dingemanse *et al.*, 2007; Réale *et al.*, 2009). Additionally, in a meta-analysis Dochtermann (2011) found a strong correlation between phenotypic and genetic correlations

Table 1 Heritability of behaviour measured in wild populations, including those from entirely wild populations (w) and those estimated from offspring raised in captivity (c).

Species	Behaviour	h^2	m^2	r_G	Reference	
<i>Ovis canadensis</i> (sheep) (w)	Boldness	0.21	–		Réale <i>et al.</i> (2000)	
<i>Parus major</i> (tits) (w)	Exploration	0.22–0.41	–	–	Dingemanse <i>et al.</i> (2002)	
<i>Gasterosteus aculeatus</i> (sticklebacks) (c)	Activity	0.05	–	S	Bell (2005)	
	Aggression	0.01	–	S		
	Boldness	0.04	–	S		
	Population 2	Activity	0.16	–		S
	Aggression	0.14	–	S		
	Boldness	0.002	–	S		
<i>Euprymna tasmanica</i> (squids) (c)	Boldness	0.21	NS	–	Sinn <i>et al.</i> (2006)	
	Antipredator context	Activity	0.67	NS		–
	Reactivity	0.89	NS	–		
	Feeding context	Boldness	NS	NS		–
	Activity	NS	NS	–		
	Reactivity	NS	NS	–		
<i>Gasterosteus aculeatus</i> (sticklebacks) (c)	Multiple traits	0.06–0.27	–	S	Dingemanse <i>et al.</i> (2009)	
	Antipredator context	Multiple traits	0.15–0.32	–		S
<i>Sialia mexicana</i> (bluebirds) (w)	Aggression	0.34	NS	S	Duckworth & Kruuk (2009)	
	Dispersal	0.52	NS	S		
<i>Ovis canadensis</i> (sheep) (w)	Docility	0.65	NS	S	Réale <i>et al.</i> (2009)	
	Boldness	0.39	NS	S		
<i>Parus major</i> (tits) (w)	Exploration	0.23	–	–	Quinn <i>et al.</i> (2009)	
<i>Marmota flaviventris</i> (marmots) (w)	Vigilance	0.08	NS	NS	Blumstein <i>et al.</i> (2010)	
	Sprint speed	0.21	NS	NS		
<i>Thamnophis ordinoides</i> (garter snakes) (c)	Anti-predator	0.54–0.65	–	S	Brodie (1993)	

(–) Parameter was not estimated; NS: parameter was estimated but found no significant; S: a significant genetic correlation was found between this trait and another personality trait.

for behavioural traits. However, the aggression-boldness syndrome is not ubiquitous and has been shown to depend on environmental context (Bell, 2005; Dingemanse *et al.*, 2007). Genetic correlations may be the result of pleiotropic relationships (Conner & Via, 1993; Blows & Hoffmann, 2005). Alternatively, natural selection for optimal trait combinations (correlational selection) may produce genetic correlations through linkage disequilibrium (Sinervo & Svensson, 2002), as hypothesized for correlated behaviours by Wolf *et al.* (2007), Stamps (2007), Biro & Stamps (2008), Duckworth & Kruuk (2009) and Houston (2010).

Environmental conditions during an individual's development can also have consistent long lasting effects (Lindstrom, 1999). These cohort effects can be important sources of trait variation especially when the environment substantially fluctuates on an annual basis. Environmental effects shared by relatives may also play a role in shaping variation in personalities and, if not accounted for, may bias estimates of heritabilities (Kruuk & Hadfield, 2007). Parental effects are a special case in which the environment provided by one or both parents results in similar offspring phenotypes (Mousseau & Fox, 1998; Mousseau *et al.*, 2009), which can cause an overestimation of additive genetic effects when the effects of a common parental environment are not considered (Falconer & Mackay, 1996; Kruuk & Hadfield, 2007). These effects can be especially strong in species where parents provide extended care to their offspring, as in the case of mammals where mothers typically nurse their young for extended periods (Reinhold, 2002). Although documented in captivity (Forstmeier *et al.*, 2004), maternal (or parental) effects have rarely been estimated for personality traits in wild populations and have never been shown to account for a substantial proportion of the variation in those traits (Table 1). The potential for maternal effects to shape evolutionary processes has received a lot of recent attention (Mousseau *et al.*, 2009) and they may play an important role in the evolution of personality traits, but this has yet to be thoroughly examined.

