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Research paper

# Corticosterone response by *Peromyscus* mice to parasites, reproductive season, and age

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ABSTRACT

A common response to parasite infestations is increased production of glucocorticoid hormones that regulate immune function. We examined relationships between ectoparasite infestations and fecal corticosterone metabolites (FCM) in deer mice (*Peromyscus maniculatus*). Furthermore, we experimentally removed fleas to determine if reductions in ectoparasites affected FCM production. Individuals were assigned to control (no flea removal) or treatment (anti-flea application, physical combing) groups and individuals were recaptured to assess changes in FCM concentrations. There was a significant and negative effect of number of anti-flea treatment applications on FCM concentrations of deer mice. However, models including host biology traits and environmental predictors had a better model fit compared to models containing ectoparasite predictors. In particular, there was a significant relationship of deer mouse FCM with date and host age, where glucocorticoid production decreased towards the end of the breeding season and increased with age. Overall, adverse events associated with reproduction and age class, rather than ectoparasites, may be more important to variation in glucocorticoids of deer mice.

## 1. Introduction

Parasites are an integral component of ecological systems; they can modify host behaviour, morphology, and physiology, and thus influence the role that their hosts play within an ecological community (Goodman and Johnson, 2011). Although there are certain cases where parasites may be beneficial to their hosts, by definition, parasites exploit their hosts and can have deleterious impacts (Maizels, 2016; Sánchez et al., 2018; Zug and Hammerstein, 2018). These effects can be direct through consequences of parasitic feeding, including tissue damage, toxins from saliva, and allergic reactions (Steen et al., 2006; Palm et al., 2012). However, parasites can also have indirect effects on their hosts, including changes in fecundity, body condition, survivorship, and behaviour (Brown et al., 1995; Newey and Thirgood, 2004; Sánchez et al., 2018; Santicchia et al., 2020).

Given the effects that parasites can have on their hosts, many studies have focused on trade-offs between parasite defence and life-history traits (Leung and Koprivnikar, 2016; Albery et al., 2019). In particular, parasite resistance is often expected to play a demanding role in host life-history, and an associated immune defence is generally accepted as a costly investment by a host (Langand et al., 1998; Zuk and Stoehr, 2002). Thus, further consumption of host resources by a parasite or requisite immune response by a host can lead to fewer resources available for other functions, such as host maintenance and reproduction (Branson, 2003).

It is common for studies to focus on the host immune system, but it is a combination of systems that interact in a host to respond to parasitism (Modha et al., 1996; Morales-Montor et al., 2001). For instance, a stimulus (such as a parasite) can generate production of a collection of cytokines, hormones, neurotransmitters, and neuropeptides expressed from receptors of immune, endocrine, and nervous cells (Besedovsky and del Rey, 1996; Corrêa-de-Santana et al., 2006). One of the main

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components of this complex network is the HPA (hypothalamic-pituitary-adrenal) axis, which modulates immune response and secretes hormones, including glucocorticoids (Buckingham et al., 1996; Corrêa-de-Santana et al., 2006).

Glucocorticoids, namely cortisol and corticosterone, have long been recognized as a critical component of the stress response in vertebrates; however, they also play an especially vital role in the immune-neuroendocrine system (Weil et al., 2006). Specifically, glucocorticoids regulate immune system functioning though a variety of mechanisms, such as enhancing or inhibiting inflammation and adaptive immunity (Cain and Cidlowski, 2017). Acute elevations can promote immune function and enhance parasite defence; however, chronic elevations in glucocorticoids can lead to reduced parasite resistance and tolerance through immunosuppression (Padgett and Glaser, 2003; Schoenle et al., 2019). Additionally, parasites may manipulate glucocorticoid concentrations to favour their own survival, leading to greater parasite loads over longer periods of time (Defolie et al., 2019). Therefore, studies on host glucocorticoid responses to parasite infestations can evaluate how parasites may act as a physiological stressor to their hosts (O'Dwyer et al., 2020).

Studies examining relationships between parasites and glucocorticoids in mammals have had inconsistent results, with some finding the expected positive correlation between glucocorticoids and parasite infestations (Brown and Fuller, 2006; Martínez-Mota et al., 2017; Seguel et al., 2019), others finding no relationship (Monello et al., 2010; Carlsson et al., 2016; Trevisan et al., 2017) and some even finding a negative correlation (Sures et al., 2002; Hufschmid et al., 2014). Additionally, the direction, shape, and strength of the relationship may vary with parasite species, as well as parasite life stage (Romeo et al., 2020). Meta-analyses of relationships between parasites and vertebrate host glucocorticoids demonstrate that a positive relationship is the most common trend (Defolie et al., 2019; O'Dwyer et al., 2020). However, the elevation of glucocorticoids may be delayed over the course of infestation (O'Dwyer et al., 2020). Co-infection of multiple parasite species is also common in wild environments and can influence infestation intensity, host immune response, and the costs of parasite infestations, which may alter host hormone profiles (Ezenwa, 2016; Schoenle et al., 2019). Furthermore, there is variation in how hosts respond to parasites due to host and environmental factors (Pedersen and Greives, 2008; Gaitan and Millien, 2016; Puehringer-Sturmayr et al., 2018). Overall, a positive correlation is expected between parasite infestations and host physiological stress as the host immune response, including increased glucocorticoids, is upregulated in response to infestation (Defolie et al., 2019).

*Peromyscus* mice are an intensively studied genus, representing the most abundant and widespread small mammal genus in North America (Bedford and Hoekstra, 2015); however, to our knowledge, the relationship between parasites and glucocorticoids in deer mice has not yet been examined. We evaluated changes in glucocorticoids of the deer mouse (*Peromyscus maniculatus*) in response to ectoparasites. Specifically, we investigated the effects of infestations of fleas (*Orchopeas leucopus*) and mites (*Neotrombicula harperi*), which are known to commonly infest deer mice (Bobbie et al., 2016; Veitch et al., 2020). We experimentally removed fleas, expecting that hosts would experience a decrease in glucocorticoids while statistically controlling for host and environmental factors. We also expected that occurrence of fleas, mites, and the interaction between the two parasites would lead to heightened glucocorticoid production in hosts. Lastly, we examined how glucocorticoid concentrations varied with host biology traits (ex. sex, body mass, reproductive status) and environmental conditions (ex. predation risk, host population size, time of year), which can affect the glucocorticoid response (Harper and Austad, 2004; Good et al., 2005; Sheriff et al., 2009; Stewart et al., 2020).

## 2. Materials and methods

### 2.1. Field methods

Deer mice (*Peromyscus maniculatus*) were sampled in Algonquin Provincial Park, Ontario, Canada (45°54′ N, 78°26′ W) from May – August 2018 across seventeen 100-m traplines with two Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) placed at each station, 10-m apart (detailed methods in Fryxell et al., 1998). Traps were baited with water-soaked sunflower seeds at dusk and checked at dawn over 3 consecutive nights on alternate weeks. Captured individuals were transferred to a handling bag and sexed. Individuals were weighed using a Pesola scale and age (juvenile, subadult, adult) was determined from body mass and from hair colour (Schmidt et al., 2019). Reproductive status was recorded as non-reproductive or reproductively active (scrotal testes for males, perforate vagina, pregnant (swollen abdomen), or lactating (prominent nipples) for females) (Millar et al., 1992; Mayfield et al., 2000). Individuals received two metal ear tags with unique alphanumeric codes (National Band and Tag Co., Newport, Kentucky, USA).

Traplines are distributed across a variety of forest habitat types (Fryxell et al., 1998). These traplines were paired by habitat type (sugar maple hardwood, cut-over mixed-wood, dense mixed-wood, conifer, white pine and white spruce, black spruce and aspen, white pine and red pine) and were assigned to a control or treatment group. The trapline with the highest number of captures the previous year was assigned to the treatment group, additional lines in the same habitat were assigned to the control group. In the treatment group, fleas were removed from mice through combing and application of 0.8-ul of Frontline Plus (Merial Limited, Duluth, GA, USA, main active ingredient: fipronil 0.29%). Treatment was reapplied to recaptures that had not been treated within 26 days, as the labels indicated that reapplication is necessary after 30 days. Flea specimens were stored in 70% ethanol for future identification. In the control group, mice were visually examined for ectoparasites, did not have their fleas removed, and did not receive Frontline Plus. The ectoparasite quantification methods used to compare between the control and treatment group is similar to those previously used on Franklin's ground squirrels (Pero and Hare, 2018).

