



Social stress in female Columbian ground squirrels: density-independent effects of kin contribute to variation in fecal glucocorticoid metabolites

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Abstract

Social interactions among conspecifics can have marked effects on individual physiology, especially through their modulation of the stress axis by affecting the production of adrenal glucocorticoids (GCs). Previous research has focused on how individual GC levels may be influenced by social status, but few studies have considered how the balance between positive (e.g. cooperation) and negative (e.g. competition) social interactions shape individual GC levels. A lack of association between individual GC levels and social factors may be confounded by opposite effects of social competition on the one hand and social cooperation on the other. We tested for these effects in the Columbian ground squirrel (*Urocitellus columbianus*), a colonial rodent. During the breeding season, females are exposed to territorial unrelated neighbors and to territorial, but more tolerant, close kin. On one hand, territoriality and competition for resources led us to predict a positive association between local colony density and female GC levels. On the other, higher tolerance of philopatric kin females and known fitness benefits led us to predict a negative association between kin numbers and female GC levels. We compared levels of fecal cortisol metabolites (FCMs) in females at two different spatial scales during lactation: local (a female's core territory during lactation, 30-m radius about her nest burrow) and colony-wide. At the local scale, female FCM levels were neither related to colony density nor to the number of co-breeding female kin, but FCM levels increased with age. At the colony scale, female FCM levels varied in a quadratic fashion with female kin numbers. FCM levels decreased by 15% from 0 to 1 co-breeding kin present and increased with > 1 kin present. Among females that had only one co-breeding kin present, daughters (and littermate sisters and mothers, but not significantly) led to a 14% reduction in FCM levels compared with females that had no kin. Our results reject the idea that local colony density is associated with increased GC levels in this species, but indicate subtle (positive and negative) effects of kin on individual GC secretion. They further call into question the importance of the nature of social relationships in modulating the stress experienced by individuals.

Significance statement

Few studies have tested how the balance between positive (e.g. cooperation) and negative (e.g. competition) social interactions shapes individual stress and glucocorticoid (GC) levels in group-living animals. In colonial Columbian ground squirrels,

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breeding females are exposed to territorial neighbors and to more tolerant close kin. We show that kin numbers have subtle (positive and negative) effects on female GC levels. Compared with breeding females with no kin, female GC levels decrease by 15% with the presence of a single co-breeding close relative, but increase with the presence of more than one co-breeding related female. Among females that have only one co-breeding kin, the presence of daughters (and littermate sisters and mothers, but not significantly) leads to a 14% reduction in female GC levels. Our results highlight how GC levels may be influenced by the specific nature of social relationships in group-living animals.

Keywords Age · Glucocorticoids · Kinship · Population density · Social environment · Stress

Introduction

In social organisms, the interactions resulting from regular contact with related and unrelated animals may have profound effects on individual physiology, health, and fitness (Sapolsky 1992; Bartolomucci 2007; Razzoli et al. 2018). Studies have highlighted both positive and negative effects of social interactions (or lack thereof) on individual metabolic rate (Stefanski and Engler 1998; Sloman et al. 2000; Willis and Brigham 2007; Cao and Dornhaus 2008), immunity (Stefanski and Engler 1998; de Groot et al. 2001), and oxidative stress (Nation et al. 2008; Zhao et al. 2013; Beaulieu et al. 2014; Lardy et al. 2016), as well as gene regulation and cellular maintenance (Kotrschal et al. 2007; Tung et al. 2012; Aydinonat et al. 2014). In particular, the so-called stress axis (the hypothalamic-pituitary-adrenal axis, HPA) may provide insight into these positive and negative effects (Harris 2020). The HPA is one of the key physiological systems mediating the relationship between the organism and its environment, permitting short-term adaptations to acute stressors, such as social conflict, and long-term evolutionary responses to particular ecological and environmental challenges. The HPA axis is a vital regulator of adaptation. Glucocorticoid (GC) hormones from the adrenal glands influence the expression of approximately 10% of the genome and their targets include genes that control metabolism, growth, repair, reproduction, and the management of resource allocation (Le et al. 2005). Because of its central role in maintaining homeostasis via the action of GC hormones (Sapolsky et al. 2000; Wingfield and Romero 2001; Landys et al. 2006), a large number of studies have considered the effects of the social environment (i.e. social interactions between conspecifics, territoriality, population density, and social status) on HPA axis activation (Boonstra and Boag 1992; Creel 2001; DeVries 2002; Carere et al. 2003; Goymann and Wingfield 2004; Dantzer et al. 2013; reviewed in Boonstra et al. 2007; Creel et al. 2013).

On one hand, social competition and conflict may increase individual stress and the activity of the HPA axis, which can often be assessed through increases in individual GCs (Goymann and Wingfield 2004; Ostner et al. 2008; Rubenstein and Shen 2009). On the other, social cooperation may help to alleviate individual stress and decrease the

activity of the HPA axis, through affiliative social networks (e.g. Wittig et al. 2008, 2016) or social and emotional support (Turner-Cobb et al. 2000; Scheiber et al. 2009; Young et al. 2014), reducing or stabilizing individual GC levels. Positive effects of the social environment on decreasing the activity of the HPA axis are expected where group-living or social cooperation among individuals is known to have positive effects on fitness, for instance by providing anti-predator benefits (Hare et al. 2015) or by decreasing rates of inter-individual aggression and/or affecting the outcome of aggression (Frigerio et al. 2005). This is likely to happen in species where stable cooperative alliances can form among social members (e.g. Young et al. 2014), where cooperative family groups are the essential units of the social system or where tolerant kin co-occur. The direction in which the social environment affects the activity of the HPA axis is thus complex and may be subtle. A lack of association between social factors and individual stress load may result from the antagonistic effects of social competition on one hand (increasing individual GCs) and social cooperation on the other (decreasing individual GCs). In this regard, concurrently evaluating the relationship between individual GC levels and aspects of the social environment pertaining both to competition (e.g. number of territorial or dominant conspecifics) and cooperation (e.g. number of social allies or nepotistic kin individuals) is likely to provide valuable information on the physiological costs and benefits of sociality.

We tested for opposing (positive and negative) effects of the social environment on the activity of the HPA axis in female Columbian ground squirrels (*Urocitellus columbianus*). Columbian ground squirrels are colonial rodents living in colonies of up to over 100 individuals (Festabianchet and Boag 1982; Murie and Harris 1988). They are a hibernating species, with a short (3–4 months) active season, during which, mature females (typically > 1 year old) breed, raise a single litter, and actively forage and fatten before subsequent hibernation (Murie and Harris 1982; Dobson et al. 1992). We specifically focused on females during lactation for three reasons. First, females are the philopatric sex in Columbian ground squirrels (King 1989a; Arnaud et al. 2012), allowing for social familiarity among colony members (Hare 1992, 1994; Raynaud and Dobson 2011) and the establishment of social relationships. Second, females during lactation are specifically territorial, defending a core-territory

limited to a ca. 30-m radius around individual nest burrows that are used for raising young (Festa-Bianchet and Boag 1982; Murie and Harris 1988). Territorial aggression is expected to have physiological effects on individuals, among which is the activation of the HPA axis and the secretion of GC hormones (Boonstra and Boag 1992; see Creel et al. 2013 for a review). Although females defend a core territory during lactation, they regularly range throughout the entire colony in their daily foraging activities during this period and are subject both to local and colony-wide (up to ca. 2–3 ha on our study sites) social environments (FSD et al., personal observations). Third, in Columbian ground squirrels, female kin overlap both spatially and temporally (King and Murie 1985; Murie and Harris 1988). The philopatry of kin provides both direct (Viblanç et al. 2010) and indirect (Dobson et al. 2012) fitness benefits for breeding females. Direct kin-related fitness benefits appear to occur mostly via increased breeding success through the production of larger litters and greater survival of young to yearling age, both at first breeding (Neuhaus et al. 2004) and over a lifetime (Viblanç et al. 2010; Dobson et al. 2012). In turn, those fitness benefits likely arise because female kin are less aggressive to one another (King 1989b; Viblanç et al. 2016a). Lowered aggression may facilitate the acquisition/maintenance of breeding territories (Harris and Murie 1984; Neuhaus et al. 2004; Arnaud et al. 2012), thus providing a safer environment for raising offspring (i.e. diminished risks of infanticide by unrelated females; Stevens 1998) and allowing females to invest more energy into reproduction (Viblanç et al. 2016b).

