BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research

**Cite this article:** Guindre-Parker S, Mcadam AG, van Kesteren F, Palme R, Boonstra R, Boutin S, Lane JE, Dantzer B. 2019 Individual variation in phenotypic plasticity of the stress axis. *Biol. Lett.* **15**: 20190260.
http://dx.doi.org/10.1098/rsbl.2019.0260

Received: 9 April 2019
Accepted: 20 June 2019

**Subject Areas:**
ecology, evolution

**Keywords:**
glucocorticoids, density, plasticity, red squirrels

**Author for correspondence:**
Sarah Guindre-Parker
e-mail: sguindre@uoguelph.ca

THE ROYAL SOCIETY PUBLISHING

**Physiology**

# Individual variation in phenotypic plasticity of the stress axis

Sarah Guindre-Parker[1,2], Andrew G. Mcadam[1], Freya van Kesteren[2], Rupert Palme[4], Rudy Boonstra[5], Stan Boutin[6], Jeffrey E. Lane[7] and Ben Dantzer[2,3]

[1]Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada
[2]Department of Psychology, and [3]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA
[4]Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria
[5]Department of Biological Sciences, University of Toronto Scarborough, Scarborough, Ontario, Canada
[6]Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada
[7]Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

SG-P, 0000-0002-6205-3752; AGM, 0000-0001-7323-2572; FvK, 0000-0002-3034-9022; RP, 0000-0001-9466-3662; SB, 0000-0001-6317-038X; JEL, 0000-0003-0849-7181; BD, 0000-0002-3058-265X

Phenotypic plasticity—one individual's capacity for phenotypic variation under different environments—is critical for organisms facing fluctuating conditions within their lifetime. North American red squirrels (*Tamiasciurus hudsonicus*) experience drastic among-year fluctuations in conspecific density. This shapes juvenile competition over vacant territories and overwinter survival. To help young cope with competition at high densities, mothers can increase offspring growth rates via a glucocorticoid-mediated maternal effect. However, this effect is only adaptive under high densities, and faster growth often comes at a cost to longevity. While red squirrels can adjust hormones in response to fluctuating density, the degree to which mothers differ in glucocorticoid plasticity across changing densities remains unknown. Findings from our reaction norm approach revealed significant individual variation not only in a female red squirrel's mean endocrine phenotype but also in endocrine plasticity in response to changes in local density. Future work on proximate and ultimate drivers of variation in endocrine plasticity and maternal effects is needed, particularly in free-living animals experiencing fluctuating environments.

## 1. Introduction

All organisms experience changes in their environment, and the ability to adjust morphology, physiology or behaviour according to environmental conditions can provide individuals with important fitness benefits [1]. Phenotypic plasticity—when one individual can produce multiple phenotypes across a gradient of environments—is thought to represent an important mechanism allowing organisms to respond to environmental changes [2]. Phenotypic plasticity may be particularly important for organisms in fine-grained environments [1], defined by spatial or temporal fluctuations of key environmental features that occur within an individual's lifespan [3,4].

North American red squirrels (*Tamiasciurus hudsonicus*, hereafter 'red squirrels') experience drastic fluctuations in their fine-grained environment, where an important aspect of their environment—local conspecific density—can vary up to fourfold within an individual's lifetime [5]. Pulses in food resources lead to periods of high density, posing a challenge for breeding individuals as the availability of vacant territories critical for offspring overwinter survival is

**Table 1.** We compared four LMMs differing in random effect structure to test for individual differences in endocrine plasticity. Fixed effects were identical in all models.

| model | random effects | covariance (intercepts and slopes) | d.f. | $AIC_C$ | ΔAIC | model weight |
|---|---|---|---|---|---|---|
| model 1: null | n.a. | n.a. | 15 | 3353.9 | 80.7 | <0.001 |
| model 2: with ID | intercept (ID) | n.a. | 16 | 3279.4 | 6.3 | 0.027 |
| model 3: with ID × density, no covariance | intercept (ID) slopes (ID × density) | no | 17 | 3273.1 | 0 | 0.630 |
| model 4: with ID × density, with covariance | intercept (ID) slopes (ID × density) | yes | 18 | 3274.3 | 1.2 | 0.343 |

low and competition for these vacancies is high [6–8]. Red squirrel mothers can prepare their young to cope with high-density conditions via an adaptive hormone-mediated maternal effect [5]: under high densities, mothers with elevated glucocorticoids during pregnancy give birth to faster-growing pups that have a greater probability of surviving their first winter [5]. In this system, this maternal effect is only adaptive under high densities [5,9], since faster growth does not improve juvenile recruitment under low density [5,10]. Offspring born under high densities have shorter lifespans [10], potentially suggesting a trade-off between growth and lifespan [5]—this would be consistent with mammalian fitness costs of compensatory growth [11–13]. Chronically elevated glucocorticoids may have negative impacts on mothers, leading to oxidative stress [14], immunosuppression [15,16] and reduced parental care [17]. We expect the optimal red squirrel maternal phenotype to include elevated glucocorticoids during periods of high density, but decreased glucocorticoids during periods of low density. While glucocorticoids are positively related to density in red squirrels [5], the degree to which individuals vary in their endocrine response to changes in density remains unclear [18–20].