For visual assessment of ectoparasites, observers spent 60-s examining the dorsal and the ventral side of the mouse for ectoparasites such as fleas (Siphonaptera) and trombiculid mites while gently combing backwards with a fine-toothed comb and blowing on the fur to expose the skin (Patterson et al., 2013). Only ectoparasites large enough for visual observation without the need for a microscope were included in this study. While combing and visual assessment in combination is a reliable method for examining fleas, lice, and ticks, it is possible that mite species can be missed through visual inspection and combing (Beaumont et al., 2019); therefore, it is possible that we were not able to identify additional mite species in this study. However, this is a common method of quantifying ectoparasite communities on small mammals that has been used in multiple studies (ex. Buchholz and Dick, 2017; Pero and Hare, 2018; Beaumont et al., 2019).

Frontline Plus is only effective at preventing infestation of fleas and ticks (Wiedemann, 2000a, 2000b; Hoffmann et al., 2016), and no other ectoparasites were removed, only quantified through visual assessments. Juvenile deer mice were not treated with Frontline Plus to avoid potential mortality risk of improper dosage and were excluded from the ectoparasite removal experiment. All methods used in this study were reviewed and approved by the Animal Care Committee (ACC) at Laurentian University, Sudbury, Ontario, Canada, protocol number 2018-03-04.

### 2.2. Taxonomic identification

A subset of ectoparasites (specifically fleas and mites) were combed from host fur and collected using tweezers to identify to species. The two

main ectoparasites species on this population of deer mice are a flea (*Orchopeas leucopus*) and mite (*Neotrombicula harperi*) (Veitch et al., 2020). Mite specimens were collected in 2016 prior to data collection for the present study. Flea specimens were collected in 2017 prior to data collection for the present study, as well as from traplines in the parasite removal treatment group in 2018. Specimens were stored in 70% ethanol. Recaptures of individuals not included in the dataset showed that fleas and mites take ~3–4 days to recolonize a host after parasite removal (Veitch, 2020). Therefore, recaptures of individuals with ectoparasites removed were not included in the dataset unless at least a week had passed since ectoparasite removal.

Representative subsamples of fleas from deer mice were prepared for detailed morphological examination and identification using balsam mounts (Richards, 1964). The rest of the flea samples from deer mice were examined using a dissecting microscope (Olympus SZ61). All flea samples were identified to species using keys to Siphonaptera (Holland, 1985; Lewis, 2000) and with the assistance of T. Galloway (Department of Entomology, University of Manitoba, Canada). The mites were identified to species by H. Proctor (Department of Biological Sciences, University of Alberta, Canada) (Bobbie et al., 2016).

### 2.3. Collection and processing of fecal glucocorticoid metabolites

We used fecal samples to evaluate glucocorticoid concentrations of deer mice. Feces can be used as a non-invasive sample that represents an integrated average measure of circulating hormone levels that are less affected by short-term fluctuations compared to blood or saliva samples (Dantzer et al., 2010). Fecal glucocorticoid metabolites are a common method to examine glucocorticoid concentrations and have been used previously to examine glucocorticoid levels in deer mice (Hayssen et al., 2002; Harper and Austad, 2004).

Feces were collected from all traps containing deer mice (no later than 19 h after defecation) as well as any fresh samples that could be collected from deer mice in the hand. The effect of time spent in the trap on corticosterone concentrations was examined through trapline order, as traplines checked later in the day would contain animals that had been in the traps for longer periods of time compared to earlier traplines. We ran a linear mixed-effects model ('nlme' package version 3.1–145) with trapline order (first trapline checked, second, etc.) as a fixed effect and individual ID number as a random effect, but there was no significant effect of trapline order ($\beta = -0.078$, $P = 0.236$) (Pinheiro et al., 2012). Feces were placed in individual Eppendorf tubes with 80% methanol, placed on ice packs in the field, and then stored at −20 °C until analysis.

Fecal corticosterone metabolites of mice in the treatment and control group were examined for ~two-week periods. Fecal samples were then labelled as "pre-" or "post-treatment". For treatment individuals, "pre-treatment" was before application of Frontline Plus and then "post-treatment" was roughly two weeks later at subsequent capture. For control individuals, "pre-" and "post-treatment" are simply ~two weeks apart. In total, 184 fecal samples collected from 59 deer mice (2.44 ± 1.15 samples/individual) over two months (June 20th to August 15th, 2018) were included in the statistical analysis.

### 2.4. Hormone extraction and analysis

Concentrations of corticosterone, the main circulating glucocorticoid in deer mice (Pedersen and Greives, 2008), and its metabolites were determined using hormone extraction and enzyme immunoassays. All materials were purchased from Fisher Scientific Inc. unless otherwise stated. Fecal samples were transferred from Eppendorf tubes to glass scintillation vials along with any methanol present, and Eppendorf tubes were subsequently rinsed with 1-ml of 100% methanol, which was also transferred into the glass vial. The samples were then evaporated to dryness under a fume hood overnight, and then weighed to obtain a fecal mass. Freshly made 80% methanol (in water; v:v) was added using a ratio of 0.005-g/ml, samples were vortexed for 10-s, and then mixed overnight on a plate shaker at 200-rpm. The vials were centrifuged at 2300-g for 10-min and the extract was transferred to a clean glass vial. The extracts were diluted 1:30 in enzyme immunoassay buffer (0.1-mM sodium phosphate buffer, pH 7.0, containing 9-g of NaCl and 1-g of bovine serum albumin per litre), and diluted for a final 1:20 dilution.

To quantify fecal corticosterone metabolite (FCM) concentrations, we used an enzyme immunoassay (detailed methods provided by Baxter-Gilbert et al., 2014; Stewart et al., 2020). Microtitre plates were coated with 0.25-µg/well goat anti-rabbit IgG polyclonal antibody (Sigma-Aldrich, Mississauga, ON, Canada; 1:200,000 in coating buffer, 50-mM bicarbonate buffer, pH 9.6) and incubated overnight at room temperature. Plates were washed with 0.05% Tween 20, 0.15-M NaCl solution and blocked with 250-µl EIA buffer for 1-hr at room temperature. Plates were then loaded with 50-µl corticosterone standard (Steraloids Q1550; 39–10,000 pg/ml), extracts, and controls, followed by 100-µl horseradish peroxidase conjugate (1:1,000,000) and 100-µl corticosterone antiserum (1:200,000; antibody lot: CJM006; C. Munro, University of California, Davis, CA, USA), all diluted in EIA buffer. Plates were incubated overnight at room temperature, and then washed and loaded with 200-µl of substrate solution (0.5-ml of 4-mg/ml tetramethylbenzidine in dimethylsulphoxide and 0.1-ml of 0.176-M $H_2O_2$ diluted in 22-ml of 0.01-M sodium acetate trihydrate [$C_2H_3NaO_2 \cdot 3H_2O$], pH 5.0). After 30-min incubation, colour reaction was stopped with 50-µl $H_2SO_4$ (1.8 M) and absorbance was measured at 450-nm using a spectrophotometer ($MRX^e$ microplate reader, Dynex Technologies, Chantilly, VA). Hormone data are presented as ng FCM/g feces. Inter- and intra-assay CV's were 9.2% and 4.4%, respectively. Serial dilutions of pooled fecal extract showed parallel displacement with the corticosterone standard curve ($r = 0.996$, $P < 0.001$). Assay sensitivity is 82-pg/ml. The corticosterone antibody (CJM006) cross-reactivities are: corticosterone (100%), deoxycorticosterone (14.25%), and other metabolites (<1%).

### 2.5. Statistical analysis

Analyses were conducted using statistical software package R version 3.5.2 (R Core Team 2018). We fitted linear mixed-effects models using the maximum-likelihood method (ML) in 'nlme' package version 3.1–145 (Pinheiro et al., 2012). To account for repeated sampling of an individual, individual ID was added as a random effect for all models. We tested for variation in FCM concentrations with our experimental removal of fleas using a linear mixed-effects model with the main effects and interaction of treatment group (control or treatment) and occasion (pre- or post-treatment), number of Frontline applications received, and date as fixed effects. We also examined the effect of flea occurrence (1 = present, 0 = absent), mite occurrence (1 = present, 0 = absent), the interaction between occurrence of the two parasites species, and date as fixed effects. Flea or mite intensity was not included in the linear mixed-effects model. We ran a linear model ('stats' package version 4.0.2) with flea intensity at first capture as the predictor, but there was no significant effect of flea intensity on fecal corticosterone metabolites ($\beta = 0.025$, $P = 0.806$). We did not have intensity measures for mites. To examine the effect of host biology traits, we ran a linear mixed-effects model with host age (subadult or adult), sex, reproductive status (non-reproductive, reproductively active), body mass, and date as fixed effects. We ran an additional model including these host biology predictors and treatment group and occasion.

