THESIS

MATERNAL SURVIVAL AND DAUGHTER DISPERSAL IN THE GOLDEN-MANTLED GROUND SQUIRREL

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ABSTRACT

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Variation in the schedule of key life events such as the age of first reproduction and the timing of dispersal arises via differential investment of resources into key functions such as maintenance, reproductive output, and growth. Individual differences scale up to affect the population as a whole; over time, such variation accumulates in genetic lineages and natural selection can shape the structure of the population. By studying the factors that influence variation at different levels of organization, we can begin to understand what ultimately shapes the diversity of life histories we observe around us.

In the first chapter of this thesis, we used a long-term observational dataset to explore how individual characteristics, social dynamics, and environmental conditions affect maternal survival in an asocial mammal, the golden-mantled ground squirrel (GMGS), *Callospermophilus lateralis*. We found that producing a litter of any size, as opposed to no litter at all, positively affected maternal annual survival probability, likely because successful females are in better body condition and able to sustain themselves and their litter. Conversely, the presence of more related breeding females as well as total females, regardless of breeding or kin status, negatively influenced survival probability. Competition among related females is likely greater than unrelated females because mothers and daughters will share space and vie for the same resources while unrelated females are less tolerant of each other and more defensive of their territories, thereby explaining why we see lower survival when the presence of kin increases but not when

the number of non-kin increases. In addition, non-breeding females present an additional challenge because they are trying to establish a new territory and reproductive females must defend their established territories against potential invaders. From an environmental perspective, survival probability was higher when the length of the growing season (i.e., number of days from first day of bare ground in the spring to last day of permanent snow-free ground in the fall) was longer and when the date of permanent snow cover was delayed. Both factors may enhance the ability of reproductive females to compensate for reproductive output by having more time to acquire resources prior to hibernation.

Our results emphasize another key facet of life history, the level of sociality. Sociality falls on a spectrum based on kin and non-kin interactions and the costs and benefits associated with those interactions. Some of the costs of living in groups include more predator attraction, more easily transmitted pathogens and parasites, and higher intensity local competition for resources. The benefits to interacting with others can include greater reproductive output and higher survival probability. When the costs of social interactions outweigh the benefits, species live asocially. In mammals, the formation of matrilines, defined as multigenerational kin clusters, is thought to be key to the evolution of sociality. Matrilineal groups serve as the transition from solitary-living to complex social groups and are formed through the production and retention of daughters via philopatry. Taken together, interactions between mothers and daughters are likely to shape decisions to disperse or remain philopatric and have fitness consequences that are different than for pairs of unrelated females in small mammals like the GMGS.

Because it appears that the presence of kin can negatively affect maternal survival probability, as shown in Chapter 1, recruiting daughters via natal philopatry could lead to mothers overlapping their home range with their daughters and effectively generating more competition as they share the same space and vie for the same resources. In the second chapter, we set out to explore whether maternal effects – when non-genetic, phenotypic responses in offspring are induced by maternal phenotypes – may drive natal dispersal, another important behavior that has several long-term effects on individual fitness. To do so, we quantified cortisol concentrations, used as an index of stress, in feces and hair to assess the relationship between daughter stress and subsequent dispersal behavior in the same population of GMGS. Although we did not find the anticipated strong correlation in cortisol concentration between mothers and daughters at the time of litter emergence, our results indicated that daughters with higher cortisol levels during early development tended to have a higher probability of dispersing away from their natal home range.

Given these results, physiology may play an essential role in offspring behavior as the effects of competition for ecological resources and agonistic social interactions both promote dispersal in other small mammals. More recently, maternal effects have been gaining attention as a means of promoting dispersal, too. Although our results did not explicitly support the idea of maternal effects at the time of offspring emergence, it will be important to evaluate the role of stress during early development (i.e., during gestation and lactation), an important time period which we were unable to address here.

Taken together, our work highlights the importance of kin relationships in an asocial hibernating, small mammal species, GMGS. Lower survival probability in the presence of kin suggests that interactions with relatives are likely a critical factor maintaining solitary living in GMGS. In addition, daughter stress during early development, potentially deriving from sibling interactions, maternal investment, and/or genetic differences, affects dispersal behavior in species with sex-biased dispersal. Furthermore**,** we see how individual variation in life histories occurs as a response to individual characteristics, social dynamics, and environmental conditions, and this variation, in turn, may have important impacts on individual fitness which may scale up to influence population growth, community dynamics, and beyond.

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SYNOPSIS

When access to resources is limited, differential allocation to components of fitness, such as survival and reproduction, can lead to trade-offs. Short-lived mammals like the golden-mantled ground squirrel (GMGS), *Callospermophilus lateralis*, tend to maximize effort towards current reproduction at the expense of survival, and a mother's individual characteristics, social dynamics, and environmental factors may influence these components of fitness. Here, we sought to estimate survival in an asocial, hibernating mammal, the GMGS. Using 25 years of data (1995-2020) collected from a population of GMGS at the Rocky Mountain Biological Research Laboratory in Gothic, Colorado, we tested for an association of several maternal characteristics (e.g., age, experience, residency status, age of first reproduction, litter size, litter mass, timing of litter emergence), social context (e.g., litter sex ratio, number of breeding females, number of related and unrelated breeding females, total number of females), and environmental context (e.g., day of bare ground, day of permanent snow cover, length of vegetative growing season, amount of winter snowfall, amount of summer rainfall, average summer temperature, number of days above 25°C) on reproductive female survival using Cox proportional hazard models. Results indicated that maternal characteristics (i.e., litter size), social dynamics (i.e., density) and environmental conditions (i.e., day of snow cover and length of growing season) all explain significant variation in annual maternal survival. The production of a litter reduces the mortality hazard while higher density of related breeding females or total number of females are associated with higher mortality hazards. Delaying the first day of

permanent snow cover and prolonging total growing season both reduced the mortality hazard. This information sheds light on sociality and could be paired with climate change data to predict effects on population size, components of fitness, and life history trade-offs.

INTRODUCTION

Variability in the allocation of resources towards current reproduction, future reproduction, or survival (i.e., ‘the principle of allocation’ (Levins, 1968)) can result in a fitness trade-off known as the ‘cost of reproduction’ (Roff, 1992; Stearns, 1992). A change in current reproductive effort affects an organism’s fitness by inversely impacting survival and/or future reproduction (Reznick, 1985; Williams, 1966). Short-lived mammals tend to maximize effort towards current reproduction at the expense of survival, whereas their long-lived counterparts may delay reproduction and instead invest in survival, given greater reproductive flexibility (i.e., “bet-hedging”) (Hamel et al., 2010; Starrfelt & Kokko, 2012; Stearns & Rodrigues, 2020). Maternal characteristics, social dynamics, and environmental factors influence female reproductive output and survival, as well as the dynamic trade-offs that may exist among these fitness traits (Williams, 1957).

Maternal characteristics that drive resource allocation for reproduction include individual characteristics (e.g., age of first reproduction, reproductive experience) that influence both reproductive output (e.g., litter size, sex ratio, offspring size and quality) as well as annual survival (Hamel, et al., 2010; Toni et al., 2020). Moore et al. (2016) studied the trade-off between age of first reproduction and fitness from inter- and intra-generational perspectives in a population of golden-mantled ground squirrels (GMGS), *Callospermophilus lateralis*, and found that delaying the age of first reproduction resulted in relatively lower individual fitness, measured as (i) lifetime reproductive success and (ii) individual fitness calculated as the dominant eigenvalue of the population projection matrix. In the bank vole (*Myodes glareolus*), offspring sex ratio is related to the mother’s previous reproductive status (Rutkowska et al., 2011), and in Columbian ground squirrels (*Spermophilus columbianus*), more experienced mothers had higher reproductive success than their inexperienced counterparts (Broussard et al., 2008). In yellow-bellied marmots (*Marmota flaviventer*), earlier timing of offspring weaning resulted in reduced adult mortality (Ozgul et al., 2010). Additional studies on yellow-bellied marmots (*Marmota flaviventer*) showed adult survival was highest for reproductive females and for individuals with prior reproductive experience (Paniw et al., 2020).

The degree of sociality plays another important role in mammal life histories. The evolution of sociality is driven by the costs and benefits of close interactions with conspecifics, with sociality occurring when the benefits outweigh the costs, and *vice versa* for asociality (Boone, 2017; Silk, 2007). Costs related to group-living include more predator attraction (Ale & Brown, 2007), increased transmission of pathogens and parasites (Cote & Poulin, 1995; Lucatelli et al., 2021), and more intense intra-specific competition (Van Baalen & Yamauchi, 2019; Stockley & Bro-Jørgensen, 2011). The benefits of social interactions include greater reproductive output (Armitage, 1987) and higher survival probability (Ebensperger et al., 2012). Sociality thus falls on a spectrum based on the degree of kin and non-kin interactions. Golden-mantled ground squirrels, the focus of this study, are classified as asocial (Armitage, 1981; Michener, 1983). Females defend territories and occupy burrows by themselves, but they may exhibit some home range overlap with related kin (i.e., mothers or daughters) (Aliperti et al., 2020; Jesmer et al., 2011).

Density and kinship together play a prominent role in shaping reproductive output and survival in GMGS. For example, the density of female GMGS within a population can dictate the sex ratio of offspring, favoring more daughters – the philopatric sex – when fewer adult females are present in the population (Wells & Van Vuren, 2017). Furthermore, density-dependent offspring sex bias, favoring the production of sons when density is high, is more pronounced when the number of kin within the local area (i.e., ‘locality’) is higher (Wells & Van Vuren, 2017). The presence of littermate sisters can also affect reproduction by delaying the age of first reproduction, but mother and other kin presence have no effect for GMGS (Wells & Van Vuren, 2018). Although kin presence affects fitness (Moore et al., 2016; Viblanc et al., 2010) and reproductive output (Wells & Van Vuren, 2017), its explicit link to survival has been less explored. Kneip et al. (2011) studied the effects of total female population density and environmental factors on survival in the same system as Wells and Van Vuren (2018) and found that survival was lower with higher squirrel densities in the year prior, and short-term survival was most heavily influenced by rainfall and predation pressure.

Survival rates of ground-dwelling, hibernating mammals are especially susceptible to the effects of environmental stochasticity on resource availability. When comparing similar-sized mammals, species that hibernate typically have higher annual survival than their non-hibernating counterparts (Turbill et al., 2011), but extended winter conditions can be detrimental to hibernating mammals if phenological mismatch leads to reduced resource availability at the time of emergence (Van Vuren & Armitage, 1991). In extremely seasonal environments, hibernation is a survival strategy that has evolved to buffer individuals during periods when resources are commonly lacking (e.g., alpine winter months). Variability in snow cover and rainfall in marmots, for example, contributes to variation in survival, likely because of their effects on resource availability (Armitage, 2013). Even though hibernation increases annual survival overall, environmental stochasticity plays an important role in resource availability during the active season when most mortality occurs (Turbill et al., 2011). In early spring, above-average precipitation can increase vegetative growth (Sharpe & Van Horne, 1998); however, rainy weather precludes ground squirrels from seeking the resources needed for survival (Kneip et al., 2011). High summer temperatures after the reproductive period also reduce foraging activity, resulting in a decline of resource acquisition (Vispo & Bakken, 1993) which could reduce the probability of over-winter survival.

Collectively, maternal characteristics interact strongly with the broader social and environmental contexts experienced to shape fitness trade-offs (Gerber et al., 2021; Rusu & Krackow, 2004; Ziv & Davidowitz, 2019). We studied a population of female GMGS, an asocial, short-lived, hibernating mammal, a species that is responsive to significant variability in maternal characteristics, social dynamics, and environmental factors, and assessed which factors had the strongest influence on maternal survival. We were particularly interested in understanding how reproductive investment varies among mothers and how it shapes their survival chances from year-to-year, within the contexts of extreme seasonality and complex social interactions.

METHODS

Focal Species

Golden-mantled ground squirrels are distributed throughout the Rocky Mountains along an elevational gradient from 1,220 to 3,965 meters above sea level (Kneip et al., 2011). Spring emergence occurs between early April and late May, and they enter hibernation in the fall, usually September or October (Bronson, 1980). Polygynous breeding takes place in early spring (Kenagy et al., 1989). Females give birth to a single litter of one to nine altricial pups per year. Golden-mantled ground squirrels can live up to seven to nine years (Bronson, 1980; Kanaziz pers. obs.).

Study Site & Data Collection

The Rocky Mountain Biological Research Laboratory (RMBL) is located in the East River Valley of Gunnison, Colorado (38°58'N, 106°59'W). The 13-hectare study site is situated at an elevation of 2,900 meters above sea level. Observations of the GMGS population at RMBL began in 1990, but the current, long-term surveys of all individuals within the population did not formally begin until 1996 (McEachern et al., 2011).

We used data collected from 1995 to 2020 on 141 females for which we had 249 observations and 131 mortality events. Females entered the study the year of their first reproduction, which occurred at age one or two for nearly all individuals. Because we only included reproductive females, any instance where an individual disappeared from the study site was classified as a mortality event as post-breeding dispersal is extremely rare.

To develop a census database for analysis, field methods involved monthly trapping events during the active season and daily observations. Once trapped, individuals were identified by ear tag, weighed, and reproductive status was evaluated based on nipple development (Wells & Van Vuren, 2017). Individuals were classified as ‘alive’ if they were trapped or seen during daily observations, but adult females who failed to return to the system were classified as ‘dead.’ For immigrant individuals that were not born in the study site but entered and established a territory later, we used timing of entrance and mass at capture as indicators of age. Immigrants who were captured late in summer and had a mass consistent with other young of the year were classified as juveniles and assigned a known age; by contrast, immigrants who were captured in spring could be yearlings or older and mass could not be used as a categorical difference, making their definitive age unknown.