Glucocorticoids are mediators of phenotypic plasticity in vertebrates [19,20], promoting phenotypic adjustments following perturbations in an animal's environment [18,21–23]. This hormone is plastic [24,25], as organisms regulate glucocorticoids in response to diverse stressors [26,27]. We take a reaction norm approach to explore within-individual variation in glucocorticoid plasticity (sometimes called 'endocrine flexibility' [18]) in red squirrel females experiencing drastic among-year environmental fluctuations. While this hormone is known to change across contexts [18], little is known about the degree to which individuals might differ in their endocrine plasticity. We measured faecal cortisol metabolites (FCM)—a non-invasive measure of adrenocortical activity [28]—which was validated previously in red squirrels to reflect exposure to stressors [29]. We determine whether female squirrels show (i) individual variation in FCM and/or (ii) individual variation in the plasticity of FCM in response to density changes.

## 2. Material and methods

### (a) Field data collection

We studied two populations (Kloo and Sulphur) that have been monitored since 1987, as part of the Kluane Red Squirrel Project in the Yukon in Canada (61° N, 138° W). Each red squirrel defends one exclusive territory over their lifespan (up to 8 years, mean ± s.d. = 3.53 ± 1.84 [30]), containing a hoard of cones from white spruce (*Picea glauca*). Seeds from cached cones sustain squirrels through winter, making territory ownership crucial for survival [6–8]. Individuals were uniquely marked with numbered ear tags and unique coloured wires. Territory ownership was assessed reliably each spring via a population-wide census (described in [31]). Populations were completely enumerated annually and territory ownership was confirmed via observations of territorial vocalizations and live-trapping (using food-baited Tomahawk Live Traps, Tomahawk, WI, USA). We calculated each individual's local spring density as the number of neighbours owning territories within the acoustic environment of the focal individual (i.e. within a 130 m radius) [32].

Between 2006 and 2014, we collected faeces opportunistically when trapping individuals (from February to September, mean time of day ± s.d. = 11:30 a.m. ± 3 h). We checked below traps for fresh faeces, which we kept on ice until they could be frozen (within 5 h) [29]. We assessed female breeding status at the time each sample was collected (pregnant $n = 573$; lactating $n = 337$; or non-breeding $n = 819$) by palpating the abdomen for fetuses and checking nipple condition [30]. While FCM during pregnancy and lactation would most likely mediate maternal effects, we also include non-breeding samples. Firstly, this increases repeated observations in our dataset. Secondly, FCM are repeatable—non-breeding FCM thus provide some information about an individual's breeding phenotype. Finally, females likely adjust their HPA-axis pre-conception in anticipation of breeding (i.e. during non-breeding periods) [29]. FCM were assayed in one of two facilities (Michigan or Toronto) following identical, previously validated protocols [29,33]. A subset of samples ($n = 128$) analysed in both laboratories were strongly positively correlated (Pearson correlation = 0.88), suggesting laboratory identity had minimal effects on FCM. Samples were thawed, lyophilized, flash-frozen and pulverized by mortar and pestle. Steroids were extracted using 80% methanol (1 ml for 0.05 g of dry faeces) [29,34], and the supernatant was used in an enzyme immunoassay to quantify glucocorticoid metabolites with a 5α-3β,11β-diol structure [29]. A sample quality control run in all assays across years ($n = 115$) showed estimates of optical density were highly repeatable ($R = 0.85$, 95% confidence intervals (CI) = 0.54–0.93). FCM are expressed as ng $g^{-1}$ of dry faeces and ln-transformed to meet assumptions of a statistical test.

### (b) Statistical analyses

Our dataset included 1729 FCM measurements collected from 153 females, where individuals were typically sampled multiple times per year (mean samples per year ± s.d. = 6.2 ± 5.1, range = 1–44) [35]. This included 57 individuals with repeated FCM measurements across multiple densities (sampled in 2 or more years, mean ± s.d. = 2.4 ± 0.73, max = 5). We did not censor individuals sampled in only one year, as including these observations helped parametrize fixed effects and did not bias estimates for random effects [36].

We examined variation in endocrine plasticity with random regression models [36,37], fitting four linear mixed models (LMMs) by maximum likelihood and identifying the best-supported model(s) using Akaike's information criterion [38,39].