Lastly, we examined the effect of environmental predictors in a linear mixed-effects model including predator occurrence, population abundance of deer mice, southern-red backed voles (*Myodes gapperi*) and woodland jumping mice (*Napaeozapus insignus*), and date as fixed effects. Population abundance was measured as captures per hundred trap nights over two-week intervals, as used by Stewart et al. (2014). Southern red-backed voles and woodland jumping mice were included in the analysis, as they are known to share similar space or food preferences and may influence resource competition (Vickery, 1979;

Schulte-Hostedde and Brooks, 1997; Merlot et al., 2004; Boonstra and Krebs, 2012). Predator occurrence was assumed from nights where traplines consisted of disturbed traps (i.e. knocked over, moved from original placement) or contained blood and tissue from small mammals. These two events (trap disturbance, presence of blood/tissue) often jointly occurred. This method accounts for mammalian predators, such as American martens (*Martes americana*), a known predator of deer mice in Algonquin Provincial Park that was seen along the traplines over the course of this study (Fryxell et al., 1999).

A $\log_{10}$-transformation was applied to the FCM variable in all models to normalize distribution of data. Assumption of multicollinearity was tested using variance inflation factors (VIF) from 'car' package version 3.0–7, all predictors had VIF $< 3$ (Zuur et al., 2009). Two-level categorical variables were coded as binary continuous variables to improve model simplicity. All continuous predictors, including transformed binary variables, were centered and scaled by their mean and standard deviation. P-values were calculated using a likelihood ratio test with the 'anova' function in the 'stats' package version 4.0.2. Akaike Information Criterion (AIC) corrected for small sample sizes and likelihood-ratio based pseudo r-squared (LR $x^2$) were calculated using the 'MuMIn' package version 1.43.15 (Barton, 2019). Evidence ratios were calculated using the 'AICcmodavg' package version 2.2–2 (Mazerolle, 2019).

## 3. Results

The final dataset included 59 mice: 27 in the treatment group and 32 in the control group, where 21 mice were infested with a flea (*Orchopeas leucopus*), 14 mice were infested with a trombiculid mite (*Neotrombicula harperi*), and 4 mice were infested with both the flea and mite. Additionally, 4 mice were infested with a botfly (*Cuterebra* sp.); however, this was not investigated in the statistical analysis due to small sample sizes. Frontline Plus was an effective treatment against fleas, with only a single individual in the treatment group infested with a flea two weeks after being treated. In contrast, the Frontline treatment had no effect on mite infestations. This was expected given that Frontline Plus is designed to remove only fleas and ticks (Wiedemann, 2000a, 2000b; Hoffmann et al., 2016). Individuals without fleas were still included in the statistical analysis as they might still have come into contact with fleas in their burrows (van der Mescht et al., 2018). Mode and average number of days between fecal collections per individual was 14 days.

All of the models except the linear mixed-effects model including host biology predictors with treatment group and occasion were substantially better than the null (LER $> 0.5$); however, the model including host biology predictors was the top and only model that demonstrated a decisive difference (LER $> 2$) (Table 1) (Snipes and Taylor, 2014). The model including environmental predictors and the model containing the experimental flea removal predictors were the second and third top models, respectively. While there was not a significant change in FCM pre-and post-treatment (flea removal with Frontline Plus), there was a significant effect of the number of treatment applications (Fig. 1, Table 2). Specifically, mice with more applications had lower FCM levels. Date also exhibited a significant negative relationship in both the model including host biology predictors and the model including environmental predictors (Fig. 2, Table 2). From the model including host biology predictors, host age had a significant and negative relationship with FCM of deer mice (Fig. 3, Table 2). There was also a high degree of individual variation in deer mice FCM concentrations (Fig. 4).

## 4. Discussion

There was not a significant or large change in FCM levels after experimental removal of fleas; however, the number of anti-flea treatment applications did have a small but significant negative effect. Furthermore, in comparison to models incorporating host traits or environmental predictors, predictors associated with ectoparasite occurrence had a poorer model fit. This suggests that ectoparasites do not induce a large change in corticosterone production in deer mice compared to host traits or environmental conditions.

While there was no pre- and post-treatment effect of the experimental removal of fleas on FCM in deer mice, there was an effect of the number of anti-flea treatment applications. This suggests that the effects of flea removal on FCM concentrations may not be apparent over short-term periods, such as the two-week intervals between pre- and post-treatment measurements. However, over longer time periods with repeated removal of fleas, a significant and negative effect was observed. Given the large degree of variation in FCM concentrations observed, it may be difficult to identify trends over shorter time periods. Therefore, it is important to consider the time scale over which variation in FCM concentrations are being examined. While number of anti-flea treatment applications could be confounded with date, as both have a negative influence on FCM concentrations, the model including both number of treatment applications and date did not have any predictors with VIF $>$ 3, suggesting that there is no concern for multicollinearity (Zuur et al., 2009). Furthermore, while we did not find a significant effect of flea removal, the treatment was only effective for three weeks and some host individuals had become re-infested with fleas by their next capture, as we were applying the treatment in four-week intervals. Therefore, the relationship between flea infestations and deer mice FCM concentrations might be stronger than what is reported here, and more apparent with continued removal of ectoparasites over longer periods of time. It is also pertinent to consider that only six deer mice were treated a second time with Frontline Plus, which provided limited data on the relationship between number of anti-flea treatment applications. However, there was still a decrease in FCM levels between no applications and a single application.

The negative relationship between the number of anti-flea treatment applications and deer mouse corticosterone concentrations was expected, given that hosts often exhibit elevated glucocorticoid production when infested with parasites (Defolie et al., 2019; O'Dwyer et al., 2020). Host elevation in glucocorticoids can divert energy away from non-essential functions, such as reproduction, and towards vital functions, such as immune defense (Lutermann et al., 2012). Acute elevation of glucocorticoids can stimulate inflammatory responses, while continued elevation defends the host against damage from parasites through the

**Table 1**
Summary of linear mixed-effects models predicting $\log_{10}$-transformed values of fecal corticosterone metabolites in deer mice. Individual ID is included as a random effect in all models (n = 59 individuals).

| Model | Parameters | K | AICc | ΔAICc | Weight | LR $x^2$ | ER | LER |
|---|---|---|---|---|---|---|---|---|
| 1) | Sex + Age + Reproductive Status + Mass + Date | 8 | 281.21 | 00.00 | 0.88 | 0.18 | 106.6 | 2.03 |
| 2) | Predator Occurrence + DM PA + RBV PA + WJM PA + Date | 8 | 286.99 | 05.78 | 0.05 | 0.14 | 5.94 | 0.77 |
| 3) | Treatment*Occasion + Frontline Application + Date | 8 | 287.69 | 06.48 | 0.03 | 0.14 | 4.17 | 0.62 |
| 4) | Flea Occurrence*Mite Occurrence + Date | 7 | 287.98 | 06.76 | 0.03 | 0.12 | 3.62 | 0.56 |
| 5) | Intercept | 3 | 290.55 | 09.34 | 0.01 | 0.05 | 1.00 | 0.00 |
| 6) | Treatment + Occasion + Sex + Age + Reproductive Status + Mass | 9 | 294.85 | 13.64 | 0.00 | 0.12 | 8.57 | 0.93 |

*, main effects and interaction; PA, index of population abundance; DM, deer mouse; RBV, southern red-backed vole; WJM, woodland jumping mice; K, number of estimated parameters; AICc, Akaike information criterion for small sample sizes; ΔAICc, difference in $AIC_c$ to the best model; LR $x^2$, likelihood-ratio based pseudo r-squared; ER, evidence ratio comparison with null model, LER, $\log_{10}$-transformed evidence ratio comparison with null model.

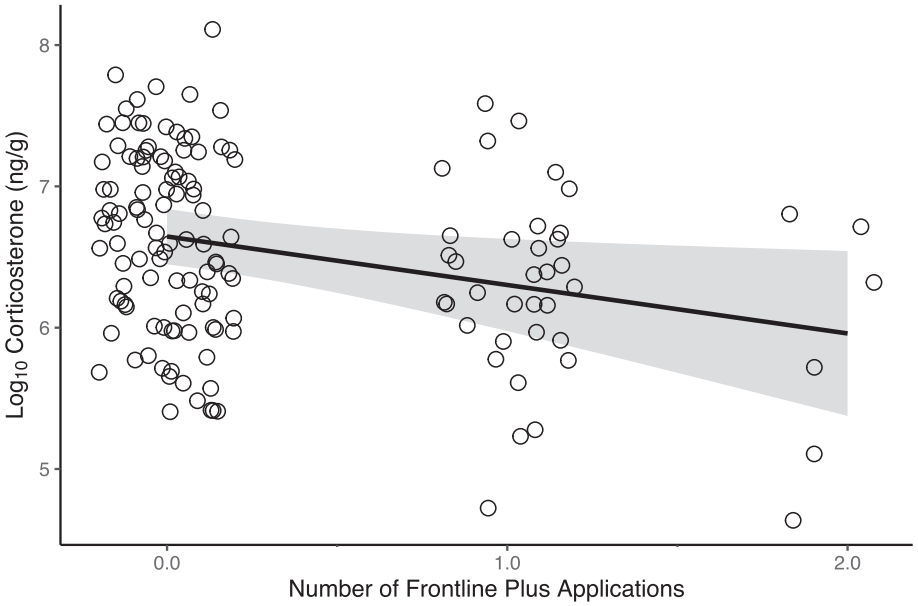


**Fig. 1.** Relationship between $log_{10}$-transformed values of fecal corticosterone metabolites (FCM) in deer mice and number of Frontline Plus applications to experimentally remove fleas (n = 59 individuals). Number of applications had a negative effect on FCM concentrations. Shown are partial residuals (circles) and the 95% confidence interval (shading) extracted from linear mixed effects model (package 'nlme'). Data points are jittered along x-axis for ease of interpretation.

