

Heritability of Maternal Investment in Golden Mantled Ground Squirrels (*Callospermophilus lateralis*)

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Abstract: Understanding the genetic and environmental influences on fitness-relevant traits in wild organisms provides insight into these organisms' abilities to track changing environmental conditions, a topic of increasing relevance in light of global climate change. In ground squirrels, success of females and their offspring is influenced by the level of maternal investment prior to weaning. Despite this, little is known about the genetic and environmental sources of variation in this trait, and no studies have estimated its heritability. We used a multi-generation maternal pedigree and records of pup and litter mass over 25 years to estimate the heritability of maternal investment in a wild population of golden-mantled ground squirrels (*Callospermophilus lateralis*). Our results suggest that variation in maternal investment in this species is primarily explained by environmental rather than genetic factors, and that the maternal environment may introduce factors causing dissimilarity in phenotype between daughter and mother.

Introduction

Global climate change threatens significantly and rapidly alter environments, particularly in high elevation regions which are sensitive to warming temperatures (Lamprecht et al. 2018, McCain 2019, Santos et al. 2017). Changes in temperature influence precipitation and habitat, and have had notable effects on the behavior and distribution of various species worldwide (Charmantier et al. 2008, Santos et al. 2017). Assessing how populations will respond to such changes is important when considering management solutions, but responses vary between species based on taxonomic, geographic, and ecological characteristics (McCain 2019). Organisms may respond to changing environmental conditions through adaptive genetic changes (microevolution) and/or phenotypic plasticity, mechanisms which differ in pace and longevity (Charmantier et al. 2008, Lane et al. 2018). Phenotypic plasticity may provide quicker adaptive responses to short term fluctuations in environment, but it can buffer against microevolution which may be necessary to ensure long term viability of populations facing continuing, directional environmental changes (Lane et al. 2018, Visser 2008). Heritability is a key metric in predicting whether microevolutionary or phenotypically plastic responses can occur (Lane et al. 2018), and when measured in fitness relevant traits, it can provide insight into the resilience of populations to changing conditions.

High-elevation hibernators such as the golden mantled ground squirrel (*Callospermophilus lateralis*) may be some of the most vulnerable mammals to negative effects from climate change (McCain 2018). In this species, hibernation often lasts for the majority of the year (8-9 months), restricting activities such as foraging and reproduction to a short active season (McKeever 1964). For reproductive female *C. lateralis*, numerous energy-demanding activities are necessary during this short time; mating, gestation, lactation, and prehibernation fattening must be completed for successful reproduction and survival to the following year. These obligations make it necessary

for females to properly allocate energy between themselves and their offspring. In altricial mammals, maternal investment is characterized by the transfer of nutrients from mother to offspring during gestation and lactation, and represents a significant, and variable, energy cost to mothers (Boyce et al 1986). Because pups are nourished entirely by mothers before weaning, total litter mass at time of emergence from the natal burrow (which coincides with time of weaning) can serve as a measure of maternal investment in ground squirrels.

Numerous factors affect juvenile ground squirrel success. It has been shown that earlier date of weaning and larger individual offspring mass are positively correlated with juvenile overwinter survival (Armitage et al 1976, Reiger 1996). Greater juvenile mass at weaning is a result of high maternal investment in the form of lactation, and earlier weaning provides juveniles with more time to forage before hibernation, increasing their chance of survival to yearling age (Wells and Van Vuren 2018). Habitat and forage quality generally decline with the progression of the season (Millesi 1999) and in some species it has been observed that late-reproducing females adjust by producing fewer, heavier offspring to offset the effects of shorter foraging time and reduced forage quality leading up to hibernation (Dobson & Michener 1995, Rieger 1996). Physical condition of females is also significant factor affecting their reproductive success; maternal weight, structural size, and body condition have been observed to positively correlate with indicators of reproductive success such as total litter size and juvenile overwinter survival (Dobson & Michener 1995, King et al 1991, Rieger 1996).