**Table 2.** We identified two top models, presenting estimates and *t*-values of fixed effects, and variances and standard deviations of random effects. We estimated 95% confidence intervals (CI) by parametric bootstrap. While we do not report *p*-values, parameters with 95% CI not overlapping with 0 are considered as significantly different from 0 (indicated with an asterisk)—this is consistent with *p*-values calculated using the Satterthwaite approximation in 'lmerTest'.

| | model 3 (ID × density, no covariance) | | | model 4 (ID × density, with covariance) | | |
|---|---|---|---|---|---|---|
| **fixed effects** | **estimate ± s.e.** | ***t*-value** | **95% CI (estimate)** | **estimate ± s.e.** | ***t*-value** | **95% CI (estimate)** |
| intercept (lactating, Toronto, 2006) | 8.42 ± 0.22 | 39.1 | 8.00–8.84* | 8.43 ± 0.22 | 39.0 | 7.98–8.84* |
| assay ID (Michigan) | 0.13 ± 0.04 | 3.23 | 0.05–0.21* | 0.13 ± 0.04 | 3.34 | 0.06–0.21* |
| breeding status (non-breeding) | 0.14 ± 0.04 | 3.29 | 0.05–0.22* | 0.14 ± 0.04 | 3.29 | 0.06–0.22* |
| breeding status (pregnant) | 0.20 ± 0.04 | 4.39 | 0.11–0.28* | 0.20 ± 0.04 | 4.38 | 0.11–0.29* |
| Julian date | 0.01 ± 0.02 | 0.87 | −0.02–0.05 | 0.14 ± 0.02 | 0.85 | −0.02–0.04 |
| Julian date squared | −0.07 ± 0.02 | −4.31 | −0.10 to −0.03* | −0.07 ± 0.02 | −4.33 | −0.10 to −0.04* |
| density | −0.01 ± 0.03 | −0.20 | −0.07 to 0.05 | −0.01 ± 0.03 | −0.21 | −0.07 to 0.04 |
| year (2007) | −0.40 ± 0.25 | −1.59 | −0.90 to 0.11 | −0.39 ± 0.25 | −1.58 | −0.85 to 0.19 |
| year (2008) | −0.65 ± 0.21 | −3.06 | −1.09 to −0.23* | −0.66 ± 0.21 | −3.11 | −1.08 to −0.20* |
| year (2009) | −1.03 ± 0.22 | −4.66 | −1.48 to −0.60* | −1.04 ± 0.22 | −4.69 | −1.47 to −0.58* |
| year (2010) | −0.83 ± 0.22 | −3.75 | −1.28 to −0.40* | −0.84 ± 0.22 | −3.79 | −1.28 to −0.37* |
| year (2011) | 0.10 ± 0.22 | 0.48 | −0.32 to 0.51 | 0.10 ± 0.22 | 0.47 | −0.31 to 0.57 |
| year (2012) | −0.90 ± 0.25 | −3.64 | −1.40 to −0.45* | −0.90 ± 0.25 | −3.63 | −1.40 to −0.40* |
| year (2014) | −0.79 ± 0.22 | −3.60 | −1.23 to −0.38* | −0.80 ± 0.22 | −3.64 | −1.22 to −0.35* |
| **random effects** | **variance** | **s.d.** | **95% CI (s.d.)** | **variance** | **s.d.** | **95% CI (s.d.)** |
| ID (intercept) | 0.035 | 0.188 | 0.122–0.238* | 0.035 | 0.188 | 0.116–0.232* |
| ID × density (slope) | 0.015 | 0.123 | 0.00000004–0.177* | 0.015 | 0.123 | 0.031–0.185* |
| residual | 0.352 | 0.593 | 0.572–0.613* | 0.352 | 0.593 | 0.570–0.612* |
| **covariance of random effects** | **correlation coefficient** | **95% CI (correlation)** | | **correlation coefficient** | **95% CI (correlation)** | |
| intercept (ID) & slope (ID × density) | n.a. | n.a. | | 0.25 | −0.37–1.00 | |