**Table 2**
Summary of scaled fixed effects of top three best fit linear mixed-effects models predicting $log_{10}$-transformed values of fecal corticosterone metabolites in deer mice. Individual ID is included as a random effect in all models (n = 59 individuals). Bolded variables are those with P < 0.05 (considered to have strong support).

| Model | Predictor Variable | Estimate | SE | F/t[a] | P |
|---|---|---|---|---|---|
| 1) Sex + Age + Reproductive Status + Mass + Date | | | | | |
| | $Sex_M$ | −0.193 | 0.129 | −1.49 | 0.218 |
| | **$Age_A$** | **−0.099** | **0.161** | **−0.61** | **0.045** |
| | Reproductive Status | −0.158 | 0.131 | −1.21 | 0.438 |
| | Mass | −0.103 | 0.080 | −1.29 | 0.440 |
| | **Date** | **−0.197** | **0.053** | **−3.75** | **<0.001** |
| 2) Predator Occurrence + DM PA + RBV PA + WJM PA + Date | | | | | |
| | Predator Occurrence | −0.031 | 0.063 | −0.48 | 0.472 |
| | DM PA | −0.025 | 0.056 | −0.44 | 0.849 |
| | RBV PA | −0.044 | 0.059 | −0.74 | 0.258 |
| | WJM PA | −0.101 | 0.056 | −1.80 | 0.260 |
| | **Date** | **−0.181** | **0.052** | **−3.47** | **<0.001** |
| 3) Treatment*Occasion + Frontline Application + Date | | | | | |
| | Treatment | 0.085 | 0.077 | 1.11 | 0.669 |
| | Occasion | −0.008 | 0.055 | −0.15 | 0.102 |
| | **Frontline Application** | **−0.152** | **0.081** | **−1.86** | **0.008** |
| | Date | −0.080 | 0.065 | −1.23 | 0.118 |
| | Treatment × Occasion | 0.059 | 0.058 | 1.04 | 0.310 |

[a] F values are presented for interactions and t values for parameter estimates.

immune response and protection of host cells, organs, and nervous system (Defolie et al., 2019). Fleas in particular can negatively affect a host directly, not only through blood loss, but also skin irritation, injection of salivary toxins, and introduction of harmful pathogens (Khokhlova et al., 2002). Furthermore, fleas are associated with indirect adverse effects on body size, survival, and reproductive success (Devevey and Christe, 2009; Patterson et al., 2013). Therefore, removal of fleas may reduce the need for parasite defence and subsequently lead to a decrease in FCM concentrations. However, given that the treatment predictor itself did not have a large or significant effect, further experimentation is required to establish whether the flea *Orchopeas leucopus* has a significant effect on deer mouse FCM concentrations.

It is also important to consider that models containing host biology traits or environmental predictors had a better fit than any of the models containing ectoparasite-related predictors. Furthermore, the model containing predictors on occurrence of the flea and mite was not included in the top three models, suggesting that infestation of these two ectoparasite species, either in isolation or in combination, were poorer predictors of FCM concentrations in deer mice. We also focused on occurrence rather than parasite intensity measures, which may influence how strong a role ectoparasites play in FCM variation and does not allow for the evaluation of non-linear relationships (Romeo et al., 2020). Additionally, we had limited data on the effect of co-infections, as only four mice were infested with both ectoparasite species. Nevertheless, all investigated models containing parasite predictors had higher AICc values compared to those examining host traits and environmental conditions. This suggests that in comparison, ectoparasites are not as influential in FCM concentrations.

One explanation for models with ectoparasite predictors exhibiting poorer model fit compared to host traits and environmental factors is that hosts likely have a combination of both resistance (ability to limit parasite burden) and tolerance (ability to limit damage of parasite burden) mechanisms (Råberg et al., 2009). Specifically, hosts with a larger investment in tolerance mechanisms for parasites may minimize

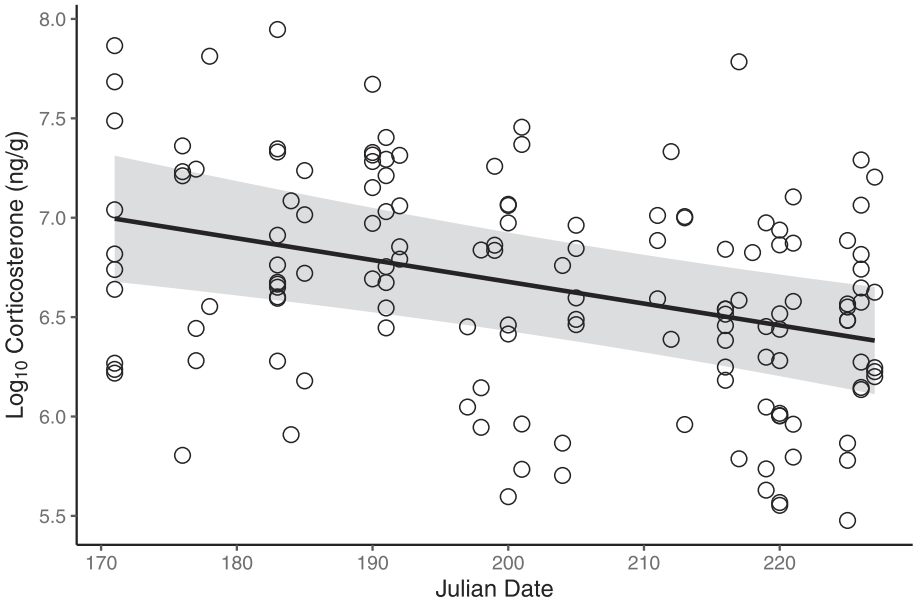


**Fig. 2.** Relationship between $\log_{10}$-transformed values of fecal corticosterone metabolites (FCM) in deer mice and Julian date (n = 59 individuals). Date had a negative effect on FCM concentrations. Shown are partial residuals (circles) and the 95% confidence interval (shading) extracted from the linear mixed effects model 1 with host biology predictors (package 'nlme').

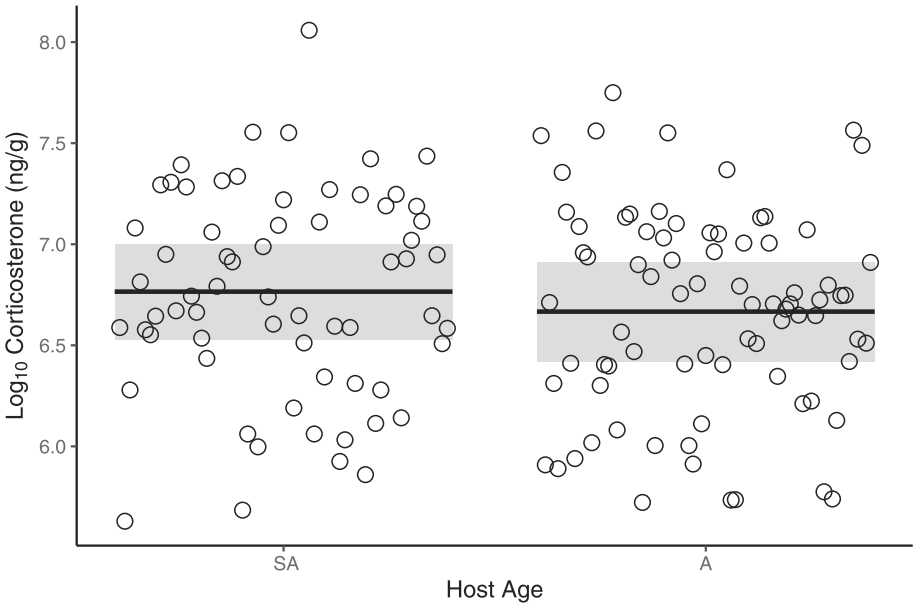


**Fig. 3.** Relationship between $\log_{10}$-transformed values of fecal corticosterone metabolites (FCM) in deer mice and age class (n = 59 individuals). Subadults had higher FCM concentrations than adults. Shown are partial residuals (circles) and the 95% confidence interval (shading) extracted from linear mixed effects model (package 'nlme'). SA, subadult; A, adult.

energetic costs of parasite infestations and associated defence, leading to minimal changes in glucocorticoid concentrations with parasite infestations (Schoenle et al., 2019; Defolie et al., 2019). Alternatively, hosts may have highly targeted immune responses against parasite species that they have had previous exposure with, leading to increased resistance and decreased physiological stress and glucocorticoid levels (St. Juliana et al., 2014; Defolie et al., 2019). This could be the case in this host-parasite system, given that there are few common ectoparasite species that deer mice were found to come into contact with, and that they would then need to develop an immune response against. Therefore, ectoparasites may not affect deer mice FCM levels as much as host biology traits or environmental factors if mice have a high investment in tolerance or a targeted immune response against the investigated parasites.