Given the above, we hypothesized that both local colony density and the presence of female co-breeding kin should affect the activity of the HPA axis in breeding females in opposite directions. The first hypothesis is supported by the peak in female territoriality during gestation and lactation (Festa-Bianchet and Boag 1982), the potential for female-related infanticide during lactation (Dobson 1990; Stevens 1998), the importance of food resources in regulating population size (Dobson and Kjelgaard 1985; Dobson 1995; Dobson and Oli 2001), and reported dispersal occurrences of females from high to low local density areas (Arnaud et al. 2012). Thus, we predicted that local conspecific density would be positively associated with female GC levels. The second, kin-related hypothesis is supported by the higher tolerance of females towards individual kin (King 1989b; Viblanç et al. 2016a) and positive kin effects on female investment in reproduction (Viblanç et al. 2010, 2016b). Here, we predicted that increasing numbers of co-breeding kin should decrease female-female competition and be negatively associated with female GC levels.

We assessed female GC levels during the territorial period of lactation by analyzing for fecal cortisol metabolites (FCMs). Metabolized GCs excreted in the feces provide a useful non-invasive method for assessing individual stress

in Columbian ground squirrels (Bosson et al. 2009), and they reflect free, biologically active levels of plasma GCs (Sheriff et al. 2010; Fauteux et al. 2017). FCMs provide a more integrated measure of individual GC levels than can be obtained through acute plasma measures and are less prone to researcher-induced biases (Sheriff et al. 2011; Palme 2019). In red squirrels (*Tamiasciurus hudsonicus*), individual perception of social density is reflected in their FCM levels (Dantzer et al. 2013). Thus, FCMs should provide a rigorous method for testing relationships between the social environment and GC levels in female Columbian ground squirrels.

Methods

Study sites and demographic monitoring

Data were collected over two consecutive years (2013 and 2014) in the Sheep River Provincial Park (Alberta, Canada), in three different colonies of Columbian ground squirrels monitored as part of long-term studies on the behavior and ecology of these animals: meadow A (50° 38' 19.80" N; 114° 39' 46.47" W; 1520 m; 3.4 ha), meadow B (50° 38' 10.73" N; 114° 39' 56.52" W; 1524 m; 2.3 ha), and Dot (50° 38' 59.74" N; 114° 39' 41.79" W; 1545 m; 3.0 ha). It was not possible to record data blind because our study involved focal animals in the field. In each year, entire ground squirrel populations (mean \pm SD = 118 \pm 68 individuals, range = 60–226) were trapped using 13 \times 13 \times 40 cm³ live-traps (Tomahawk Live Trap, Hazelhurst, WI, USA) baited with a knob of peanut butter (taken from the tip of a knife) as individuals emerged from hibernation (Skippy®, Hormel Foods, LLC). Each ground squirrel was given a pair of uniquely numbered ear tags (Monel #1 National Band & Tag Co., Newport, KY, USA) for permanent identification. In addition, each individual was given a unique dorsal mark using black human hair dye (Clairol® Hydrience N°52 Black Pearl, Clairol Inc., New York, USA) for identification during field observations. Each female was monitored from emergence of hibernation through the first emergence of the pups from nest burrows at about the time that they were weaned. Details on the long-term monitoring of the colonies are given elsewhere (Hare and Murie 1992; Raveh et al. 2010, 2011; Rubach et al. 2016). Briefly, identification of the mating date for all breeding females allowed estimation of the timing of parturition (+ 24 days after mating) and weaning (+ 27 days after birth) (see Murie and Harris 1982). In the field, nest burrows were identified from repeated visual observations of females entering burrows with mouthfuls of dry grass nesting material, and complete litters were caught and marked at these burrows near the time of weaning (Raveh et al. 2010). For virtually all adult females,

we recorded complete information on individual age and life history since the time of birth.

Feces sampling and FCM assays

Sample collection Fecal samples were collected during lactation by baiting live-traps with a small amount of peanut butter and deploying them close to focal individuals (see above). Traps were systematically cleaned before being deployed to ensure fecal samples corresponded to targeted individuals. Fecal samples were always collected within minutes of capture, animals on the study sites being target-trapped. In Columbian ground squirrels, an acute stressor causes FCM levels to increase $7 \pm (\text{SE}) 0.82$ h later (the gut passage time) (Bosson et al. 2009). Thus, we are confident that GC levels measured in these animals were not affected by trapping, since time of capture invariably put FCM-critical timing to the previous night or morning (samples collected at the first capture of the day). Fecal samples were most often collected either directly into 2-mL sterile vial as the female defecated or from the floor of the trap. In this latter case, the female was always observed defecating in the trap and the feces collected immediately. We insured no fecal sample was contaminated by urine upon collection. Samples that were contaminated were systematically discarded. Hence, there was no possible confusion of fecal samples nor cross-contamination in the field. We systematically recorded hour of sample collection. Because FCM levels are likely to vary according to the time of sampling in the day, we insured that sampling hour had no significant effect on FCM levels prior to analyses ($t = -0.09$; $P = 0.93$). Fecal samples were immediately stored on ice packs when in the field and transferred to a -20 °C freezer within no more than a couple of hours after sampling. At the end of the field season, samples were shipped on dry ice to the University of Toronto and stored at -80 °C until analyses. Overall, we were able to acquire 126 fecal samples for 92 females.

FCM assays Fecal cortisol metabolites were determined as previously validated and described in Columbian ground squirrels (Bosson et al. 2009). Briefly, lyophilized fecal samples were frozen in liquid nitrogen and pulverized with a small grinding pestle. We weighed 0.030 ± 0.001 g of the sample and extracted FCMs by vortexing it (30 min at 1450 rpm; Barnstead Thermolyne Maxi-Mix III, IA) in 1 mL of 80% methanol (v/v). FCMs (ng/g dried feces) were determined using a 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one enzyme immunoassay (EIA), specifically designed to measure metabolites with a 5α - $3\beta,11\beta$ -diol structure (Touma et al. 2003). Cross-reactivities of the antibody used in this EIA are given elsewhere (Touma et al. 2003). All samples were run in duplicate on fifteen 96-well plates. Low value ($\sim 70\%$ binding) and high value ($\sim 30\%$ binding) pooled samples were run on each plate as controls. Intra-assay coefficients of variation

were $5.9 \pm 1.1\%$ (low pool) and $4.6 \pm 1.3\%$ (high pool), and the mean inter-assay coefficient of variation based on the pools was $5.5 \pm 1.2\%$.