Personality traits and a behavioural syndrome were recently identified in a population of red squirrels (*Tamiasciurus hudsonicus* Erxleben; Boon *et al.*, 2007), for which an extensive pedigree has been established (e.g. Réale *et al.*, 2003; McFarlane *et al.*, 2011). Significant phenotypic correlations between aggressive behaviour towards a mirror-image, activity in an open-field arena and activity in response to handling (i.e. docility) indicated the presence of a behavioural syndrome where aggressive squirrels tended to be more active and less docile (Boon *et al.*, 2007), which is similar to the aggression-boldness syndrome that has been identified in many taxa (Tulley & Huntingford, 1988; Koolhaas *et al.*, 1999; Careau *et al.*, 2010).

Boon *et al.* (2007) also found that natural selection on activity and aggression fluctuated in direction and mag-

nitude across years, a mechanism by which genetic and phenotypic variation in red squirrel personality traits could be maintained. Understanding how patterns of behavioural variation respond to natural selection, however, depends also on the levels of genetic variation and covariation in these traits. We, therefore, estimated heritabilities, maternal effects and sources of environment variation (e.g. cohort, permanent environmental and residual effects) in aggression, activity and docility, as well as genetic correlations among these traits for this population of red squirrels using a Bayesian animal model approach (Hadfield, 2010).

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). *T. hudsonicus* is a small (150–250 g), diurnal, semi-arboreal rodent in the family Sciuridae that is present in much of forested North America (Steele, 1998). Individuals of both sexes defend exclusive year-round territories (Smith, 1968). This territoriality allows for complete enumeration of the study population through targeted trapping and behavioural observations. Though trappability is related to red squirrel personality traits (Boon *et al.*, 2008), the ability to target individuals minimizes sampling bias by ensuring that all individuals in the population are sampled (Biro & Dingemanse, 2009). Each squirrel in the study area was 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 once immediately after parturition, then again when pups were approximately 25 days age, to tag pups and collect tissue samples for paternity analysis (Lane *et al.*, 2008).

We measured personality traits of individuals from three study areas. The Kloo and Sulphur study areas have been monitored continuously since 1989. In contrast, the Agnes study area has been monitored only recently. Since the autumn of 2004, *ad libitum* peanut butter has been experimentally provided to every squirrel in the Agnes study area between October and May of each year.

Following Boon *et al.* (2007), we used three behavioural tests to measure red squirrel personality traits. The first test, an open field (OF) test, was used to measure an individual's activity, exploration and behavioural stress response in a novel environment (Walsh & Cummins, 1976; Martin & Réale, 2008). The second test was a mirror-image stimulation (MIS) that measured aggression in response to the individual's reflection (Svendsen & Armitage, 1973). The third test, conducted during routine handling events, measured docility as the struggle rate of an individual confined in a mesh handling bag. We performed 556 OF and MIS trials on 183 female and 183 male red squirrels and 3122 struggle rate tests on 291

female and 301 male squirrels over a 4-year period (2005 and 2008–2010). The mean number of OF and MIS trials per individual was 1.4 (range 1–5) with a mean interval of 261 days (range 12–1435) and the mean date of trials was July 2 (range April 29–September 24). The mean number of struggle rate trials per individual was 6.2 (range 1–44) with a mean interval of 56 days (range 0–1436 days) and the mean date of trials was June 22 (range April 29–September 24). Individual squirrels were tested within 1 h of being trapped on their territory. To measure docility, the captured squirrel was 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. If the ground was wet or snowy a foam or cloth barrier was positioned between the squirrel and the ground. After the docility test was performed, ear-tag numbers, mass, and reproductive status were recorded. If the squirrel was to be tested in the OF or MIS test, the squirrel was then transferred into the arena through a sliding door to begin the OF trial. The testing arena for the OF and MIS tests was a 60 cm × 80 cm × 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. A 45 cm × 30 cm mirror at one end of the arena was exposed during the MIS portion of the session. OF and MIS trials were both performed in the same testing session. OF performance was tested first so that it would also serve as a habituation period for the MIS trial. After 7.5 min the mirror was exposed to start the 5-min MIS trial. At the conclusion of the session the squirrel was released where it was trapped, the number of faeces deposited in the arena counted and the arena was cleaned with 70% ethanol. These procedures were the same as those performed by Boon *et al.* (2007).