So, as a result of limited resources, female ground squirrels are faced with a life-history tradeoff that determines their reproductive success. On one side, there is pressure for females to invest heavily in their offspring before weaning to ensure juvenile survival. On the other, energy that a female devotes to nursing young is energy taken away from her, subtracting from her own vitality. An optimal balance between female investment in offspring vs. self is likely to be different under different environmental conditions, and the ability of females to adjust their investment allocation in response to changing conditions depends on the trait's environmental and genetic underpinnings (Roff 2003). Despite this, little is known about the sources of variation in maternal investment between individuals, and no attempts have been made to estimate the heritability of this trait in ground squirrels. Knowledge of this trait's heritability could provide insight into the vulnerability or resilience of this species to changing conditions, particularly in the midst of global climate change. Here, we use a multi-generation maternal pedigree and records of offspring mass, spanning 25 years, to estimate the heritability of maternal investment in a wild population of golden-mantled ground squirrels.

Methods

Field Methods

For this study, a long-term dataset on the population of golden-mantled ground squirrels occupying the Rocky Mountain Biological Laboratory townsite was utilized. The dataset is continuing, and includes unique identities of every individual squirrel in the population from 1995-2020, all maternal relationships, and masses of each pup at time of weaning. The following paragraph describes the field protocols used to collect this information from the wild population.

At the beginning of each summer, every individual in the population was trapped using Tomahawk live traps (model 201) and, if not already tagged, given permanent ear tags with unique identification numbers. Unique dye marks were also given to all individuals for identification through binoculars. The population was closely observed throughout each summer, with particular vigilance during the time of litter emergence. Maternity was determined through direct observation of litter emergence from burrows occupied by known reproductive females. Masses of juveniles at time of weaning were obtained by trapping and weighing pups upon emergence from the natal burrow (which coincides with time of weaning). All new pups were ear tagged and marked, ensuring identification of every individual in the population.

All methods were conducted according to protocols approved by the Animal Care and Use Committees of the University of California, Davis (Protocol No. 19642) and the Rocky Mountain Biological Laboratory, permitted by Colorado Parks and Wildlife (Colorado Scientific Collecting Permit No. TR334) and following guidelines approved by the American Society of Mammalogists (Sikes, 2016). Squirrels were handled using cone handling bags, a technique which has been shown to reduce visible signs of stress and to minimize handling time (Mantor et al 2014). Typically, under these protocols, squirrel mortality rate as a result of trapping is very low (0.1-0.2%) and no additional injuries occur (Wells and Van Vuren 2017).

Statistical Analyses

Maternal investment was quantified as total litter mass at time of emergence from the natal burrow, representing the total investment of mothers into their offspring during gestation and lactation. To ensure that total litter mass was not influenced by pup foraging, only litters that were weighed completely within 48 hours of litter emergence were used in our analyses. Heritabilities were estimated using a parent-offspring regression method, in which narrow-sense heritability (h^2) is reported as the slope of the regression line of mothers' phenotypes plotted against those of their daughters (Fernandez and Miller 1985). Because females often produced multiple litters over their lifetime, mean values for total litter mass and litter size were used to ensure that each mother-daughter pair ($N = 18$) was used only once in the analysis. In cases where individual females produced multiple litters over their lives, intra-female repeatability was analyzed using a linear regression method, in which values of litter mass for females' first litter were plotted against values for their second litter. Relationships between the following pairs of factors were examined using linear regression: average pup mass & litter size, average pup mass & litter mass, female's average litter mass & her mass as a pup, and female's average litter size & size of her natal litter.

Results

Over 24 years, 61 complete litters from 44 female golden-mantled ground squirrels were weighed at emergence. Of these females, there were 18 known mother-daughter pairs in which records of one or more complete litter were available for both the mother and daughter. The mean total litter mass ($\pm$ SD) was 378.0 ± 77.5 g, ranging from 204g to 615g. The number of pups per litter ranged from 3 to 8, with an average of 5.

