MATERNAL EFFECTS IN COLUMBIAN GROUND SQUIRRELS

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MATERNAL EFFECTS IN COLUMBIAN GROUND SQUIRRELS

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THESIS ABSTRACT

MATERNAL EFFECTS IN COLUMBIAN GROUND SQUIRRELS

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The phenotype of an organism is the physical expression of its genotype and is a result of both the genetic makeup of an organism and the environment it experiences. An individual's phenotype can also be affected by the phenotype of its mother. A maternal effect occurs when the phenotype of the mother influences the phenotype of her offspring, independent of the offspring's genotype or non-maternal aspects of its environment.

In this study, maternal effects on pup growth and size in Columbian ground squirrels were assessed. A cross-fostering study was used to determine the contributions of maternal effects and direct genetic effects to variation in pup traits. Maternal effects were responsible for a large proportion of the variation in offspring growth rate and weaning weight, as indicated by the high associations in these traits between unrelated littermates (growth rate: $R^2 = 0.58$; weight: $R^2 = 0.64$). Heritability estimates for both

offspring traits were zero, suggesting that maternal effects are a more important determinant of offspring phenotype than are genetic effects.

Relationships between maternal phenotype and offspring growth rate and weaning weight and the influences of these traits on survival of pups over-winter were also examined. Litter size and maternal traits (body condition, timing of reproduction, structural size, and change in mass during reproduction) explained 69% of the variation in offspring weaning weight and 72% of the variation in offspring growth rate. Only maternal body condition and litter size had significant effects on offspring weaning weight (condition: p = 0.34; litter size: p = -0.85) and growth (condition: p = 0.28; litter size: p = -0.88). Pup growth rate was the only variable with an effect on pup survival to yearling age. Influences of maternal investment in young did not appear to persist to yearling age, as suggested by the lack of association between maternal traits and size of offspring at emergence from hibernation the following spring (R^2 = 0.34).

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INTRODUCTION

Maternal effects are any phenotypic variation that is due to the phenotype of the mother and exclusive of: 1) the genotype of the offspring (Bernardo 1996a, Roff 1998), 2) the non-maternal components of the offspring's environment (Lacey 1998), and 3) the interaction of offspring genotype with its non-maternal environment (Bernardo 1996b). Until relatively recently, maternal effects were regarded as potentially confounding factors in ecological and evolutionary studies of phenotypic variation and researchers attempted to design experiments that controlled for maternal effects (Bernardo 1996a). In the past several years literature pertaining to the topic of maternal effects has increased dramatically as researchers have become aware of the potential ecological and evolutionary ramifications of maternal effects themselves. For example, maternal care during early offspring development may increase survival probability of young through reproductive age, which could enhance both maternal and offspring fitness.

In this study we examined aspects of maternal effects in Columbian ground squirrels. Specifically, we sought to determine if maternal effects are responsible for observed variation in offspring phenotypes (*viz*., growth rate and weaning weight). We investigated patterns of maternal investment on offspring growth and size in the wild and searched for evidence of persistence of maternal influences on juvenile traits to yearling age.

The first objective was addressed by conducting a cross-fostering study, whereby neonates were transferred between pairs of litters. Thus, each litter consisted of a mother's own pups and fostered pups. By examining relationships between unrelated littermates and between genetic siblings raised by different mothers, it was possible to disentangle contributions of both maternal effects and direct genetic effects (*viz*., heritability) to offspring traits. We predicted that if maternal effects had an influence on development, then unrelated littermates would be more closely related in growth rate and weaning weight than to genetic siblings that were raised in different litters. However, if heritability was more important than maternal effects in pup development, we expected genetic siblings raised in different litters to be more similar in traits than unrelated littermates.

The second objective of the study was to describe specific effects of maternal traits on offspring phenotype and their persistence on offspring the following year. We first examined patterns of maternal investment in offspring growth rate and weaning weight using a multivariate approach. We then searched for influences of maternal traits and offspring traits on survival of pups to yearling age and on pup weight at yearling age, as an indication of persistence of maternal influences on pup phenotypes.

CHAPTER ONE

MATERNAL EFFECTS ON OFFSPRING WEIGHT AND GROWTH RATE IN THE COLUMBIAN GROUND SQUIRREL: A CROSS-FOSTERING APPROACH

Abstract. The external expression of a trait is influenced by both the genetic composition of the individual as well as the environment it experiences. Maternal effects are a specific source of this environmental variation and occur when the phenotype of the mother influences the phenotype of her offspring, independent of the offspring's genotype. A potentially important maternal effect in Columbian ground squirrels is development of young prior to their first hibernation. The goal of this study was to determine the relative contributions of the genetic composition of offspring and maternal effects to pup weaning mass and growth rate in mass during the lactation period. A cross-fostering technique was employed to separate maternal effects from direct genetic effects on offspring phenotype. Maternal effects contributed significantly to variation in both weaning weight ($R^2 = 0.64$) and growth rate ($R^2 = 0.58$). The estimated heritability for weaning weight and growth rate was zero. This indicates that the environment provided by the mother has more of an influence on pup weight than direct genetic inheritance.

INTRODUCTION

Natural selection acts on phenotypic variation (Stearns 1992), which is comprised of both genotypic and environmental factors (Bull 1987). Genotypic variance is due to differences among individuals in their genetic composition. The environmental component of phenotypic variation is due to all non-genetic sources and arises when individuals experience different environments, such as nutritional or climatic differences (Falconer and Mackay 1996). One particularly interesting source of environmental variation is maternal effects.

Maternal effects are phenotypic influences of the mother on the phenotype of her offspring (Falconer and Mackay 1996, Bernardo 1996a, Mousseau and Fox 1998) and have been explored in a wide range of taxa, including plants (reviewed in Roach and Wulff 1987), insects (reviewed in Mousseau and Dingle 1991, Kyneb and Toft 2006), amphibians (Parichy and Kaplan 1992, Kaplan 1998), fish (Heath and Blouw 1998), birds (reviewed in Price 1998, Blums et al. 2002), reptiles (Kolbe and Janzen 2001) and mammals (Ellis et al. 2000, McAdam et al. 2002). Maternal effects can be mediated through behaviors, such as nutritional provisioning of young pre-and post-partum (Mather and Jinks 1971), oviposition or nest site selection (Kolbe and Janzen 2001), preparation of nests and burrows, and protection against predators (Clutton-Brock 1991). Maternal influences also can occur physiologically, through transmission of pathogens, antibodies (Mather and Jinks 1971) and hormones (Mousseau and Dingle 1991, Clark and Galef 1995) from mother to offspring. The result is modification of offspring traits, such as sex ratio (Trivers and Willard 1973, Nager et al. 1999), litter size (Campbell and

Slade 1995, Dobson et al. 1999), propagule size (reviewed in Bernardo 1996b), and offspring quality (Ellis et al. 2000). Offspring quality impacts juvenile performance, such as growth and competitive ability, and survival (Bernardo 1996a).

The potential for maternal influences on offspring phenotype should be especially high during early offspring development, which is often the case (Shaw and Byers 1998). In many mammalian species, young are altricial at birth and are dependent on parental provisioning, allowing ample opportunities for maternal effects to occur. In Columbian ground squirrels, *Spermophilus columbianus*, young develop in a nest between birth and weaning and mothers provide the sole source of nutrition during this time. Thus, development of young, as reflected by growth rate and body mass, should be strongly influenced by maternal effects. However, variation in body mass and growth rate may also be attributable to differences in the genetic composition of young.

In this study, a cross-fostering technique was used to explore the relative contributions of direct genetic effects and maternal effects to offspring weaning weights and growth rate in body mass in Columbian ground squirrels. Cross-fostering is commonly applied to studies regarding evolution of clutch size in birds and can be readily applied to any species exhibiting parental care (Bernardo 1996a). Recent studies have successfully used this method to detect maternal effects on juvenile growth rates, survival, and progeny size (McAdam et al. 2002, Pelayo and Clark 2003, Crespi and Lessig 2004). By using the cross-fostering technique, it is possible to discern direct effects of genes and indirect effects, due to the environment provided by the mother, on offspring phenotype (Roff 1998).

If weaning weights and growth rates are influenced by maternal effects, we predict that unrelated individuals within cross-fostered litters will be similar in growth rate and weight at weaning and heritability of these traits should be low. That is, the proportion of the offspring phenotype attributable to the additive effects of genes should be low. However, if maternal effects are not influencing pup phenotype, heritability estimates should be large and unrelated littermates will be different with respect to these traits.