We quantified each squirrel's behaviour during the trials by scoring the videotaped trials using JWatcher Video 1.0 (Blumstein & Daniel, 2007) and the same ethogram as described in Boon *et al.* (2007). During the OF trial, we recorded latencies, rates and proportions of time the squirrel spent engaged in a variety of activities that we did not consider to be unique behaviours or traits, but which we hoped would collectively represent behaviours such as activity or aggression. These behavioural measurements included the proportion of time spent walking, sniffing, chewing (gnawing at arena sides), rearing, grooming, scanning (a clear movement of the head in a side to side manner), and still. These measurements were mutually exclusive. We also recorded the proportion of time spent hanging from the arena lid, which is mutually exclusive with walking, rearing, grooming, and still but not mutually exclusive to sniffing, chewing, or scanning. In addition to these proportions, we recorded rates of jumping and rates of interactions with the false holes. The measurements recorded during

MIS trials were proportions of time spent in the third of the arena closest to the mirror (front), and farthest from the mirror (back), and the proportion of time spent stretching towards the mirror. We also recorded the rate of aggressive contact with the mirror (attacks), the rate of grunting vocalizations, the rate of crouches (tail positioned over head with hairs erect) and the latency in seconds until the first attack and first approach towards the mirror. A. K. Boon performed and analysed all trials in 2005 (study 1). Trials from 2008, 2009, and 2010 (study 2) were performed by Taylor and four assistants and analysed by Taylor and five assistants. We tested the inter-observer reliability of our measurements by calculating the correlation between two observers' scores of the same trial (56 trials were scored by multiple observers). We removed measurements with a reliability of < 0.7 (see Supporting Information Table S1; Martin & Bateson, 1993).

Maternity has been determined with certainty by ear-tagging juveniles prior to first emergence from their natal nest since 1989 on the Kloo and Sulphur study areas and since 2002 on the Agnes study area; adoption in red squirrels is extremely rare (Gorrell *et al.*, 2010). Tissue samples for paternity analysis have been collected since 2003. Paternity was assigned based on 16 microsatellite loci using CERVUS 3.0 with matches accepted at 95% or greater probability and no more than one mismatch (detailed in Gunn *et al.*, 2005; Lane *et al.*, 2008). The complete pedigree included 7086 individuals, 819 of which were informative for the docility phenotype, and 451 were informative for OF and MIS phenotypes (Supporting Information Table S2).

Statistical analysis

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). We, therefore, used principal component analysis to reduce the redundancy among our measurements and to identify the dominant axes of behavioural variation in the OF and MIS trials. Principal components were calculated separately for the OF and MIS measurements using a correlation matrix (Table 2; results). We obtained very similar loadings to Boon *et al.* (2007), confirming that the correlation matrices are consistent across years so principal components were calculated for all years combined. All further analyses used the scores calculated from the first principal component loadings for each trial and will be referred to as OF PC1 and MIS PC1.

Table 2 First principle component loadings for behaviours from an open field arena test (OF PC1) and a mirror-image stimulation test (MIS PC1) in North American red squirrels.

Behaviour	OF PC1	Behaviour	MIS PC1
Walk	0.49	Front	0.49
Jump rate	0.44	Attack rate	0.37
Hole rate	0.31	Back	-0.41
No. pellets	0.29	Attack latency	-0.47
Hang	0.25	Approach latency	-0.48
Chew	0.24		
Groom	-0.06		
Still	-0.52		
SD	1.71		1.67
% total variance	36.36		55.67

Behaviours were measured as percentage of time unless otherwise noted. Latencies were log transformed prior to principle component analysis. Additional principle component axes are provided in Supporting Information Table S3.