Kin numbers

For each breeding female, we used long-term matrilineal genealogies to calculate her number of co-reproductive close kin or non-kin. We counted as close kin her mother, daughter(s), and littermate sister(s). Among sisters, we only considered littermates as close kin (i.e. females born in the same litter) based on previous findings that non-littermate sisters do not appear to be recognized as close kin in this species (Hare and Murie 1996), and only littermates, mothers, and daughters appear to provide genial neighbor benefits in terms of direct and indirect fitness (Viblanco et al. 2010; Dobson et al. 2012). During lactation, female Columbian ground squirrels actively defend a core territory of ca. 30 m surrounding their nest-burrows to protect their young. During this period, however, they regularly range throughout the entire colony in their daily foraging activities (FSD et al., personal observations). Females are thus exposed both to local and colony-wide social environments, and for each female, we calculated the number of co-reproductive close kin and the overall number of conspecifics (including close kin) occurring at these two different spatial scales: local and colony-wide. The local scale comprised a radius of 30 m around a given female's nest burrow. For this, we used the location of female nest burrows and, for a given female, counted all the kin and non-kin nest burrows located within a 30-m radius of her own. The second spatial scale was global, and we counted all co-reproductive kin present in the colony (colonies ranged from 2.3 to 3.4 ha in our study). We subsequently evaluated the relationships between social environments and female FCM levels at those two different scales.

Data analyses

Variation in female FCM levels related to local conspecific and co-breeding kin numbers

We used a linear mixed model (LMM) to test for the relationships between female FCM levels and local conspecific and local co-reproductive kin numbers within a 30-m radius. Breeding female FCM levels (ln-transformed) were specified as the dependent variable, and conspecific and co-breeding kin numbers were specified as independent variables in the model. We further included female age to test for potential age-related effects on female GC levels. Female ID within colony and year were included as random factors in the model to account for repeated measures on individuals in different years and repeated measures within the same colony. Thus, the model was specified as follows:

$$\ln(\text{FCM}) \sim n_{\text{conspecifics}} + n_{\text{kin}} + \text{age} + (1|\text{colony} : \text{ID}) \\ + (1|\text{year})$$

Variation in female FCM levels related to overall co-breeding kin numbers

A similar LMM was used to test for the relationships between female FCM levels and co-breeding kin numbers at a colony scale. In this model, it made little sense to test for a population density effect on female FCM levels since the number of conspecifics at the population level is identical for all females in a given year and meadow. Although we originally predicted a negative linear effect of kin numbers on female FCM levels, visual inspection of the data suggested a non-linear effect of kin numbers on female FCM levels. A non-linear effect might occur if there is some optimal kin number such that increasing kin numbers up to that optimal point allows decreasing territorial aggression (King 1989b; Viblanc et al. 2016a) and reducing the activity of the HPA axis, but results in kin competition (e.g. for food resources; Dobson and Kjelgaard 1985; Dobson 1990) and increased activation of the HPA axis beyond. Thus, we included a quadratic term for kin numbers as an independent variable in the model. Female ID within colony and year were included as random factors in the model to account for repeated measures on individuals in different years and repeated measures within the same colony. Here, the model was specified as follows:

$$\ln(\text{FCM}) \sim n_{\text{kin}} + n_{\text{kin}}^2 + \text{age} + (1|\text{colony} : \text{ID}) + (1|\text{year})$$

Nature of kin environment and relationship with female FCM levels

In light of the previous analyses, potentially highlighting a special effect of having one kin present in the population on female FCM levels (see Results), we tested if FCM levels varied depending on the nature of the 1 kin relationship to breeding females. For all females that had only one close-kin co-breeder, we identified whether that individual was a mother, a daughter, or a littermate sister. We then ran a LMM including female FCM levels as the dependent variable of interest, the nature of the kin relationship (mother, daughter, littermate sister, no kin) as the independent variable. Here also, female ID within colony and year were included as random factors in the model to account for repeated measures on individuals in different years, and repeated measures within the same colony. The model was thus specified as follows:

$$\ln(\text{FCM}) \sim \text{kin category}_{[\text{No kin}/\text{mother}/\text{daughter}/\text{littermate sister}]} + \text{age} \\ + (1|\text{colony} : \text{ID}) + (1|\text{year})$$

All analyses were performed in R 3.6.2. (R Core Team 2019). The approach with linear mixed models was conducted using the “lme4” v. 1.1.20 package (Bates et al. 2015) with the alpha level set to 0.05. FCM levels were ln-transformed prior to analyses to meet normality assumptions. However, average values in the text are reported based on the raw data. Conditional and marginal R^2 values for mixed-effect models were computed with the “MuMIn” package v. 1.42.1 (Barton 2019). The marginal R^2 represents the variance explained by fixed factors in the model, whereas the conditional R^2 represents the variance explained by both fixed and random factors in the model. For all models, we insured that residual distribution did not substantially deviate from normal distributions using qq-plots (“fitdistrplus” package in R; Delignette-Muller and Dutang 2015). Independent variables were checked for collinearity using Variance Inflation Factors (VIFs) (suggested cut-off VIF > 3; Zuur et al. 2010). Results are provided as means \pm 1 SE.

Results

Variation in female FCM levels in relation to conspecifics and kin numbers at a local scale

Contrary to our predictions, within a 30-m radius of a female’s nest burrow, a female’s FCM level was not positively related to local conspecific density or negatively to local kin density (Table 1; see Online Supplementary Material 1). Female age, however, was positively related to female FCM levels (Table 1): the older a female, the higher her FCM levels. At a local spatial scale, a female’s age was not significantly associated with kin density (Pearson’s correlation; $r = 0.12$, $t = 1.38$, $p = 0.17$) or conspecific density ($r = 0.03$, $t = 0.39$, $p = 0.69$). Although co-breeding close kin numbers and conspecific density were obviously correlated at the local scale ($r = 0.40$, $t = 4.91$, $p < 0.001$), there was no indication of substantial collinearity in the model (all VIFs < 1.23). Indeed, testing for the relationship between kin/conspecific density and female FCMs levels (accounting for age) in separate models led to the same results (see Online Supplementary Material 2).

Variation in female FCM levels in relation to overall kin numbers at a colony scale

At a colony scale, once controlling for female ID and colony as random factors, 12.68% of residual variance in female FCM levels was explained by the number of co-breeding close kin and female age (marginal $R^2 = 12.68\%$; conditional $R^2 = 33.91\%$). Breeding female FCM levels varied in a quadratic fashion (estimate \pm SE: -0.15 ± 0.07 kin + 0.06 ± 0.03 kin²) with the number of close co-breeding kin present in the

Table 1 Linear mixed model estimates for the relationships between female FCM levels and female age, number of conspecifics, and number of close kin within a 30-m radius of the nest burrow. Female identity within colony and year were included as random effects in the model.