Neither litter mass nor litter size (in number of pups) were found to be significantly heritable. In the case of litter mass, a significantly negative value for heritability was estimated. From a mother-daughter regression, the narrow-sense heritability of total litter mass ($\pm$ SE) in females was estimated to be -0.75 ± 0.23 ($F = 10.35$, $df = 17$, $P = 0.005$). Narrow-sense heritability of litter size ($\pm$ SE) was estimated to be -0.11 ± 0.27 which was not significantly different from zero ($F = 0.16$, $df = 18$, $P = 0.7$). Average pup mass was negatively associated with the number of pups in a litter ($F = 7.48$, $df = 56$, $P = 0.008$), but positively associated with litter mass ($F = 4.18$, $df = 57$, $P = 0.045$). A female's average litter mass was not significantly associated with her mass as a pup ($F = 1.61$, $df = 24$, $P = 0.22$), but a significantly positive association was observed after the removal of a single outlier ($F = 13.93$, $df = 23$, $P = 0.0011$). For females producing multiple litters, no significant repeatability was observed in litter mass ($F = 1.40$, $df = 11$, $P = 0.26$). The size of a female's natal litter was not significantly associated with her average litter mass as an adult ($F = 2.43$, $df = 21$, $P = 0.13$).

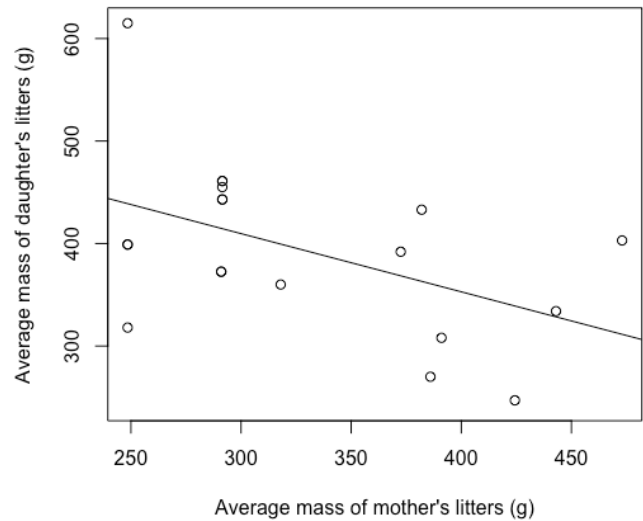


Figure 1. Total litter mass is not heritable. The mother-daughter resemblance was significantly negative ($p < 0.01$), with narrow sense heritability estimated to be -0.75 ± 0.23 .

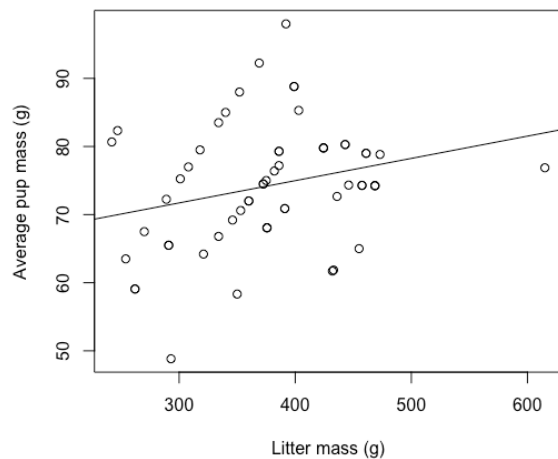


Figure 2. Average pup mass and total litter mass are positively associated ($p < 0.05$).

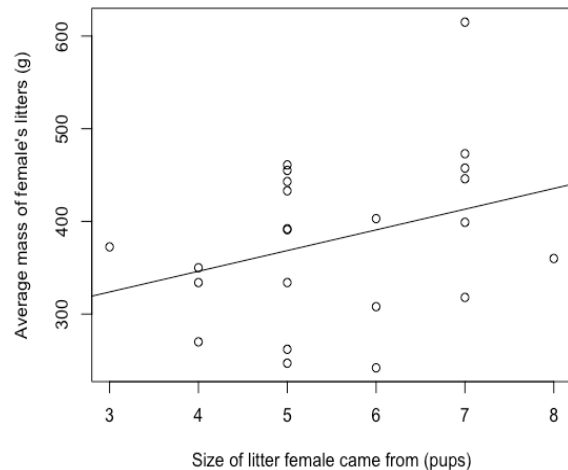


Figure 3. No statistically significant relationship was observed between females' average litter masses and the sizes (in number of pups) of the litters they came from ($p > 0.05$).