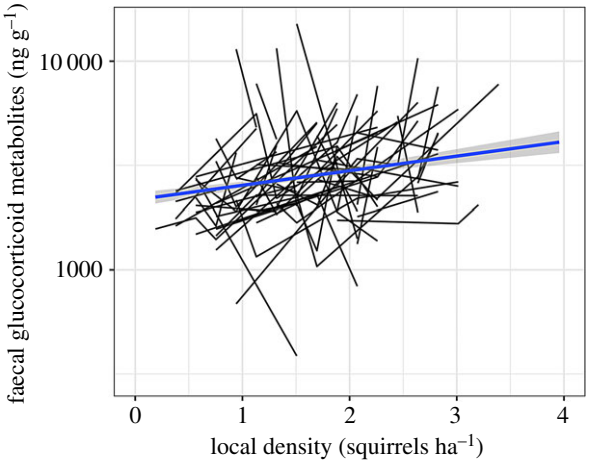


**Figure 1.** Reaction norms for repeatedly sampled female red squirrels ($n$ = 57). Each line indicates one individual's reaction norm, connecting mean annual endocrine phenotype across densities. The emboldened line with shaded 95% CI indicates the population-wide relationship between faecal glucocorticoid metabolites (ng g$^{-1}$) and local density (squirrels ha$^{-1}$), where the $y$-axis is on a ln-scale. (Online version in colour.)

We performed LMMs in R [40] using 'lme4' (v. 1.1-17). Diagnostic plots revealed model residuals were normally distributed and not heteroscedastic. We identified the top model(s) using 'bbmle' (v. 1.0.20) to calculate model $AIC_C$ scores and model weights. Lower $AIC_C$ scores indicate stronger support, and models within two $AIC_C$ units fit a dataset similarly well [39].

We built a null model including the following variables, because they are known to influence FCM in red squirrels [5,29]: breeding status, linear and quadratic effects of Julian date, the facility of analysis, local spring density and year as fixed effects. Controlling for age did not alter the conclusions of the study and since age was not a significant predictor, we excluded it from our models (as in [5]). There were no nonlinear effects of predictors (beyond the quadratic effect of sampling date). We standardized continuous fixed effects (i.e. with mean = 0, s.d. = 1), and checked for multicollinearity (variance inflation factors less than 2). Model 1 (null) included only fixed effects. Model 2 built on the null model, adding a random intercept for each individual. Models 3 and 4 added a random slope for density to model 2, which allowed us to test for individual variation in endocrine plasticity across changes in density [36]. Model 3 assumed there was no correlation between random intercepts and slopes, whereas model 4 allowed for random intercepts and slopes to be correlated. This tested whether an individual's endocrine phenotype affected the likelihood they exhibited weaker or stronger plasticity.

## 3. Results

Female red squirrels had FCM levels from 247 to 46 573 ng g$^{-1}$ and experienced local densities ranging from 0.19 to 3.96 squirrels per hectare. We identified two equivalent top models with a combined weight of 97%, both including random intercepts and slopes (models 3 and 4; table 1). Model 4 also included a negative correlation between intercepts and slopes, though this correlation was not statistically significant (the 95% CI overlapped with 0; table 2). The other two models had a ΔAIC of 6 or more, indicating they were not supported by the data (table 1).

The top models suggest female red squirrels show individual variation in FCM, as well as individual variation in endocrine plasticity across changes in density (figure 1). We did not find support for a correlation between random intercepts and slopes (table 2). Thus, an individual's tendency to have elevated FCM was independent of the degree to which they exhibited endocrine plasticity (figure 2). The fixed effects in both models supported previous findings in this system, where FCM changed across years and breeding status and declined nonlinearly with Julian date (table 2) [5,29].

## 4. Discussion

This study highlights three key results about endocrine variation in free-living red squirrels. First, individuals differed consistently in FCM. Second, females differed in their endocrine plasticity in response to changes in density. Over half of females had elevated FCM as population density increased (56% of females, slope greater than 0.01), whereas 9% of females showed little change (−0.01 < slope < 0.01) and 35% of females showed a decline in FCM with increasing density (slope less than −0.01). Finally, our results suggest that an individual's mean FCM phenotype does not covary with their plasticity in FCM in response to changes in their social environment.

Our results add to a growing body of literature supporting significant individual variation in glucocorticoid plasticity. House sparrows (*Passer domesticus*) showed individual variation in the degree to which glucocorticoids declined with age [41] or with food availability [24], where some individuals responded strongly to changes in age or food availability, while others showed little response. Similarly, free-living male chimpanzees (*Pan troglodytes*) showed repeatable individual variation in urinary glucocorticoid responses to circadian changes [42]. Our study is the first to examine variation in endocrine plasticity along a natural gradient of ecological conditions, providing important insights into how organisms differ in their ability to track environmental changes. Future studies characterizing endocrine plasticity in free-living animals will be critical to better predict how individuals, populations or species cope with changing environments.