Variables of interest and hypothesized relationships with survival

Maternal characteristics. The census dataset and corresponding measurements were used to compile a list of variables related to maternal characteristics, including individual attributes as well as metrics of reproductive investment. Data were recorded in each year of the study for all female GMGS who were alive in that year. The ***age*** of each female was counted as the number of years since birth (i.e., a newborn juvenile was viewed as age 0; a yearling was age 1, etc.) with the expectation that increasing age would improve survival as ***experience*** (the difference between maternal age and age of first reproduction) also increased. ***Resident status*** was determined based on whether the individual was born within the study site ('resident') or immigrated into the site from a different population ('immigrant'). Resident mothers should have better survival because site familiarity gives them an advantage in resource acquisition and predator avoidance as compared to their immigrant counterparts who came into the study site from a different population (typically as a juvenile or yearling). The ***age of first reproduction*** was recorded for all residents, and, if an immigrant joined the system as a known juvenile, then her age of first reproduction was also recorded. By delaying age of first reproduction, a female can devote more resources to individual growth rather than invest in reproductive output which should benefit her survival probability. ***Litter size*** was a count of the number of offspring that emerged from the natal burrow. We hypothesize that a smaller litter size (i.e., fewer offspring per litter) should be associated with higher maternal survival as maternal care is apportioned among fewer offspring. The ***litter mass*** was the cumulative mass of all offspring within a litter at the time of weaning, and higher litter mass should be associated with higher annual survival, as both may indicate that mothers had access to better resources. The ***day of litter emergence***, a proxy of maternal reproductive timing, was standardized by the day of bare ground which represented the first day of spring in which there was no longer permanent snow cover. This day was standardized by the number of days before or after April 30 of each year with day one beginning on May 1 and negative values indicating snowmelt prior to April 30. Earlier litter emergence should improve maternal survival as mothers will have longer to prepare for hibernation after the reproductive period.

Social context. The social environment is shaped by both reproductive output that can alter future social context as well as current density-related factors that are especially important in asocial species like the GMGS. The proportion of daughters in each litter was used to calculate ***sex-ratios***. Producing more daughters per litter (i.e., a female-biased sex-ratio) should be associated with lower survival probability as it increases the opportunity for daughter philopatry which could increase kin overlap and intensify intraspecific competition. Female population size was considered on a local scale as the number of reproductive and non-reproductive individuals within one of six discrete 'localities' within the study site (Wells & Van Vuren, 2017). Higher local density of both kin and non-kin is hypothesized to result in lower maternal survival probability because of increased competition. Of particular interest was the breeding population as mothers are responsible for acquiring enough resources to sustain themselves as well as their offspring. Thus, the ***proportion of breeding females*** within the year for the maternal locality was also assessed (Wells & Van Vuren, 2017) with the logic that a higher density of breeding females will negatively affect maternal survival due to increased competition. Kin presence of other ***related females*** was calculated because, although interactions between related individuals tend to be less agonistic (Ferron, 1985), females will incur more competition for resources when they overlap home ranges with related kin (Aliperti et al., 2020). Such overlap leads to more intense intraspecific competition which will likely translate to lower maternal survival probability, especially as the density of related females increases. Increasing the density of ***unrelated females*** should also be related to lower survival because interactions against non-kin are more aggressive (Ferron, 1985) which negatively affects survival (Cubaynes et al., 2014). Overall, higher ***local population density*** will likely result in lower survival probability because of increased intraspecific competition for resources and aggression.

Environmental context. Weather variables pertaining to snow data were obtained courtesy of Billy Barr (2020) and variables for rainfall and temperature were derived from the National Weather Service Forecast Office for Crested Butte, Colorado (NOAA, 2020). The ***first day of bare ground*** marked the onset of vegetative growth; early snowmelt is associated with drought and reduced primary productivity (Sloat et al., 2015). To determine the end point of the active season, the ***day of first snow cover*** served as the initial day of snow after which the ground stayed continuously covered; this day was also standardized by the number of days past April 30. An intermediate day of first snow cover will have the greatest positive effect on squirrel survival as early snow cover might not give squirrels time to acquire enough resources to survive over-winter, but late snow cover may reduce thermal cover, leaving burrows less insulated. The duration of days between the day of bare ground and the day of snow cover for each year represented the ***length of the vegetative growing season*** (Schwartz & Armitage, 2005). An intermediate length of the growing season should optimize survival as individuals need enough time to acquire the resources needed for over-wintering but not so long that drought conditions prevail and reduce vegetative quality.

Total winter snowfall was recorded in centimeters from September to July beginning in the winter prior to the year assessed (i.e., total snowfall associated with the 1995 active season was calculated based on the snowfall from September 1994 through July 1995). Too little snowfall reduces snowpack and increases the risk of freezing to death (Cordes et al., 2020), but too much snowfall can lengthen the duration of hibernation which could be fatal if squirrels do not acquire enough body fat to endure an extended hibernation (Armitage, 2013); thus, an intermediate amount of snowfall is expected to optimize survival.

During the months of June and July of each year, the ***total amount of summer rainfall*** was recorded in centimeters (Kneip et al., 2011). Rainfall is necessary for vegetative growth, but it reduces the amount of time squirrels spend above-ground acquiring resources and can cause burrow flooding. Conversely, low rainfall totals can lead to drought-induced food limitations which, in turn, reduce the chances of over-winter survival for hibernating squirrels (Farand et al., 2002). To balance these aspects, survival is expected to be highest when there is an intermediate amount of rainfall. Summer temperature can also affect the length of time squirrels spend actively acquiring resources. The ***average summer temperature*** during June and July was calculated in degrees Celsius. Lower summer temperatures should improve survival as higher temperatures reduce foraging time and increase energy expenditure as squirrels must avoid overheating (Cordes et al., 2020; Fletcher et al., 2012). Alternatively, higher summer temperature can cause vegetative quality to decline by causing drought (Armitage, 2013). Further, the ***number of summer days with temperatures above 25 degrees Celsius*** were counted during June and July (if data were missing, then it was assumed the temperature was less than 25^{o}C) as temperatures higher than that reduce above-ground activity in at least one other species of ground dwelling squirrels (i.e., alpine marmots, *Marmota marmota*) (Turk & Arnold, 1984), and may do so for GMGS, too. Both of these activities reduce the time available for acquiring resources which we expect will have a negative effect on survival.

Data Analysis

Because all females in the study site are detected and observed every year, from first occurrence in the study site until death, the assumption of perfect detection is met for the life of each tracked individual; thus, one can use known-fate survival models for estimating annual survival rates (e.g., Wintrebert et al. 2005). We used known-fate Cox proportional hazard models (CPH; Cox, 1972), an extension of the non-parametric Kaplan-Meier model (1958), to estimate annual survival as a function of the covariates of interest discussed above (e.g., Aubry et al., 2011). Cox proportional hazard models are semi-parametric and allow the hazard to fluctuate with time while measuring the effects of covariates on either age- or time- specific survival estimates (Klein, 1992). Cox proportional hazard models make no assumption about the shape of the underlying mortality hazard (a.k.a., the 'force' of mortality) over time. Each covariate within the model is assumed to act multiplicatively (i.e., proportionally) on the baseline mortality hazard at each time step (e.g., Bradburn et al., 2013), such that:

$$h_0(t) \times h(t, X_i) = \exp(\sum_{i=1}^{p} \beta_i X_i)$$

where h_0 refers to the baseline hazard (i.e., the hazard's value when all covariate values are null), p denotes the number of parameters in the model, the βs denotes a set of estimated parameters, and the Xs represents the data, or series of covariate values for each individual i such as $X = (X_1, X_2, \ldots X_i)$, and t denotes time (in this case, time elapsed since first reproduction rather than actual age). X_i can either consist of one unique value per individual (e.g., the age at first reproduction) or can be a vector of values (i.e., one value per year lived for each individual as, for example, with time-specific reproductive investment).

All analyses were performed in RStudio (R Core Team, 2019), using the 'survival' package (Therneau & Grambsch, 2000) as well as the 'survminer' (Kassambara et al., 2020) and

‘AICcmodavg’ (Mazerolle, 2020) libraries. The function ‘cox.zph’ was used to assess the proportional hazard assumptions of the model to ensure covariates weighed evenly on the mortality hazard (e.g., Aubry et al., 2011).

RESULTS

Maternal Characteristics. We tested for an association of different parameterizations of age (i.e., continuous; 1, 2, 3+; 1,2, 3, 4+), experience (i.e., continuous; 0, 1+), resident status (i.e., resident, immigrant), age at first reproduction (i.e., continuous; 1, 2+), litter size (i.e., continuous; 0, 1+; 0, 1-3, 4-5, 6+), litter mass (i.e., continuous; lite, moderate, heavy), and day of litter emergence (i.e., continuous; early, moderate, late) on maternal survival. Only one of the best performing models evaluated for maternal characteristics (Table 1.1), litter size, was associated with a positive relationship with annual survival probability (Table 1.2). Litter size (0, 1+) outperformed both a categorical (0, 1-3, 4-5, 6+) as well as a continuous parameterization in explaining variability in maternal survival (Table 1.4 (i)). Litter size (0, 1+) indicated that producing a litter with any number of offspring, as compared to the reference group of producing zero offspring, reduced the mortality hazard by 52% (exp (β) Hazard Ratio (HR) = 0.48; 95% CI = 0.33, 0.68; p-value <0.0001; Table 1.4; Figure 1.1).

Social context. Using different parametrizations of sex ratio (i.e., more, equal, fewer daughters), breeding female density (i.e., continuous; 0-1, 2+; 0, 1-2, 3+), unrelated, related, and total breeding female density (i.e., continuous; 0-1, 2+; 0, 1-2, 3+) we tested for an association with maternal survival. Among the top models (Table 1.3), we found that increasing related breeding female density and total female density had a significant negative effect on maternal survival (Table 1.4). Looking at the density of related breeding females, kin presence treated as a continuous variable ranked better than other structures of kin presence ((0-1, 2+) or (0, 1-2, 3+)) for describing differences in maternal survival (Table 1.4). Kin (continuous) included a range of 0-5 individuals, and results indicated that living near breeding kin was positively associated with mortality, as every additional related female led to a 13% increase in the mortality hazard (HR = 1.13; 95% CI = 0.99, 1.23; p-value = 0.08; Table 1.4). We found similar results for total local female density in that the population size (continuous) model outcompeted the discrete population size models ((0-1, 2+) and (0, 1-2, 3+)) for representing variation in maternal survival (Table 1.3). Population density ranged from 0-11 individuals, and we found that increasing the local female density by one unit was associated with an increased mortality hazard by 7% (HR = 1.07; 95% CI = 1.00, 1.14; p-value = 0.07; Table 1.4 (viii)) regardless of reproductive status or relatedness to other individuals in the population.

Environmental context. We evaluated continuous versus categorical parameterization (i.e., early, moderate, late; short, medium, long) of first day of bare ground, first day of permanent snow cover, length of the vegetative growing season, amount of winter snowfall, amount of summer rainfall during June and July, average summer temperature during June and July, and number of days above 25ºC for an association with maternal survival. The best performing models (Table 1.5) that had a significant association with annual survival probability included the date of snow cover and the length of the growing season (Table 1.6). Snow cover treated as a continuous variable ranked better than the categorical parameterization (i.e., early, moderate, late) model in accounting for variation in maternal survival (Table 1.5). The first date of permanent snow cover ranged from October 17 to November 21 in a given year, yet GMGS enter hibernation as early as late August, especially at higher elevations (Bartels & Thompson, 1993). Model results indicated that delays in the timing of permanent snow cover was associated with a 2% decline in the mortality hazard (HR = 0.98; 95% CI = 0.96, 1.00; p-value = 0.02; Table 1.6) for each additional day that the study site remained snow-free. Likewise, length of the growing season (i.e., continuous) outperformed the categorical parameterization (i.e., short, medium, long) in explaining variability in maternal survival (Table 1.5). Season length varied from 135-204 days, and results showed that lengthening the growing season by one day was associated with a 1% decline in the mortality hazard (HR = 0.99; 95% CI = 0.98, 1.00; p-value = 0.02; Table 1.6 (iii)).

DISCUSSION

Maternal Characteristics. It was surprising that among the maternal characteristics that we tested, only litter size had an effect on annual survival and was linked to a decline in mortality hazard when a mother successfully produced a litter. As a short-lived species, few GMGS individuals may reach the point at which senescence begins to affect survival which could explain the lack of effect between age and annual survival (Turbill & Ruf, 2010). Likewise, breeding experience may not have affected GMGS annual survival because, although they are iteroparous, their short lifespan means that all mothers tend to attempt breeding each subsequent year after their first reproductive attempt. This differs from the reproductive strategies of long-lived species who may not breed every year yet experience higher survival with increasing experience (Aubry et al., 2011). We only included individuals once they made their first attempt at breeding, thus any individuals that died before they had any reproductive experience were not included in our analysis, and their effect on annual survival was not considered. However, we did find that, amongst females that had recruited and were included in our analysis, those that skipped breeding (n = 6) or failed to wean a litter (n = 66) had a much higher chance of mortality than females that bred and successfully weaned a litter (n = 167). Females that skipped breeding or were unsuccessful in weaning a litter were most likely in poor condition and unable to sustain the energetic investment required for reproduction which resulted in a cost to survival for both mothers and offspring (Festa-Bianchet & King, 1991; Murie & Dobson, 1987). Our results support the growing body of work that maternal survival of ground squirrels may be more affected by body condition and environmental conditions than the cost of producing a litter (Festa-Bianchet & King, 1991; Neuhaus, 2000; Risch et al., 2007).