MATERIALS AND METHODS

Study organism

Columbian ground squirrels are small, iteroparous, hibernating rodents with a short active season, in which females have to mate, give birth, lactate, and acquire fat stores before the 8-9 month hibernation period (Murie and Boag 1984, Festa-Bianchet and King 1991, Dobson et al. 1992, Wilson and Ruff 1999). Females mate a few days after spring emergence from hibernation and produce one litter per year (Hare and Murie 1992). Life histories are extremely plastic as evidenced by early maturation in females, increased survival, larger litter size, and heavier individuals in populations supplemented with food (Dobson and Murie 1987). Population size is regulated, at least in part, by availability of food resources (Dobson 1995, Dobson and Oli 2001).

Study location

Field studies of Columbian ground squirrels were conducted during the summers from 1992-2006. This study includes data only from years 1993, 1994, 1999, 2001, and 2003; the years when pups were cross-fostered. This population inhabits a meadow (elevation of 1550m; 110°W 50°N) along the Sheep River in the Sheep River Provincial Park, Alberta, Canada.

Trapping and experimental manipulation

Ground squirrels were captured at spring emergence (April-May) by placing live-traps (Tomahawk #201 collapsible chipmunk trap, 16 x 5 x 5 cm^3) baited with peanut butter near the opening of the burrow. All individuals were marked with numbered fingerling ear tags and were given unique body markings using hair dye (Lady Clairol Hydrience #52 black pearl). Weight, using a Pesola scale, reproductive status, and zygomatic arch breadth were recorded for all captured individuals. During June and early July, juveniles were captured at emergence from natal burrows using the same techniques as described above. Time of litter emergence was estimated by adding 51 days to the mating date: 24 days from mating to parturition (Murie and Harris 1982) and 27 days from parturition to litter emergence (Murie 1992). Mating date was determined by examination of external morphology or by observations of pre- and post-copulatory behavior. For example, prior to mating the vulva becomes swollen and within 1 to 2 days following copulation the vulva is flaccid (Murie and Harris 1982). Although most copulation occurred underground, pre- and post-copulatory behavior was observed

frequently. Males entering burrows with estrous females followed by female aggression towards the male or cessation of male interest in a female (viz., sniffing the anogenital region, kissing) indicated that copulation had occurred.

Females that copulated were captured prior to parturition (May-June), weighed, and housed in the laboratory until birth (1-10 days). Females were kept in polycarbonate microvent rat cages (Allentown Caging Equipment Company; 267 x 483 x 203 mm^3) on pine chip bedding and given newspaper for nest building material. To obscure vision from neighboring females and to simulate the burrow environment, cages were covered in black plastic bags and stored in a field lab maintained at ambient temperature. Squirrels were fed a high protein horse feed (minimum crude protein 13%, oats, barley, wheat, and compressed vegetable material in a molasses mix) ad libitum and lettuce and apple twice daily. Females were checked for pups 3-4 times per day from 0530-2230 hrs. Females and neonates were weighed at least 4 hours after birth (to ensure that parturition was complete) using a Pesola scale (mothers) or a Mettler balance (neonates). Neonates were marked by removing a small amount of tissue from the outer left or right toe bud or from the end of the tail. This allowed identification of pups when they emerged from natal burrows. Litters were then paired and one to three neonates from each litter were exchanged. The same number of pups was cross-fostered between litters and each mother retained at least one of her own pups. Mothers were released on the meadow by opening the cage containing mother and pups. Mothers entered the nest burrow, usually within 20 minutes. Pups were then placed in the nest burrow opening and the opening was observed until the mother retrieved her pups. Nest burrows are inconspicuous

burrows that females create before giving birth. Mothers give birth to and nurse pups in the nest burrow. Nest burrows were discovered by observing females stocking them with nesting material and were flagged prior to placing females in the lab.

Statistical analysis

Differences in pup traits among years were examined by conducting analysis of variance (ANOVA) tests. Pup traits included weaning weight, birth weight, and growth rate. Pup data were averaged within litters to retain independence of data points. Weaning weights, birth weights, and growth rates did not differ significantly among years (respectively, $R^2 = 0.10$, $F_{[4, 46]} = 1.28$, $P = 0.29$; $R^2 = 0.13$, $F_{[3, 37]} = 1.8$, $P = 0.16$; $R^2 = 0.06$, $F_{[3, 36]} = 0.79$, $P = 0.51$). Therefore, offspring data were pooled among years. Analyses were run using only 2001 (the year with the greatest sample size; $n = 30$ cross-fostered litters) and all years combined ($n = 51$ cross-fostered litters). Results did not differ; thus, results from all years combined are presented.

Pooling data among years invokes multiple records for some females ($n = 9$ females), violating the assumption of data independence. However, records for females were considered independent because studies show that reproduction in one year does not influence litter size (Risch et al. 1995), litter mass (Dobson et al. 1999, Broussard et al. 2005a) or female survival to the following year (Murie and Dobson 1987). In addition, average offspring mass for females that were older than 1 year, did not significantly affect average offspring mass the following year (Spearman rank correlation; $r = 0.17$, $n = 92$, $P = 0.10$).

Regressions were employed to analyze relationships between traits of foster pups (e.g. pups that were fostered into that mother's litter; hereafter referred to as foster pups) and non-fostered pups (e.g. pups that remained with the birth mother; hereafter referred to as non-fostered pups). Only females that had at least one of her own and at least one fostered pup survive to weaning were used in analyses. When more than one foster or own pup survived to weaning, traits were averaged within litters. Growth rate indicates weight gain in grams per day during the lactation period and was calculated by subtracting mass at birth from mass at weaning and dividing by length (in days) of the lactation period. Duration of lactation is equal to the date of litter emergence minus litter birth date. Litter emergence date gives an approximate date for the termination of the lactation period (Anderson et al. 1976, Murie and Dobson 1987, Michener 1989, Rieger 1996). Since litter size has been shown to be correlated with pup weights (Michener 1989, Dobson et al. 1999), regressions were run both including and excluding litter size at weaning as a covariate.

Estimates of heritability for weaning weight and growth rate were calculated from relationships between genetic siblings. Weaning weights and growth rates were standardized by taking residuals obtained from analysis of variance (ANOVA) tests of fostered sibling and non-fostered sibling weaning weights and growth rates using size of their respective litter at weaning as the grouping factor. When more than one fostered sibling or non-fostered sibling survived to weaning, weaning weights and growth rates were averaged within litters. Regressions used standardized weaning weights and growth rates of fostered siblings (e.g. siblings that were fostered to another litter; hereafter

referred to as fostered sibling) on non-fostered siblings (e.g. siblings that remained with the natal mother; hereafter referred to as non-fostered sibling). The slope of the line generated by the regression of related individuals provided an estimate of the amount of additive genetic variance of a trait as a proportion of the total phenotypic variance (e.g. heritability; Falconer and Mackay 1996). In Columbian ground squirrels, siblings share either 25% or 50% of their genes because multiple paternities within a litter are possible (Murie 1995); therefore, the slope of the regression line only estimates 0.25 (for half sibs) or 0.50 (for full sibs) of the heritability of the trait . Slopes of the lines and standard errors can be corrected by multiplying by 2 and 4. This gives a range within which heritability of offspring weight should fall.

All analyses were performed using SAS statistical software for Windows (SAS, 2002). Significance level for all tests was $\alpha = 0.05$. Data were tested for normality by performing Shapiro-Wilks tests. All variables were normally distributed. Linearity and homoscedasticity were examined graphically. Durbin-Watson tests were used to detect autocorrelation of residuals.

RESULTS

Litter size at weaning and non-fostered pup weaning weights explained a significant amount of variation in foster pup weaning weights ($R^2 = 0.65$, d.f. = 2,48, $P < 0.0001$; means ± 1 SE: litter size: 3.22 ± 0.11; non-fostered pup weights: 104.47 g ± 2.34; foster pup weights: 102.88 g ± 2.35). Non-fostered pup weaning weight alone explained only slightly less variation in foster pup weaning weight and the association

remained highly significant (Fig. 1; $R^2 = 0.64$, d.f. = 1,49, $P < 0.0001$). Non-fostered pup growth rate was 3.57 g/day ± 0.09 and foster pup growth rate was 3.51 g/day ± 0.09. Non-fostered pup growth rate explained 58% of the variation in foster pup growth rate (Fig. 2; d.f. = 1,35, $P<0.0001$). Inclusion of litter size at weaning into the regression model as a covariate did not result in explanation of more of the variation in foster pup growth rate ($R^2 = 0.58$, d.f. = 2,34, $P < 0.0001$).