We estimated the variance components for the squirrel struggle rate, and their first principal components scores for the OF and MIS trials (interpreted as docility, activity and aggressiveness, respectively; see results and Boon *et al.*, 2007) using a mixed-effect 'animal model', which allows for variance structures associated with pedigrees (Henderson, 1984; Lynch & Walsh, 1998; Kruuk, 2004; Wilson *et al.*, 2009). We fitted the animal models using a Markov Chain Monte Carlo for Generalized Linear Mixed Models (MCMCglmm) analysis in the R statistical package (Hadfield, 2010; R Development Core Team, 2011). We assumed that all study areas functioned as a single population and, therefore, estimated common variance components across all populations in all analyses. Phenotypic (V_P), additive genetic (V_A), maternal (V_M), permanent environmental (V_I) and cohort (V_C) variances and their covariances were estimated using a trivariate MCMCglmm model with individual, additive genetic, dam and birth year as random effects. Variance components were estimated as the mode of the posterior distribution and 95% credible intervals are given. Covariances were rescaled as correlations ($r = \text{Cov}_{a,b} / \sqrt{V_a V_b}$). The variances for aggression and activity were not transformed into coefficients of variation because they were estimated from principal component scores and so had mean values of 0. Heritabilities, maternal effects, permanent environmental effects were calculated for each MCMC sample by dividing the relevant variance component by the total phenotypic variance ($V_A + V_M + V_I + V_C$) and the mode and 95% credible intervals of these posterior distributions are reported. Repeatabilities were estimated as the mode of a posterior distribution generated by dividing the among-individual variance (genetic and nongenetic) by the sum of among- and within-individual variances (Lessells & Boag, 1987) for each MCMC sample.

Covariances were supported when 95% credible intervals excluded zero. This is not applicable to variances

because they are bounded above zero, so we determined support of variances by comparing deviance information criterion (DIC) values of the fitted models (DIC values for each model are provided in the Supporting Information Tables S5 and S6). DIC can be viewed as the Bayesian equivalent to the Akaike information criterion (AIC) and the rules of thumb developed for AIC (Burnham & Anderson, 1998) appear to also work well for DIC (Spiegelhalter *et al.*, 2002). In all cases the most complete models were within 2 DIC of the best model (Supporting Information Tables S5 and S6) so we report values from the models that include all random effects to avoid biased parameter estimates.

Because missing values are not allowed in random effects, unique dams were generated for individuals whose dam was unknown and treated as founders in the pedigree. This generation of dams allowed us to use all the information available in our estimates of the variance components, but assumes that all individuals with unknown dams were unrelated.

To account for the effects of habituation we included as covariates for the OF and MIS scores a fixed effect term for lifetime and yearly trial number. For struggle rate we included lifetime and yearly handling event, which included handling events where no struggle test was performed, because handling for routine data collection is similar to the struggle rate trial. We also included a quadratic term for trial numbers to account for a non-linear response to repeated trials. To control for effects of seasonality and study area we included day of year and study area as continuous and categorical fixed effects, respectively. 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 natural selection.

Priors for the reported models were slightly informative and generated by partitioning the variance in phenotype evenly among each random term and were given a low degree of belief ($V = \text{diag}(n) * V_P / r$, $\text{nu} = 0.2$; Hadfield, 2010). Altering the priors so that V_P was not evenly distributed had no effect on the results. We evaluated convergence by visually inspecting time series plots of the model parameters and assessing autocorrelation values (all were < 0.1 for reported results). The posterior distribution of the animal models was sampled every 500 iterations after a burn-in period of 50 000 iterations for a total of 2000 samples.

Results

Red squirrel responses to the open field arena varied from active to sedentary behaviour and the major axis of

Table 3 Effects of habituation, day of year and study area on red squirrel docility, aggression and activity.

	Docility	Aggression	Activity
Intercept	15.494 (13.614–17.944)	–0.187 (–1.666 to 0.943)	0.784 (–0.588 to 1.834)
Life trial no.	– 0.057 (–0.088 to –0.011)	0.650 (–0.285 to 1.705)	– 0.841 (–1.929 to –0.100)
Life trial no. 2	0.00043 (0.00005–0.00078)	–0.101 (–0.357 to 0.108)	0.148 (–0.050 to 0.377)
Year trial no.	– 0.204 (–0.312 to –0.138)	–0.118 (–1.894 to 1.852)	–0.962 (–3.045 to 0.405)
Year trial no. 2	0.004 (0.002–0.005)	–0.012 (–0.651 to 0.383)	0.234 (–0.237 to 0.749)
Day of year	0.010 (0.004–0.018)	–0.002 (–0.007 to 0.003)	0.005 (0.001–0.010)
Kloo	1.484 (0.201–2.503)	0.295 (–0.124 to 0.840)	0.565 (0.102–0.972)
Sulphur	0.784 (–0.506 to 2.227)	0.617 (0.046–1.019)	1.020 (0.563–1.467)