When studying the effect of resident status on annual survival, we expected that site familiarity would give residents a substantial advantage in terms of survival (i.e., more efficient resource acquisition, better predator avoidance) over immigrants (Clutton-Brock & Lukas, 2012). A limitation in our study was that we were only able to compare residents and immigrants within the study site, but we were unable to track dispersers that left the study and may have had a different mortality hazard during the dispersal period. It is expected that dispersal is a risky behavior with high mortality during the act of dispersal (Byrom & Krebs, 1999; Garrett et al., 1988). As it stands in our study, the lack of difference in survival between residents and immigrants indicates that, once a mother is able to establish a territory within a population and have her first reproductive event, her survival probability from that point onward is the same regardless of her prior residency status. However, if mortality is high during the dispersal period, then that could represent a cost to dispersal, suggesting that there is, in fact, a benefit to survival in remaining philopatric, but this was not feasible to fully address in our study. As for the age of first reproduction, we expected that survival would be higher when individuals delayed reaching reproductive maturity (Descamps et al., 2006), but we found no difference in annual survival. Our finding differs slightly from Moore et al. (2016) who found that delaying the age of first reproduction causes a significant decline in fitness, possibly through a decline in survival, for species with faster-paced life histories, but claimed that such an effect may be counteracted by the benefits of early maturity. My results showed no effect on survival because of delayed reproduction. Again, this could be in part due to the design of our study in which we only included females once they became reproductively active; individuals in poor body condition who delayed reproduction, but did not survive to ever breed, were not included in our analysis.

Litter mass represents the amount of resource investment that mothers put into their litters. Heavier litters indicate greater investment which we thought would weaken maternal condition and therefore reduce maternal survival. However, we found no effect of litter mass on annual survival which suggests that mothers invest a practical "amount" per litter given their individual body condition (i.e., a mother in poorer condition will invest less than a mother in better condition who can invest more) as evidenced by the positive correlation between increasing maternal body fat (i.e., condition) and increasing litter mass (Robbins et al., 2012). In other words, GMGS mothers may invest as much as they can into their litters without detracting from their own body condition, nor risking a decline in their own survival. Because body condition was not evaluated as part of this study, our results highlight the importance of further exploration involving body condition.

We expected that mothers would have higher survival when they had more time after the reproductive period to prepare for hibernation to improve their body condition by gaining fat deposits necessary for over-winter survival (Dark, 2005). Thus, earlier timing of litter emergence was anticipated to improve maternal survival. Instead, we found no significant effect on annual maternal survival regardless of when litters emerged. However, timing of emergence may have a more influential effect on offspring survival (Armitage et al., 1976; Rieger, 1996). Later emerging Uinta ground squirrel (*Spermophilus armatus*) offspring tend to emerge at a heavier weight (Rieger, 1996), but GMGS rate of mass gain is significantly reduced for juveniles that emerge later in the summer (Wells & Van Vuren, 2018).

Social context. Sex ratio did not affect maternal survival despite the hypothesis that the production of more daughters would generate increased competition for resources. While raising more of the philopatric sex can indeed lead to more intense competition (Clark, 1978), GMGS mothers have male-biased litters when female kin density is high (Wells & Van Vuren, 2017), which may help compensate for the anticipated effect of higher female density. By adjusting the sex ratio of their litters, mothers may preemptively avoid the opportunity that daughters will generate more competition, thereby explaining the lack of effect we found in our survival model.

When investigating the effects of local density on maternal survival, it was not surprising that breeding kin density and total density negatively affected maternal survival. As an asocial species, we expected that one of the costs GMGS would incur by interacting with conspecifics would be a reduction in survival, likely due to increased resource competition (Stockley & Bro-Jørgensen, 2011). Breeding females are likely more sensitive to more intense competition for resources as they are primarily considered income breeders, relying on daily energy intake (Stearns, 1992) after parturition (Broussard et al., 2005; Wells & Van Vuren, 2017). The local density of related females also played a critical role in shaping maternal survival. Golden-mantled ground squirrel females are known to establish and defend territories (Armitage, 1981; Michener, 1983), but they do exhibit some degree of home range overlap with other females (Jesmer et al., 2011). Further work investigating the relatedness of females that overlap home ranges has demonstrated that related females, specifically mother-daughters but not sisters, will overlap home ranges more than unrelated females (Aliperti et al., 2020). Related females must experience some fitness benefit by overlapping home ranges (i.e., inclusive fitness (Armitage, 1987)), but our results suggest that they experience a cost to their survival as kin density increases, which may be partially explained by increased competition for resources within a shared home range. When we consider the total population density, the cost to survival manifests again, likely because of the intensity of the competition associated with relatedness and reproductive status. Previously, we delineated that kin presence had a more negative effect on maternal survival than non-kin presence, but we now see that non-reproductive females, specifically pre-breeding yearlings who might be trying to establish a territory, could present a challenge to reproductive females who have to defend their established territories against invading pre-breeders vying for resources. The extra energy required for territory defense may leave reproductive females, who are already investing heavily in reproduction, more susceptible to a greater risk of mortality. These results emphasize the asocial aspect of the GMGS life history as we see that, although related females may tolerate each other within their home ranges, they do so at a cost to their own survival.

Environmental context. The first day of bare ground in the spring and the last snow-free day before permanent cover in the winter were used to determine the length of the growing season, so it is interesting that only delaying the day of snow cover positively affected survival, but an overall lengthening of the growing season also positively influenced survival. The day of bare ground fluctuated within a 58-day range while the day of permanent snow cover fluctuated within a 35-day range, yet variability within that narrower window exhibited a stronger effect on maternal survival. It is likely that the day of bare ground was less influential on survival because hibernating ground squirrels flexibly respond to environmental variation and may match their phenology to changing environmental conditions (Sheriff et al., 2011; Williams et al., 2014).

This suggests that, although timing of bare ground may vary each year, squirrels likely vary the timing of their emergence to match conditions, thereby enabling them to time resource acquisition with vegetative growth and minimizing the effect of spring emergence on survival.

Our results support Kneip et al.'s (2011) finding that the date of bare ground did not affect GMGS survival. However, our results slightly contradict other work that maintains that a shorter active season is better for survival as mortality risk is lower during the hibernation period (because of e.g., reduced predation, reduced cellular-aging) (Bieber et al., 2014; Kirby et al., 2019; Turbill et al., 2011). While a shorter active season may benefit males, juveniles, and non-reproductive females, we specifically studied reproductive females who must meet the energetic demands of reproduction by allocating more time to foraging (Macwhirter, 1991). Thus, reproductive females may incur a survival benefit from having a longer active season during which they can acquire more resources, compensate for reproductive output, and better prepare for hibernation.

None of the explicit weather variables that we evaluated had an effect on annual survival. For our study, we looked at winter snowfall as a representation of snow cover (Armitage, 2013), but did not consider snowpack (Ikeda et al., 2021). We know that snow conditions can affect fitness in other ways: snowpack is an important condition necessary for maintaining appropriate hibernacula temperature for over-winter survival (Tafani et al., 2013) and is known to affect reproductive output (e.g., litter size (Tafani et al., 2013)), but we did not detect a relationship between snowfall and annual survival. In the future, it may be beneficial to study the effect of snowpack rather than simply snowfall.

During the active season, moderate weather conditions for rainfall and temperature, particularly with fewer days above 25°C (Turk & Arnold, 1984), should produce favorable conditions that optimize survival (Schwartz & Armitage, 2005). Our analysis did not detect an effect on survival for either rainfall or temperature within each year. This differed slightly from the results of Kneip et al. (2011) who found a significant negative effect of rainfall on juvenile survival and suggested a potential positive effect on adult survival. Given the results of both studies, it appears important to consider age and potentially sex in relation to rainfall and survival. Because our study only included reproductive females who have established territories, adults may be more efficient at acquiring resources throughout the active season as opposed to juveniles who have fewer days to forage and gain mass necessary for over-winter survival.

Within this population of GMGS, variability in maternal survival is affected by maternal characteristics, specifically reproductive investment, as well as by particular social dynamics and environmental contexts experienced by mothers. In sum, the mortality hazard was reduced when mothers successfully produced a litter, but the mortality hazard increased as the density of related breeding females as well as total female density increased. Mothers also experienced a reduced mortality hazard when the day of permanent snow cover was delayed and when the length of the vegetative growing season was longer.

Conclusions. Collectively, our results provide further insight into the impacts of various maternal, social, and environmental components on maternal survival. Our results may enable us to predict future population-level effects of changing conditions as we anticipate that continuing climate-mediated environmental variability will affect maternal survival. It also begs the question of how these results might change depending on the age, sex, and/or status of other members within the population beyond adult, reproductive females. Our study enhances understanding of the spectrum of sociality by shedding light on how relatedness and associated interactions may affect maternal survival, a key component of local population growth and dynamics in short-lived territorial species, where females tend to remain philopatric.

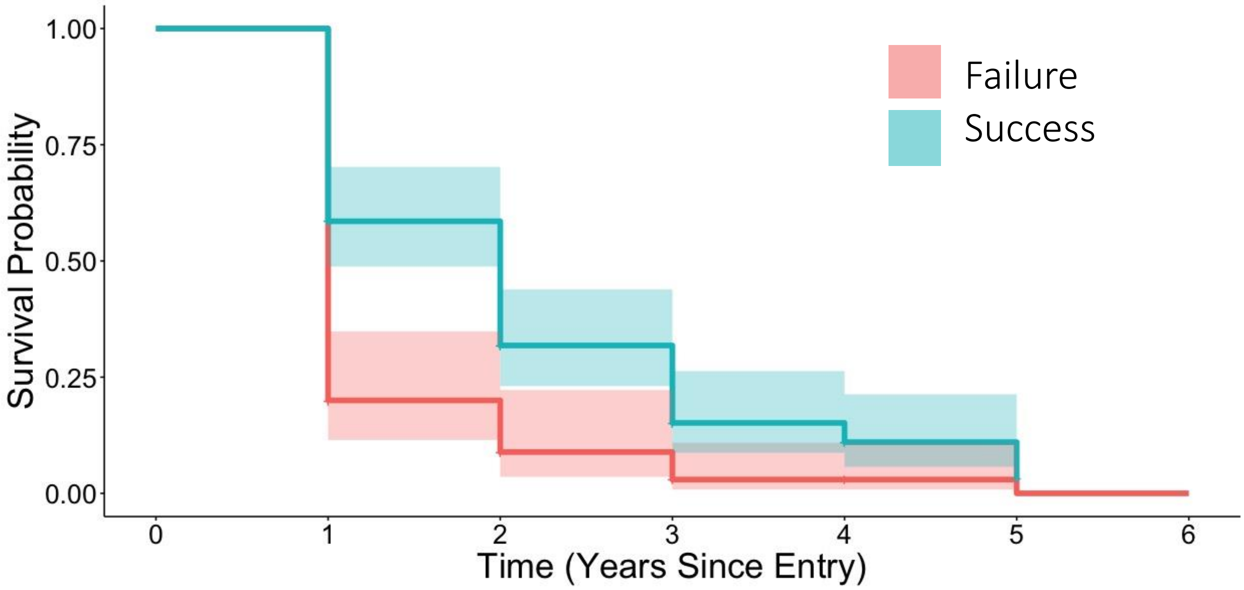

Figure 1.1. Golden-mantled ground squirrel mothers who successfully produced a litter (blue) have higher survival probability over time than mothers who failed to produce a litter (pink).

Table 1.1 Model selection results for Cox proportional hazard models evaluating the effect of maternal characteristics on annual survival. We considered different parameterizations of age (i), experience (defined as the number of years since an individual's first reproductive event) (ii), residency (classified as resident if born in the study site or immigrant if immigrated in from another population) (iii), age at first reproduction (iv), litter size (v), collective litter mass (vi), and day of litter emergence (vii).

	Model Set	K[1]	Log Likelihood[2]	AICc[3]	Delta AICc[4]	AICc Weight[5]	Cumulative Weight[6]
(i)	Age (1, 2, 3, 4+)	3	-363.23	730.58	0.00	0.48	0.48
	Age (Continuous)	1	-363.30	730.62	0.04	0.47	0.95
	Age (1, 2, 3+)	2	-365.43	734.92	4.34	0.05	1.00
(ii)	Experience (0, 1+)	1	-272.86	547.74	0.00	0.51	0.51
	Experience (Continuous)	1	-272.90	547.83	0.09	0.49	1.00
(iii)	Residency	1	-	-	0.00	1.00	1.00
(iv)	Age (1, 2+)	1	-274.71	551.45	0.00	0.55	0.55
	Age (Continuous)	1	-274.92	551.86	0.41	0.45	1.00
(v)	Size (0, 1+)	1	-494.41	990.83	0.00	0.77	0.77
	Size (Continuous)	1	-496.19	994.39	3.56	0.13	0.90
	Size (0, 1-3, 4-5, 6+)	3	-494.37	994.84	4.01	0.10	1.00
(vi)	Mass (Continuous)	1	-80.79	163.63	0.00	0.70	0.70
	Mass (Lite, Moderate, Heavy)	2	-80.59	165.35	1.72	0.30	1.00
(vii)	Emergence (Early, Moderate, Late)	2	-273.27	550.61	0.00	0.75	0.75

Emergence (Continuous)	1	-275.42	552.86	2.25	0.25	1.00

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[1] K represents the number of parameters in the model.

[2] Log Likelihood is used to predict how likely a given model is based on the available data.

[3] AICc is a score of the information presented by the model and adjusted for small sample size.

[4] Delta AICc calculates the difference in AICc score between the top model and the model being assessed.

[5] AICc Weight demonstrates the predicative power of a given model in relation to the full set of models under consideration.

[6] Cumulative Weight shows the additive effect of the AICc weights as more model AICc values are considered.