The relationships between growth rate and weaning weight of non-fostered and foster pups within litters could have been due to an initial relationship between foster and non-fostered pup birth weights that persisted to weaning rather than due to maternal effects during postnatal development. However, non-fostered pup birth weight could only explain a small and insignificant amount of variation in foster pup birth weight, both when litter size at weaning was included as a covariate and when litter size was excluded (respectively; $R^2 = 0.06$, d.f. = 2,35, $P = 0.32$; $R^2 = 0.01$, d.f. = 1,36, $P = 0.63$). This suggests that the relationship between traits of unrelated littermates arose during the lactation period.

For both the regression of foster pup weaning weight on non-fostered pup weaning weight and foster pup growth rate on non-fostered pup growth rate, the slopes of the estimated regression lines (weight: b = 0.80 ± SE 0.09; growth rate: b = 0.76 ± SE 0.11) were significantly different from a line of slope of 1.0, which is predicted if foster pups and non-fostered pups are identical in weaning weight and growth rate (weight: $F_{[1,49]} = 5.27$, $P = 0.03$; growth rate: $F_{[1,35]} = 4.71$, $P = 0.04$). This indicates some

differentiation between foster pup and non-fostered pup weaning weights and growth rates.

The differentiation between non-fostered pups and foster pups could be due to initial differences in birth weights that persisted to weaning or to mothers differentially investing in pups she gave birth to. At birth, foster pups weighed an average of 12.24 g ± 0.17 and non-fostered pups weighed 11.98 g ± 0.14. Birth weights of foster pups were not significantly different from non-fostered pups (paired t-test: $t = 1.38$, d.f. = 37, $P = 0.18$). Weaning weights for both foster pups and non-fostered pups were not correlated with their birth weights (foster pups: $r = 0.12$, $n = 39$, $P = 0.29$; non-fostered pups: $r = 0.27$, $n = 40$, $P = 0.09$).

If mothers were differentially investing in their own young over the fostered young, we would expect deviations of data points from the line of slope 1.0 to be non-normally distributed or for non-fostered young to have increased survival to yearling age. Deviations were estimated by taking the distance of a perpendicular line from each data point to a line with a slope of 1.0 and an intercept of 0. T-tests were then performed to test if the deviations were significantly different from 0. Deviations of the data points from a line with a slope of 1.0 did not differ significantly from 0 for both weaning weight ($t = -1.08$, d.f. = 50, $P = 0.29$) and growth rate ($t = -0.64$, d.f. = 36, $P = 0.53$), indicating that females do not differentiate between her own pups and the foster pups. A mixed model logistic regression was used to test survival differences in non-fostered and foster pups. Litter number, which was arbitrarily assigned to each litter, was included in the model as a random variable to control for data on multiple pups within the same litter.

Survival of pups to yearling age was not related to whether they were fostered or not ($F_{[1,162]} = 0.06$, $P = 0.80$).

Non-fostered siblings weighed an average of 103.74 g ± 2.40 at weaning and fostered siblings weighed 107.42 g ± 3.25. For siblings (full or half), standardized weaning weights of non-fostered siblings were not significantly associated with fostered sibling weights (Fig. 3; $R^2 = 0.03$, d.f. = 1,44, $P = 0.22$). The slope of the estimated regression line was b = -0.23 ± SE 0.18, which represents an estimate of heritability if pups were related by 100%. Growth rate of non-fostered siblings also did not explain a significant amount of the variation in fostered sibling growth rate (Fig. 4; $R^2 = 0.10$, d.f. = 1,33, $P = 0.07$; means ± 1 SE: non-fostered siblings: 3.60 g/day ± 0.11; fostered siblings: 3.54 g/day ± 0.14). The estimated slope of the line was b = -0.40 ± SE 0.21. Since both slopes are negative, resulting in a heritability estimate of 0 for both weaning weight and growth rate, it was not necessary to correct for the actual relatedness of *S. columbianus* siblings.

DISCUSSION

Cross-fostering is a technique used to separate maternal and genetic contributions to phenotypic variation (Roff 1998). This study employed the cross-fostering method to determine if maternal effects are responsible for variation in offspring weaning weights and growth rates. The regression of weaning weights of unrelated individuals (Fig. 1) raised in the same litter provides an estimate of the amount of variation in weight that is due to a common environment provided by the mother to the exclusion of direct genetic

variance. We found a highly significant association between foster and non-fostered weaning weights, suggesting that maternal effects contribute to offspring weight. Murie et al. (1998) also found that weaning weight did not differ between unrelated littermates.

The relationship between weaning weights of foster and non-fostered pups, however, could reflect an initial relationship between foster and non-fostered pups in weights at birth that persisted through the nursing period rather than maternal effects. However, no association was found between birth weights of foster pups and non-fostered pups. Therefore, during the period of maternal investment (e.g. lactation), foster pups and non-fostered pup weights became associated, suggesting that maternal effects have a strong contribution to offspring weaning weights.

Non-fostered pup growth rate also explained a significant amount of the variation in foster pup growth rate (Fig. 2), which suggests that the similarity of weights at weaning may be mediated through maternal contribution in the form of nutrient provisioning during lactation. Our results corroborate other studies that have shown significant maternal effects on the growth rate of body mass in rodents (Rutledge et al. 1972, Riska et al. 1984, McAdam et al. 2002).

Growth rates and weaning weights were not identical between unrelated littermates as indicated by the significant difference between the estimated slopes of the regression lines and the predicted slope of 1.0. This could be caused by initial differences in non-foster and foster pup birth weights that persisted to weaning or by the ability of mothers to discriminate between their own and other young. However, foster and non-foster pups were similar in birth weights and birth weight was not correlated

with weaning weight for both non-fostered and foster pups. This suggests that the differentiation between non-fostered pup and foster pup weaning weight and growth rate was not due to differences in birth weight that persisted to weaning. Also, the deviations from the lines of slope 1.0 for both weaning weight and growth rate were not significantly different from zero, which indicates that mothers did not give preferential treatment to their own young. The lack of a relationship between whether a pup was fostered or not and survival to yearling age also suggests that mothers do not discriminate their own young from fostered young. This result is consistent with a two-year study by Murie et al. (1998), that found no difference in survival of juveniles that were fostered and juveniles that remained with the natal mother through the nursing period. The significant difference between the estimated regression lines and a line of slope equal to 1.0 could be due to the use of ordinary least squares (OLS) regression. When X and Y values are not independent the slope of the regression line estimated by the OLS method tends to be underestimated (Green 2001).

Estimates of the amount of variation in offspring phenotype due to maternal effects could potentially be confounded by non-maternal environmental effects, such as differences in temperature or climate. The time period in which maternal effects were examined in this study occurred during lactation, when pups remain underground in natal burrows. Litters could experience differences in temperature due to differences in the location and depth of natal burrows. However, locations of natal burrows are selected and the burrows created by the mother, and thus, could be considered a maternal effect.

The regressions of weaning weights and growth rates of unrelated littermates are based on the underlying assumption that prenatal maternal effects do not contribute to pup weight. A study by Moore *et al.* (1970) on mice showed that prenatal effects on pup weight were small and did not persist past one week following birth. However, Rhees *et al.* (1999) found that uterine maternal effects had significant and persistent contributions to offspring body weight and growth rate. In Richardson's ground squirrels (*Spermophilus richardsonii*) mothers invest much more energy in pups during lactation than to developing embryos during gestation (Michener 1989). Assuming this also occurs in Columbian ground squirrels, as it does in other rodent species (McClure 1987), the propensity for prenatal maternal effects on weaning body mass and growth rate are likely small relative to postnatal maternal effects.

Since pups were not always cross-fostered on the parturition date, some pups spent one to two days with the birth mother before cross-fostering occurred. Thus, relationships between unrelated littermates may be influenced by maternal investment by the natal mother prior to cross-fostering. However, if this were the case the result would be either a lack of a significant association between unrelated littermates or a weaker association than was actually found. Thus, the actual relationship between fostered pups and the mother's own pups within a litter may be slightly stronger than was found in this study.