Year trial number is the trial number counted from the start of each year, while life trial number is the trial number counted over each individual's entire life. Trial numbers for docility include all handling events, even those in which docility was not measured. 95% credible intervals are given in parentheses and those that exclude zero are indicated in bold. The effects of the Kloo and Sulphur study areas are assessed relative to the Agnes study area.

behavioural variation (Table 2) was best described as activity in a novel environment. After release into the arena active individuals immediately began walking, sniffing, jumping, hanging and chewing while sedentary individuals remained still with longer latency until first movement (Table 2). This variation in activity was captured by the first principal component (OF PC1) and explained 32% of the behavioural variation in the open field test (Table 2). Many individuals were able to hang from the top corners of the arena by clinging to seams in the walls of the arena. This hanging was often accompanied by chewing directed at the walls and corner of the arena. The second principal component for the open field test separated individuals that spent much of the trial hanging and chewing from those who did not and explained 16% of the behavioural variation (Supporting Information Tables S3 and S4 for all PC loadings). Because the second principal component explained relatively little variation and its biological relevance is not readily apparent we did not perform any further analyses on this axis of variation.

Red squirrel behaviour in the arena immediately and noticeably changed upon exposure to their mirror image. Individuals varied in their response with some immediately approaching and attacking the mirror and others

retreating to the opposite end of the arena and adopting a passive posture. This variation in behaviour was reflected in the first principal component for the mirror image stimulation trial (MIS PC1) which differentiated aggressive interactions directed at the mirror (e.g. approaching and attacking the mirror) from avoidance (e.g. retreating from the mirror and staying in the back of the arena) and explained 56% of the total variation in behavioural response to the mirror image (Table 2).

Red squirrels varied in their response to restraint in the mesh handling bag with some individuals struggling for the entire 30 s of the test and others remaining entirely still. We have interpreted this behaviour as a measure of docility.

The complete univariate models for struggle rate, which included permanent environment, additive genetic, dam and cohort as random effects, had the lowest DIC score and the complete models for OF PC1 and MIS PC1 were within 2 DIC of the best models (Supporting Information Table S5). The most complete trivariate model also had the lowest DIC score (Supporting Information Table S6). Because our focus was on achieving the best estimates for each (co)variance we evaluated the most complete trivariate model to avoid estimates that may be confounded in the reduced models (see Supporting

Table 4 Heritability ($h^2 = V_A/V_P$), maternal effects ($m^2 = V_M/V_P$), permanent environmental effects ($PE = V_{PE}/V_P$), cohort effects ($C = V_C/V_P$), repeatability ($[V_A + V_M + V_{PE} + V_C]/V_P$), and the mean trait value. [Correction added 2 February 2012 after online publication: brackets added to equation for repeatability].

	h^2	m^2	PE	C	Repeatability	Mean
Docility	0.09 (0.05–0.19)	0.07 (0.03–0.10)	0.16 (0.08–0.21)	0.07 (0.03–0.17)	0.41 (0.36–0.49)	18.65
Aggression	0.12 (0.03–0.22)	0.09 (0.03–0.18)	0.07 (0.03–0.20)	0.07 (0.03–0.23)	0.44 (0.33–0.56)	–
Activity	0.08 (0.03–0.19)	0.15 (0.05–0.26)	0.08 (0.04–0.21)	0.09 (0.04–0.27)	0.51 (0.40–0.63)	–
	CV_A	CV_M	CV_{PE}	CV_C		
Docility	15.54 (9.37–18.54)	10.67 (7.21–13.81)	16.99 (12.78–19.97)	10.24 (6.75–18.89)		

Variances were estimated using a trivariate model. Variances are calculated as the mode of the posterior distribution with 95% credible intervals in parentheses and are bound above zero. Coefficients of variation ($CV = 100 \times \text{standard deviation}/\text{mean}$) are given for docility. Because aggression and activity are scores from a principal component analysis using a correlation matrix the trait means are 0 and coefficients of variation cannot be calculated.

Table 5 Additive genetic and phenotypic variances, covariances and correlations (G-matrix and P-matrix) of red squirrel personality traits.