The prevalence of individual variation in glucocorticoid plasticity across studies suggests individuals frequently differ in their abilities to respond to environmental challenges, though the proximate mechanism underlying these differences remains unknown. In red squirrels, individuals that do not increase FCM under elevated densities could have responded plastically in downstream targets of glucocorticoids (e.g. changing receptor densities or corticosteroid-binding globulins [43]). A second possibility is that individuals showing little change in glucocorticoids across densities might be constrained in their ability to regulate glucocorticoid secretion [18]—animals with elevated glucocorticoids may already be operating at their physiological maximum and may be unable to increase circulating concentrations further. If this were the case, however, we would expect to find a negative correlation between intercepts and slopes (which was not supported). A third possibility is that individuals differ in their ability to perceive local density—individuals underestimating density could fail to upregulate glucocorticoids under high-density conditions.

More broadly, it is unclear whether individual differences in endocrine plasticity arise from genetic, early-life or environmental effects. Circulating glucocorticoids are shaped in part by additive genetic effects [44–47], though the heritability of glucocorticoid plasticity has not been examined [18]. Early-life exposure to fluctuating environments [48], maternal glucocorticoids [49] and reduced parental care [50] all shape

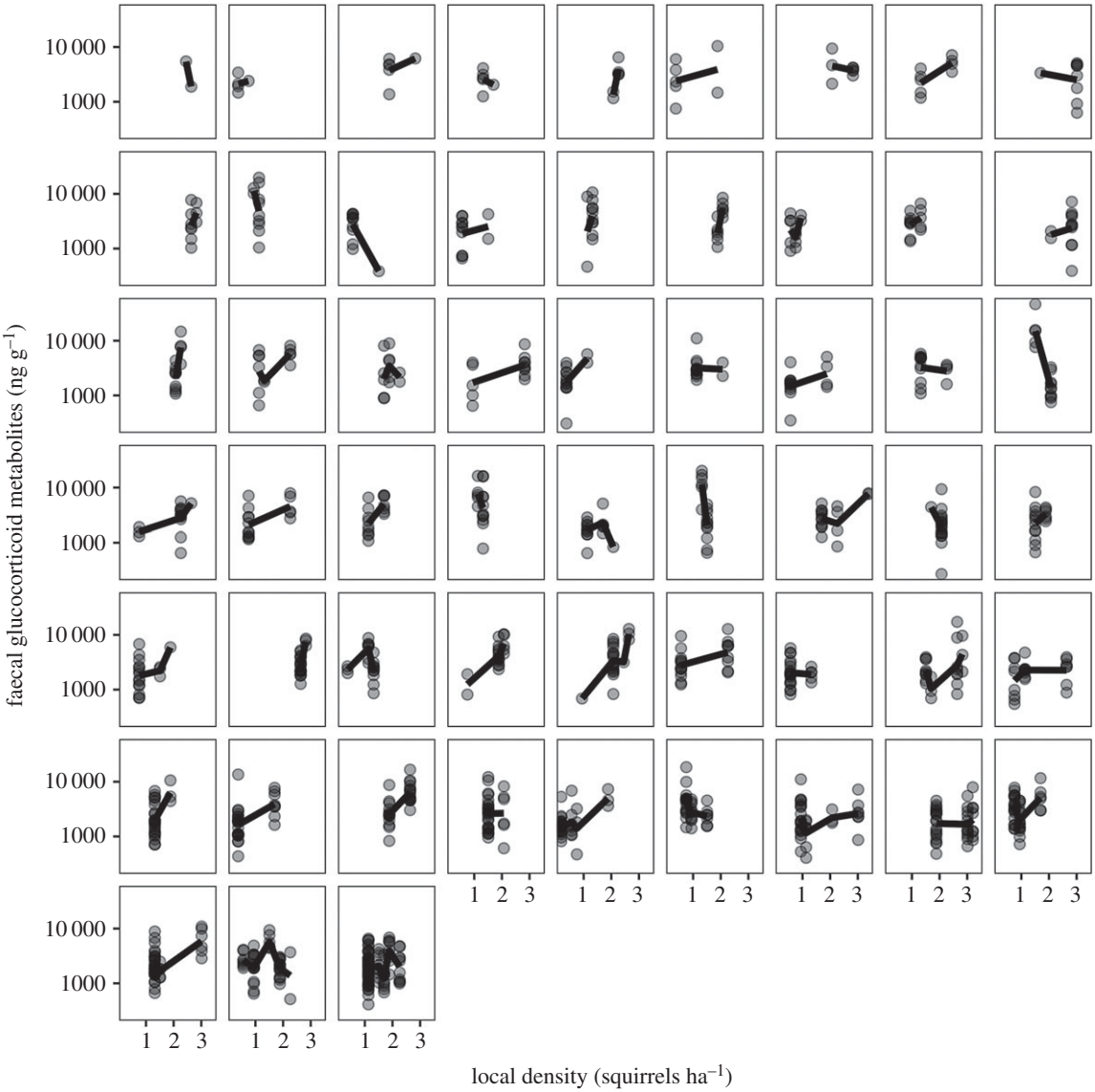


**Figure 2.** Individual reaction norms of female red squirrels. Each panel depicts one female's reaction norm, with points representing a single endocrine sample. Lines connect mean annual faecal glucocorticoid metabolites (ng g$^{-1}$) across changes in density (squirrels ha$^{-1}$), where the *y*-axis is on a ln-scale. Panels are ordered by increasing sample size for hormone measurements collected from a given individual.