Heritability of a character is estimated by comparing values of the trait between relatives. But, if relatives are raised in the same environment, the heritability estimate may be biased because it includes both environmental and genetic effects on the trait in

question (Smith and Wettermark 1995) . In our study, cross-fostering eliminates the shared environment between siblings during the energetically costly lactation period, and thus, allows for examination of genetic relatedness at the exclusion of potentially confounding maternal effects. Of course, maternal effects during gestation are possible, so this estimate of heritability may still include some environmental variation. Heritability estimates can also be biased by dominance when comparing traits of full-sibs. However, this would result in inflation of the heritability estimate, therefore estimates derived from regressions involving full-sibs are a maximum estimate (Falconer and Mackay 1996).

In our study, heritability of juvenile weaning weight and growth rates was estimated as zero. Low heritabilities are often calculated from traits that have higher fitness consequences (Falconer and Mackay 1996, Merila and Sheldon 2000). Although the relationships between weaning weights or growth rates and reproductive success have not been examined in *S. columbianus*, the impact of weight on survival, a correlate of fitness, has been explored. Weight attained by juveniles prior to hibernation has a significant impact on over-winter survival (Murie and Boag 1984) and over-winter survival of juveniles accounts for the majority of variation in a female's lifetime reproductive success (King et al. 1991).

Future research involving maternal effects on offspring mass and growth rate should attempt to discern between environmental and genetic maternal effects. Since maternal effects are phenotypic contributions to offspring, they themselves consist of both environmental and genetic components (Krist 2004). An evolutionary response to

selection occurs only when the trait consists of heritable variation (Stearns 1992); thus, genetic maternal effects can have important evolutionary consequences. For example, selection can act on heritable maternal effects, which can alter the relationship between the additive genetic variation and phenotypic variation of a trait and result in changes in evolutionary rates (Wolf et al. 1998).

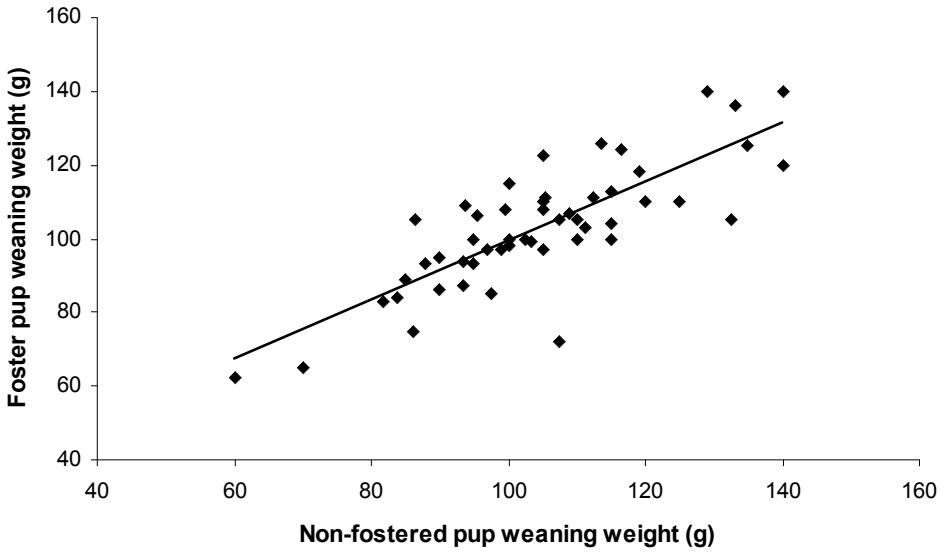


Fig. 1 Relationship between weights of unrelated littermates at weaning. Litters are comprised of both foster pups and non-fostered pups. Foster pups are pups that were transferred from another litter and non-fostered pups refer to pups that remained with the birth mother. Weights were averaged within litters if more than one foster or non-fostered pup survived to weaning. Weight is measured in grams. A mother's own pup weight explained 64% of the variation in foster pup weight ($P<0.0001$).

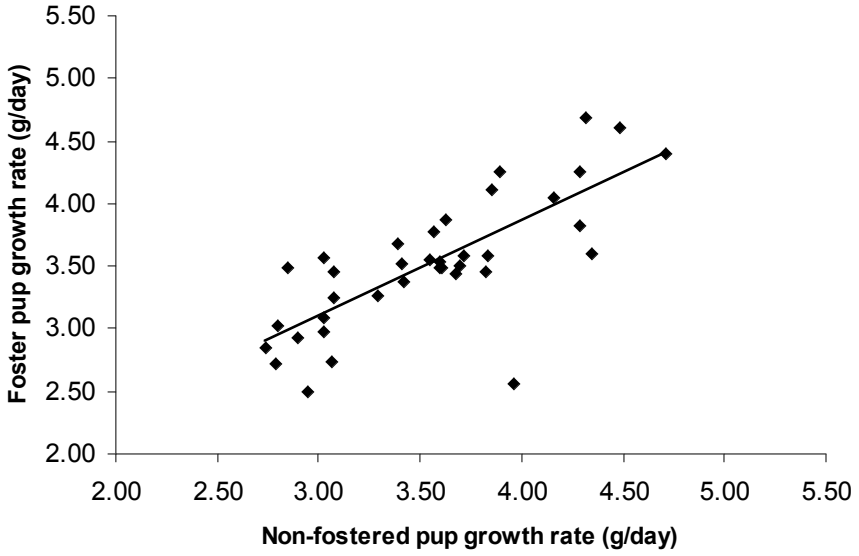


Fig. 2 Plot of association between foster and non-fostered pup growth rates. Growth rates were calculated by subtracting weight at birth from weight at weaning and dividing by duration of the lactation period. Lactation duration is the number of days elapsed from birth to litter emergence. Foster pups are pups that were fostered into that litter. Non-fostered pups are pups that were not cross-fostered. Growth rates were averaged when there were multiple foster or non-fostered pups in a litter at weaning. The relationship between foster and non-fostered growth rates was positive and significant ($R^2 = 0.58$, $P<0.0001$).

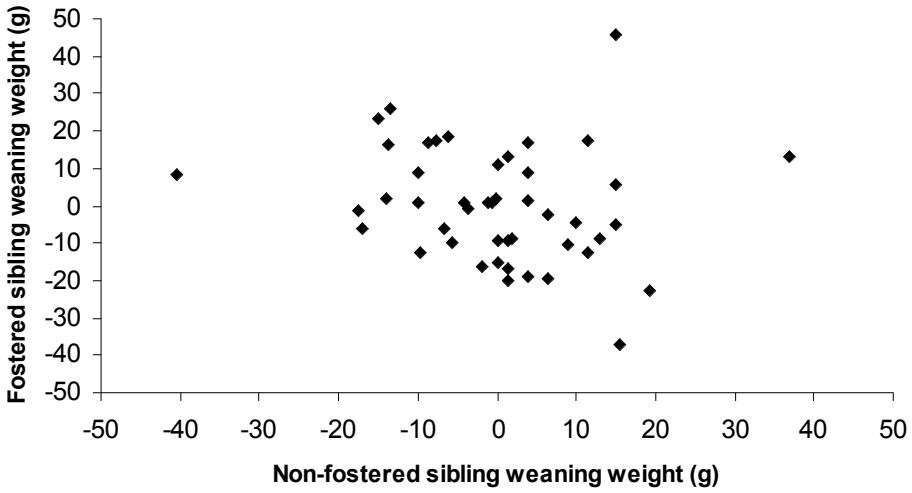


Fig. 3 Regression of fostered sibling weaning weight on non-fostered sibling weaning weight. Non-fostered siblings are pups that remained with the natal mother. Fostered siblings are the siblings of the non-fostered pups that were fostered into another litter after birth. Weights were averaged if more than one fostered sibling or own sibling survived to weaning. Weaning weights standardized for litter size at weaning were used in the regression, which were residuals obtained from ANOVA with litter size at weaning as the class variable. Non-fostered sibling weaning weight only explained 3% of the variation in fostered sibling weaning weight (df = 1,44, $P = 0.22$).