Table 1.2 Hazard ratios and 95% confidence intervals obtained from the best performing Cox proportional hazard models examining the effects of maternal characteristics, specifically age (i), experience (ii), resident status (iii), age at first reproduction (iv), litter size (v), collective litter mass (vi), and day of litter emergence (vii) on maternal annual survival.

	Selected Models (See Table 1a.)	Hazard Ratio	95% Confidence Interval	p-value[1]
(i)	Age (=1, Reference)	1.00	-	-
	Age (=2)	1.12	0.65, 1.93	0.69
	Age (=3)	1.60	0.62, 4.15	0.33
	Age (=4+)	0.37	0.09, 1.43	0.15
(ii)	Experience (=0, Reference)	1.00	-	-
	Experience (1+)	0.66	0.20, 2.17	0.49
(iii)	Resident	1.00	-	-
	Immigrant	1.10	0.75, 1.60	0.63
(iv)	Age (=1, Reference)	1.00	-	-
	Age (=2+)	1.18	0.73, 1.89	0.50
(v)	Size (=0, Reference)	1.00	-	-
	Size (=1+)	0.48	0.33, 0.68	<0.0001
(vi)	Mass (=33†, Reference)	1.00	-	-
	Mass (>33, Continuous)	1.00	1.00, 1.00	0.94
(vii)	Emergence (=Moderate, Reference)	1.00	-	-
	Emergence (=Early)	0.81	0.39, 1.70	0.58
	Emergence (=Late)	1.43	0.78, 2.60	0.25

[1] The p-value is from a test on the null hypothesis assuming that there is no difference in the risk of mortality between the reference variable and a given variable; if p-value < 0.05, the null hypothesis is rejected and a difference between variables is deemed significant.

[†] The reference value of 33 represents the smallest litter mass (grams) recorded in the long-term dataset. Results were evaluated on a continuous scale ranging from 33g to 615g.

Table 1.3 Model selection results for Cox proportional hazard models testing for the effect of social context factors on annual survival. We considered different parameterizations of litter sex ratio (i), local density of breeding females (ii), local density of related breeding female (iii), local density of unrelated breeding females (iv), and local population density of total breeding and non-breeding females (v).

	Model Set	K[1]	Log Likelihood[2]	AICc[3]	Delta AICc[4]	AICc Weight[5]	Cumulative Weight[6]
(i)	Ratio	2	-	-	0.00	1.00	1.00
(ii)	Breeders (0-1, 2+)	1	-483.02	968.06	0.00	0.51	0.51
	Breeders (Continuous)	1	-483.55	969.13	1.06	0.30	0.82
	Breeders (0, 1-2, 3+)	2	-483.03	970.11	2.05	0.18	1.00
(iii)	Kin (Continuous)	1	-477.65	957.31	0.00	0.48	0.48
	Kin (0-1, 2+)	1	-478.01	958.04	0.73	0.33	0.82
	Kin (0, 1-2, 3+)	2	-477.59	959.23	1.92	0.18	1.00
(iv)	Non-Kin (Continuous)	1	-479.01	960.04	0.00	0.43	0.43
	Non-Kin (0-1, 2+)	1	-479.02	960.07	0.02	0.42	0.85
	Non-Kin (0, 1-2, 3+)	2	-479.04	962.12	2.08	0.15	1.00
(v)	Population (Continuous)	1	-482.30	966.62	0.00	0.63	0.63
	Population (0-1, 2+)	1	-483.44	968.89	2.28	0.20	0.83

Population (0, 1-2, 3+)	2	-482.61	969.27	2.65	0.17	1.00

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[1] K represents the number of parameters in the model.

[2] Log Likelihood is used to predict how likely a given model is based on the available data.

[3] AICc is a score of the information presented by the model and adjusted for small sample size.

[4] Delta AICc calculates the difference in AIC score between the top model and the model being assessed.

[5] AICc Weight demonstrates the predicative power of a given model in relation to the full set of models under consideration.

[6] Cumulative Weight shows the additive effect of the AICc weights as more model AICc values are considered.

Table 1.4 Hazard ratios and 95% confidence intervals obtained from the best performing Cox proportional hazard models testing for the effect of social context, specifically litter sex ratio (i), local density of breeding females (ii), local density of related breeding female (iii), local density of unrelated breeding females (iv), and local population density of total breeding and non-breeding females (v).

	Selected Models (See Table 2a.)	Hazard Ratio	95% Confidence Interval	p-value[1]
(i)	Ratio (=Equal, Reference)	1.00	-	-
	Ratio (=Fewer Daughters)	0.95	0.49, 1.86	0.89
	Ratio (More Daughters)	1.31	0.71, 2.41	0.39
(ii)	Breeders (=0-1, Reference)	1.00	-	-
	Breeders (=2+)	1.28	0.89, 1.85	0.19
(iii)	Kin (=0, Reference)	1	-	-
	Kin (>0, Continuous)	1.13	0.99, 1.23	0.08
(iv)	Non-Kin (=0, Reference)	1.00	-	-
	Non-Kin (>0, Continuous)	0.98	0.87, 1.11	0.78
(v)	Population (=0, Reference)	1.00	-	-
	Population (>0, Continuous)	1.07	1.00, 1.14	0.07

[1] The p-value is from a test on the null hypothesis assuming that there is no difference in the risk of mortality between the reference variable and a given variable; if $p\text{-value} < 0.05$, the null hypothesis is rejected and a difference between variables is deemed significant.

Table 1.5 Model selection results for Cox proportional hazard models testing for the effect of environmental factors on annual survival. We considered different parameterizations of first day of bare ground (i), first day of permanent snow cover (ii), length of the growing season (iii), amount of winter snowfall (iv), amount of summer rainfall during June and July (v), average summer temperature during June and July (vi), and number of days above 25°C during June and July (vii).

	Model Set	K[1]	Log Likelihood[2]	AICc[3]	Delta AICc[4]	AICc Weight[5]	Cumulative Weight[6]
(i)	Bare (Early, Moderate, Late)	2	-522.60	1049.26	0.00	0.79	0.79
	Bare (Continuous)	1	-524.94	1051.89	2.64	0.21	1.00
(ii)	Covered (Continuous)	1	-522.38	1046.77	0.00	0.53	0.53
	Covered (Early, Moderate, Late)	2	-521.50	1047.05	0.28	0.47	1.00
(iii)	Length (Continuous)	1	-522.84	1047.70	0.00	0.58	0.58
	Length (Short, Medium, Long)	2	-522.16	1048.36	0.66	0.42	1.00
(iv)	Snow (Low, Moderate, High)	2	-501.83	1007.71	0.00	0.51	0.51
	Snow (Continuous)	1	-502.87	1007.75	0.05	0.49	1.00
(v)	Rain (Continuous)	1	-476.01	954.04	0.00	0.61	0.61
	Rain (Low, Moderate, High)	2	-475.44	954.94	0.90	0.39	1.00

(vi)	Temperature (Continuous)	1	-524.80	1051.62	0.00	0.59	0.59
	Temperature (Low, Moderate, High)	2	-524.13	1052.31	0.69	0.41	1.00
(vii)	25°C Days (Continuous)	1	-524.90	1051.82	0.00	0.79	0.79
	25°C Days (Low, Moderate, High)	2	-525.24	1054.52	2.70	0.21	1.00

[1] K represents the number of parameters in the model.

[2] Log Likelihood is used to predict how likely a given model is based on the available data.

[3] AICc is a score of the information presented by the model and adjusted for small sample size.

[4] Delta AICc calculates the difference in AIC score between the top model and the model being assessed.

[5] AICc Weight demonstrates the predicative power of a given model in relation to the full set of models under consideration.

[6] Cumulative Weight shows the additive effect of the AICc weights as more model AICc values are considered.

Table 1.6 Hazard ratios and 95% confidence intervals obtained from the best performing Cox proportional hazard models testing for the effect of environmental factors, specifically first day of bare ground (i), first day of permanent snow cover (ii), length of the growing season (iii), amount of winter snowfall (iv), amount of summer rainfall during June and July (v), average summer temperature during June and July (vi), and number of days above 25°C during June and July (vii).

	Selected Models (See Table 3a.)	Hazard Ratio	95% Confidence Interval	p-value[1]
(i)	Bare (=Moderate, Reference)	1.00	-	-
	Bare (=Early)	0.78	0.49, 1.24	0.29
	Bare (=Late)	1.33	0.90, 1.97	0.15
(ii)	Covered (=170, Reference)	1.00	-	-
	Covered (>170, Continuous)	0.98	0.96, 1.00	0.02
(iii)	Length (=135, Reference)	1.00	-	-
	Length (>135, Continuous)	0.99	0.98, 1.00	0.02
(iv)	Snow (=Moderate, Reference)	1.00	-	-
	Snow (=Low)	1.13	0.73, 1.75	0.60
	Snow (=High)	1.39	0.92, 2.09	0.12
(v)	Rain (=3.12, Reference)	1.00	-	-
	Rain	0.96	0.90, 1.03	0.29

	(>3.12, Continuous)			
(vi)	Temperature (=10.69, Reference)	1.00	-	-
	Temperature (>10.69, Continuous)	1.11	0.92, 1.35	0.27
(vii)	25°C Days (=6, Reference)	1.00	-	-
	25°C Days (>6, Continuous)	1.01	0.99, 1.03	0.31

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[1] The p-value is from a test on the null hypothesis assuming that there is no difference in the risk of mortality between the reference variable and a given variable; if p-value < 0.05, the null hypothesis is rejected and a difference between variables is deemed significant.

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CHAPTER 2: NATAL DISPERSAL IS ASSOCIATED WITH HIGHER PRE-WEANING CORTISOL LEVELS IN THE GOLDEN-MANTLED GROUND SQUIRREL

SYNOPSIS

Stress is an acute or chronic physiological state with markers that may be passed on from mother to offspring and have lasting impacts on future generations. Our study sheds light on the mechanisms by which maternal stress may shape daughter dispersal in a population of golden-mantled ground squirrels, *Callospermophilus lateralis,* at Rocky Mountain Biological Research Laboratory, Colorado. Golden-mantled ground squirrels are asocial, hibernating mammals that give birth in burrows where the mothers suckle offspring until weaning occurs at emergence from the natal burrow. Weeks later, offspring either disperse from their natal home range or stay and establish territories (i.e., remain philopatric). While sons tend to disperse, the behavior of daughters varies. Cortisol can be transmitted during gestation and lactation from mother to offspring and deposited in developing hair follicles and feces of the young. We collected hair from offspring and fecal samples from mothers and offspring upon litter emergence and quantified cortisol concentrations as an index of stress. Sons and daughters had similar cortisol concentrations in both hair and fecal samples. There was no relationship between maternal cortisol concentration and average daughter concentration within a litter for either sample type. However, dispersing daughters tended to have higher cortisol concentrations in hair than their philopatric counterparts. Collectively, these results demonstrate that high cortisol concentrations in offspring can help predict dispersal trends, but maternal stress at offspring emergence was not directly correlated to offspring cortisol concentrations. Daughter stress, as indexed by hair

cortisol concentration during early development, may be a cue for facultative natal dispersal in this species.

INTRODUCTION

Natal dispersal or philopatry, a key behavior that has long-term effects on an individual's life history, is shaped by a suite of ecological (Solomon, 2003; Waser & Jones, 1983), social (Clutton-Brock & Lukas, 2012; Le Galliard et al., 2006), and physiological factors (Aguilera-Miller & Álvarez-Castañeda, 2019; Goossens et al., 2020; Greenwood, 1980). Competition for ecological resources and agonistic social interactions both negatively affect the profitability of remaining in an area, prompting some individuals to disperse. When natal philopatry occurs in female mammals, mothers, daughters, and non-kin females all compete for resources, especially food (Clark, 1978; Li & Kokko, 2019). Thus, escaping kin competition is a primary driver prompting dispersal (Cote & Clobert, 2010; Waser et al., 2013). In addition, agonistic behavior among individuals can negatively affect resource acquisition, reproduction, and offspring care, making it advantageous for females of lower social rank to leave voluntarily rather than wait until eviction by dominant females (Clutton-Brock & Lukas, 2012; Ferron, 1985; Stockley & Bro-Jørgensen, 2011). Moving to an unfamiliar site makes resource acquisition less efficient whereas site familiarity increases efficiency and may reduce competition with other philopatric individuals (Clutton-Brock & Lukas, 2012; Solomon, 2003).

While juvenile mammals use resource competition and social interactions as cues for dispersal, they also receive physiological cues from their mother during the early stages of development that play a critical role in encouraging philopatry or dispersal (Dufty et al., 2002; Goossens et al., 2020). In Belding's ground squirrels (*Spermophilus beldingi*), early exposure to gonadal steroids prompted natal dispersal by stimulating exploratory and social behaviors (Holekamp et al., 1984). Social behavior is also affected by hypothalamic-pituitary-adrenal (HPA) axis function which is associated with the production of glucocorticoids (Creel et al., 2013), another type of steroid hormone that has been linked to altered brain activity due to prenatal exposure to stressors (McGowan & Matthews, 2018; Pascual et al., 2015; Welberg & Seckl, 2009). Other work in Belding's ground squirrels (*Spermophilus beldingi*) related to maternal effects has linked glucocorticoids to changes in offspring resource acquisition and anti-predator behavior (Mateo, 2014), but little research has specifically examined the effects of maternal stress (via glucocorticoids) on influencing philopatry or dispersal in small mammals. Glucocorticoids have pleiotropic effects and represent a small portion of the numerous physiological responses to a stressor (MacDougall-Shackleton et al., 2019). Nevertheless, glucocorticoids are useful as a proxy of HPA activity that is often associated with the stress response (Reeder & Kramer, 2005; Welberg & Seckl, 2009). Here, we use glucocorticoid concentrations, specifically cortisol, as an index of maternal effects (non-genetic, phenotypic responses in offspring induced by maternal phenotypes (Maestripieri & Mateo, 2009)) and use 'stress' to broadly reference a number of stressors perceived by an individual (e.g., predation, social dynamics, nutrition, etc.) in our work on the golden-mantled ground squirrel (GMGS), *Callospermophilus lateralis*, a hibernating income-breeder with sex-biased, juvenile dispersal.