σ^2 = within-group variance; τ_{00} = between-group variance, ICC = intraclass correlation coefficient. Sample size along with marginal and conditional R^2 are presented. Significant values for $p < 0.05$ are given in italics

		ln FCMs _(ng per g dried feces)			
Fixed effects					
	Estimates ± SE	CI	Statistic	<i>p</i>	
Intercept	6.10 ± 0.09	5.92–6.28	66.11	< 0.001	
Local kin numbers	0.01 ± 0.06	– 0.12–0.13	0.15	0.883	
Local colony density	0.01 ± 0.01	– 0.01–0.03	0.71	0.482	
Age	0.05 ± 0.01	0.02–0.07	3.63	< 0.001	
Random effects					
σ^2	0.06				
τ_{00} colony:ID	0.01				
τ_{00} year	0.01				
ICC _{colony:ID}	0.15				
ICC _{year}	0.10				
Fecal samples/Individuals	126/92				
Marginal R^2 /Conditional R^2	0.097/0.323				

population (Table 2A; Fig. 1). FCM levels decreased by 15.11% on average between 0 (613.9 ± 24.8 ng FCM/g) and 1 (521.2 ± 23.4 ng FCM/g) close kin but increased rapidly thereafter by 12.91% on average between 1 and 2 (588.5 ± 34.6 ng FCM/g) close kin present. Here also, female FCM levels were positively related to female age (Table 2A;

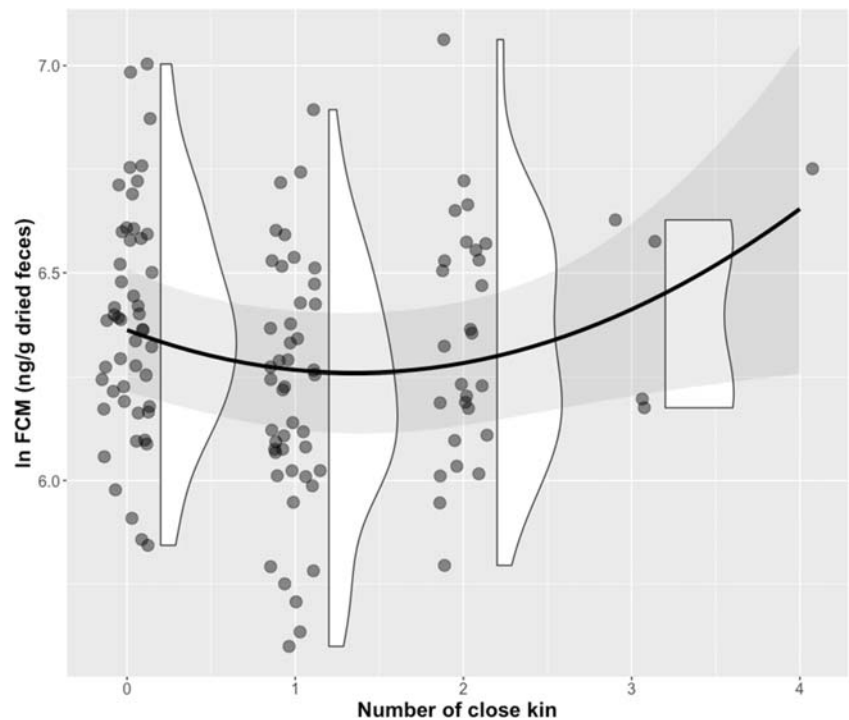
Fig. 2). It is noteworthy that few females had 3 to 4 co-breeding close kin present so that sample sizes for those categories were small (Fig. 1). However, an analysis on females that had only 0, 1, or 2 kin present led to a similarly significant quadratic effect of kin numbers on female FCM levels (Table 2B; see Online Supplementary Material 3).

Table 2 Linear mixed model estimates for the relationships between female FCM levels and female age, number of close kin, and number of close kin² in the colony. Female identity within colony and year were included as random effects in the model. σ^2 = within-group variance; τ_{00} = between-group variance, ICC = intraclass correlation coefficient.

A) Model including all females. B) Model restricted to females that had 0, 1, or 2 kin individuals present, removing potential outlier effects of low sample sizes for females with 3 ($N = 4$) or 4 ($N = 1$) kin present. Sample size along with marginal and conditional R^2 are presented. Significant values for $p < 0.05$ are given in italics

	A) Model with all values of co-breeding close kin				B) Model with a maximum value of co-breeding close kin of 2			
Fixed effects								
	Estimates ± SE	CI	Statistic	<i>p</i>	Estimates ± SE	CI	Statistic	<i>p</i>
(Intercept)	6.19 ± 0.09	6.01–6.38	66.59	< 0.001	6.21 ± 0.09	6.04–6.38	71.09	< 0.001
Kin numbers	–0.15 ± 0.07	– 0.29–0.02	– 2.25	0.026	– 0.24 ± 0.11	– 0.45–0.03	– 2.25	0.026
Kin numbers ²	0.06 ± 0.03	0.01–0.11	2.24	0.027	0.11 ± 0.05	0.00–0.21	1.98	0.050
Age	0.04 ± 0.01	0.02–0.07	3.20	0.002	0.04 ± 0.01	0.01–0.07	2.97	0.004
Random effects								
σ^2	0.06				0.06			
τ_{00} colony:id	0.01				0.02			
τ_{00} year	0.01				0.01			
ICC _{colony:id}	0.14				0.19			
ICC _{year}	0.11				0.07			
Fecal samples/Individuals	126/92				121/91			
Marginal R^2 /Conditional R^2	0.127/0.339				0.117/0.347			

Fig. 1 Quadratic relationship between close kin numbers and lactating female fecal cortisol metabolite (FCM) levels (ln-transformed) in Columbian ground squirrels (*Urocitellus columbianus*). The estimated effect and 95% CI from the linear mixed model is plotted. Violin plots show the distribution of data ($n = 126$ fecal samples; $N = 92$ females)



Nature of the kin environment and relationship with female FCM levels

For females that had only one co-breeding kin present in the colony and for which we had FCM levels, 19 had a co-breeding mother, 17 a co-breeding sister, and 9 a co-breeding

daughter. Females with different types of close kin exhibited different values of FCMs during the lactation period (Table 3). Whereas the presence of a mother or sister did not seem to significantly affect female FCM levels, females with a co-breeding daughter present had 14% lower FCM levels than females that had no kin present in the population (Table 3; Fig. 3).

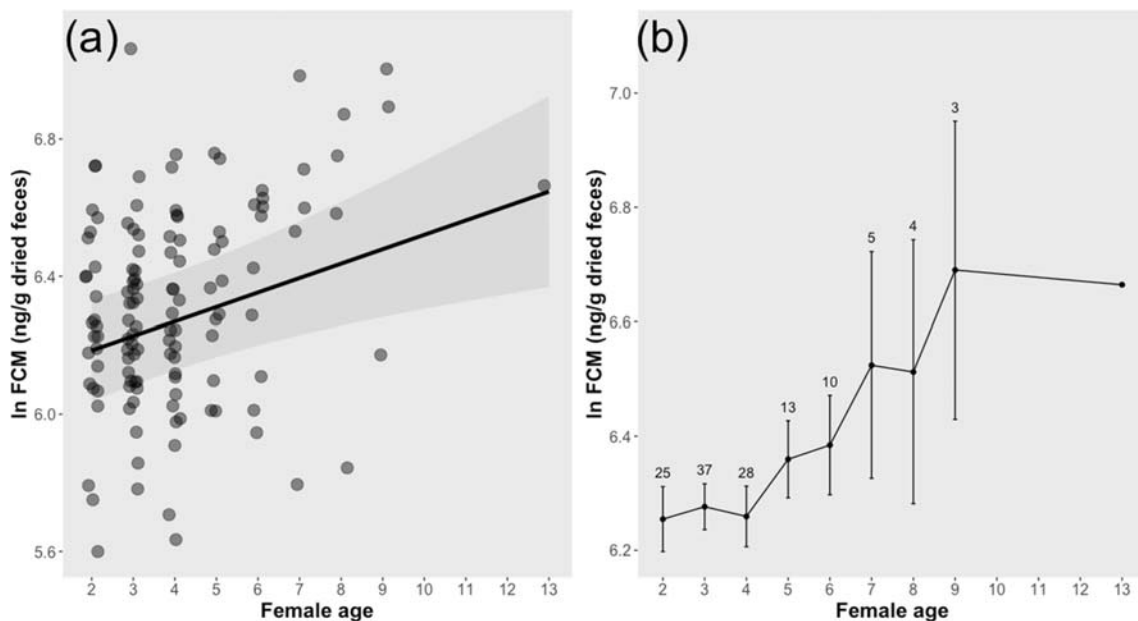


Fig. 2 Relationships between female age and fecal cortisol metabolite (FCM) levels (ln-transformed) in Columbian ground squirrels (*Urocitellus columbianus*). **a** Estimated effect and 95% CI from the linear

mixed model ($n = 126$ fecal samples; $N = 92$ females); **b** mean levels \pm SE (fecal sample sizes are indicated above the bars). Note different scales on the y-axis