the glucocorticoid phenotype of offspring [51–53] and could similarly shape variation in endocrine plasticity. Future research on endocrine plasticity is needed to understand (i) the proximate mechanism generating variation in glucocorticoid plasticity and (ii) the evolutionary causes and consequences of variation in glucocorticoid plasticity.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.s284591 [35]

Authors' contributions. B.D., A.G.M. and S.G.-P. conceived of the research question. B.D., S.B., A.G.M., and J.E.L. supervised and funded the field data collection. B.D., F.v.K., R.P., and R.B. contributed to the endocrine laboratory work. S.G.-P. conducted analyses with guidance from A.G.M. and B.D. S.G.-P., A.G.M. and B.D. wrote the first draft of the manuscript and all authors contributed to subsequent drafts. All authors agreed to be accountable for the work and approved the final version.

Competing interests. We declare we have no competing interests.

Funding. Data collection was supported by the Natural Sciences and Engineering Research Council, the Northern Scientific Training Program, the National Science Foundation and the Ontario Ministry of Research and Innovation. S.G.-P. was funded by a postdoctoral fellowship from NSERC.

Acknowledgements. We thank the Champagne and Aishihik First Nations for allowing us to conduct research within their traditional territory. We also thank the teams who contributed to data collection and anonymous reviewers for their constructive feedback. This is publication number 100 of the Kluane Red Squirrel Project.

## References

1. Levins R. 1968 *Evolution in changing environments*. Princeton, NJ: Princeton University Press.
2. Hoffmann AA, Sgro CM. 2011 Climate change and evolutionary adaptation. *Nature* **470**, 479–485. (doi:10.1038/nature09670)
3. Chevin LM, Hoffmann AA. 2017 Evolution of phenotypic plasticity in extreme environments. *Phil. Trans. R. Soc. B* **372**, 20160138. (doi:10.1098/rstb.2016.0138)
4. Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEBB, Sheldon BC. 2008 Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320**, 800–804. (doi:10.1126/science.1157174)
5. Dantzer B, Newman AEM, Boonstra R, Palme R, Boutin S, Humphries MM, McAdam AG. 2013

Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science* **340**, 1215–1217. (doi:10.1126/science.1235765)