A significant and negative trend was observed between date and deer mice FCM, suggesting that glucocorticoid production varied on a temporal scale. Seasonal variation is often the rule rather than the exception, especially given the importance of glucocorticoids in regulating many physiological processes (Borniger et al., 2017; Cain and Cidlowski, 2017). For instance, photoperiod and temperature are physiological signals for glucocorticoid secretion in deer mice (Demas and Nelson, 1996; Borniger et al., 2017). Deer mice also vary their diet seasonally, which can influence metabolism or gut passage time, and consequently alter FCM concentrations (Jameson, 1952; Goymann, 2012). Generally,

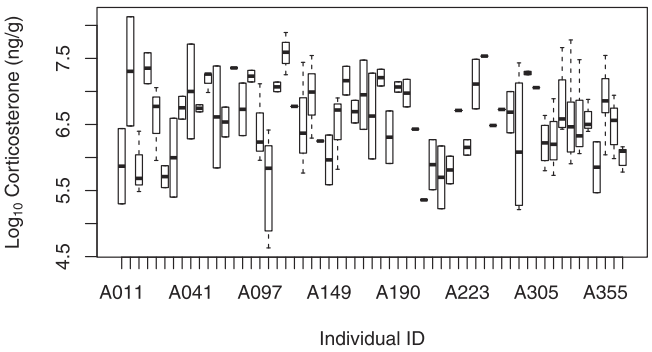


**Fig. 4.** Relationship between $\log_{10}$-transformed values of fecal corticosterone metabolites in deer mice and individual ID number (n = 59 individuals). Shown are the median (black line), interquartile range (box) and minimum and maximum values (bars).

it is well known that predictable changes in corticosterone production have evolved as a response to environmental conditions to help individuals adjust their energetic demands over different seasons (Reeder and Kramer, 2005). Therefore, there may be some seasonal or environmental component to changes in FCM levels observed here. In particular, the effect of date might be related to changes in the breeding season of deer mice (May to early August), with females producing multiple litters within a season (Fairbairn, 1977a). While we did not find a relationship between deer mice reproductive status and FCM, there may be an increase in glucocorticoids during the reproductive season due to associated adverse events (preparative hypothesis) or due to elevated energetic costs (energy mobilization hypothesis). This was not the expected trend for *Peromyscus* mice, where FCM often increase from spring to summer (Harper and Austad, 2001; Stewart et al., 2020). However, high glucocorticoids during breeding periods have been observed in many small mammal species (Dantzer et al., 2016; Edwards et al., 2016; Desantis et al., 2018; but see Romero et al., 2008; Delehanty and Boonstra, 2011). The preparative hypothesis suggests that glucocorticoid concentrations prime cardiovascular, immune, cognitive, and metabolic systems during time periods with an increased frequency of adverse events (Romero, 2002). During the breeding season, deer mice often exhibit an increase in territory defence and conspecific aggression, and females experience greater mortality risk due to energetic demands of breeding (Petticrew and Sadleir, 1974; Fairbairn, 1977b). We may expect that in June, there is still a higher degree of aggressive behaviour and reproduction-related energetic costs compared to August when the breeding season has ended.

Another explanation for the greater levels of FCM during the breeding season is the energy mobilization hypothesis. Under this hypothesis, glucocorticoid concentrations are expected to be higher during energetically costly time periods, such as during the breeding season (Romero, 2002). *Peromyscus* mice have short lifespans and therefore few reproductive opportunities (Jacquot and Vessey, 1998). Consequently, they may invest more in each reproductive attempt and exhibit greater FCM concentrations during the reproductive season to promote mobilization of energy reserves (Sheriff et al., 2011; Vitousek et al., 2019). This could be the case for females undergoing the energetic costs of pregnancy and lactation, but also for males experiencing associated costs of elevated testosterone and territory defence (Millar, 1975; Ketterson and Nolan, 1999; Romero, 2002). Furthermore, exogenous testosterone can lead to subsequent increases in glucocorticoid concentrations (Schoech et al., 1999). Thus, we may expect higher FCM concentrations in deer mice during the breeding season due to associated adverse events (preparative hypothesis) or due to elevated energetic costs (energy mobilization hypothesis).

FCM concentrations in deer mice significantly varied with age class, where subadults had higher levels compared to adults. This may be related to immune system maturation, which often develops with age in mammals (Holt and Jones, 2000; Simon et al., 2015). Given that glucocorticoids reduce immunocompetence (Webster et al., 2002), we may then expect adults (with greater immunocompetence) to exhibit lower levels of glucocorticoid production. However, it is important to note that immunocompetence does deteriorate with age (Holt and Jones, 2000). Another explanation is that the higher FCM concentrations may be related to dispersal. Subadults are the age class in deer mice that disperse from their natal area (King, 1968) and may experience additional negative stimuli and associated elevations in FCM concentrations. For example, dispersal may lead to greater predation risk and conspecific aggression (Metzgab, 1967; Mayer et al., 2020). Furthermore, corticosterone often increases prior to and remains elevated during dispersal, as corticosterone can mediate locomotor activity (Belthoff and Dufty, 1998). Therefore, the small but significant difference in FCM concentrations between subadult and adult deer mice may be due to variation in immunocompetence or dispersal rates.

There was a high degree of individual variation in FCM concentrations, a trend that has been previously established in literature (St. Juliana et al., 2014; Stedman et al., 2017). This result also highlights the importance of collecting multiple measurements from individuals, as single observations are often uninformative as interpretations of individual differences (Baugh et al., 2014). Glucocorticoid production can be influenced by a large number of genetic, environmental, and social factors (Touma and Palme, 2005; St. Juliana et al., 2014), and a large amount of variation can make it difficult to discern factors that influence glucocorticoid production. Furthermore, this may explain the low effect sizes found in all the investigated models. We did find significant trends of variation in FCM concentrations with age, date, and the number of anti-flea treatment applications. This suggests that these predictors might influence corticosterone production in deer mice; however, it is important to note the potential difficulty in identifying predictors of FCM variation.

FCM levels in deer mice did vary with anti-flea treatment applications, indicating that fleas may represent a physiological stressor to deer mice. However, models containing host biology traits and environmental conditions were a better model fit of deer mice FCM concentrations compared to ectoparasites. In particular, deer mice FCM concentrations significantly varied with age class and date. Therefore, stimuli associated with the breeding season and host dispersal may play a larger role in glucocorticoid production of deer mice.

## CRediT authorship contribution statement

**Jasmine S.M. Veitch:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Jeff Bowman:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Gabriela Mastromonaco:** Methodology, Resources, Writing - review & editing. **Albrecht I. Schulte-Hostedde:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

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## References


Albery, G.F., Watt, K.A., Keith, R., Morris, S., Morris, A., Kenyon, F., Nussey, D.H., Pemberton, J.M., 2019. Reproduction has different costs for immunity and parasitism in a wild mammal. Funct. Ecol. 34, 229–239. https://doi.org/10.1111/1365-2435.13475.

Barton, K., 2019. Package 'MuMIn'.

Baugh, A.T., van Oers, K., Dingemanse, N.J., Hau, M., 2014. Baseline and stress-induced glucocorticoid concentrations are not repeatable but covary within individual great tits (*Parus major*). Gen. Comp. Endocr. 208, 154–163. https://doi.org/10.1016/j.ygcen.2014.08.014.

Baxter-Gilbert, J.H., Riley, J.L., Mastromonaco, G.F., Litzgus, J.D., Lesbarrères, D., 2014. A novel technique to measure chronic levels of corticosterone in turtles living around a major roadway. Conserv. Physiol. 2, cou036. https://doi.org/10.1093/conphys/cou036.

Beaumont, J., Beaumont, A., Waterman, J., 2019. Estimation of ectoparasites in an African ground squirrel. Proc. Manit. Undergrad. Sci. Eng. Res. 5, 10–13. https://doi.org/10.5203/pmuser.201951696.

Bedford, N.L., Hoekstra, H.E., 2015. The natural history of model organisms: *Peromyscus* mice as a model for studying natural variation. Elife 4, e06813. https://doi.org/10.7554/eLife.06813.001.