Table 3 Mean values (\pm SE) for fecal cortisol metabolites (FCM) for females that had only one co-breeding kin in the colony. The nature of the one kin relationship was either the mother, a daughter, or a littermate

Kin category	FCMs _(ng per g dried feces)	ln (FCMs)	Tukey HSD	Individual fecal samples per year (<i>N</i>)	
				2013	2014
No kin	613.92 \pm 24.83	6.38 \pm 0.04	a	29	20
Mother	563.68 \pm 32.96	6.30 \pm 0.06	a,b	9	10
Daughter	527.03 \pm 66.04	6.21 \pm 0.11	b	4	5
Littermate sister	470.59 \pm 34.17	6.12 \pm 0.07	a,b	5	12

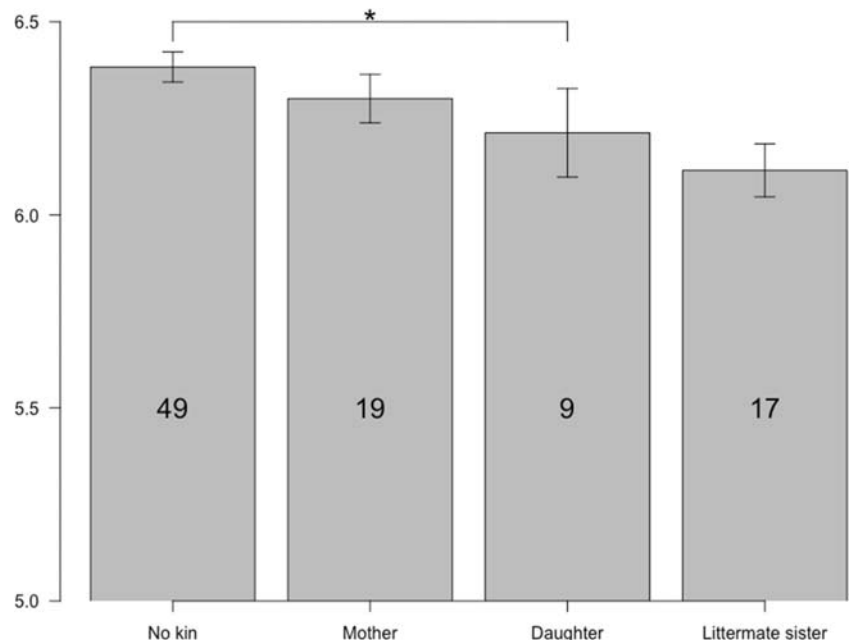
Discussion

Within animal groups, social conflict and cooperation might impose different tolls on individuals, with varying consequences on the functioning of the HPA axis and the secretion of GC, so-called “stress,” hormones (reviewed in Creel et al. 2013). Whereas numerous studies have considered the positive (e.g. Scheiber et al. 2009; Frigerio et al. 2005; Young et al. 2014; Ludwig et al. 2017) or negative (e.g. Goymann and Wingfield 2004; Ostner et al. 2008) relationships between social environments and individual GC levels in group-living species, few have concurrently investigated the joint effect of socially aggressive and socially tolerant environments on the stress axis of free-living vertebrates (Dantzer et al. 2017). Here, we tested the hypothesis that local colony density and the presence of co-breeding kin should affect female HPA axis activity in opposite directions in the Columbian ground squirrel. We expected that female GC levels would increase with high local conspecific density (a reflection of increased competition) and decrease with more

co-breeding kin (a reflection of increased cooperation). Such effects could be expected because of (1) local competition on one hand (high female territoriality, risks of infanticide carried out by lactating females, importance of food resources in regulating demographics, and reported dispersal of females from high density areas (Festa-Bianchet and Boag 1982; Dobson and Kjelgaard 1985; Dobson 1990; Arnaud et al. 2012)) and (2) kin-related direct and indirect fitness benefits on the other (Neuhaus et al. 2004; Viblanc et al. 2010; Dobson et al. 2012), likely through reduced rates of aggression (King 1989b; Viblanc et al. 2016a), kin acting as “genial neighbors” to one another.

Contrary to our predictions, our analysis conducted at the local spatial scale of a 30-m radius around a female’s nest burrow (viz. the area actively defended during lactation; Festa-Bianchet and Boag 1982) did not suggest that an increase in local colony density resulted in an increase in female FCM levels. Similarly, at a local scale, increasing co-breeding close kin numbers did not seem to be associated with a decrease in FCM levels. Interestingly, however, though the kin

Fig. 3 Female fecal cortisol metabolite (FCM) levels (ln-transformed) for females with zero or one co-breeding kin in the study (either no kin, a mother present, a daughter present, or a littermate sister present). Values are given as means \pm SE. Significant differences to no-kin levels are given by the asterisk. All other values were not significantly different from each other (Tukey HSD; see Table 3). Fecal sample sizes are indicated in the bars ($n = 94$ fecal samples; $N = 74$ females)



effect was lacking at a local scale, it existed at a population scale, though unexpectedly, this effect was not linear. Whereas we expected a negative relationship between kin numbers and female FCM levels, the data showed a negative quadratic effect of the social kin environment on female FCM levels. Female FCM levels were high when no kin were present, decreased by 15% when 1 co-breeding close kin was present and increased when more than one kin were present. Although a quadratic function appeared to provide the best fit to the data, it should be noted that the sample size of individuals with 3 ($n = 4$) and 4 ($n = 1$) close kin was low. Nonetheless, when considering only female with 0, 1, and 2 kin present (for which there was adequate sample sizes), the quadratic effect remained but was weaker.

The fact that the kin effect was not clear at a local level, but appeared at a colony level, suggests that kin advantages extend beyond the reduction of territorial aggression on female core territories per se. Indeed, although the core of female aggression is located within a 30-m radius (Festa-Bianchet and Boag 1982), females may encounter kin individuals well beyond 30-m of their nest burrow (Viblanç et al. 2010; Arnaud et al. 2012). At a colony scale, kin environments may not only reduce aggression rates during daily commutes to and from foraging sites (King 1989b; Viblanç et al. 2016a) but also facilitate emigration movements and territorial establishment (Arnaud et al. 2012). This advantage is likely to occur even over the course of a single breeding season, as females are known to change their nest burrow locations, sometimes more than once during lactation (FSD et al., pers. obs.). On the other hand, the positive relationship between female FCMs and kin numbers beyond one kin is likely to reflect social competition for resources (Dobson and Kjelgaard 1985; Dobson 1995) and an upregulation of the HPA axis (but see caveat expressed above).