6. Larsen KW, Boutin S. 1994 Movements, survival, and settlement of red squirrel (*Tamiasciurus hudsonicus*) offspring. *Ecology* **75**, 214–223. (doi:10.2307/1939395)
7. LaMontagne JM, Williams CT, Donald JL, Humphries MM, McAdam AG, Boutin S. 2013 Linking intraspecific variation in territory size, cone supply, and survival of North American red squirrels. *J. Mammal.* **94**, 1048–1058. (doi:10.1644/12-MAMM-A-245.1)
8. Boutin S, Tooze Z, Price K. 1993 Post-breeding dispersal by female red squirrels (*Tamiasciurus hudsonicus*): the effect of local vacancies. *Behav. Ecol.* **4**, 151–155. (doi:10.1093/beheco/4.2.151)
9. McAdam AG, Boutin S. 2003 Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution* **57**, 1689–1697. (doi:10.1111/j.0014-3820.2003.tb00374.x)
10. Descamps S, Boutin S, Berteaux D, McAdam AG, Gaillard JM. 2008 Cohort effects in red squirrels: the influence of density, food abundance and temperature on future survival and reproductive success. *J. Anim. Ecol.* **77**, 305–314. (doi:10.1111/j.1365-2656.2007.0)
11. Metcalfe NB, Monaghan P. 2003 Growth versus lifespan: perspectives from evolutionary ecology. *Exp. Gerontol.* **38**, 935–940. (doi:10.1016/S0531-5565(03)00159-1)
12. Rollo CD. 2002 Growth negatively impacts the life span of mammals. *Evol. Dev.* **4**, 55–61. (doi:10.1046/j.1525-142x.2002.01053.x)
13. Metcalfe NB, Monaghan P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260. (doi:10.1016/S0169-5347(01)02124-3)
14. Costantini D, Marasco V, Møller AP. 2011 A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J. Comp. Physiol. B* **181**, 447–456. (doi:10.1007/s00360-011-0566-2)
15. Martin LB. 2009 Stress and immunity in wild vertebrates: timing is everything. *Gen. Comp. Endocrinol.* **163**, 70–76. (doi:10.1016/j.ygcen.2009.03.008)
16. Berger S, Martin II LB, Wikelski M, Romero LM, Kalko EKV, Vitousek MN, Rödl T. 2005 Corticosterone suppresses immune activity in territorial Galapagos marine iguanas during reproduction. *Horm. Behav.* **47**, 419–429. (doi:10.1016/j.yhbeh.2004.11.011)
17. Lynn SE. 2016 Endocrine and neuroendocrine regulation of fathering behavior in birds. *Horm. Behav.* **77**, 237–248. (doi:10.1016/j.yhbeh.2015.04.005)
18. Taff CC, Vitousek MN. 2016 Endocrine flexibility: optimizing phenotypes in a dynamic world? *Trends Ecol. Evol.* **31**, 1–13. (doi:10.1016/j.tree.2016.03.005)
19. Hau M, Casagrande S, Ouyang JQ, Baugh AT. 2016 Glucocorticoid-mediated phenotypes in vertebrates: multilevel variation and evolution. *Adv. Study Behav.* **48**, 41–115. (doi:10.1016/bs.asb.2016.01.002)
20. Guindre-Parker S. 2018 The evolutionary endocrinology of circulating glucocorticoids in free-living vertebrates: recent advances and future directions across scales of study. *Integr. Comp. Biol.* **58**, 814–825. (doi:10.1093/icb/icy048)
21. Angelier F, Wingfield JC. 2013 Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *Gen. Comp. Endocrinol.* **190**, 118–128. (doi:10.1016/j.ygcen.2013.05.022)
22. Meylan S, Miles DB, Clobert J. 2012 Hormonally mediated maternal effects, individual strategy and global change. *Phil. Trans. R. Soc. B* **367**, 1647–1664. (doi:10.1098/rstb.2012.0020)
23. Wingfield JC, Kitaysky AS. 2002 Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integr. Comp. Biol.* **42**, 600–609. (doi:10.1093/icb/42.3.600)
24. Lendvai AZ, Ouyang JQ, Schoenle LA, Fasanello V, Haussmann MF, Bonier F, Moore IT. 2014 Experimental food restriction reveals individual differences in corticosterone reaction norms with no oxidative costs. *PLoS ONE* **9**, e110564. (doi:10.1371/journal.pone.0110564)
25. Baugh AT, van Oers K, Naguib M, Hau M. 2013 Initial reactivity and magnitude of the acute stress response associated with personality in wild great tits (*Parus major*). *Gen. Comp. Endocrinol.* **189**, 96–104. (doi:10.1016/j.ygcen.2013.04.030)
26. Madliger CL, Love OP. 2016 Do baseline glucocorticoids simultaneously represent fitness and environmental quality in an aerial insectivore? *Oikos* **125**, 1824–1837. (doi:10.1111/oik.03354)
27. de Bruijn R, Romero LM. 2018 The role of glucocorticoids in the vertebrate response to weather. *Gen. Comp. Endocrinol.* **269**, 1–23. (doi:10.1016/j.ygcen.2018.07.007)
28. Palme R. 2019 Non-invasive measurement of glucocorticoids: advances and problems. *Physiol. Behav.* **199**, 229–243. (doi:10.1016/j.physbeh.2018.11.021)
29. Dantzer B, McAdam AG, Palme R, Fletcher QE, Boutin S, Humphries MM, Boonstra R. 2010 Fecal cortisol metabolite levels in free-ranging North American red squirrels: assay validation and the effects of reproductive condition. *Gen. Comp. Endocrinol.* **167**, 279–286. (doi:10.1016/j.ygcen.2010.03.024)
30. McAdam AG, Boutin S, Sykes AK, Humphries MM. 2007 Life histories of female red squirrels and their contributions to population growth and lifetime fitness. *Ecoscience* **14**, 362–369. (doi:10.2980/1195-6860(2007)14[362:LHOFRS]2.0.CO;2)
31. Berteaux D, Boutin S. 2000 Breeding dispersal in female North American red squirrels. *Ecology* **81**, 1311–1326. (doi:10.1890/0012-9658(2000)081[1311:BDIFNA]2.0.CO;2)
32. Smith CC. 1968 The adaptive nature of social organization in the genus of three squirrels *Tamiasciurus*. *Ecol. Monogr.* **38**, 31–64. (doi:10.2307/1948536)
33. Dantzer B, Mcadam AG, Palme R, Humphries MM, Boutin S, Boonstra R. 2011 Maternal androgens and behaviour in free-ranging North American red squirrels. *Anim. Behav.* **81**, 469–479. (doi:10.1016/j.anbehav.2010.11.021)
34. Touma C, Sachser N, Möstl E, Palme R. 2003 Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen. Comp. Endocrinol.* **130**, 267–278. (doi:10.1016/S0016-6480(02)00620-2)
35. Guindre-Parker S, Mcadam AG, van Kesteren F, Palme R, Boonstra R, Boutin S, Lane JE, Dantzer B. 2019 Data from: individual variation in phenotypic plasticity of the stress axis. Dryad Digital Repository. (https://doi.org/10.5061/dryad.s284591)
36. Martin JGA, Nussey DH, Wilson AJ, Réale D. 2011 Measuring individual differences in reaction norms in field and experimental studies: a power analysis of random regression models. *Methods Ecol. Evol.* **2**, 362–374. (doi:10.1111/j.2041-210X.2010.00084.x)
37. Nussey DH, Wilson AJ, Brommer JE. 2007 The evolutionary ecology of individual phenotypic plasticity in wild populations. *J. Evol. Biol.* **20**, 831–844. (doi:10.1111/j.1420-9101.2007.01300.x)
38. Burnham KP, Anderson DR. 2002 *Model selection and multimodel inference*. 2nd edn. New York, NY: Springer-Verlag.
39. Symonds MRE, Moussalli A. 2010 A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav. Ecol. Sociobiol.* **65**, 13–21. (doi:10.1007/s00265-010-1037-6)
40. R Core Team. 2017 *R: a language and environment for statistical computing*. Vienna, Austria: Foundation for Statistical Computing. See https://www.R-project.org/.
41. Lendvai ÁZ, Giraudeau M, Bókony V, Angelier F, Chastel O. 2015 Within-individual plasticity explains age-related decrease in stress response in a short-lived bird. *Biol. Lett.* **11**, 20150272. (doi:10.1098/rsbl.2015.0272)
42. Sonnweber R *et al.* 2018 Circadian rhythms of urinary cortisol levels vary between individuals in wild male chimpanzees: a reaction norm approach. *Front. Ecol. Evol.* **6**, 1–11. (doi:10.3389/fevo.2018.00085)
43. Breuner CW, Orchinik M, Hahn TP, Meddle SL, Moore IT, Owen-Ashley NT, Sperry TS, Wingfield JC. 2003 Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R594–R600. (doi:10.1152/ajpregu.00748.2002)
44. Jenkins BR, Vitousek MN, Hubbard JK, Safran RJ. 2014 An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. *Proc. R. Soc. B* **281**, 20141302. (doi:10.1098/rspb.2014.1302)
45. Bairos-Novak K, Ryan C, Freeman A, Anderson W, Hare JF. 2018 Like mother, like daughter: heritability of female Richardson's ground squirrel *Urocitellus richardsonii* cortisol stress responses. *Curr. Zool.* **64**, 153–163. (doi:10.1093/cz/zox014)
46. Evans MR, Roberts ML, Buchanan KL, Goldsmith AR. 2006 Heritability of corticosterone response and