Maternal 'stress' can be triggered by predation (Hik et al., 2001), density (Dantzer et al., 2013), kin interactions (Sosa et al., 2020), social rank (Goymann & Wingfield, 2004), and nutritional availability (Love et al., 2013). Mothers pass their environmental experiences to their offspring via 'maternal effects' during gestation and lactation (Maestripieri & Mateo, 2009; Mateo 2014; Meise et al., 2016). Early influences of maternal effects on development can shape offspring fitness and serve as an adaptive strategy to prepare offspring for the environment they will experience upon emergence from the natal burrow (Dantzer et al., 2013). Examples of maternal effects induced by stress include faster offspring growth, higher metabolic rates, and reduced reproductive output (Love et al., 2013). For income breeders, the majority of maternal reproductive energy is acquired on a daily basis rather than coming from stored energy reserves (Kenagy, et al., 1989). Thus, lower quality diet and limited resource availability during gestation have been linked to relatively higher maternal glucocorticoid levels (Love et al., 2013; also see Appendix 1). Mothers pass accumulated cortisol, the primary glucocorticoid circulating in rodents (Mateo & Cavigelli, 2005; Boonstra et al., 2001), through the placenta during pregnancy (Takahashi et al., 1998; Weinstock, 2008; Welberg et al., 2000; Williams et al., 1999) and via maternal milk during lactation (Dettmer et al., 2014; Hollanders et al., 2017). Corticosteroids provide hormonal cues that serve as a direct reflection of the current environment experienced by the mother, and provide an indicator to offspring of the environmental conditions they will enter upon emergence (Love et al., 2013; Welberg & Seckl, 2009).

During both the prenatal and postnatal phases of development, glucocorticoids are integrated into the growth of hair follicles (Koren et al., 2019). Golden-mantled ground squirrel offspring are born in burrows with short vibrissae (whiskers) and a few hairs on the top of their heads. They develop a full pelage after approximately 40 days (McKeever, 1964) which generally precedes their initial emergence from the natal burrow but occurs prior to the natal dispersal period. Sampling offspring hair takes advantage of the way cortisol passes through the placenta during gestation and milk during lactation and is taken up into juvenile hair follicles (Koren et al., 2019; Meise et al., 2016) as all nutrients necessary for pelage growth are provided by mothers to her young before emergence (Crill et al., 2019; Heimbürge et al., 2019). Glucocorticoids can also be quantified from feces (Mateo & Cavigelli, 2005; Boonstra et al., 2001) as an index of stress experienced within the 6-12 hours prior to sampling (Harper & Austad, 2000). Collection of hair and fecal samples are minimally invasive techniques and are not confounded by short-term spikes in hormone levels associated with handling and sampling (Koren et al., 2019; Mateo & Cavigelli, 2005).

Here, we use the GMGS to test whether maternal 'stress' affects natal dispersal by measuring cortisol concentration in hair and fecal samples collected from mothers and offspring to test the hypothesis that higher cortisol experienced during early development represents a maternal effect associated with a higher probability of daughter dispersal. To do this, i) we first tested for a difference in cortisol concentration between sons and daughters in hair and fecal samples with the expectation that sons would have elevated levels of cortisol when compared to daughters, because males almost always disperse. We expect that this sex-biased dispersal trend may be, in part, driven by elevated cortisol concentrations in males and used as a cue for dispersal. ii) We then evaluated the correlation between average cortisol concentration for daughters within a litter and maternal cortisol concentration for fecal samples at natal emergence. Our expectation was that mothers with higher cortisol concentration would produce litters with higher-than-average cortisol concentration in daughters because mothers pass hormones directly to their offspring during gestation and lactation. iii) Finally, to address the question of whether or not daughters who have relatively higher cortisol levels during early development are more likely to disperse from their natal home range, we assessed the relationship between cortisol concentration in daughter hair – our metric of maternal stress – and daughter dispersal, expecting that higher cortisol concentrations would be associated with a higher probability of daughter dispersal. We also examined the influence of maternal age, timing of litter emergence, and sex ratio within a litter on daughter hair cortisol concentrations.

We expected cortisol concentration to be higher for young mothers as concentration varies by age group for large and small mammals (Azevedo et al., 2019; del Rosario et al., 2011).

In other territorial mammals such as the gelada (*Theropithecus gelada*), mothers of lower social dominance rank and no prior reproductive experience tended to have higher glucocorticoid levels during gestation (Carrera et al., 2020). Over the course of the active season, GMGS cortisol levels are initially high, decrease during the first half of the above-ground period, but then rise again and reach a seasonal maximum at the end of the active period (Boswell et al., 1994); hence, we expected that individuals from later emerging litters would have an increased dispersal likelihood due to increased cortisol levels. We anticipated that larger litters would stimulate dispersal as individuals should attempt to avoid competition with siblings within the natal range. Female-biased litters should also stimulate dispersal as GMGS littermate sisters do not share home ranges (Aliperti, 2020) and may delay each other's reproductive maturity when multiple remain philopatric (Wells & Van Vuren, 2018).

METHODS

Focal Species. Golden-mantled ground squirrels are distributed along an elevational gradient between 1,220 and 3,965 meters above sea level in the Rocky Mountains (Kneip et al., 2011). They are omnivorous and dig their own shallow burrows which they use for hibernation, rearing young, storing food, and avoiding predators. Males emerge from hibernation first, and polygynous breeding begins within days of female emergence (Kenagy et al., 1989). Golden-mantled ground squirrels have a gestation period of about 28 days (Cameron, 1967) after which mothers raise 1-9 pups for an additional 30 days before offspring are ready to emerge from their natal burrow and begin the weaning process (Phillips, 1981; Wells et al., 2017). Dispersal occurs as early as two weeks after weaning (Wells & Van Vuren, unpublished data). Offspring always leave their natal burrow, but the extent to which they remain philopatric or disperse to an entirely new population can vary (Jesmer et al., 2011).

Study Site. We studied a population of free-living GMGS at the Rocky Mountain Biological Research Laboratory (RMBL), located in the East River Valley of Gunnison, Colorado (38°58'N, 106°59'W). The 13-hectare study site is situated at an elevation of 2,900 meters above sea level and primarily consists of high elevation meadows with stands of willow (*Salix* spp.), aspen (*Populus tremuloides*), and spruce (*Picea* spp.) (McEachern et al., 2011; Kneip et al., 2011).

Field Methods. Beginning on June 1st and continuing once a month until hibernation, all GMGS were live trapped in baited Tomahawk traps strategically placed in areas of known squirrel activity. Once trapped, resident individuals were identified and weighed, and reproductive status and body condition were evaluated. New immigrant individuals were similarly processed when captured. When pups emerged, natal burrows were targeted for trapping and all pups in each litter were trapped and processed within 48 hours of their initial emergence.

Dispersal was monitored by making daily observations of individuals within the study site and noting their location (within one of 4,289 7mx7m grid squares that overlay the study site (Aliperti, 2020)) and behavior (e.g., foraging, running, alert, etc.). Individuals who remained within their natal range, defined by their mother’s locality (one of six discrete sites of centralized squirrel activity within the overall study system (Wells & Van Vuren, 2017)) for the remainder of the active season were classified as ‘philopatric.’ Those that were observed within the study system but in a different locality than where they were born were considered ‘dispersed.’ Nearby populations were scoured twice after the primary dispersal period to check for additional dispersed individuals that traveled beyond the study system, but none were detected.

Sample Collection and Processing. During the study years 2016 to 2020, hair samples were collected from captured individuals by plucking hair follicles from the rump of each individual pup within 48 hours of their initial emergence. Samples were placed in a dry envelope and stored at room temperature until further processing. Fecal samples from offspring and mother GMGS were collected from 2019-2020 during trapping sessions. Samples collected in these years were frozen immediately until further processing for analysis.

<u>Hair Extractions.</u> Hair cortisol extraction and quantification were performed by adapting the protocol described by Davenport et. al (2006), using methanol to remove surface contaminants. Before hormone extraction and quantification, hair samples were finely ground to facilitate analysis. For the first set of analyses (n = 28 total, but only a subset of 18 samples were used for analysis based on sample quantity, age, sex, and litter association), hormone extraction and processing occurred at Utah State University (USU) where a retsch ball mill with steel beads was used to grind the hair samples. Hormone extraction for the second set of analyses (n = 125, but only a subset of 89 samples were used for analysis based on sample quantity, age, sex, and litter association) occurred at Colorado State University where grinding was done using steel beads in PowerBead Tubes (Qiagen, Germantown, MD, USA) before being shipped overnight to USU for extraction.

Extraction protocols were the same for all hair samples. Ground samples were transferred to pre-weighed, 1.5 mL Eppendorf tubes and then reweighed. A weight-based volume of methanol was added (1mL/50 mg) to ground hair samples for extraction with a lower volume limit of 50 µL (50 µL was added to samples that had methanol volume calculations lower than

50 µL to ensure a high enough methanol volume for extraction. Samples were vortexed at 200 rpm overnight, then vortexed vigorously the following morning to ensure adequate extraction. Following extraction, samples were centrifuged at 2000 rpm for 10 minutes. The extraction methanol was removed with a Hamilton syringe and the volume of recovered methanol was recorded. The recovered methanol was dried in a glass tube using Nitrogen gas. After complete drying, the remaining hormone concentrate was resuspended in either 100 or 200 µL of assay diluent, depending on the initial mass of the sample.

Hair Enzyme-Linked Immunoassays. We validated 25 µL of hair extract for use with an enzyme-linked immunosorbent assay kit (ELISA, Salimetrics, State College, PA, USA) for cortisol according to the manufacturer’s protocol of dilution- and spike- recovery validation and optimized for the golden-mantled ground squirrel. The cortisol ELISA is based on the competitive binding reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine and the bound cortisol enzyme. The amount of cortisol enzyme conjugate detected in the reaction is inversely proportionate to the amount of cortisol present in the hair sample. The minimum detectable value was 0.012 µg/dL.

A volume of 25 µL of each sample was run in duplicate on a 96-well plate for the squirrel hair samples (n = 171), according to manufacturer guidelines (Assay # 1-3002; Salimetrics, State College, PA, USA). Raw assay output values were calculated by interpolation to a four-point standard curve. Final cortisol concentrations (µg/g) were calculated as follows:

$$\frac{Raw\ assay\ value\ cortisol\ (\mu\text{g/dL})}{Ground\ hair\ weight\ (g)} \times \frac{MeOH\ extraction\ volume\ (\text{1mlL/50mg})}{MeOH\ recoverd\ (\mu\text{L})} \times \frac{Diluent\ added\ (\mu\text{L})}{Volume\ in\ assay\ (\mu\text{L})}$$

Any samples that were not on the standard curve were rerun with an optimized volume into the assay (higher or lower volume); calcualtions were adjusted as necessary to account for this. Spikes, an analyte added in a known amount, were added to the sample matrix to confirm measurement of only the cortisol hormone without interference from unwanted binding. Intra- and inter- assay variation were calculated to check the coefficient of variability and ensure consistency among sample replicates. Assay results indicated average linearity for samples yielded an $R^2 = 1.000$; spike recovery with a known interference averaged 96.088%. Mean intra-assay variation across six plates and 171 samples was 5.221% and mean inter-assay validation was 8.047%.

Fecal Extractions. We extracted steroid metabolites from fecal samples using a phosphate and methanol wet extraction buffer from Shideler et al. (1993), modified to increase yields of estradiol, progesterone, and testosterone from feces (Bauman and Hardin, 1998). The modification involved increasing final methanol concentration from 20% to 50% and adding sodium azide to inhibit bacterial growth (Bauman and Hardin, 1998). We weighed 20 mL scintillation vials (Wheaton, Milville, NJ, USA), added fecal material, and inundated with 5.0 mL of working fecal extraction buffer (50:50 buffer:methanol). Using a clean spatula, we broke up the fecal pellet to incorporate the buffer and vortexed for 20 seconds. Vials were placed on a shaker at 200 rpm for a minimum of 16 hours. The liquid was decanted into 12 x 75 mm glass tubes (Fisherbrand, Ontario, Canada) after the scintillation vials were allowed to settle for an hour. Following the decanting, the tubes were centrifuged for an hour at 3,500 rpm at 4°C (Beckman Coulter Allegra™ 6R Centrifuge, Indianapolis, IN, USA). Tubes were decanted again into clean 1.5 mL microcentrifuge tubes (Fisherbrand) and stored at -80°C. The remaining solution in the glass tubes was poured back into original scintillation vials and placed in a drying oven to determine fecal weight used per sample for conversion calculations.

Fecal Enzyme-Linked Immunoassays. We validated 20 µL of fecal extract for use with an enzyme-linked immunosorbent assay kit (ELISA, Enzo Life Sciences, Farmingdale, NY, USA) for cortisol following the manufacturer's protocol and optimized for the golden-mantled ground squirrel, equating to a dilution of 1:5. The cortisol ELISA is based on competitive binding between mouse monoclonal antibodies and fecal cortisol metabolites that takes place on a goat anti-mouse immunoglobulin microtiter plate. We assayed each fecal sample (n = 148 total, but only a subset of 79 samples were used for analysis based on age, sex, and litter association) in duplicate across 96-well plates according to manufacturer guidelines (Product # ADI-900-071; Enzo Life Sciences, Farmingdale, NY, USA). Intra- and inter- assay variation were used to determine certainty for comparison between samples. Spikes were added to quantify binding of cortisol and test for interference from unwanted hormone binding. Minimum detectable value was 0.05672 ng/mL with an intra-assay variation across 148 cortisol assays of 2.184% and with an inter-assay variation of 10.875%. For cortisol, average linearity for samples yielded an R^2 = 1.000 and interference tests with known spikes yielded an average recovery of 86.118%.