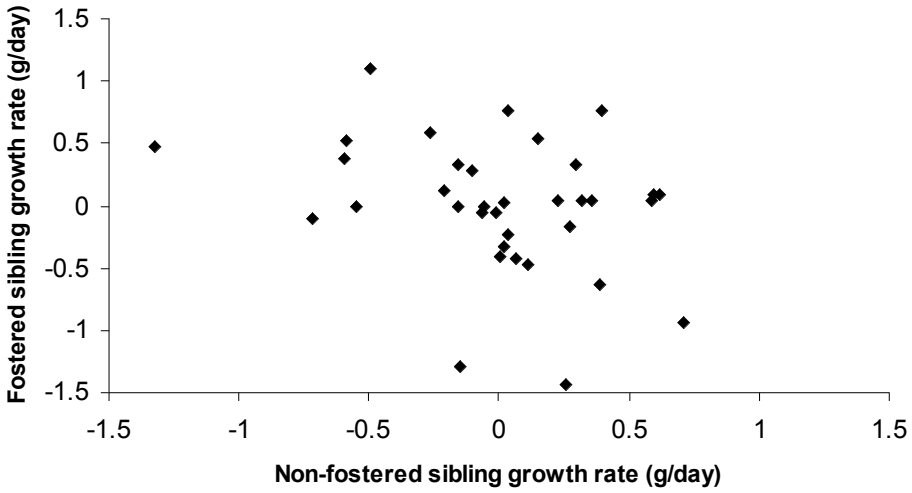


Fig. 4 Relationship between non-fostered sibling growth rate and fostered sibling growth rate. Non-fostered siblings are pups that remained with the natal mother. Fostered siblings are the siblings of non-fostered pups and were transferred into another litter after birth. Growth rates were calculated as the difference between the weaning weight and birth weight and dividing by length of the lactation period. Duration of lactation was estimated as the litter emergence date minus the litter birth date. Data standardized for litter size was used in the regression and was obtained by extracting the residuals from an ANOVA using respective litter size at weaning as the grouping variable. Non-fostered sibling growth rate did not explain a significant amount of variation in fostered sibling growth rate ($R^2 = 0.10$, $df = 1,33$, $P = 0.07$).

CHAPTER TWO

EFFECTS OF MATERNAL CHARACTERISTICS ON OFFSPRING WEIGHT, GROWTH RATE, AND SURVIVAL IN COLUMBIAN GROUND SQUIRRELS

Abstract. Offspring phenotype is influenced by an individual's own genetic composition, its environment and the phenotype of its mother. Therefore, variation in maternal characteristics may substantially alter phenotypic expression of her offspring. In this study we tested possible relationships between maternal body condition, structural size, change in mass during reproduction, timing of reproduction, and litter size on offspring traits. Offspring traits were weaning weight and rate of weight gain during the nursing period. To assess if maternal investment in offspring traits extended beyond the period of maternal care, we looked for associations between offspring and maternal traits and over-winter survival of pups and pup mass at spring emergence from hibernation the following year. Maternal variables explained a significant amount of variation in both average pup weaning weight (R^2 = 0.69) and growth rate (R^2 = 0.72) but not pup spring mass (R^2 = 0.34). Patterns of investment on growth rate and weaning weight were similar. Maternal body condition and litter size explained the most variation in pup growth rate (condition: p = 0.28; litter size: p = -0.88) and weaning weight (condition: p = 0.34; litter size: p = -0.85). Pup growth rate had a positive effect on survival to yearling age ($F_{[1, 111]}$ = 4.57, *P*

= 0.03), whereas pup weaning weight and maternal characteristics had no effect on survival.

INTRODUCTION

Maternal effects occur when a mother's phenotype impacts the phenotype of her offspring (Bernardo 1996a). For example, a mother's access to high quality food resources may affect her condition, which can influence body mass or growth rate of her offspring. Growth rate and weight during early postnatal development are important because they are often related to survival, especially through the first winter (Clutton-Brock et al. 1982, Murie and Boag 1984, Côté and Festa-Bianchet 2001). Growth rate and weaning weight can also have persistent effects, as indicated by their correlation to adult body size (Clutton-Brock et al. 1982, Myers and Master 1983). Larger adult size can result in greater reproductive success, possibly through stronger competitive abilities and differential procurement of mates (Clutton-Brock et al. 1982).

Variation in offspring phenotypes due to maternal effects can be caused by variation in maternal behaviors, such as parental care or timing of reproduction or through specific maternal traits that impact offspring traits. Several factors, such as maternal size, parity, age, and timing of the reproductive season are known to influence offspring weight and growth rate in mammals. In many mammalian species maternal body mass is positively correlated with offspring mass (Skogland 1984, Hoogland 1995, Côté and Festa-Bianchet 2001) and growth rate (Mattingly and McClure 1982, Myers and Master 1983, Bowen et al. 2001), which could be attributed to the enhanced ability of larger females to acquire resources and provide food for their young (Mattingly and McClure 1982).

In some mammalian species, offspring born earlier in the reproductive season have lower mass gain during lactation (Zwank and Zeno 1986) and timing of reproduction is negatively correlated with offspring mass (Fairbanks 1993, Côté and Festa-Bianchet 2001). In ground-dwelling sciurids, however, females that reproduce earlier often give birth to offspring that are lighter than later born individuals (Dobson and Michener 1995, Rieger 1996). Mating earlier may be advantageous because it allows females and juveniles more time to grow and acquire fat reserves before the onset of the winter months (Murie and Boag 1984, Millesi et al. 1999, Côté and Festa-Bianchet 2001).

Dobson et al. (1999) examined the influences of maternal body size and timing of reproduction on offspring mass, litter size, and litter mass in Columbian ground squirrels, but found that maternal characteristics did not explain significant amounts of variation in average offspring mass. The lack of significance is surprising considering maternal effects constitute a large proportion of the variation in both offspring weaning mass and growth rate during lactation (Skibiel 2007). In addition, Dobson et al. (1999) did not find an association between offspring survival and pup weaning mass. The lack of significant patterns between maternal traits and offspring weight and between weight and survival may be attributed to the location of the population. The population studied occurs at a much lower elevation than the population in this study (695 m versus 1550 m in the current study) and differences in life history traits among ground squirrel populations at different elevations have been documented (Murie and Harris 1982, Dobson and Murie 1987, Dobson et al. 1992). In addition, Dobson et al. (1999) did not take number of

offspring into account in the analyses, which could have influenced the results. Litter size has significant influences on offspring size (see results) and therefore should be controlled for statistically. Thus, further examination of the effects of maternal traits on offspring traits and survival in Columbian ground squirrels is warranted.

The first objective of this study was to test causal hypotheses of relationships between maternal size, change in mass during the reproductive season, and offspring weaning weight and growth rate, which was conducted through path analysis. Since mass can reflect both physiological condition and/or structural size (Dobson 1992, Dobson and Michener 1995, Dobson et al. 1999), body mass was partitioned into these two constituents. Body condition is a measure of the energetic composition of an organism in the form of muscle mass (Perrins and McCleery 2001), fat reserves, protein, and water (Dobson 1992, Schulte-Hostedde et al. 2005), whereas structural size represents appendage length or skeletal size (Dobson 1992). Since females of the same structural size can vary greatly in the amount of energy stores in the body, analyzing the influence of both maternal structural size and body condition may shed light on patterns of variation in reproductive output (Dobson and Michener 1995).

Since mothers in better condition, mothers with higher weight gain during reproduction, or larger mothers likely have more or better quality resources available to them, they should invest more of the available energy into reproduction (Hirschfield and Tinkle 1975, Price 1998). Thus, we predicted that females in better condition, larger females, and females with higher gain in mass during reproduction would raise offspring with greater weaning weights and higher growth rates during lactation. We also expected

a positive influence of reproductive timing on offspring weaning weight and growth rate.

The second objective was to test the hypothesis that maternal influences on offspring traits persist to yearling age. This was accomplished by examining influences of maternal and offspring traits on survival to yearling age and on spring mass of pups at emergence from their first hibernation. We predicted that survival and spring emergence mass of pups would be positively associated with offspring weaning weight, growth rate, maternal condition, mass gain, and structural size. We predicted a negative association between mating date and survival of offspring and between mating date and spring emergence mass of pups the following year.

MATERIALS AND METHODS

Study location

Field studies of Columbian ground squirrels were conducted during the years 1992-2006. The population inhabited a meadow (110°W 50°N; elevation, 1550 m) adjacent to Gorge Creek in the Sheep River Provincial Park, Alberta, Canada.

Field methods

Ground squirrels were captured at spring emergence (April-May) by placing live-traps (Tomahawk #201 collapsible chipmunk trap, 16 x 5 x 5 cm^3) baited with peanut butter near burrow openings. All individuals were marked with numbered fingerling ear tags, weighed using a Pesola scale, and given unique body markings using hair dye (Lady Clairol Hydrience #52 black pearl). Zygomatic arch breadth was measured in 1993,

1994, and 2003-2006. In June and early July, juveniles were captured at emergence from natal burrows using the same techniques described above and also were marked with unique numbered ear tags. Time of litter emergence was estimated by adding 51 days to the mating date: 24 days from mating to parturition (Murie and Harris 1982) and 27 days from parturition to litter emergence (Murie 1992). Mating date was determined by examination of external reproductive morphology (Murie and Harris 1982) or by observations of pre-copulatory behavior, such as male interest in the female prior to but not following copulation, males entering burrows with estrous females, or by observation of a copulatory plug. If a juvenile was captured the following spring after emergence from a hibernaculum it was recorded as surviving its first hibernation.