	Genetic			Phenotypic		
	Docility	Aggression	Activity	Docility	Aggression	Activity
Docility	8.40 (3.05–11.95)	–0.49 (–0.81 to 0.07)	–0.45 (–0.71 to 0.20)	63.35 (58.02–72.33)	–0.12 (–0.25 to –0.01)	–0.20 (–0.30 to –0.08)
Aggression	–0.50 (–1.71 to 0.21)	0.35 (0.11–0.74)	0.68 (0.12–0.87)	–1.61 (–3.68 to –0.13)	3.25 (2.72–3.94)	0.40 (0.26–0.50)
Activity	–0.45 (–1.39 to 0.27)	0.11 (0.01–0.47)	0.23 (0.09–0.57)	–2.48 (–4.34 to –0.96)	1.31 (0.77–1.63)	2.89 (2.43–3.63)

Variances are indicated along the diagonal, the upper triangle contains correlations and the lower triangle covariances. Variances are calculated as the mode of the posterior distribution with 95% credible intervals in parentheses and are bounded above zero. Correlations and covariances that were different from zero (based on 95% credible intervals) are indicated in bold.

Information Appendix S1 for all univariate model parameters and Supporting Information Appendix S2 for all trivariate model parameters).

For all three behavioural measures (OF PC1; MIS PC1; struggle rate) the addition of identity as a random effect greatly improved the fit of the model (Supporting Information Tables S5 and S6). Along with substantial repeatabilities (Table 4), this demonstrates that consistent individual differences in behaviour exist for all three of these variables that we have interpreted as representing activity, aggression and docility (see above). Red squirrels were less active with repeated lifetime trials and less docile with repeated yearly and lifetime handling events; however, there was no effect of trial number on aggression (Table 3). There was a quadratic component to the effect of handling events on docility such that the effect of each successive handling event diminished. There was a small positive effect of day of year on activity and docility. Red squirrels on the Kloo and Sulphur study areas were more active, and squirrels on the Sulphur study area were more aggressive than squirrels on the Agnes study area.

Heritabilities were low for all traits (0.09–0.12) as were cohort effects (0.07–0.09), maternal effects on aggression and docility (0.07–0.09) and permanent environmental

effects on activity and aggression (0.07–0.08). However, maternal effects on activity (0.15) and permanent environmental effects on docility (0.16) were nearly twice as strong as the other effects (Table 4).

Activity and aggression were positively phenotypically correlated ($r_P = 0.40$; 0.26–0.50), while docility was negatively correlated with both activity ($r_P = -0.20$; –0.30 to –0.08) and aggression ($r_P = -0.12$; –0.25 to –0.01), confirming the presence of a behavioural correlation (Table 5). The genetic correlations were in the same direction as the phenotypic correlations but varied in strength. There was a strong positive genetic correlation between aggression and activity ($r_G = 0.68$; 0.12–0.87), and a moderate negative correlation between aggression and docility ($r_G = -0.49$; –0.81 to 0.07) and between activity and docility ($r_G = -0.45$; –0.71 to 0.20), though the credible intervals between docility and the other traits overlapped with zero (Table 5). There were also a maternal effect correlations ($r_M = 0.58$; 0.01–0.81) and a permanent environmental effect correlation ($r_{PE} = 0.61$; 0.03–0.83) between activity and aggression and a permanent environmental effect correlation between activity and docility ($r_{PE} = -0.45$; –0.74 to 0.01) that overlapped with zero (Table 6). We did not find any support for cohort effects correlations (Table 6).

Table 6 Maternal, permanent environmental and cohort (birth year) variances, covariances and correlations of red squirrel personality traits.