Discussion

Our results indicate that genetics is not a significant determinant of maternal investment in *C. lateralis*, revealed by a negative estimate for narrow-sense heritability (h^2) of total litter mass at emergence. The lack of repeatability in litter mass within females producing multiple litters further supports this. Previously, negative estimates for heritability have been written off as abstract consequences of mathematics (see Johnston et al. 2010), but Steinsaltz et al. (2018) propose that such negative heritabilities could represent true biological processes and should not be rejected. In the case of parent-offspring regression, h^2 of a trait is estimated as the slope of the corresponding regression line (Fernandez and Miller 1985). A positive slope indicates that offspring generally have similar phenotype to that of their parents. A slope not significantly different from zero indicates that parent phenotype has no effect on offspring phenotype. Following this train of thought, a negative slope indicates that offspring generally have *dissimilar* phenotype to that of their parents. In the case of this study, the observed negative h^2 indicates that females born to mothers who produced heavier litters were likely to produce lighter litters themselves. Before accepting this as a true inter-generational characteristic of maternal investment in this species, it is important to investigate biological explanations for this effect.

Low heritability generally implies that variation in a trait is better explained by environmental rather than genetic factors (Fernandez and Miller 1985). If a heritability of zero indicates that a trait is influenced entirely by environmental factors, then a negative heritability may suggest that the parental environment introduces factors driving offspring phenotype away from that of the parents. In our case, this would mean that some characteristic(s) of heavy litters may affect pups in such a way to cause them to have light litters. One possible explanation for this effect could be as follows: heavier litters, with many pups, tend to consist of smaller pups, and females who were smaller as pups tend to have lighter litters as adults. Some aspects of this hypothesis are supported by our results and by literature, but it is invalidated by our observation that litter mass and average pup mass are positively associated; pups from heavier litters do not tend to be smaller. Though, we did find that average pup mass is negatively associated with litter size (in number of pups). This aligns with the conclusions of Dobson & Michener (1995) and Rieger (1996), who reported that ground squirrel litters tended to consist of fewer heavier pups, or more lighter pups. Thus, maternal investment may remain somewhat consistent amidst varying numbers of pups per litter. We also observed that females who were lighter as pups may tend to have lighter litters as adults. Though this relationship was not statistically significant, the removal of a single outlier restored significance. This could reflect a true biological feature of this species, and would align with evidence that, in mammals, patterns of growth and nutrition early in life often influence adult phenotype (Bartke et al 2016, Wells 2018).

Another possible explanation for the tendency of females from heavy litters to produce light litters is that higher litter mass could correspond to increased competition between littermates, which may be a detriment to pup fitness. Though no studies have investigated the relationship between litter mass and intra-litter competition, neonates of polytocous mammals (such as ground squirrels) compete for access to teats during maternal lactation (Skok & Povše 2019) and share common foraging territory for several weeks after emergence, making it plausible that litter size could influence intra-litter competition. However, we found no significant evidence that

females' natal litter size influenced their future litter masses. Further investigation into the influence of intra-litter competition on ground squirrel development will be necessary to fully understand its effects on pup fitness and adult phenotype.

Our results indicate that, in *C. lateralis*, variation in maternal investment is primarily explained by environmental rather than genetic factors, and that the maternal environment may introduce factors causing dissimilarity in phenotype between daughter and mother. Low heritability of maternal investment could suggest the ability of female *C. lateralis* to adapt to changing conditions on a time scale too short for adaptive mutations to occur, and this plasticity could be advantageous in response to short-term variation in environmental conditions such as resource availability. But, climate change threatens to produce extended periods of directional environmental change, in which case microevolutionary responses may be necessary for the long term resilience of populations (Lane et al. 2018, McCain 2019). A lack of heritable variation in this trait could decrease the ability of selection to drive adaptive genetic changes in populations (Lane et al. 2018, Visser 2008). In light of our findings, further research investigating the influence of specific environmental variables on maternal investment would strengthen our understanding of adaptive plasticity in this trait and shed light onto the vulnerability or resilience of this species to a changing environment.