In 1993, 1994, 1996, 1999, 2000, and 2003-2006, females that copulated were captured prior to parturition (May-June), weighed, and housed in the laboratory until birth (1-9 days). Females were kept in polycarbonate microvent rat cages (Allentown Caging Equipment Company; 267 x 483 x 203 mm^3) on pine chip bedding and were supplemented with newspaper nesting material. To obscure vision from neighboring females and to simulate the burrow environment, cages were covered in vented black plastic bags and stored in a temperature controlled room. Squirrels were fed a high protein horse feed (oats, barley, wheat, and compressed vegetable material in a molasses mix; 13% crude protein) ad libitum and lettuce and apple twice daily. Cages were checked for pups 3-4 times per day from 0530-2230 hrs. Females and neonates were weighed at least 4 hours after birth (to ensure that parturition was complete). Neonates were marked by removing a small amount of tissue from the outer right or left toe bud on

a hind limb or from the tail. The nail does not grow on the toe where tissue was removed and a knot appears at the end of a tail that had tissue removed, allowing for identification of pups at emergence from the natal burrows. Mothers were released on the meadow by opening the cage containing mother and pups and in the event that the mother did not retrieve the pups from the cage, pups were placed in the natal burrow following immergence of the mother into that burrow.

Variables

Maternal characteristics examined included body condition, structural size, mass change during reproduction and timing of reproduction. Both body mass and zygomatic arch breadth were measured at first capture after spring emergence from hibernation. Since zygomatic arch breadth is associated with other skeletal measurements (Dobson et al. 1999), zygomatic arch breadth was used as an estimate of structural size. In this study females mated, on average, 3.66 days following first capture after spring emergence; therefore measures of mass and zygomatic arch breadth approximate size at the beginning of the gestation period. Body condition was estimated from the residuals of the regression of spring emergence mass on zygomatic arch breadth. Change in mass over the reproduction period is the difference between female's body mass at spring emergence and her mass at the time of pup emergence from the nest burrow. Emergence of pups from nest burrows is an indication of litter weaning and cessation of lactation (Anderson et al. 1976, Murie and Dobson 1987, Michener 1989, Rieger 1996). Timing of reproduction is the julian date that mating occurred. Offspring traits included weaning

weight and growth rate during the nursing period, and pup spring emergence mass the following year. Growth rate was calculated by subtracting weight at birth from weight at weaning and dividing by the length of the lactation period. When more than one pup within a litter survived to weaning, weaning weight and growth rate where averaged within litters. Spring emergence mass of pups is the weight at first capture of offspring following their first hibernation. For analysis of the influence of maternal variables on pup spring emergence mass, mass of pups was averaged within litters when more than one pup within a litter survived to yearling age.

Multiple records for some females were considered independent because 1) other studies show that reproduction in one year does not influence reproduction in the following year (Risch et al. 1995, Dobson et al. 1999, Broussard et al. 2005b) and 2) weaning weight in one year was not associated with weaning weight the following year, litter size in one year was not correlated to litter size the next year, and growth rate in one year was not associated with growth rate the following year (Spearman rank correlation; respectively, $r = 0.17$, $n = 92$, $P = 0.10$; $r = 0.06$, $n = 92$, $P = 0.59$; $r = -0.07$, $n = 22$, $P = 0.77$).

Statistical analysis

All analyses were conducted using SAS statistical software for Windows (SAS Institute 2002). Tests employed included general linear models (PROC GLM, used for analysis of variance [ANOVA]), correlations (PROC CORR), mixed models (PROC MIXED used for logistic regression) and path analysis (STB option of PROC REG). VIF

and COLLIN options of PROC REG were utilized to detect biases due to co-linearity of independent variables. Significance level for all tests was $\alpha = 0.05$. Data were assessed for normality graphically or by performing Shapiro-Wilks tests.

General linear models and Tukey tests for multiple comparisons of means were used to explore differences in maternal characteristics and offspring traits among females of different ages. Yearlings were not included in analyses because only 9 yearlings during the 15 year study raised a litter to weaning and only 2 of those yearlings exhibited characteristics necessary to conduct the path analyses. Yearlings were not pooled with other age groups because studies have found differences in life history traits between yearlings and older females in Columbian ground squirrels (Dobson et al. 1999, Broussard et al. 2005a). Females aged 7-10 were pooled due to small sample sizes.

Path analysis was used to determine the interrelationships among maternal and offspring traits (Fig. 1). Since tradeoffs between litter size and offspring traits were evident (Table 2), litter size was controlled for statistically by including it as an independent variable in the path analyses. Path analysis (Li 1981) involves first diagramming a hypothetical model, *a priori*, that incorporates causal relationships among the variables. The null hypothesis of no causal relationship is tested by the significance of path coefficients. Path coefficients are standardized partial regression coefficients which indicate the magnitude of the effect of the independent variable on the dependent variable, while all other variables are held statistically invariant (Li 1981, Pedhazur 1982, Sokal and Rohlf 1995). Standardized path coefficients allow for the inclusion of

variables measured on different scales, such as mass (in grams) and timing of reproduction (in days).

As in all multiple regression techniques, co-linearity of independent variables in path analysis can result in erroneous conclusions. Co-linearity of independent variables can inflate standard errors of path coefficients, increasing the probability of a type II error, and can also inflate values of the path coefficients. Inflation of standard errors due to co-linearity can be determined by examining variance inflation factors (VIFs) (Petraitis et al. 1996). VIFs greater than 10 indicate biases of standard errors due to collinearity of independent variables (Myers 1990). Biases in values of path coefficients can be detected through condition indices. Condition indices are derived from the eigenvalues of the correlation matrices of independent variables (Petraitis et al. 1996). Condition indexes between 5 and 10 suggest weak biases due to co-linearity while condition indexes between 30 and 100 indicate strong biases (Belsley et al. 1980).

As in Dobson et al. (1999), years were subdivided according to timing of the reproductive season and path analyses were conducted separately for years of early and years of late reproduction, adjusting variables for effects of years among early years and among late years. Years were categorized as early (years 1992, 1994-2000, 2005) if the mean date of mating for that year was before the mean date of mating for all years in the study. Late years (years 1993, 2001-2003, 2005, 2006) were years in which mean mating date was after the mean mating date of the entire study. Mean Julian date for early years was 117 and mean date for late years was 122. Since patterns of maternal investment did not differ between early and late years, all years were combined to perform path analyses.

Differences in maternal and offspring characteristics were first examined for variation among years in the combined data set using general linear models.

Possible relationships between offspring growth rate and weaning weight and spring emergence mass of pups were examined by conducting Spearman rank correlations. When more than one pup in a litter survived to yearling age (n = 27 litters out of 116 litters with surviving pups), one pup was randomly selected for inclusion in the analysis. This resulted in removal of 31 pups out of 109 pups with data on spring emergence mass.

Mixed model logistic regression was used to assess influences of maternal traits and offspring growth rate and weaning weight on survival to yearling age. Year and litter identification number (litter ID) were included in the model as random variables. This statistically removed the effect of the year on offspring survival and controlled for non-independent data, due to use of multiple pups within litters. Litter ID was a number arbitrarily assigned to each litter.

RESULTS

Spring mass, date of mating, and zygomatic arch breadth differed significantly among females of different ages, while mass change during reproduction, body condition, litter size at weaning, average weaning weight, average growth rate of offspring, and average pup spring emergence mass did not. On average, two year olds were lighter at spring emergence, had later mating dates, and had narrower zygomatic arch breadths than females in other age classes (Table 1). Tukey's test also revealed a significant difference

in zygomatic arch breadth between the 3-year old and 6-year old age classes. Because females older than 2-years did not differ in any maternal or offspring traits (except zygomatic arch breadth, above) and because 2-year olds did not differ from older females in any of the dependent variables (*viz.* offspring traits) used in the path analyses, females of all age classes were pooled for further analyses.