	Maternal			Permanent environmental		
	Docility	Aggression	Activity	Docility	Aggression	Activity
Docility	3.96 (1.81–6.63)	–0.22 (–0.65 to 0.25)	–0.31 (–0.67 to 0.16)	10.03 (5.68–13.86)	–0.26 (–0.71 to 0.19)	–0.45 (–0.74 to 0.01)
Aggression	–0.24 (–0.91 to 0.33)	0.24 (0.10–0.59)	0.58 (0.01–0.81)	–0.53 (–1.53 to 0.44)	0.29 (0.10–0.70)	0.61 (0.03–0.83)
Activity	–0.41 (–1.13 to 0.21)	0.16 (–0.03 to 0.43)	0.47 (0.16–0.78)	–0.84 (–1.66 to 0.06)	0.05 (–0.05 to 0.44)	0.34 (0.12–0.64)
Cohort effect						
	Docility	Aggression	Activity			
Docility	3.64 (1.59–2.41)	–0.02 (–0.65 to 0.58)	–0.16 (–0.70 to 0.49)			
Aggression	–0.02 (–1.40 to 1.09)	0.20 (0.09–0.89)	0.16 (–0.55 to 0.73)			
Activity	–0.07 (–1.59 to 0.97)	0.01 (–0.32 to 0.38)	0.30 (0.09–0.95)			

Variances are indicated along the diagonal, the upper triangle contains correlations and the lower triangle covariances. Variances are calculated as the mode of the posterior distribution with 95% credible intervals in parentheses and are bound above zero. Correlations that were different from zero (based on 95% credible intervals) are indicated in bold.

Discussion

Theoretical studies of the evolution or maintenance of animal personalities often make the assumption that personalities are heritable (reviewed in Dingemanse & Wolf, 2010). For instance Wolf *et al.* (2007) proposed that correlational selection between life-history and personality traits can give rise to suites of personality traits maintained by frequency-dependent selection. Similarly, Stamps and Biro (Stamps, 2007; Biro & Stamps, 2008) hypothesized that selection will favour certain combinations of productivity (e.g. growth rates, or fecundity) and behaviour leading to stable personalities. If variable selection is to contribute to the generation and maintenance of personalities, then the personality traits on which selection acts must be heritable.

We found low heritabilities that were smaller than typically found for behavioural traits (mean $h^2 = 0.30 \pm 0.02$; reviewed by Mousseau & Roff, 1987; Stirling *et al.*, 2002) and substantially lower than our estimates of repeatability, which sets the upper bound for heritability (Hoffmann, 2000; Dohm, 2002; Bell *et al.*, 2009). Small heritabilities may be the consequence of stabilizing or directional selection eroding additive genetic variation or due to large environmental variances (Barton & Turelli, 1989; Roff, 1997).

We found support for habituation in activity and docility, but not for aggression. The novelty of the open-field arena is an important component of the testing environment, which is altered with repeated exposure and may explain why individuals grew less active with subsequent trials (Archer, 1973; Martin & Réale, 2008). Red squirrels also grew less docile with increased handling events both within year and over their lifetime showing that the intensity of their reaction to handling decreased with experience.

Red squirrels experience large yearly fluctuations in their main food source, seeds from white spruce cones (*Picea glauca*; McAdam & Boutin, 2003; LaMontagne *et al.*, 2005; Boutin *et al.*, 2006), and fluctuations in these resources have long lasting cohort effects on red squirrel fitness and life-history traits (Descamps *et al.*, 2008). We found support for small cohort effects (approximately equal to V_A) on all three personality traits. Permanent environmental effects represented a larger proportion of individual variation in docility (approximately twice V_A) but low amounts of variation in activity and aggression (approximately equal to V_A). Permanent environmental effects represent the effects of an individual's environment that are consistent over the individual's lifetime (Roff, 1997). Individual red squirrels typically maintain a consistent territory over their lifetime (Smith, 1968; but see Boutin *et al.*, 1993; Price & Boutin, 1993) and the environmental effect of the quality of their territory could contribute to permanent environmental effects on behaviour. However, because

red squirrels rarely change territories (Bertheaux & Boutin, 2000) 'individual' and 'territory' are too confounded to reliably distinguish in the analysis of these personality data.