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Data Analysis. All statistical analyses were conducted in RStudio (R Core Team, 2019). Diagnostic plots were used when appropriate to check a number of model assumptions such as linearity, normality of residuals, and homoscedasticity (Crawley, 2005). In the event that assumptions were not met, alternative tests (i.e., Mann-Whitney U) and transformations (i.e., logarithmic) were performed.

i) Due to small sample size and a non-normal distribution of the data, we used a non-parametric Mann-Whitney U test to assess whether there was a statistically significant difference in offspring cortisol concentration between sons and daughters for both fecal and hair samples.

ii) Linear models (lm) were used to evaluate the relationship between average daughter cortisol concentration for a litter and the maternal concentration for that litter, where both variables were log-transformed to limit overdispersion in the data and ensure normality of the residuals (Arnold & Dittami, 1997).

iii) Logistic regression tested for an effect of cortisol concentration on the probability of dispersal versus philopatric behavior of daughters and compared with other models that accounted for either single or additive effects of other covariates of interest, such as maternal age, timing of litter emergence, litter sex ratio, and litter size. Cortisol concentrations were standardized for comparison. We used a two-sample t-test to test the hypothesis of no difference in cortisol concentration in hair and fecal samples between philopatric and dispersing daughters.

Inference. For the Mann-Whitney U and two-sample t-tests, we present the test statistic and associated p-values for the test of the null hypothesis (where $\alpha = 0.05$) as well as 95%

confidence intervals. An information-theoretic approach was used for model selection and inference (Burnham & Anderson, 2002) to compare generalized linear models. We generally compared a global model with additive effects of each covariate of interest to a constant model (i.e., model of no effect or intercept-only model). Within the model set, individual models were ranked according to Akaike's Information Criterion corrected for small sample sizes (AICc) (Burnham & Anderson, 2002). Lower AICc values and higher AICc weights were indicative of better fitting models to the biological processes that gave rise to the data, and these better performing models were selected for discussion (Burnham & Anderson, 2002). Among best performing model(s), we further discuss the significance of each effect by presenting regression coefficients, 95% confidence intervals, and associated p-values for the test of the null hypotheses that the regression coefficients are not different from 0 (with a threshold significance level α of 0.05).

RESULTS

i) Mean cortisol concentrations between sons and daughters were neither different in hair (W = 10014.5, p = 0.262; Figure 2.1) nor in fecal samples (W = 514.0, p = 0.587; Figure 2.2). These results suggest that there are not significant differences in offspring cortisol concentration between sexes during early development, or at the time of emergence, regardless of sample type.

ii) We found no significant relationship between maternal cortisol concentration in feces and the average cortisol concentration of the daughters in her litter for hair ($\beta = -0.172$; 95% CI = 3.907, 5.197; SE = 0.329; p = 0.621; $R^2 = 0.043$; Figure 2.3) or fecal ($\beta = -0.176$; 95% CI = 6.346, 7.046; SE = 0.179; p = 0.348; $R^2 = 0.089$; Figure 2.4) samples, indicating that related mothers and daughters do not have correlated cortisol levels at litter emergence.

iii) Evaluating the relationship between cortisol concentration and daughter dispersal was of primary interest, while accounting for any additional effects of maternal age, timing of litter emergence, litter sex ratio, litter size, and their additive effects (Table 2.1). The performance of the global model, (AICc = 56.25; Table 2.1) indicated that all variables examined influence dispersal to some degree. A model that included standardized cortisol (AICc weight = 28%) performed similarly to the null model (delta AICc = 0.00, AICc weight = 28%) and ranked better than models that included an effect of maternal age (delta AICc = 1.55, AICc weight = 13%), litter emergence (delta AICc = 1.83, AICc weight = 11%), sex ratio (delta AICc = 2.05, AICc weight = 10%), or litter size (delta AICc = 2.25, AICc weight = 9%). A one standard deviation unit increase in cortisol concentration (range = 1.00 – 483.78 μg/g) leads to a 60.5% increase in the odds of a daughter dispersing away from her natal home range ($\beta = -0.605$, SE = 0.445; Table 2.2; Figure 2.5). When comparing the mean hair cortisol concentration between philopatric and dispersed daughters, dispersers ($\bar{x} = 140.364$; 95% CI = 81.200, 199.528) tend to have higher cortisol concentrations than philopatric ($\bar{x} = 79.167$; 95% CI = 26.160, 132.174) daughters, although the difference is not statistically significant ($t = 1.462$, $p = 0.154$; Figure 2.5).

DISCUSSION

Our results indicate that offspring, regardless of sex, have similar cortisol levels during the early stages of development as well as at the timing of emergence from the natal burrow, but mothers and daughters differ in their stress levels. Looking strictly at daughters, as sons usually disperse while daughters are facultatively philopatric, the trend suggests that daughters with higher cortisol concentrations during early development are more likely to disperse from their natal home range after weaning than daughters with lower cortisol concentrations.

Despite our prediction that sons would have a higher cortisol concentration than daughters due to sex-biased dispersal trends, we found similar concentrations between sexes, suggesting that male and female offspring receive the same amount and/or process cortisol similarly during early development. However, our study did not address how offspring respond to stressors and process cortisol after emergence, as males may exhibit increased cortisol levels post-weaning such as other mammals like the southern elephant seal (Ferreira et al., 2005). Sex differences in post-weaning stress response have also been demonstrated in Sprague–Dawley CD rats where acute stress had a higher increase in males than females (Pisu et al., 2016). The reason for such differences is likely mediated by gonadal hormones interacting with the HPA axis (Goel et al., 2014). Post-weaning experiences, paired with sex-differentiated HPA axis function, may sex bias cortisol levels in response to stressors (Azevedo et al., 2019; Levine, 2002). There is a need to quantify cortisol levels of both males and females post-emergence from the natal burrow to test whether timing of stress exposure, and associated shifts in cortisol production, may serve as cues for dispersal.

Although sons and daughters within a litter have similar cortisol concentrations during early development, we found substantial variation in cortisol concentration among litters. The reason for elevated indices of stress in some litters, but not others, remains ambiguous as we found no relationship between maternal and offspring cortisol concentrations in fecal samples at natal emergence. We considered other potential drivers of dispersal behavior and found that litter size was the variable least likely to influence daughter dispersal. Larger litters have been associated with lower corticosterone, another type of glucocorticoid, in juvenile Long-Evans laboratory rats, potentially due to adaptive down-regulation of the HPA axis in response to increased nutritional competition amongst siblings (Rödel et al., 2010). However, litter size does not appear to extend its effect to ground squirrel dispersal as evidenced by our results and further supported by a study on female dispersal in the round-tailed ground squirrel (*Spermophilus tereticaudu*) (Dunford, 1977). Our inference may be quite limited by small sample sizes; additional years of data collection may help confirm our findings.

Litter sex ratio also had little influence on daughter dispersal, supporting similar findings in voles (*Microtus* spp.) (Bondrup-Nielsen, 1992; Lambin, 1994). However, these results are contradicted by studies suggesting that testosterone exposure (i.e., from more male siblings in a litter) during early development in Belding's ground squirrels (*Spermophilus beldingi*) (Holekamp et al., 1984) and male-biased litters in grey-sided voles (*Clethrionomys rufocanu*) (Ims, 1990) tend to increase female dispersal.

Timing of litter emergence was not a strong predictor of dispersal behavior either. Emergence timing does affect post-weaning fecal cortisol concentration in Common hamsters (*Cricetus cricetus*) as offspring born later in the year had higher post-weaning cortisol concentrations, presumably because a reduced above-ground activity period causes greater energetic output while acquiring resources (Siutz & Millesi, 2012). Although our study did not directly consider the relationship between cortisol and emergence timing in GMGS, results suggest that timing of emergence does not extend to influence dispersal.

Our results suggest that daughters born into litters with older mothers (i.e., more experienced, established, and familiar with the site) had a somewhat higher probability of remaining philopatric, but small sample sizes, again, may have limited the strength of this relationship. Nonetheless, this further brings into question the role of the mother in “allowing” philopatry, as older and more dominant females, in addition to having lower glucocorticoid levels (Carrera et al., 2020), are more likely to occupy higher quality habitats and have space for their offspring (Mykytowycz, 1960) as mother and daughter GMGS will overlap home ranges to some extent (Aliperti, 2020). Resource availability, which is often affected by population density, is referred to as a key factor influencing offspring dispersal via maternal effects, but we found no differences in maternal access to vegetation (see Appendix 1), implying that resource availability was not a factor that drove offspring dispersal decisions in this system.

Daughter cortisol concentration during early development was our best predictor of daughter dispersal behavior as higher concentrations were indicative of higher probability of dispersal. Although we found no evidence of a relationship between maternal stress and litter stress at offspring emergence from the natal burrow, we were unable to test for a relationship between mothers and daughters during early development because of when our samples were collected. Indeed, we were able to use hair from offspring as a long-term measure of stress accumulation during gestation and lactation (Heimbürge et al., 2019; Koren et al., 2019; Meise et al., 2016); however, hair samples from mothers collected at the same time as offspring hair samples would not solely reflect the period of early development, but also stress accumulation since the last molt in the previous summer. As an alternative, we were able to collect fecal samples from both mothers and offspring at the time of litter emergence and compare the concentrations of those samples. Fecal samples only represent stress accumulation within the previous 6-12 hours (Harper & Austad, 2000), so maternal samples were likely affected by short-term stressors (i.e., social interaction, predator sighting, human presence while trapping her litter, etc. (Delehanty & Boonstra, 2009; Mateo, 2007; Sosa et al., 2020)) that may not reflect more consistent, baseline levels of the mother. As such, we were unable to test for a relationship between mothers and daughters during early development, and instead focused on testing for a relationship at the time of offspring emergence from the natal burrow. This may have affected our findings and clouded a potential relationship involving maternal effects as they relate to offspring dispersal.

When conditions are perceived as unfavorable (i.e., indexed by higher cortisol concentrations) (Bauer et al., 2013; Hik et al., 2001), daughters are more likely to disperse from their natal home range in search of a territory that may have more suitable conditions. While we know cortisol concentration within daughters during early development does hold some predictive power related to daughter dispersal behavior, it is only marginal. Interestingly, we found that such concentrations did not track maternal cortisol concentrations prior to litter emergence. As part of ongoing research, we would like to test how daughters may use the above-ground, post-weaning period after natal emergence as an exploratory time to gather independent information that could drive their own dispersal decision (Holekamp, 1986). This highlights the need to quantify cortisol concentration at different stages of development in order to develop a more comprehensive view of what other factors may be driving dispersal behavior (Mateo, 2014).

Conclusions. We found that i) sons and daughters exhibited similar levels cortisol, an index of stress, during early development and at the time of natal emergence, contradicting our expectation that males, as the dispersing sex, would have higher cortisol levels; ii) maternal fecal cortisol and the average cortisol concentration for daughters within her litter were not associated at the time of natal emergence despite our prediction that maternal and daughter concentrations would be positively correlated; iii) dispersing daughters tended to have higher cortisol concentrations during early development than their philopatric counterparts, supporting our hypothesis that stressed daughters would be more likely to leave their natal range after weaning. Hormones during early development can affect behavior and have long-lasting implications on an individual’s life-history and fitness, including the decision to disperse or remain philopatric. Factors driving variation in hormone levels need to be further distilled in order to more fully understand how pre- and post- natal hormone exposure may program individuals and alter their behavior. Maternal stress, passed from mothers to offspring as altered cortisol concentration, may not have served as a maternal effect determining offspring dispersal behavior in the GMGS, but there are other potential avenues that need to be explored.

TABLE 2.1. Model selection results for generalized linear models evaluating the effect of cortisol (standardized hormone concentration used as an index of stress), maternal age (how many years old an offspring's mother is), litter emergence (the first day of offspring emergence from the natal burrow), sex ratio (the ratio of daughters to sons in a litter standardized as a z-score), litter size (the number of offspring that emerged from the natal burrow), and their additive interactions on the probability of daughter philopatry. Models are listed in order of Akaike weight. Cortisol was selected as our top-performing model, but it shared the same AICc weight as the null model, indicating model uncertainty.

Model Name	**K[1]**	**Log Likelihood[2]**	**AICc[3]**	**Delta AICc[4]**	**AICc Weight[5]**	**Cumulative Weight[6]**
Cortisol	2	-21.04	46.49	0.00	0.28	0.28
Constant	1	-22.18	46.49	0.00	0.28	0.56
Maternal Age	2	-21.82	48.05	1.55	0.13	0.69
Litter Emergence	2	-21.95	48.32	1.83	0.11	0.81
Sex Ratio	2	-22.07	48.55	2.05	0.10	0.91
Litter Size	2	-22.17	48.75	2.25	0.09	1.00
Global	6	-20.45	56.25	9.76	0.00	1.00

[1] K represents the number of parameters in the model.

[2] Log Likelihood is used to predict how likely a given model is based on the available data.

[3] AICc is a score of the information presented by the model and adjusted for small sample size.

[4] Delta AICc calculates the difference in AIC score between the top model and the model being assessed.

[5] AICc Weight demonstrates the predicative power of a given model in relation to the full set of models under consideration.

[6] Cumulative Weight shows the additive effect of the AICc weights as more model AICc values are considered.