Belthoff, J.R., Dufty, A.M., 1998. Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. Anim. Behav. 55, 405–415. https://doi.org/10.1006/anbe.1997.0625.

Besedovsky, H.O., del Rey, A., 1996. Immune-neuro-endocrine interactions: facts and hypotheses. Endocr. Rev. 17, 64–102. https://doi.org/10.1210/edrv-17-1-64.

Bobbie, C.B., Schmidt, E., Foley, J., Schulte-Hostedde, A.I., 2016. The presence of parasitic mites on small mammals in Algonquin Provincial Park, Ontario. Canada. Can. J. Zool. 95, 61–65. https://doi.org/10.1139/cjz-2016-0085.

Boonstra, R., Krebs, C.J., 2012. Population dynamics of red-backed voles (*Myodes*) in North America. Oecologia 168, 601–620. https://doi.org/10.1007/s00442-011-2120-z.

Borniger, C.J., Cisse, Y.M., Nelson, R.J., Martin, L.B., 2017. Seasonal variation in stress responses, in: Fink, G. (Ed.) Stress: Neuroendocrinology and Neurobiology. Handbook of Stress Series (Vol. 2), Academic Press, United Kingdom, pp. 411–419.

Branson, D.H., 2003. Effects of a parasite mite on life-history variation in two grasshopper species. Evol. Ecol. Res. 5, 397–409.

Brown, C.R., Brown, M.B., Rannala, B., 1995. Ectoparasites reduce long-term survival of their avian host. P. Roy. Soc. B 262, 313–319. https://doi.org/10.1098/rspb.1995.0211.

Brown, T.T., Fuller, C.A., 2006. Stress and parasitism of white-footed mice (*Peromyscus leucopus*) in dry and floodplain environments. Can. J. Zool. 84, 1833–1839. https://doi.org/10.1139/z06-183.

Buchholz, M.J., Dick, C.W., 2017. Ecology of rodent-ectoparasite associations in South-Central Kentucky. Northeast. Nat. 24, 97–109. https://doi.org/10.1656/045.024.0201.

Buckingham, J.C., Loxley, H.D., Christian, H.C., Philip, J.G., 1996. Activation of the HPA axis by immune insults: roles and interactions of cytokines, eicosanoids, and glucocorticoids. Pharmacol. Biochem. Be. 54, 285–298. https://doi.org/10.1016/0091-3057(95)02127-2.

Cain, D.W., Cidlowski, J.A., 2017. Immune regulation by glucocorticoids. Nat. Rev. Immunol. 17, 233–247. https://doi.org/10.1038/nri.2017.1.

Carlsson, A.M., Mastromonaco, G., Vandervalk, E., Kutz, S., 2016. Parasites, stress and reindeer: infection with abomasal nematodes is not associated with elevated glucocorticoid levels in hair or faeces. Conserv. Physiol. 4, cow058. https://doi.org/10.1093/conphys/cow058.

Corrêa-de-Santana, E., Paez-Pereda, M., Theodoropoulou, M., Nihei, O.K., Gruebler, Y., Bozza, M., Arzt, E., Villa-Verde, D.M.S., Renner, U., Stalla, J., Stalla, G.K., 2006. Hypothalamus–pituitary–adrenal axis during *Trypanosoma cruzi* acute infection in mice. J. Neuroimmunol. 173, 12–22. https://doi.org/10.1016/j.jneuroim.2005.08.015.

Dantzer, B., McAdam, A.G., Palme, R., Fletcher, Q.E., Boutin, S., Humphries, M.M., Boonstra, R., 2010. Fecal cortisol metabolite levels in free-ranging North American red squirrels: assay validation and the effects of reproductive condition. Gen. Comp. Endocr. 167, 279–286. https://doi.org/10.1016/j.ygcen.2010.03.024.

Dantzer, B., Santicchia, F., van Kesteren, F., Palme, R., Martinoli, A., Wauters, L.A., 2016. Measurement of fecal glucocorticoid metabolite levels in Eurasian red squirrels (*Sciurus vulgaris*): effects of captivity, sex, reproductive condition, and season. J. Mammal. 97, 1385–1398. https://doi.org/10.1093/jmammal/gyw095.

Defolie, C., Merkling, T., Fichtel, C., 2019. Patterns and variation in the mammal parasite–glucocorticoid relationship. Biol. Rev. 95, 74–93. https://doi.org/10.1111/brv.12555.

Delehanty, B., Boonstra, R., 2011. Coping with intense reproductive aggression in male arctic ground squirrels: the stress axis and its signature tell divergent stories. Physiol. Biochem. Zool. 84, 417–428. https://doi.org/10.1086/660809.

Demas, G.E., Nelson, R.J., 1996. Photoperiod and temperature interact to affect immune parameters in adult male deer mice: (*Peromyscus manicuiatus*). J. Biol. Rhythm. 11, 94–102. https://doi.org/10.1177/074873049601100202.

Desantis, L.M., Bowman, J., Vijayan, M.M., Burness, G., 2018. Seasonal changes in acute stressor-mediated plasma glucocorticoid regulation in New World flying squirrels. Gen. Comp. Endocr. 266, 78–86. https://doi.org/10.1016/j.ygcen.2018.04.026.

Devevey, G., Christe, P., 2009. Flea infestation reduces the life span of the common vole. Parasitology 136, 1351–1355. https://doi.org/10.1017/S0031182009990746.

Edwards, P.D., Palme, R., Boonstra, R., 2016. Seasonal programming, not competition or testosterone, drives stress-axis changes in a partially-semelparous mammal. Horm. Behav. 85, 96–101. https://doi.org/10.1016/j.yhbeh.2016.08.007.

Ezenwa, V.O., 2016. Helminth–microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. Parasite Immunol. 38, 527–534. https://doi.org/10.1111/pim.12348.

Fairbairn, D.J., 1977a. Why breed early? a study of reproductive tactics in *Peromyscus*. Can. J. Zool. 55, 862–871. https://doi.org/10.1139/z77-112.

Fairbairn, D.J., 1977b. The spring decline in deer mice: death or dispersal? Can. J. Zool. 55, 84–92. https://doi.org/10.1139/z77-009.

Fryxell, J.M., Falls, J.B., Falls, E., Brooks, R.J., 1998. Long term dynamics of small-mammal populations in Ontario. Ecology 79, 213–225. https://doi.org/10.1890/0012-9658(1998)079[0213:LTDOSM]2.0.CO;2.

Fryxell, J.M., Falls, J.B., Falls, E.A., Brooks, R.J., Dix, L., Strickland, M.A., 1999. Density dependence, prey dependence, and population dynamics of martens in Ontario. Ecology 80, 1311–1321. https://doi.org/10.1890/0012-9658(1999)080[1311:DDPDAP]2.0.CO;2.

Gaitan, J., Millien, V., 2016. Stress level, parasite load, and movement pattern in a small-mammal reservoir host for Lyme disease. Can. J. Zool. 94, 565–573. https://doi.org/10.1139/cjz-2015-0225.

Good, T.C., Harris, K.K., Ihunnah, C.A., 2005. Corticosteroids as potential mechanism regulating variability in reproductive success in monogamous oldfield mice (*Peromyscus polionotus*). Physiol. Behav. 86, 96–102. https://doi.org/10.1016/j.physbeh.2005.06.030.

Goodman, B.A., Johnson, P.T., 2011. Ecomorphology and disease: cryptic effects of parasitism on host habitat use, thermoregulation, and predator avoidance. Ecology 92, 542–548. https://doi.org/10.1890/10-0516.1.

Goymann, W., 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. Methods Ecol. Evol. 3, 757–765. https://doi.org/10.1111/j.2041-210X.2012.00203.x.

Harper, J.M., Austad, S.N., 2001. Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). Gen. Comp. Endocrin. 123, 337–344. https://doi.org/10.1006/gcen.2001.7682.

Harper, J.M., Austad, S.N., 2004. Fecal corticosteroid levels in free-living populations of deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Clethrionomys gapperi*). Am. Midl. Nat. 152, 400–410. https://doi.org/10.1674/0003-0031(2004)152[0400:FCLIFP]2.0.CO;2.

Hayssen, V., Harper, J.M., DeFina, R., 2002. Fecal corticosteroids in agouti and non-agouti deer mice (*Peromyscus maniculatus*). Comp. Biochem. Phys. A 132, 439–446. https://doi.org/10.1016/S1095-6433(02)00086-7.

Hoffmann, S., Horak, I.G., Bennett, N.C., Lutermann, H., 2016. Evidence for interspecific interactions in the ectoparasite infracommunity of a wild mammal. Parasite Vector 9, 58. https://doi.org/10.1186/s13071-016-1342-7.