Determining whether phenotypic correlations adequately reflect underlying genetic correlations requires large sample size (Kruuk, 2004) and consequently the credible intervals around our estimates were wide. The phenotypic correlations between docility and aggression ($r_P = -0.12$) and docility and activity ($r_P = -0.20$) were low and the 95% credible intervals for genetic, maternal, permanent environmental and cohort correlations overlapped with zero. However, the overlap was small for the genetic correlation between docility and aggression ($r_G = -0.49$) and the permanent environmental effect correlation between docility and activity ($r_{PE} = -0.45$). We were able to detect a more strongly supported genetic correlation ($r_G = 0.68$), a maternal effects correlation ($r_M = 0.58$) and a permanent environmental correlation ($r_{PE} = 0.61$) between aggression and activity. Together these results show that the degree to which genetic and other correlations can be inferred from phenotypes alone can depend on the particular traits being considered even within a single class of traits within a single species (Kruuk & Hadfield, 2007). Furthermore, other sources of covariation such as maternal and permanent environmental covariation may be important components of observed phenotypic correlations between behaviours. Here we were unable to assess whether these genetic correlations were due to pleiotropy or linkage disequilibrium, but are currently performing selection analyses to determine whether contemporary patterns of correlational selection are consistent with the strong positive genetic correlations that we found among activity and aggression.

We found stronger maternal effects for activity than docility and aggression and maternal effects on activity and aggression were correlated. The maternal effects correlation shows that the effect of maternal environment on activity is strongly correlated with the effect on aggression, but as maternal effects were weak the magnitude of these correlations may be misleading as other sources of variation play a large role in determining the phenotypes of offspring. Maternal effects have been found for personality traits in captive populations (Forstmeier *et al.*, 2004; Van Oers *et al.*, 2004) and have been examined in a few wild populations (Table 1); however, to our knowledge, this is the first documentation of maternal effects on a personality trait in a wild population. We did not have the power to further separate the maternal effects into maternal environmental and maternal genetic effects so cannot reject the possibility that part of the maternal variance estimated here is of genetic origin (Wilson & Réale, 2006).

Maternal hormonal responses to environmental variation and consequent differential early hormone exposure of offspring (i.e. hormone-mediated maternal

effects) could have persistent consequences on personality traits and generate the correlations between personality traits that we observed. For example, increased early exposure to androgens can increase aggression (Mann & Svare, 1983; Dloniak *et al.*, 2006; Eising *et al.*, 2006) or simultaneously increase both aggression and activity (Pasterski *et al.*, 2007). In contrast, heightened early exposure to glucocorticoids may decrease activity (Koolhaas *et al.*, 1999; Wilcoxon & Redei, 2007). The effects of early exposure to heightened androgens or glucocorticoids on personality traits can persist into adulthood (Eising *et al.*, 2006) and perhaps across generations through epigenetic programming of neuroendocrine traits (Meaney, 2001; Champagne, 2008; Weinstock, 2008). We are currently investigating the hormonal responses of female red squirrels to environmental variation and associations between maternal hormone concentrations and the behavioural attributes of their offspring.

The persistence of consistent individual variation and covariation in behaviour across a wide range of taxa has led to many adaptive hypotheses that explicitly or implicitly assume sufficient underlying genetic variation for these personalities to evolve or be maintained (e.g. Stamps, 2007; Wolf *et al.*, 2007; Biro & Stamps, 2008; Houston, 2010). Here we have not only provided evidence of the genetic basis to personality in red squirrels, but have also identified maternal effects as a potentially important source of variation in the personality of a wild vertebrate. Further studies of the inheritance of personality traits in a variety of wild organisms are needed before general patterns will emerge regarding differences in the magnitude of genetic, maternal and environmental sources of variation among personality traits. These might also shed further light on the differences that appear to exist between sources of variation in behaviours in the wild (Stirling *et al.*, 2002) and other traits typically studied from a quantitative genetic perspective (Houle, 1992). Such studies are needed if we are to evaluate the potential for current and future models to describe the evolution of persistent individual differences in behaviour of wild animals.

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Supporting information

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

Table S1 Between-observer reliability, means and variances of specific behaviours recorded during open field and mirror image stimulation tests.

Table S2 Summary statistics for the entire multi-generational red squirrel pedigree, pedigree of individuals informative for docility and pedigree of individuals informative for activity and aggression.

Table S3 Principle component loadings for behaviours from an open field arena test in North American red squirrels.

Table S4 Principle component loadings for behaviours from a mirror-image stimulation test in North American red squirrels.

Table S5 Candidate univariate models of sources of variation in behaviour in North American red squirrels.

Table S6 Candidate trivariate animal models of sources of (co)variation of the behavioural traits aggression, activity and docility.

Appendix S1 Full univariate model parameters for sources of variation in behaviour in North American red squirrels.

Appendix S2 Full trivariate model parameters for sources of variation in behaviour in North American red squirrels.

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