TABLE 2.2. Parameter estimates from the generalized linear evaluating the effect of cortisol (standardized hormone concentration used as an index of stress), maternal age (how many years old an offspring's mother is), litter emergence (the first day of offspring emergence from the natal burrow), sex ratio (the ratio of daughters to sons in a litter standardized as a z-score), litter size (the number of offspring that emerged from the natal burrow), and their additive interactions on the probability of daughter philopatry. Models are listed in order of rank according to AICc selection.

Model Name	**β Coefficient[1]**	**95% CI[2]**	**p-value[3]**
Cortisol[4]	-0.605	-1.660, 0.166	0.174
Constant	<0.001	-0.700, 0.700	1.000
Maternal age	0.245	-0.315, 0.851	0.400
Litter size	-0.058	-0.745, 0.620	0.865
Litter emergence	-0.023	-0.097, 0.044	0.505
Sex ratio	-0.247	-1.323, 0.774	0.635
Global	Cortisol: -0.005	-0.014, 0.002	0.195
	Age: 0.063	-1.094, 1.239	0.914
	Size: -0.027	-1.039, 0.954	0.956
	Emergence: -0.033	-0.151, 0.078	0.564
	Ratio: -0.346	01.911, 1.126	0.645

[1] Beta (β) coefficients estimate effect sizes and represent the expected change of the response variable for every one-unit increase of the predictor variable.

[2] 95% Confidence interval (CI) indicates the range of values that likely contains the true mean of the parameter vlaue.

[3] p-value is from a test on the null hypothesis assuming that there is no difference in the risk of mortality between the reference variable and a given variable; if p-value < 0.05, the null hypothesis is rejected and a difference between variables is deemed significant.

[4] Cortisol was standardized for comparison.

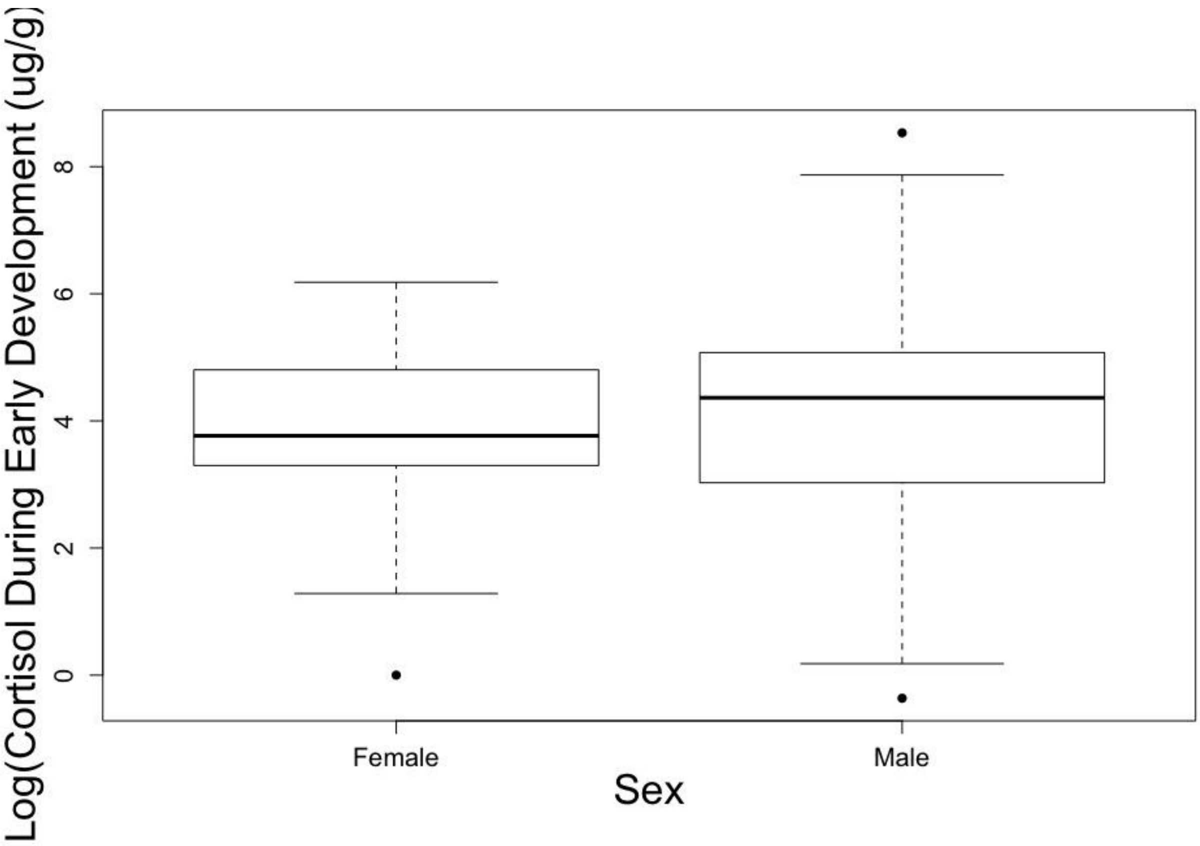

Figure 2.1. Cortisol concentration, transformed on the logarithmic scale, accumulated in offspring golden-mantled ground squirrel hair during the early stages of development does not differ significantly between male and female offspring.

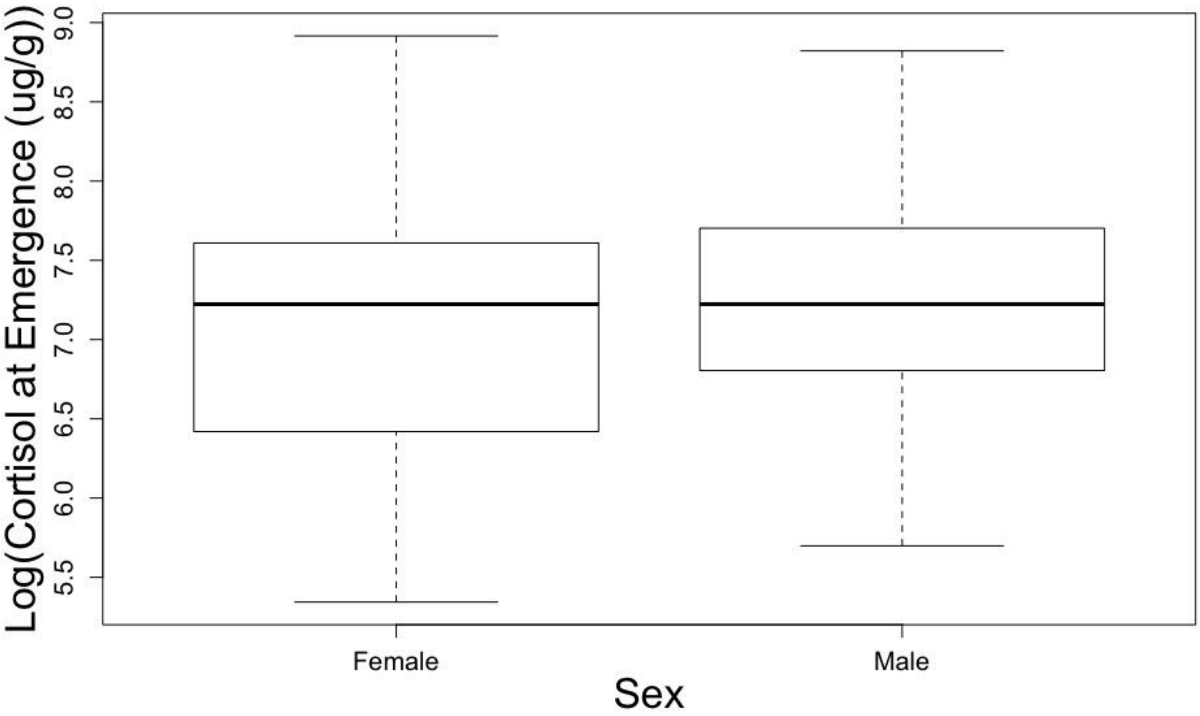

Figure 2.2. Cortisol concentration, transformed on the logarithmic scale, in offspring fecal samples collected at the time of offspring emergence does not differ significantly between male and female golden-mantled ground squirrel offspring.

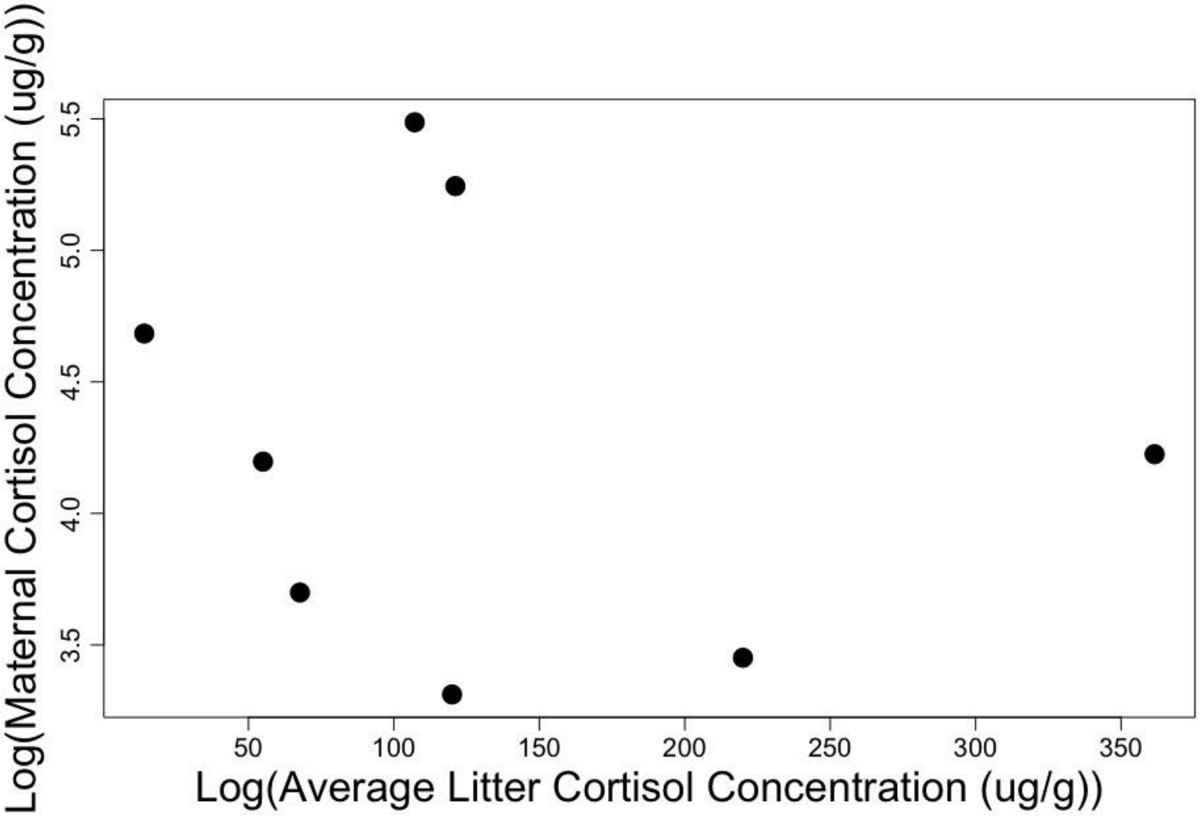

Figure 2.3. Cortisol concentrations, serving as a proxy of stress, were not significantly different between fecal samples in mothers, collected at the time of offspring emergence, and the average concentration of hair in the daughters within her litter, collected at the time of offspring emergence.

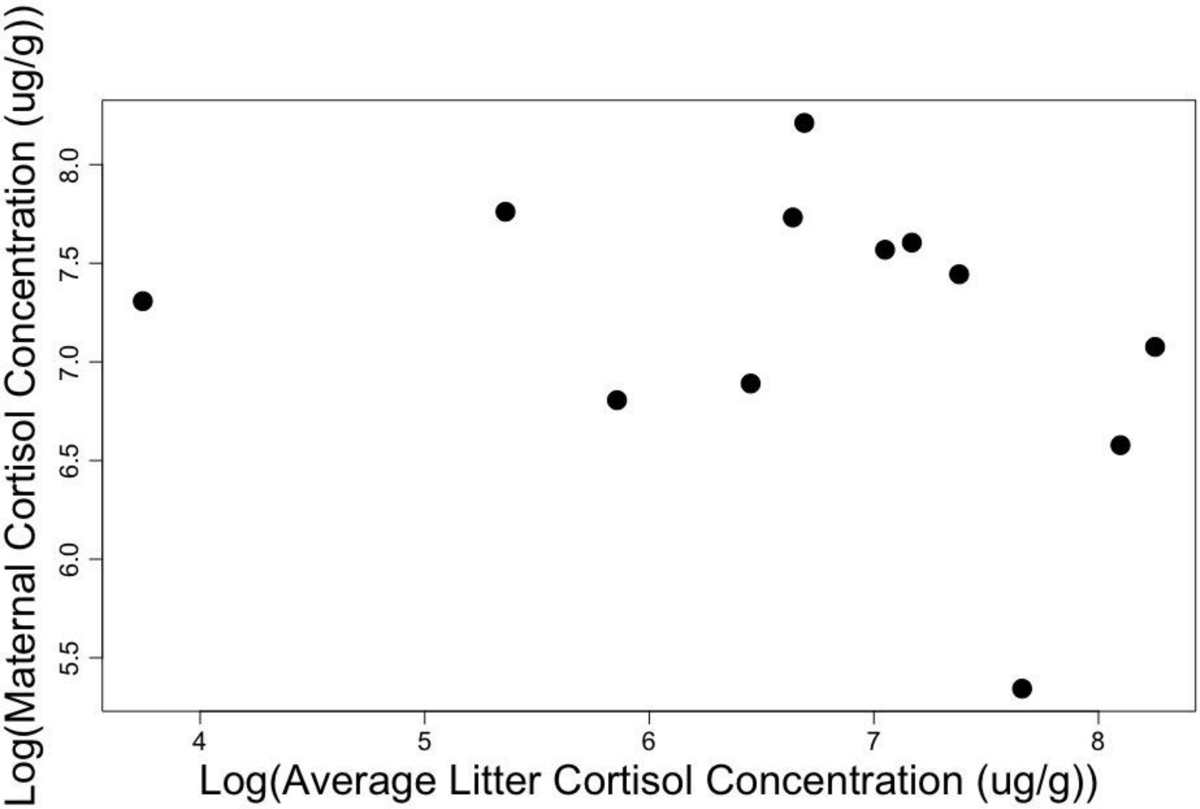

Figure 2.4. Cortisol concentration in fecal samples, used as a proxy of stress experienced during the previous 6-12 hours, were not significantly different between mothers and the average concentration of the daughters within her litter at the time of litter emergence from the natal burrow.