Significant variation among years was detected for mating date ($F_{[13, 146]} = 5.87$, $P < 0.0001$), mother's mass near the time of pup weaning ($F_{[11, 99]} = 3.88$, $P < 0.0001$), zygomatic arch breadth ($F_{[7, 110]} = 3.72$, $P = 0.001$), and maternal spring mass ($F_{[13, 160]} = 1.89$, $P = 0.03$), but not for mother's body condition at spring emergence ($F_{[7, 109]} = 1.04$, $P = 0.41$), litter size at weaning ($F_{[12, 92]} = 0.76$, $P = 0.69$), pup weaning weight ($F_{[12, 92]} = 1.09$, $P = 0.38$), offspring growth rate ($F_{[5, 52]} = 0.43$, $P = 0.82$), or spring emergence mass of pups the following year ($F_{[8, 35]} = 1.67$, $P = 0.14$). Therefore, spring mass, mating date, mother's mass at pup weaning, and zygomatic arch breadth were standardized for years by using residuals extracted from the ANOVA, where year was the grouping factor. Mass change during reproduction was calculated by subtracting mother's spring mass from mother's mass at pup weaning, both of which were adjusted for year effects. Of the females that raised a litter to weaning, 99% gained mass from spring emergence to the time of pup weaning (n = 248). All variables were normally distributed, except average pup growth rate, which was log transformed for subsequent analyses.

Physiological condition of females was estimated by extracting residuals from the regression of spring mass on zygomatic arch breadth. Spring mass increased with

zygomatic arch breadth (R^2 = 0.41, n = 118, P < 0.0001). Residuals were normally distributed (W = 0.99, n = 118, P = 0.30). Therefore, residuals were used as an index of body condition in analyses.

Maternal traits explained 69% of the variation in offspring weaning weight, which was significant (n = 66, P <0.0001). A female's body condition and litter size had significant effects on offspring weaning weight (condition: p = 0.34, P = 0.0007; litter size: p = -0.85, P <0.0001; Fig. 2a). Effects of mating date and structural size on weaning weight approached significance (mating date: p = 0.14, P = 0.07; structural size: p = 0.14, P = 0.07). Similar patterns were evident for maternal effects on pup growth rate. Maternal traits explained a high and significant amount of variation in average pup growth rate (R^2 = 0.72, n = 55, P < 0.0001). Maternal body condition at spring emergence and litter size contributed significantly to variation in offspring growth rate (respectively; p = 0.28, P = 0.009; p = -0.88, P < 0.0001; Fig. 2b). There was a trend for a positive effect of mating date and structural size on growth rate, but these paths were not significant (mating date: p = 0.14, P = 0.07; structural size: p = 0.15, P = 0.07).

Maternal traits did not explain a significant amount of variation in average pup spring mass (R^2 = 0.34, n = 28, P = 0.08), although sample size was small. Due to lack of significance, results of the path analysis are not presented. In addition, there was a lack of an association between a pup's weaning weight and its spring emergence mass (r = 0.16, n = 87, P = 0.14) and between a pup's growth rate and its mass at spring emergence (r = 0.33, n = 32, P = 0.07).

Of the independent variables included in the path analysis, body condition and change in mass were highly negatively correlated (Table 2, Fig. 2). Females in better condition at spring emergence had lower mass gain during reproduction. Growth rate and weaning weight were also highly negatively correlated with litter size, growth rate was highly positively correlated with weaning weight, and spring emergence of pups from hibernation was positively associated with growth rate (Table 2).

Despite inter-correlations among independent variables, co-linearity was not likely a cause of strong bias in estimation of path coefficients or their standard errors. The maximum VIF for the path analysis of weaning weight was 1.84, for growth rate was 1.95, and for pup spring mass was 1.49, which are well under the value of 10 recommended by Petraitis *et al.* (1996). In addition, the highest condition indices for path models were 6.47, 6.43, and 6.94 (weaning weight, growth rate, and pup spring mass, respectively). Condition indices ranging from 5 to 10 indicate slight inflation of path coefficients while condition indices greater than 30 suggest high inflation of path coefficients (Belsley et al. 1980).

Pup growth rate during the nursing period had a positive effect on survival ($F_{[1, 111]} = 4.57$, $P = 0.03$). However, pup weaning weight did not affect survival to yearling age ($F_{[1, 247]} = 3.54$, $P = 0.06$). Neither litter size at weaning nor any of the maternal traits had an effect on pup survival (litter size: $F_{[1, 136]} = 1.22$, $P = 0.27$; mating date: $F_{[1, 136]} = 2.93$, $P = 0.09$; structural size: $F_{[1, 136]} = 1.41$, $P = 0.24$; mass change during reproduction: $F_{[1, 136]} = 1.18$, $P = 0.28$; condition: $F_{[1, 136]} = 0.07$, $P = 0.79$).

DISCUSSION

Before testing causal relationships of maternal traits on offspring traits, age-specific differences in maternal variables were evaluated. Unlike other studies of Columbian ground squirrels that only found differences in maternal traits between yearlings and older females (Dobson et al. 1999, Broussard et al. 2003), we found differences between 2-year olds and older females in structural size, timing of reproduction, and spring emergence mass. Populations of Columbian ground squirrels inhabiting higher latitudes and higher elevations consist of few breeding yearlings (Dobson and Murie 1987). Thus, 2 year olds are likely primiparous females, which tend to be lighter at spring emergence and emerge later from hibernation (Table 1).

Path analysis resulted in different conclusions regarding influences of maternal traits on offspring mass and growth rate than did simple correlations. In correlation analysis maternal body condition at spring emergence was not associated with offspring weaning weight or growth rate but was significant in path analyses. Significant effects appearing in path analyses but not correlation is likely due to the path analysis testing individual pathways by holding other paths statistically invariant. This controls for non-significant correlations among independent variables, which otherwise might mask significant paths (Dobson et al. 1999).

Maternal characteristics explained a significant amount of variation in pup weaning weight and growth rate. Path analysis revealed a significant positive effect of a mother's body condition on both weaning weight and growth rate (Fig. 2). This corroborates findings in a study of Richardson's ground squirrels where a positive

correlation was found between average offspring mass and maternal body condition (Dobson and Michener 1995). As expected in the path analyses, litter size was significantly negatively correlated with both weaning weight and growth rate. In many mammalian species tradeoffs exist between offspring size or growth and number, such that litter size increases at the expense of individual size or growth (Cameron 1973, Michener 1989, Rieger 1996).

The lack of significant paths from structural size and change in mass to weaning weight and growth rate was somewhat surprising. In Richardson's ground squirrels, maternal mass gain from estrus to birth had positive effects on neonate mass (Dobson and Michener 1995) and in Columbian ground squirrels both yearling and older mothers that gained more weight during reproduction produced larger litters (Dobson et al. 1999). Females that gained more weight during reproduction were expected to raise larger individual pups with higher growth rates because the additional energy acquired could be invested in the litter. Larger females were expected to produce larger pups with higher mass gain, possibly reflecting better competitive ability to obtain higher quality territories (Murie and Harris 1988, Boag and Wiggett 1994). It appears, however, that body condition is more important in determining pup weaning weight and growth rate than is gain in mass or structural size.

Timing of reproduction has been shown to influence offspring mass in several species of ground dwelling sciurids. In both Richardson's ground squirrels (Dobson and Michener 1995) and Uinta ground squirrels (Rieger 1996), earlier reproduction resulted in lighter offspring but larger litters. A seasonal decline in litter size, such that as the

active season progresses litter size becomes smaller, with a concomitant increase in individual offspring size as the season progresses, was expected in this population of Columbian ground squirrels. However, we found a lack of a significant relationship between mating date and offspring mass and growth rate. In another study of Columbian ground squirrels, reproductive timing was not found to significantly influence litter size when data from all years of the study were combined (Dobson et al. 1999). However, when data were sorted by timing of the reproductive season, a significant relationship between mating date and litter size in years of early breeding was revealed. In this population of Columbian ground squirrels, there was no difference in patterns of investment when years were categorized by the timing of the breeding season. Thus, there appears to be no effect of reproductive seasonality, either timing of breeding within a season or timing of the breeding season among years, on offspring weaning weight or growth rate.

While standard errors of path coefficients were not likely biased in the path analyses [variance inflation factors were within the range suggested by Petraitis *et al.* (1996)], the highest condition indices for both path analyses were around 6, suggesting slight inflation of path coefficients. High condition indices were for paths from maternal change in mass during the reproductive season to offspring weaning weight and growth rate. Biases due to co-linearity should have elevated path coefficients for these paths, which was opposite the evident pattern. Since path coefficients were still not significant, despite possible inflation, conclusions regarding the effect of maternal mass change on offspring traits were not influenced by this bias.