In our study, the negative effect of kin numbers on female FCMs was limited to the presence of one kin. This is consistent with previous findings that the greatest effect of kin in mediating changes in energy allocation from somatic towards reproductive allocation occurred from a shift of having no kin to having one kin present (Viblanç et al. 2016b). Considering the nature of the kin relationship for females having only one co-breeding kin, we found that females with a co-breeding daughter experienced a decrease in their FCM levels compared to females with no kin. Thus, co-breeding daughters, but not littermate sisters or mothers, appeared to provide a substantial advantage in terms of decreasing GC levels. It should be noted, however, that females with co-breeding sisters had 23% (though not significantly) lower FCM levels than females with no kin around. One explanation for this result is that part of the variation in FCM levels was accounted by year effects since most females with co-breeding sisters occurred in 2014 ($n = 12$) vs. 2013 ($n = 5$), whereas other categories were fairly balanced between years (see Table 3), and

overall FCM levels were slightly lower in 2014 ($\ln\text{-FCM}_{2013} = 6.37 \pm 0.03$ ng/g dried feces vs. $\ln\text{-FCM}_{2014} = 6.24 \pm 0.04$ ng/g dried feces). Previous studies in Columbian ground squirrels have suggested that kin advantages mediated through mother-daughter relationships might include territory bequeathal, mothers dispersing to avoid competition with philopatric daughters (Harris and Murie 1984). However, evidence from female dispersal movements suggests that mothers are more likely to accommodate and tolerate daughters (Wiggett and Boag 1992; Arnaud et al. 2012) than provide advantages in terms of territory bequeathal. Thus, differences between kin in terms of FCM levels may perhaps be explained by age-related social dominance patterns, with dominant mothers being equivalent to not having any kin around and subordinate daughters or equally ranked sisters posing a lowered source of stress for breeding females. This idea is supported by the fact that received aggression decreases but elicited aggression generally increases with age, suggesting a pattern of age-related dominance in female Columbian ground squirrels (Viblanç et al. 2016a).

Interestingly, investigations into the relationships between individual GC levels and the presence of kin in the social environment have led to mixed results in other social mammals. In closely related Richardson ground squirrels (*Urocitellus richardsonii*) for instance, the disappearance of mothers from the population had no impact on FCM levels of their offspring shortly after weaning, and removing related neighbors from adjacent territories did not appear to substantially affect the FCM levels of breeding females (Freeman et al. 2019). In contrast, for cooperatively breeding meerkats (*Suricata suricatta*), when parents are the dominant breeding pair, subordinate individuals seem to benefit from living in social groups in the form of lower GC levels, in comparison with subordinate individuals that live in social groups with an unrelated dominant pair (Dantzer et al. 2017). In our study species and overall, the effects of kin on female FCM levels were rather limited and detectable only over a narrow range and context of close kin availability at the colony, but not local, scale. These findings reinforce previous suggestions that Columbian ground squirrel societies are typified by somewhat egalitarian and inclusive social constructs that transcend boundaries dictated by kinship alone (Hare 1992, 1994; Hare and Murie 1996, 2007; Fairbanks and Dobson 2010). Taken together, those results raise the intriguing question of the extent to which variation in social lifestyles (i.e. from more egalitarian to despotic constructs) may shape the stress load experienced by individuals. In this regard, a comparative interspecific study of GCs in relation to the social environment may be useful to evaluate the physiological toll imposed by various social constructs on individuals, all while controlling for other sources of GC variation such as climate, food availability, or predation (Rubenstein and Shen 2009;

Dantzer et al. 2017). For instance, positive effects of kin on individual GC levels may be more pronounced in matrilineal species where social systems are characterized by more despotic relationships or stronger dominance hierarchies than in the Columbian ground squirrel. Among others, the diversity of social systems in rodents and particular sciurids make them good models for future investigations into such questions (Wolff and Sherman 2008).

FCM levels increased with age in female Columbian ground squirrels. In vertebrates, increasing GC levels with age have been suggested to reflect two concurrent mechanisms: (1) increasing metabolic and reproductive demands with age (Crespi et al. 2013) and (2) progressive deterioration of the HPA axis in senescing individuals (Gupta and Morley 2011). For instance, perturbed regulation of the HPA axis leading to high GC production have been documented with increasing age in humans (Sherman et al. 1985; Van Cauter et al. 1996; Chahal and Drake 2007), dogs (Reul et al. 1991), and rats (Scaccianoce et al. 1990). Our results in Columbian ground squirrels are likely to reflect those two concurrent mechanisms: reproductive effort in breeding females is known to increase until about 5 years of age, with females of 6 years old and above starting to exhibit reproductive senescence (Broussard et al. 2003). This is consistent with the pattern of increase in FCM levels observed in this study that could be linked to increased metabolic demands up to a point and reflect senescence of the HPA axis beyond that point.

Increased circulating GC levels are often viewed as indicative of chronic stress with potentially detrimental effects (but see Boonstra (2013)), such as on the immune system (Sapolsky et al. 2000) or oxidative stress (Costantini et al. 2011). However, the primary function of glucocorticoids under acute conditions is energy mobilization (Sapolsky et al. 2000) and increased levels of maternal glucocorticoids may have adaptive transgenerational consequences (Avishai-Eliner et al. 2001; Cottrell and Seckl 2009; Jensen 2013; Sheriff and Love 2013). In red squirrels, females subject to both experimental and natural increases in conspecific density are known to exhibit increased FCM levels compared with controls, with positive consequences on their offspring growth rates and survival in a competitive social environment (Larsen and Boutin 1994; Dantzer et al. 2013). Thus, increased maternal FCM levels may appear as advantageous for the young to adapt to future social environments, despite potentially negative impacts on maternal immunity, oxidative stress, and fitness due to pleiotropic effects of GCs. This may also be the case in the Columbian ground squirrel and remains to be tested.

To conclude, our results suggest complex relationships between the social kin environment and individual stress levels in a wild colonial mammal, revealing the existence of a social trade-off between advantages and costs to social conspecifics in terms of GC levels. Whether those complex relationships

translate into significant fitness costs or set a threshold for optimal group size remains to be seen.

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Author contributions VAV, FSD, and RB designed the study. VAV, FSD, C B, CS, and PN collected the data. CuB and RB conducted laboratory analyses. SS, FSD, CS and VAV analyzed the data. RP provided antibodies and expertise on FCM measurement. SS and VAV wrote the manuscript. All authors commented on the paper.

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Data availability The data related to this paper are accessible as figshare doi: <https://doi.org/10.6084/m9.figshare.11949078>

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures carried out in the field and laboratory were approved by Auburn University (IACUC protocol # 2013–2263) and the University of Calgary. Authorization for conducting research and collecting samples in the Sheep River Provincial Park was obtained from Alberta Environment and Parks (research permits # 51774, 51,801, 54,950, 54,951) and Alberta Fish & Wildlife (research and collection permits # 13–027 and 14–048).