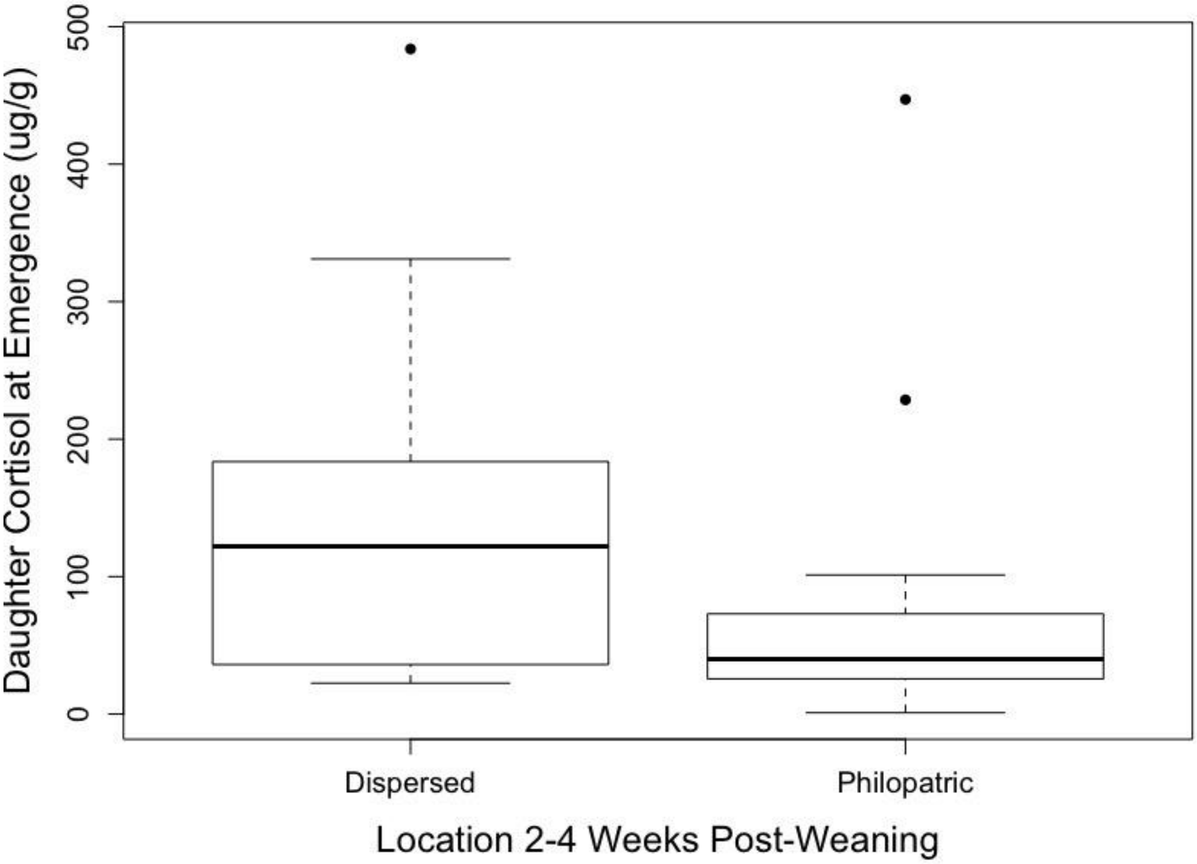

Figure 2.5. Hair cortisol concentration during early development of dispersing daughters was marginally higher than the cortisol concentration of philopatric daughters given their expected behavior post-weaning.

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APPENDIX 1.

In order to evaluate the nutritional resources available at the Rocky Mountain Biological Laboratory (RMBL), vegetation surveys were conducted (Curtis et al., 2015) within each golden-mantled ground squirrel (GMGS) mother's home range in 2019 and 2020. Using daily observational data, the top two foraging locations (7m x 7m grid square) of each reproductive female (n = 10 in 2019; n = 14 in 2020) during lactation were selected for vegetative sampling using a multiple-plot method (Cain, 1943). Each sampling plot was 1m x 1m in size. The plants rooted within each selected plot were identified to species (Peet et al., 1998), and the proportion of cover of each species was determined (Cain, 1943). Plants were also classified by vegetation type: forb, graminoid, or shrub. Percentage of bare ground was calculated when applicable. Data were used to determine vegetation differences among reproductive female foraging areas.

For all vegetation types, data were not normally distributed, so Kruskal-Wallis tests, a non-parametric alternative, were used to test for differences in vegetation types amongst plots. Neither forbs ($p = 0.47$, 0.78), graminoids ($p = 0.30$, 0.41), shrubs ($p = 0.46$, 0.41), nor ground cover ($p = 0.54$, 0.56) were different amongst plots within each year (2019, 2020, respectively) (Table A.1; Figure A.1; Figure A.2).

TABLE A.1. Model results from the Kruskal-Wallis test. Available vegetation amongst the top foraging plots did not differ for golden-mantled ground mothers within each year.

Year	Vegetation	p-value[1]	Chi-squared[2]	Degrees of Freedom[3]
2019	Forb	0.47	18.77	19
	Graminoid	0.30	21.75	19
	Ground	0.46	19.00	19
	Shrub	0.54	4.07	5
2020	Forb	0.78	20.33	26
	Graminoid	0.41	27.00	26
	Ground	0.41	27.00	26
	Shrub	0.56	3.00	4

[1] p-value is from a test on the null hypothesis assuming that there is no difference in the risk of mortality between the reference variable and a given variable; if p-value < 0.05, the null hypothesis is rejected and a difference between variables is deemed significant.

[2] Chi-squared values calculate relatedness between variables to determine if the differences in distribution of categorical variables are different from each other.

[3] Degrees of freedom report the number of categories available for testing minus one.

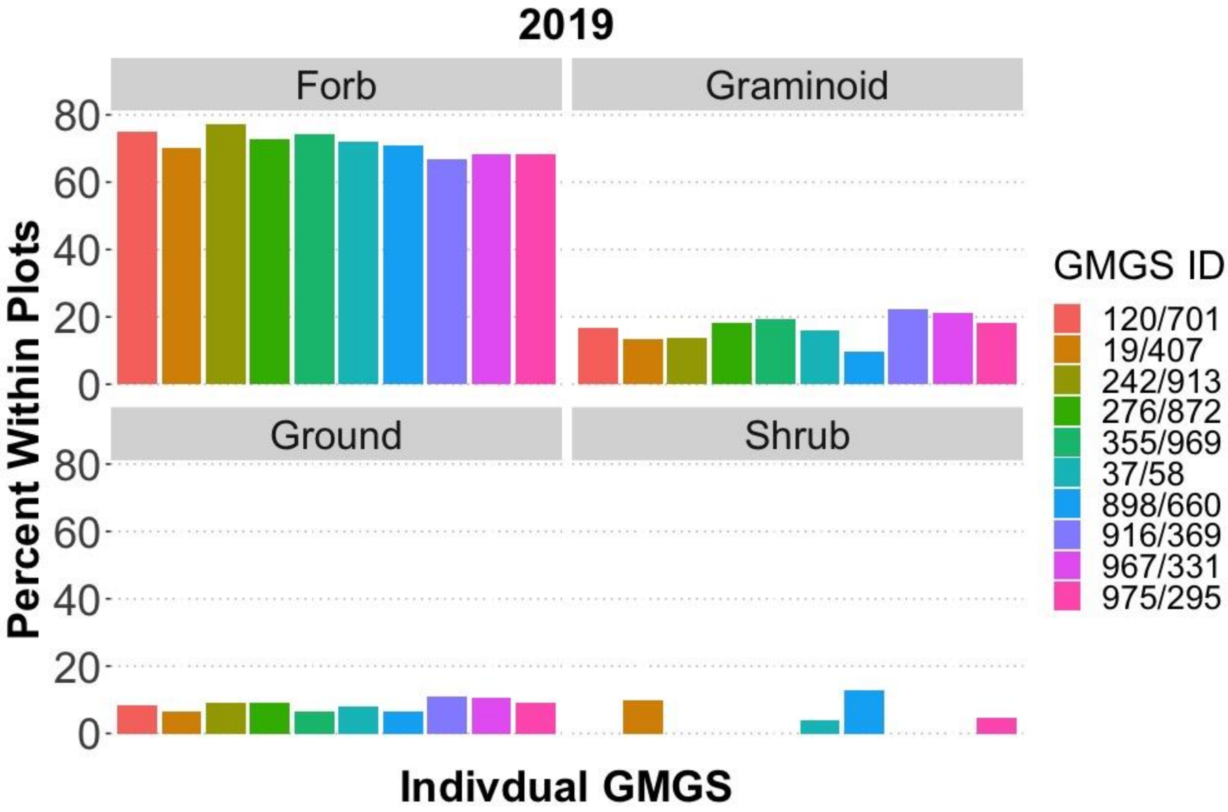

Figure A.1. Vegetation availability, in terms of the percent of each type of vegetation or cover, was similar in the top two foraging locations of each golden-mantled ground squirrel (GMGS) mother (n = 10) in 2019. Each mother had a unique identification number used to color code the vegetation available within her most used foraging location.

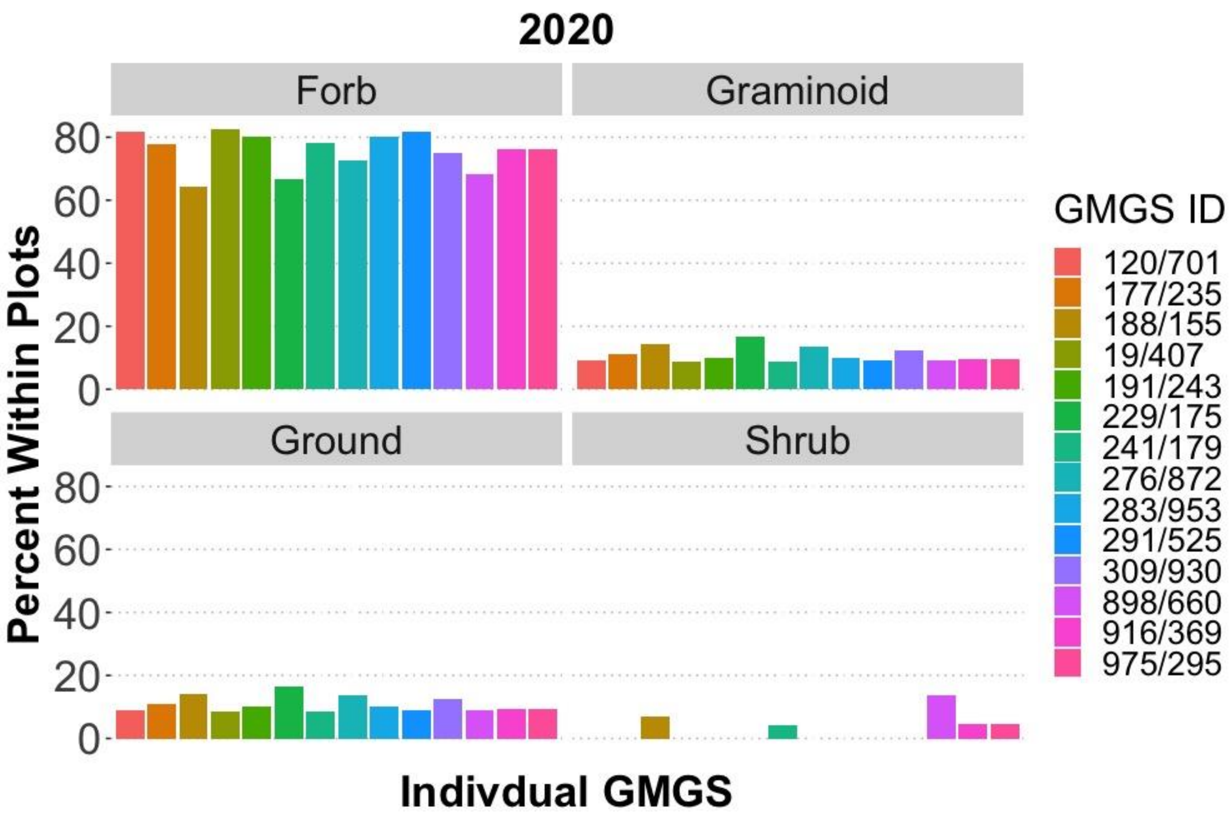

Figure A.2. Vegetation availability, in terms of the percent of each type of vegetation or cover, was similar in the top two foraging locations of each golden-mantled ground squirrel (GMGS) mother (n = 14) in 2020. Each mother had a unique identification number used to color code the vegetation available within her most used foraging location.