changes in life history traits during selection in the zebra finch. *J. Evol. Biol.* **19**, 343–352. (doi:10.1111/j.1420-9101.2005.01034.x)

47. Stedman JM, Hallinger KK, Winkler DW, Vitousek MN. 2017 Heritable variation in circulating glucocorticoids and endocrine flexibility in a free-living songbird. *J. Evol. Biol.* **30**, 1724–1735. (doi:10.1111/jeb.13135)
48. Sheriff MJ, Krebs CJ, Boonstra R. 2010 The ghosts of predators past: population cycles and the role of maternal programming under fluctuating predation risk. *Ecology* **91**, 2983–2994. (doi:10.1890/09-1108.1)
49. Love OP, Williams TD. 2008 Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. *Horm. Behav.* **54**, 496–505. (doi:10.1016/j.yhbeh.2008.01.006)
50. Liu D *et al.* 2010 Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science* **277**, 1659–1662. (doi:10.1126/science.277.5332.1659)
51. Love OP, McGowan PO, Sheriff MJ. 2013 Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Funct. Ecol.* **27**, 81–92. (doi:10.1111/j.1365-2435.2012.02040.x)
52. Schöpper H, Palme R, Ruf T, Huber S. 2011 Chronic stress in pregnant guinea pigs (*Cavia aperea f. porcellus*) attenuates long-term stress hormone levels and body weight gain, but not reproductive output. *J. Comp. Physiol. B* **181**, 1089–1100. (doi:10.1007/s00360-011-0591-1)
53. Schöpper H, Palme R, Ruf T, Huber S. 2012 Effects of prenatal stress on hypothalamic–pituitary–adrenal (HPA) axis function over two generations of guinea pigs (*Cavia aperea f. porcellus*). *Gen. Comp. Endocrinol.* **176**, 18–27. (doi:10.1016/j.ygcen.2011.12.010)