References

- Arnaud CM, Dobson FS, Murie JO (2012) Philopatry and within-colony movements in Columbian ground squirrels. *Mol Ecol* 21:493–504
- Avishai-Eliner S, Eghbal-Ahmadi M, Tabachnik E, Brunson KL, Baram TZ (2001) Down-regulation of hypothalamic corticotropin-releasing hormone messenger ribonucleic acid (mRNA) precedes early-life experience-induced changes in hippocampal glucocorticoid receptor mRNA. *Endocrinology* 142:89–97
- Aydonat D, Penn DJ, Smith S, Moodley Y, Hoelzl F, Knauer F, Schwarzenberger F (2014) Social isolation shortens telomeres in African Grey parrots (*Psittacus erithacus erithacus*). *PLoS One* 9: e93839

- Bartolomucci A (2007) Social stress, immune functions and disease in rodents. *Front Neuroendocrinol* 28:28–49
- Barton K (2019) MuMIn: multi-model inference. R Package Version 1(43):15 <https://CRAN.R-project.org/package=MuMIn>
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Beaulieu M, Mboumba S, Willaume E, Kappeler PM, Charpentier MJ (2014) The oxidative cost of unstable social dominance. *J Exp Biol* 217:2629–2632
- Boonstra R (2013) Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct Ecol* 27:11–23
- Boonstra R, Boag P (1992) Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones. *J Anim Ecol* 61:339–352
- Boonstra R, Barker JM, Castillo J, Fletcher QE (2007) The role of the stress axis in life-history adaptations of rodents. In: Wolff JO, Sherman PW (eds) *Rodent societies: an ecological and evolutionary perspective*. University of Chicago Press, Chicago, pp 139–149
- Bosson CO, Palme R, Boonstra R (2009) Assessment of the stress response in Columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. *Physiol Biochem Zool* 82:291–301
- Broussard D, Risch T, Dobson F, Murie J (2003) Senescence and age-related reproduction of female Columbian ground squirrels. *J Anim Ecol* 72:212–219
- Cao TT, Dormhaus A (2008) Ants under crowded conditions consume more energy. *Biol Lett* 4:613–615
- Carere C, Groothuis TGG, Möstl E, Daan S, Koolhaas JM (2003) Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. *Horm Behav* 43:540–548
- Chahal H, Drake W (2007) The endocrine system and ageing. *J Pathol* 211:173–180
- Costantini D, Marasco V, Møller AP (2011) A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J Comp Physiol* 181:447–456
- Cottrell E, Seckl J (2009) Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci* 3:19
- Creel S (2001) Social dominance and stress hormones. *Trends Ecol Evol* 16:491–497
- Creel S, Dantzer B, Goymann W, Rubenstein DR (2013) The ecology of stress: effects of the social environment. *Funct Ecol* 27:66–80
- Crespi EJ, Williams TD, Jessop TS, Delehanty B (2013) Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Funct Ecol* 27:93–106
- Dantzer B, Newman AE, Boonstra 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
- Dantzer B, Bennett NC, Clutton-Brock TH (2017) Social conflict and costs of cooperation in meerkats are reflected in measures of stress hormones. *Behav Ecol* 28:1131–1141
- de Groot J, Ruis MA, Scholten JW, Koolhaas JM, Boersma WJ (2001) Long-term effects of social stress on antiviral immunity in pigs. *Physiol Behav* 73:145–158
- Delignette-Muller ML, Dutang C (2015) Fitdistrplus: an R package for fitting distributions. *J Stat Softw* 64:1–34
- DeVries AC (2002) Interaction among social environment, the hypothalamic–pituitary–adrenal axis, and behavior. *Horm Behav* 41:405–413
- Dobson FS (1990) Environmental influences on infanticide in Columbian ground squirrels. *Ethology* 84:3–14
- Dobson FS (1995) Regulation of population size: evidence from Columbian ground squirrels. *Oecologia* 102:44–51
- Dobson FS, Kjelgaard JD (1985) The influence of food resources on population dynamics in Columbian ground squirrels. *Can J Zool* 63:2095–2104
- Dobson FS, Oli MK (2001) The demographic basis of population regulation in Columbian ground squirrels. *Am Nat* 158:236–247
- Dobson FS, Badry MJ, Geddes C (1992) Seasonal activity and body mass of Columbian ground squirrels. *Can J Zool* 70:1364–1368
- Dobson FS, Viblanc VA, Arnaud CM, Murie JO (2012) Kin selection in Columbian ground squirrels: direct and indirect fitness benefits. *Mol Ecol* 21:524–531
- Fairbanks BM, Dobson FS (2010) Kinship does not affect vigilance in Columbian ground squirrels (*Urociellus columbianus*). *Can J Zool* 88:266–270
- Fauteux D, Gauthier G, Berteaux D, Bosson C, Palme R, Boonstra R (2017) Assessing stress in Arctic lemmings: fecal metabolite levels reflect plasma free corticosterone levels. *Physiol Biochem Zool* 90:370–382
- Festa-Bianchet M, Boag DA (1982) Territoriality in adult female Columbian ground squirrels. *Can J Zool* 60:1060–1066
- Freeman AR, Wood TJ, Bairos-Novak KR, Anderson WG, Hare JF (2019) Gone girl: Richardson's ground squirrel offspring and neighbours are resilient to female removal. *R Soc Open Sci* 6:190904
- Frigerio D, Weiß BM, Scheiber IB, Kotschal K (2005) Active and passive social support in families of Greylag geese (*Anser anser*). *Behaviour* 142:1535–1557
- Goymann W, Wingfield JC (2004) Allostatic load, social status and stress hormones: the costs of social status matter. *Anim Behav* 67:591–602
- Gupta D, Morley JE (2011) Hypothalamic-pituitary-adrenal (HPA) axis and aging. *Compr Physiol* 4:1495–1510
- Hare JF (1992) Colony member discrimination by juvenile Columbian ground squirrels (*Spermophilus columbianus*). *Ethology* 92:301–315
- Hare JF (1994) Group member discrimination by Columbian ground squirrels via familiarity with substrate-borne chemical cues. *Anim Behav* 47:803–813
- Hare JF, Murie JO (1992) Manipulation of litter size reveals no cost of reproduction in Columbian ground squirrels. *J Mammal* 73:449–454
- Hare JF, Murie JO (1996) Ground squirrel sociality and the quest for the 'holy grail': does kinship influence behavioral discrimination by juvenile Columbian ground squirrels? *Behav Ecol* 7:76–81
- Hare JF, Murie JO (2007) Ecology, kinship, and ground squirrel sociality. In: Wolff JO, Sherman PW (eds) *Rodent societies: an ecological and evolutionary perspective*. University of Chicago Press, Chicago, pp 345–355
- Hare AJ, Waheed A, Hare JF, Anderson WG (2015) Cortisol and catecholamine responses to social context and a chemical alarm signal in juvenile lake sturgeon, *Acipenser fulvescens*. *Can J Zool* 93:605–613
- Harris BN (2020) Stress hypothesis overload: 131 hypotheses exploring the role of stress in tradeoffs, transitions, and health. *Gen Comp Endocrinol* 288:113355
- Harris M, Murie J (1984) Inheritance of nest sites in female Columbian ground squirrels. *Behav Ecol Sociobiol* 15:97–102
- Jensen P (2013) Transgenerational epigenetic effects on animal behaviour. *Prog Biophys Mol Biol* 113:447–454
- King WJ (1989a) Spacing of female kin in Columbian ground squirrels (*Spermophilus columbianus*). *Can J Zool* 67:91–95
- King WJ (1989b) Kin-differential behaviour of adult female Columbian ground squirrels. *Anim Behav* 38:354–356
- King WJ, Murie JO (1985) Temporal overlap of female kin in Columbian ground squirrels (*Spermophilus columbianus*). *Behav Ecol Sociobiol* 16:337–341
- Kotschal A, Ilmonen P, Penn DJ (2007) Stress impacts telomere dynamics. *Biol Lett* 3:128–130
- Landys MM, Ramenofsky M, Wingfield JC (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen Comp Endocrinol* 148:132–149