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Appendix: supplemental figures

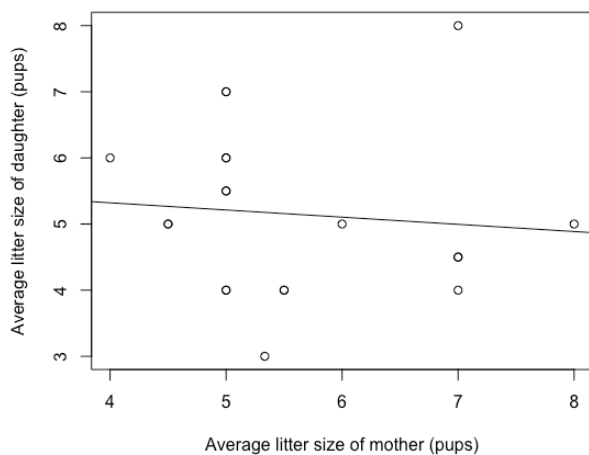


Figure 4. Litter size was not significantly heritable, with a calculated h^2 of -0.11 ± 0.27 ($p > 0.05$).

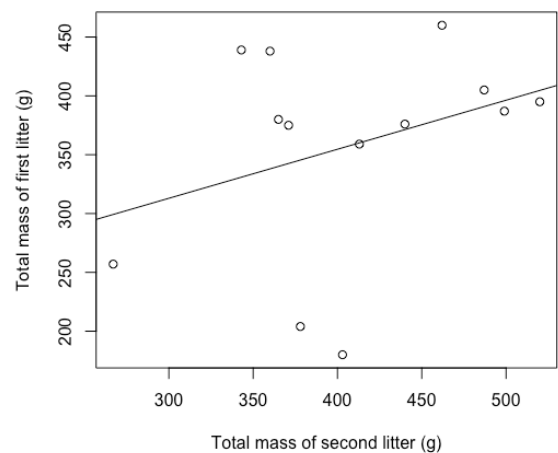


Figure 5. For females producing multiple litters, no significant repeatability was observed in litter mass ($p > 0.1$).

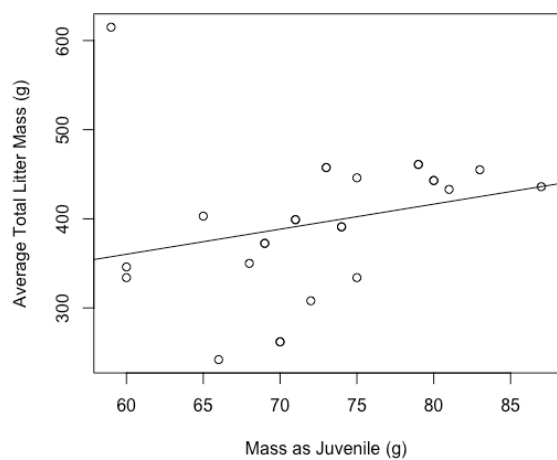


Figure 6. A female's average litter mass was not significantly associated with her mass as a pup ($p > 0.1$), but a significantly positive association was observed after the removal of a single outlier ($p = 0.001$).

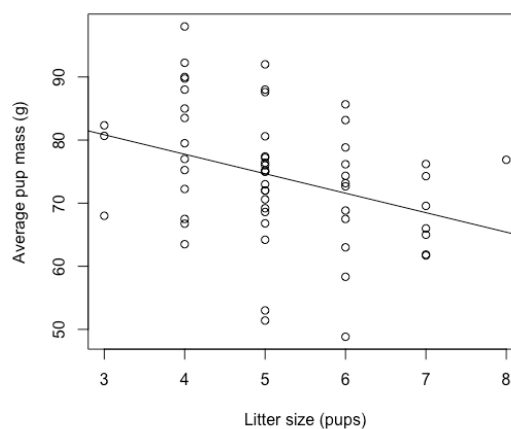


Figure 7. Average pup mass was negatively associated with the number of pups in a litter ($p < 0.01$).



Figure 8. Map of study area. Red line indicates boundary of study population.