Holland, G.P., 1985. The fleas of Canada, Alaska and Greenland (Siphonaptera). Mem. Entomol. Soc. Can. 117, 3–632. https://doi.org/10.4039/entm117130fv.

Holt, P.G., Jones, C.A., 2000. The development of the immune system during pregnancy and early life. Allergy 55, 688–697. https://doi.org/10.1034/j.1398-9995.2000.00118.x.

Hufschmid, J., Beveridge, I., Handasyde, K.A., 2014. Haematology and serum biochemistry of adult free-ranging mountain brushtail possums (*Trichosurus cunninghami*), including correlations with season, sex, age, habitat type and disease status. Aust. J. Zool. 61, 430–445. https://doi.org/10.1071/ZO12097.

Jacquot, J.J., Vessey, S.H., 1998. Recruitment in white-footed mice (*Peromyscus leucopus*) as a function of litter size, parity, and season. J. Mammal. 79, 312–319. https://doi.org/10.2307/1382867.

Jameson, E.W., 1952. Food of deer mice, *Peromyscus maniculatus* and *P. boylei*, in the northern Sierra Nevada. California. J. Mammal. 33, 50–60. https://doi.org/10.2307/1375640.

Ketterson, E.D., Nolan Jr, V., 1999. Adaptation, exaptation, and constraint: a hormonal perspective. Am. Nat. 154, S4–S25. https://doi.org/10.1086/303280.

Khokhlova, I.S., Krasnov, B.R., Kam, M., Burdelova, N.I., Degen, A.A., 2002. Energy cost of ectoparasitism: the flea *Xenopsylla ramesis* on the desert gerbil *Gerbillus dasyurus*. J. Zool. 258, 349–354. https://doi.org/10.1017/S0952836902001498.

King, J.A., 1968. Biology of *Peromyscus* (Rodentia), second ed. American Society of Mammalogists, Stillwater, Okla.

Langand, J., Jourdane, J., Coustau, C., Delay, B., Morand, S., 1998. Cost of resistance, expressed as a delayed maturity, detected in the host–parasite system *Biomphalaria glabrata/Echinostoma caproni*. Heredity 80, 320. https://doi.org/10.1046/j.1365-2540.1998.00291.x.

Leung, T.L., Koprivnikar, J., 2016. Nematode parasite diversity in birds: the role of host ecology, life history and migration. J. Anim. Ecol. 85, 1471–1480. https://doi.org/10.1111/1365-2656.12581.

Lewis, R.E., 2000. A taxonomic review of the North American genus *Orchopeas* Jordan, 1933 (Siphonaptera: Ceratophyllidae: Ceratophyllinae). J. Vector Ecol. 25, 164–189.

Lutermann, H., Bodenstein, C., Bennett, N.C., 2012. Natural parasite infection affects the tolerance but not the response to a simulated secondary parasite infection. PLoS ONE 7, e52077. https://doi.org/10.1371/journal.pone.0052077.

Maizels, R.M., 2016. Parasitic helminth infections and the control of human allergic and autoimmune disorders. Clin. Microbiol. Infec. 22, 481–486. https://doi.org/10.1016/j.cmi.2016.04.024.

Martínez-Mota, R., Garber, P.A., Palme, R., Gillespie, T.R., 2017. The relative effects of reproductive condition, stress, and seasonality on patterns of parasitism in wild female black howler monkeys (*Alouatta pigra*). Am. J. Primatol. 79, e22669 https://doi.org/10.1002/ajp.22669.

Mazerolle, M.J., 2019. Package 'AICcmodavg'.