- Lardy S, Rey B, Salin K, Voituron Y, Cohas A (2016) Beneficial effects of group size on oxidative balance in a wild cooperative breeder. *Behav Ecol* 27:132–149
- Larsen KW, Boutin S (1994) Movements, survival, and settlement of red squirrel (*Tamiasciurus hudsonicus*) offspring. *Ecology* 75:214–223
- Le PP, Friedman JR, Schug J, Brestelli JE, Parker JB, Bochkis IM, Kaestner KH (2005) Glucocorticoid receptor-dependent gene regulatory networks. *PLoS Genet* 1:159–170
- Ludwig SC, Kapetanopoulos K, Kotschal K, Wascher CAF (2017) Effects of mate separation in female and social isolation in male free-living Greylag geese on behavioural and physiological measures. *Behav Process* 138:134–141
- Murie JO, Harris M (1982) Annual variation of spring emergence and breeding in Columbian ground squirrels (*Spermophilus columbianus*). *J Mammal* 63:431–439
- Murie JO, Harris MA (1988) Social interactions and dominance relationships between female and male Columbian ground squirrels. *Can J Zool* 66:1414–1420
- Nation DA, Gonzales JA, Mendez AJ, Zaias J, Szeto A, Brooks LG, Paredes J, D'Angola A, Schneiderman N, McCabe PM (2008) The effect of social environment on markers of vascular oxidative stress and inflammation in the Watanabe heritable hyperlipidemic rabbit. *Psychosom Med* 70:269–275
- Neuhaus P, Broussard D, Murie J, Dobson F (2004) Age of primiparity and implications of early reproduction on life history in female Columbian ground squirrels. *J Anim Ecol* 73:36–43
- Ostner J, Heistermann M, Schülke O (2008) Dominance, aggression and physiological stress in wild male Assamese macaques (*Macaca assamensis*). *Horm Behav* 54:613–619
- Palme R (2019) Non-invasive measurement of glucocorticoids: advances and problems. *Physiol Behav* 199:229–243
- R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna <http://www.R-project.org>
- Raveh S, Heg D, Dobson FS, Coltman DW, Gorrell JC, Balmer A, Neuhaus P (2010) Mating order and reproductive success in male Columbian ground squirrels (*Urocitellus columbianus*). *Behav Ecol* 21:537–547
- Raveh S, Heg D, Viblanc VA, Coltman DW, Gorrell JC, Dobson FS, Balmer A, Neuhaus P (2011) Male reproductive tactics to increase paternity in the polygynandrous Columbian ground squirrel (*Urocitellus columbianus*). *Behav Ecol Sociobiol* 65:695–706
- Raynaud J, Dobson FS (2011) Scent communication by female Columbian ground squirrels, *Urocitellus columbianus*. *Behav Ecol Sociobiol* 65:351–358
- Razzoli M, Nyuyki-Dufe K, Gurney A, Erickson C, McCallum J, Spielman N, Marzullo M, Patricelli J, Kurata M, Pope EA (2018) Social stress shortens lifespan in mice. *Aging Cell* 17:e12778
- Reul JM, Rothuizen J, de Kloet ER (1991) Age-related changes in the dog hypothalamic-pituitary-adrenocortical system: neuroendocrine activity and corticosteroid receptors. *J Steroid Biochem Mol Biol* 40: 63–69
- Rubach K, Wu M, Abebe A, Dobson FS, Murie JO, Viblanc VA (2016) Testing the reproductive and somatic trade-off in female Columbian ground squirrels. *Ecol Evol* 6:7586–7595
- Rubenstein DR, Shen S-F (2009) Reproductive conflict and the costs of social status in cooperatively breeding vertebrates. *Am Nat* 173: 650–661
- Sapolsky RM (1992) Do glucocorticoid concentrations rise with age in the rat? *Neurobiol Aging* 13:171–174
- Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21:55–89
- Scaccianoce S, Di Sciullo A, Angelucci L (1990) Age-related changes in hypothalamo-pituitary-adrenocortical axis activity in the rat. *Neuroendocrinology* 52:150–155
- Scheiber IB, Kotschal K, Weiß BM (2009) Benefits of family reunions: social support in secondary greylag goose families. *Horm Behav* 55: 133–138
- Sheriff MJ, Love OP (2013) Determining the adaptive potential of maternal stress. *Ecol Lett* 16:271–280
- Sheriff MJ, Krebs CJ, Boonstra R (2010) Assessing stress in animal populations: do fecal and plasma glucocorticoids tell the same story? *Gen Comp Endocrinol* 166:614–619
- Sheriff MJ, Dantzer B, Delehanty B, Palme R, Boonstra R (2011) Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166:869–887
- Sherman B, Wysham W, Pfoh B (1985) Age-related changes in the circadian rhythm of plasma cortisol in man. *J Clin Endocrinol Metab* 61:439–443
- Sloman K, Motherwell G, O'connor K, Taylor A (2000) The effect of social stress on the standard metabolic rate (SMR) of brown trout, *Salmo trutta*. *Fish Physiol Biochem* 23:49–53
- Stefanski V, Engler H (1998) Effects of acute and chronic social stress on blood cellular immunity in rats. *Physiol Behav* 64:733–741
- Stevens SD (1998) High incidence of infanticide by lactating females in a population of Columbian ground squirrels (*Spermophilus columbianus*). *Can J Zool* 76:1183–1187
- 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
- Tung J, Barreiro LB, Johnson ZP, Hansen KD, Michopoulos V, Toufexis D, Michelini K, Wilson ME, Gilad Y (2012) Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *Proc Natl Acad Sci U S A* 109:6490–6495
- Turner-Cobb JM, Sephton SE, Koopman C, Blake-Mortimer J, Spiegel D (2000) Social support and salivary cortisol in women with metastatic breast cancer. *Psychosom Med* 62:337–345
- Van Cauter E, Leproult R, Kupfer DJ (1996) Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab* 81:2468–2473
- Viblanc VA, Arnaud CM, Dobson FS, Murie JO (2010) Kin selection in Columbian ground squirrels (*Urocitellus columbianus*): littermate kin provide individual fitness benefits. *Proc R Soc Lond B* 277: 989–994
- Viblanc VA, Pasquaretta C, Sueur C, Boonstra R, Dobson FS (2016a) Aggression in Columbian ground squirrels: relationships with age, kinship, energy allocation, and fitness. *Behav Ecol* 27:1716–1725
- Viblanc VA, Saraux C, Murie JO, Dobson FS (2016b) Kin effects on energy allocation in group-living ground squirrels. *J Anim Ecol* 85:1361–1369
- Wiggert D, Boag DA (1992) The resident fitness hypothesis and dispersal by yearling female Columbian ground squirrels. *Can J Zool* 70: 1984–1994
- Willis CK, Brigham RM (2007) Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behav Ecol Sociobiol* 62:97–108
- Wingfield JC, Romero LM (2001) Adrenocortical response to stress and their modulation in free-living vertebrates. In: McEwen BS (ed) *Handbook of physiology, section 7: the endocrine system, vol 4: Coping with the environment: neural and endocrine mechanisms*. Oxford University Press, New York, pp 211–234
- Wittig RM, Crockford C, Lehmann J, Whitten PL, Seyfarth RM, Cheney DL (2008) Focused grooming networks and stress alleviation in wild female baboons. *Horm Behav* 54:170–177
- Wittig RM, Crockford C, Weltring A, Langergraber KE, Deschner T, Zuberbühler K (2016) Social support reduces stress hormone levels in wild chimpanzees across stressful events and everyday affiliations. *Nat Commun* 7:13361
- Wolff JO, Sherman PW (eds) (2008) *Rodent societies: an ecological and evolutionary perspective*. University of Chicago Press, Chicago