Mayer, M., Frank, S.C., Zedrosser, A., Rosell, F., 2020. Causes and consequences of inverse density-dependent territorial behaviour and aggression in a monogamous mammal. J. Anim. Ecol. 89, 577–588. https://doi.org/10.1111/1365-2656.13100.
Mayfield, R.L., Van Vuren, D., Johnson, M.L., 2000. Demography of an insular endemic rodent, *Peromyscus maniculatus santacruzae*, on Santa Cruz Island. Southwest. Nat. 45, 508–513. https://doi.org/10.2307/3672599.
Merlot, E., Moze, E., Bartolomucci, A., Dantzer, R., Neveu, P.J., 2004. The rank assessed in a food competition test influences subsequent reactivity to immune and social challenges in mice. Brain Behav. Immun. 18, 468–475. https://doi.org/10.1016/j.bbi.2003.11.007.
Metzgab, L.H., 1967. An experimental comparison of screech owl predation on resident and transient white-footed mice (*Peromyscus leucopus*). J. Mammal. 48, 387–391. https://doi.org/10.2307/1377771.
Millar, J.S., 1975. Tactics of energy partitioning in breeding *Peromyscus*. Can. J. Zool. 53, 967–976. https://doi.org/10.1139/z75-112.
Millar, J.S., Derrickson, E.M., Sharpe, S.T.P., 1992. Effects of reproduction on maternal survival and subsequent reproduction in northern *Peromyscus maniculatus*. Can. J. Zool. 70, 1129–1134. https://doi.org/10.1139/z92-158.
Modha, J., Roberts, M.C., Kusel, J.R., 1996. Schistosomes and serpins: a complex business. Parasitol. Today 12, 119–121. https://doi.org/10.1016/0169-4758(96)80672-7.
Monello, R.J., Millspaugh, J.J., Woods, R.J., Gompper, M.E., 2010. The influence of parasites on faecal glucocorticoid metabolite levels in raccoons: an experimental assessment in a natural setting. J. Zool. 282, 100–108. https://doi.org/10.1111/j.1469-7998.2010.00717.x.
Morales-Montor, J., Mohamed, F., Ghaleb, A.M., Baig, S., Hallal-Calleros, C., Damian, R. T., 2001. In vitro effects of hypothalamic-pituitary-adrenal axis (HPA) hormones on *Schistosoma mansoni*. J. Parasitol. 87, 1132–1139. https://doi.org/10.1645/0022-3395(2001)087[1132:IVEOHP]2.0.CO;2.
Newey, S., Thirgood, S., 2004. Parasite–mediated reduction in fecundity of mountain hares. P. Roy. Soc. B 271, S413–S415. https://doi.org/10.1098/rsbl.2004.0202.
O'Dwyer, K., Dargent, F., Forbes, M.R., Koprivnikar, J., 2020. Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: a meta-analysis. J. Anim. Ecol. 89, 519–529. https://doi.org/10.1111/1365-2656.13123.
Padgett, D.A., Glaser, R., 2003. How stress influences the immune response. Trends Immunol. 24, 444–448. https://doi.org/10.1016/S1471-4906(03)00173-X.
Palm, N.W., Rosenstein, R.K., Medzhitov, R., 2012. Allergic host defences. Nature 484, 465–472. https://doi.org/10.1038/nature11047.
Patterson, J.E., Neuhaus, P., Kutz, S.J., Ruckstuhl, K.E., 2013. Parasite removal improves reproductive success of female North American red squirrels (*Tamiasciurus hudsonicus*). PLoS ONE 8, e55779. https://doi.org/10.1371/journal.pone.0055779.
Pedersen, A.B., Greives, T.J., 2008. The interaction of parasites and resources cause crashes in a wild mouse population. J. Anim. Ecol. 77, 370–377. https://doi.org/10.1111/j.1365-2656.2007.01321.x.
Pero, E.M., Hare, J.F., 2018. Costs of Franklin's ground squirrel (*Poliocitellus franklinii*) ectoparasitism reveal adaptive sex allocation. Can. J. Zool. 96, 585–591. https://doi.org/10.1139/cjz-2017-0129.
Petticrew, B.G., Sadleir, R.M.F.S., 1974. The ecology of the deer mouse *Peromyscus maniculatus* in a coastal coniferous forest. I. Population dynamics. Can. J. Zool. 52, 107–118. https://doi.org/10.1139/z74-015.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2012. nlme: Linear and nonlinear mixed effects models.
Puehringer-Sturmayr, V., Wascher, C.A., Loretto, M.C., Palme, R., Stoewe, M., Kotrschal, K., Frigerio, D., 2018. Seasonal differences of corticosterone metabolite concentrations and parasite burden in northern bald ibis (*Geronticus eremita*): the role of affiliative interactions. PLoS ONE 13, e0191441. https://doi.org/10.1371/journal.pone.0191441.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R Programming Software, Vienna, Austria.
Råberg, L., Graham, A.L., Read, A.F., 2009. Decomposing health: tolerance and resistance to parasites in animals. Philos. T. R. Soc. A 364, 37–49. https://doi.org/10.1098/rstb.2008.0184.
Reeder, D.M., Kramer, K.M., 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. J. Mammal. 86, 225–235. https://doi.org/10.1644/BHE-003.1.
Richards, W.R., 1964. A short method for making balsam mounts of aphids and scale insects. Can. Entomol. 96, 963–966. https://doi.org/10.4039/Ent96963-7.
Romeo, C., Wauters, L.A., Santicchia, F., Dantzer, B., Palme, R., Martinoli, A., Ferrari, N., 2020. Complex relationships between physiological stress and endoparasite infections in natural populations. Curr. Zool. https://doi.org/10.1093/cz/zoaa029.
Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocr. 128, 1–24. https://doi.org/10.1016/S0016-6480(02)00064-3.
Romero, L.M., Meister, C.J., Cyr, N.E., Kenagy, G.J., Wingfield, J.C., 2008. Seasonal glucocorticoid responses to capture in wild free-living mammals. Am. J. Physiol.-Reg. I. 294, R614–R622. https://doi.org/10.1152/ajpregu.00752.2007.
Sánchez, C.A., Becker, D.J., Teitelbaum, C.S., Barriga, P., Brown, L.M., Majewska, A.A., Hall, R.J., Altizer, S., 2018. On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. Ecol. Lett. 21, 1869–1884. https://doi.org/10.1111/ele.13160.
Santicchia, F., Wauters, L.A., Piscitelli, A.P., Van Dongen, S., Martinoli, A., Preatoni, D., Romeo, C., Ferrari, N., 2020. Spillover of an alien parasite reduces expression of costly behaviour in native host species. J. Anim. Ecol. https://doi.org/10.1111/1365-2656.13219.
Schmidt, E., Mykytczuk, N., Schulte-Hostedde, A.I., 2019. Effects of the captive and wild environment on diversity of the gut microbiome of deer mice (*Peromyscus maniculatus*). ISME J. 13, 1293–1305. https://doi.org/10.1038/s41396-019-0345-8.
Sheriff, M.J., Bosson, C.O., Krebs, C.J., Boonstra, R., 2009. A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). J. Comp. Physiol. B. 179, 305–313. https://doi.org/10.1007/s00360-008-0314-4.
Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166, 869–887. https://doi.org/10.1007/s00442-011-1943-y.
Seguel, M., Perez-Venegas, D., Gutierrez, J., Crocker, D.E., DeRango, E.J., 2019. Parasitism elicits a stress response that allocates resources for immune function in South American fur seals (*Arctocephalus australis*). Physiol. Biochem. Zool. 92, 326–338. https://doi.org/10.1086/702960.
Schoech, S.J., Ketterson, E.D., Nolan Jr, V., 1999. Exogenous testosterone and the adrenocortical response in dark-eyed juncos. Auk 116, 64–72. https://doi.org/10.2307/4089454.
Schoenle, L.A., Moore, I.T., Dudek, A.M., Garcia, E.B., Mays, M., Haussmann, M.F., Cimini, D., Bonier, F., 2019. Exogenous glucocorticoids amplify the costs of infection by reducing resistance and tolerance, but effects are mitigated by co-infection. P. Roy. Soc. B 286, 20182913. https://doi.org/10.1098/rspb.2018.2913.
Schulte-Hostedde, A.I., Brooks, R.J., 1997. An experimental test of habitat selection by rodents of Algonquin Park. Can. J. Zool. 75, 1989–1993. https://doi.org/10.1139/z97-831.
Simon, A.K., Hollander, G.A., McMichael, A., 2015. Evolution of the immune system in humans from infancy to old age. P. Roy. Soc. B 282, 20143085. https://doi.org/10.1098/rspb.2014.3085.
Snipes, M., Taylor, D.C., 2014. Model selection and Akaike Information Criteria: An example from wine ratings and prices. Wine Econ. Policy, 3, 3-9. Model selection and Akaike Information Criteria: An example from wine ratings and prices.
St. Juliana, J.R., Khokhlova, I.S., Wielebnowski, N., Kotler, B.P., Krasnov, B.R., 2014. Ectoparasitism and stress hormones: strategy of host exploitation, common host–parasite history and energetics matter. J. Anim. Ecol. 83, 1113–1123. https://doi.org/10.1111/1365-2656.12217.
Stedman, J.M., Hallinger, K.K., Winkler, D.W., Vitousek, M.N., 2017. Heritable variation in circulating glucocorticoids and endocrine flexibility in a free-living songbird. J. Evolution. Biol. 30, 1724–1735. https://doi.org/10.1111/jeb.13135.
Steen, N.A., Barker, S.C., Alewood, P.F., 2006. Proteins in the saliva of the Ixodida (ticks): pharmacological features and biological significance. Toxicon 47, 1–20. https://doi.org/10.1016/j.toxicon.2005.09.010.
Stewart, F.E., Brooks, R.J., McAdam, A.G., 2014. Seasonal adjustment of sex ratio and offspring masculinity by female deer mice is inconsistent with the local resource competition hypothesis. Evol. Ecol. Res. 16, 153–164.
Stewart, N.D., Mastromonaco, G.F., Burness, G., 2020. No island-effect on glucocorticoid levels for a rodent from a near-shore archipelago. PeerJ 8, e8590. https://doi.org/10.7717/peerj.8590.
Sures, B., Scheef, G., Klar, B., Kloas, W., Taraschewski, H., 2002. Interaction between cadmium exposure and infection with the intestinal parasite *Moniliformis moniliformis* (Acanthocephala) on the stress hormone levels in rats. Environ. Pollut. 119, 333–340. https://doi.org/10.1016/S0269-7491(01)00340-2.
Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. NY Acad. Sci. 1046, 54–74. https://doi.org/10.1196/annals.1343.006.
Trevisan, C., Montillo, M., Prandi, A., Mkupasi, E.M., Ngowi, H.A., Johansen, M.V., 2017. Hair cortisol and dehydroepiandrosterone concentrations in naturally *Taenia solium* infected pigs in Tanzania. Gen. Comp. Endocr. 246, 23–28. https://doi.org/10.1016/j.ygcen.2017.03.007.
van der Mescht, L., Warburton, E.M., Khokhlova, I.S., Stanko, M., Vinarski, M.V., Korallo-Vinarskaya, N.P., Krasnov, B.R., 2018. Biogeography of parasite abundance: latitudinal gradient and distance decay of similarity in the abundance of fleas and mites, parasitic on small mammals in the Palearctic, at three spatial scales. Int. J. Parasitol. 48, 857–866. https://doi.org/10.1016/j.ijpara.2018.04.005.
Veitch, J.S.M., 2020. Ectoparasitism of Rodent Hosts in Algonquin Provincial Park, Ontario, Canada: Infestation Patterns, Host Glucocorticoids, and Species co-occurrence (Master's thesis). Laurentian University of Sudbury https://zone.biblio.laurentian.ca/handle/10219/3460.
Veitch, J.S.M., Bowman, J., Schulte-Hostedde, A.I., 2020. Parasite species co-occurrence patterns on *Peromyscus*: Joint species distribution modelling. Int. J. Parasitol. Parasites Wildl. 12, 199–206. https://doi.org/10.1016/j.ijppaw.2020.04.011.
Vickery, W.L., 1979. Food consumption and preferences in wild populations of *Clethrionomys gapperi* and *Napaeozapus insignis*. Can. J. Zool. 57, 1536–1542. https://doi.org/10.1139/z79-201.
Vitousek, M.N., Johnson, M.A., Downs, C.J., Miller, E.T., Martin, L.B., Francis, C.D., Donald, J.W., Fuxjager, M.J., Goymann, W., Hau, M., Husak, J.F., 2019. Macroevolutionary patterning in glucocorticoids suggests different selective pressures shape baseline and stress-induced levels. Am. Nat. 193, 866–880. https://doi.org/10.1086/703112.
Webster, J.I., Tonelli, L., Sternberg, E.M., 2002. Neuroendocrine regulation of immunity. Annu. Rev. Immunol. 20, 125–163. https://doi.org/10.1146/annurev.immunol.20.082401.104914.
Weil, Z.M., Martin, L.B., Nelson, R.J., 2006. Interactions among immune, endocrine, and behavioural response to infection. In: Morand, S., Krasnov, B.R., Poulin, R. (Eds.), Micromammals and Macroparasites: From Evolutionary Ecology to Management. Springer Science & Business Media, Springer, Tokyo, pp. 443–473.
Wiedemann, C., 2000a. Studies on the efficacy of Fipronil (Frontline®) against ectoparasites. I. Flea control. Tierärztliche Umschau 55, 118–123.

Wiedemann, C., 2000b. Studies on the efficacy of Fipronil (Frontline®) against ectoparasites. II. Tick infestations. Tierärztliche Umschau 55, 211–216.
Zug, R., Hammerstein, P., 2018. Evolution of reproductive parasites with direct fitness benefits. Heredity 120, 266–281. https://doi.org/10.1038/s41437-017-0022-5.
Zuk, M., Stoehr, A.M., 2002. Immune defense and host life history. Am. Nat. 160, S9–S22. https://doi.org/10.1086/342131.
Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer Science & Business Media.