Maternal traits did not explain a significant amount of the variation in mass of pups at spring emergence from hibernation. However, this could be attributed to the small sample size. Offspring growth rate and weaning weight were also not associated with pup spring mass. These results suggest that maternal influences on growth rates and weaning weights of their young dissipate over time. This opposes the findings for red deer and deer mice that juvenile body size or growth is correlated to adult body size (Clutton-Brock et al. 1982, Myers and Master 1983).

Although offspring traits were not associated with mass of the pup at spring emergence, they may be indicators of survival. Pups that grew faster during the nursing period survived more than slower growing pups. Pup weaning weight also showed a positive trend, whereby heavier pups had a better chance of survival to yearling age, although this association was not significant. Unlike the lower elevation population studied by Dobson et al. (1999), we found that litter size and maternal characteristics did not have an effect on the over-winter survival of pups.

In this population of Columbian ground squirrels, mothers invest in their offspring by altering growth rates and weaning weights of their young. Particularly, mothers in better condition at spring emergence from hibernation produce pups that grow faster during the period of maternal care and weigh more at weaning than pups born from mothers in poor body condition. We also found that effects of maternal investment on offspring growth rate and weaning weight do not appear to persist to yearling age, in that they are not associated with pup mass at emergence from hibernation. However, growth rate appears to be especially important because of its influence on offspring survival.

Pups that grow faster during the nursing period survive more than pups that grow at a slower rate.

These results are different from the maternal investment patterns described by Dobson et al. (1999). Dobson et al. (1999) found that both yearling and older females alter reproductive output by producing larger litters rather than producing heavier young. None of the maternal characteristics we examined were correlated with litter size at weaning, suggesting that mothers do not increase reproductive output by altering litter size. In addition, Dobson et al. (1999) suggested that litter size was the best predictor of survival to yearling age whereas our results suggest that offspring growth rate is the best predictor of survival. It is currently unclear if differences in maternal investment patterns between the two populations are due to differences in location of the populations or due to differences in treatment of variables in statistical analyses. Further research is necessary to determine cause of differences in maternal investment patterns among populations of Columbian ground squirrels.

TABLE 1. Averages of maternal characteristics and offspring traits for each age class ± 1 standard error of the mean. Sample sizes are in parentheses. Females age 7-10 were pooled due to low sample sizes. Mass change is the difference between spring emergence mass and mass at litter emergence. Body condition was estimated from residuals of the regression of mass at spring emergence on zygomatic arch breadth. Growth rate was calculated by subtracting birth weight from weaning weight and dividing by the duration of the lactation period. F-values, degrees of freedom, and P-values correspond to results of ANOVAs testing differences in variables among age classes. P-values were significant at $\alpha = 0.05$.

	Age class (years)								
Variable	2	3	4	5	6	7-10	*F*	*d.f.*	*P*
Spring mass* (g)	389 ± 4.7 (79)	425 ± 5.1 (56)	434 ± 6.4 (48)	442 ± 9.7 (48)	443 ± 9.3 (27)	445 ± 7.4 (39)	16.2	5, 291	<0.0001
Mating date*	122 ± 0.7 (67)	119 ± 0.6 (55)	119 ± 1.0 (48)	118 ± 0.8 (47)	117 ± 1.0 (28)	117 ± 1.1 (38)	4.9	5, 277	0.0003
Mass change	86 ± 7.5 (41)	81 ± 8.8 (39)	73 ± 8.5 (33)	110 ± 8.6 (40)	83 ± 22.7 (26)	97 ± 12.8 (30)	1.5	5, 203	0.19

Structural size* (mm)	33.2 ± 0.16 (35)	33.9 ± 0.16 (19)	34.2 ± 0.11 (21)	34.4 ± 0.14 (21)	34.9 ± 0.19 (7)	34.5 ± 0.16 (15)	13.8	5, 112	<0.0001
Body condition	-0.20 ± 6.5 (35)	6.33 ± 7.88 (19)	3.20 ± 6.91 (20)	-0.32 ± 7.33 (21)	-16.03 ± 15.92 (7)	-8.11 ± 12.35 (15)	0.53	5, 111	0.75
Litter size	2.63 ± 0.11 (41)	2.83 ± 0.16 (35)	2.75 ± 0.16 (32)	2.86 ± 0.16 (37)	3.33 ± 0.25 (24)	3.08 ± 0.22 (25)	2.0	5, 188	0.08
Weaning weight (g)	109 ± 3.9 (41)	110 ± 3.4 (35)	114 ± 3.1 (32)	116 ± 3.4 (36)	109 ± 3.7 (24)	109 ± 5.4 (25)	0.7	5, 187	0.63
Growth rate (g/day)	3.81 ± 0.21 (18)	3.82 ± 0.33 (11)	4.34 ± 0.24 (9)	3.85 ± 0.20 (17)	3.56 ± 0.18 (9)	3.85 ± 0.31 (13)	0.7	5, 71	0.60
Pup spring mass (g)	245 ± 6.3 (13)	270 ± 7.1 (21)	255 ± 7.3 (16)	257 ± 8.9 (19)	255 ± 10.6 (10)	265 ± 13.2 (10)	1.03	5, 83	0.41

*Significant differences between 2-year olds and all other age classes (Tukey multiple range tests). Tukey's test also showed a significant difference between 3-year olds and 6-year olds in structural size.

TABLE 2. Correlations of variables used in path analyses. P-values are located under the correlation coefficients. Sample size is in parentheses. P-values less than 0.05 were considered significant. Mass change is the difference between spring emergence mass and mass at litter emergence. Body condition was estimated from residuals of the regression of mass at spring emergence on zygomatic arch breadth. Growth rate is the gain in mass during lactation divided by lactation length. Structural size, mating date, and mass change are year standardized. Growth rate is log transformed.

	Mating date	Mass change	Zygomatic Breadth (mm)	Wean weight (g)	Growth rate (g/day)	Litter size	Pup spring mass (g)
Condition	-0.02 0.81 (110)	-0.62 <0.0001 (66)	0.01 0.88 (117)	0.11 0.35 (70)	0. 21 0.38 (55)	0.12 0.31 (70)	-0.10 0.58 (30)
Mating date	--	0.14 0.17 (107)	-0.16 0.09 (111)	0.01 0.90 (105)	0.04 0.74 (58)	0.001 0.99 (105)	-0.21 0.17 (44)
Mass change		--	0.15 0.22 (66)	-0.08 0.42 (101)	-0.17 0.20 (58)	0.17 0.10 (101)	-0.17 0.27 (42)
Zygomatic breadth			--	0.04 0.74 (70)	0.006 0.96 (55)	0.18 0.13 (70)	0.004 0.98 (30)
Weaning weight				--	0.96 <0.0001 (58)	-0.65 <0.0001 (105)	0.25 0.10 (44)

Growth rate	--	-0.80 <0.0001 (58)	0.46 0.04 (21)
Litter size		--	-0.24 0.12 (44)

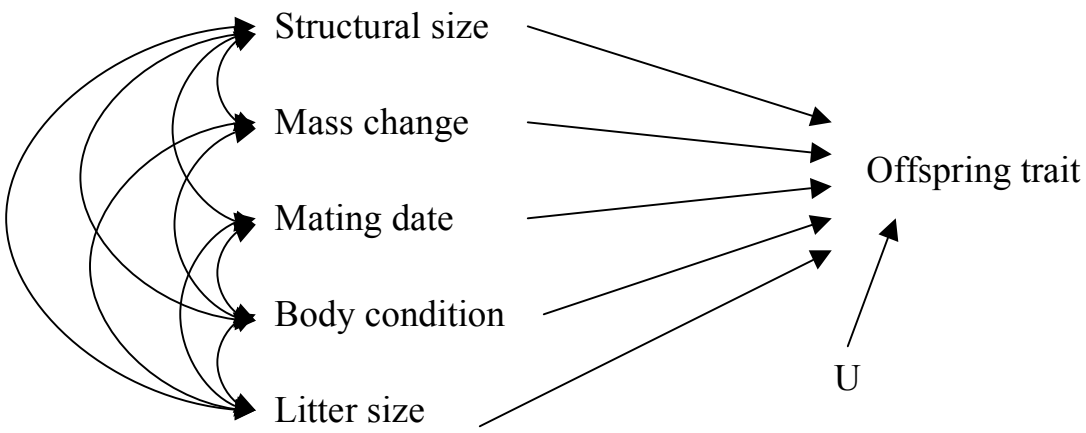
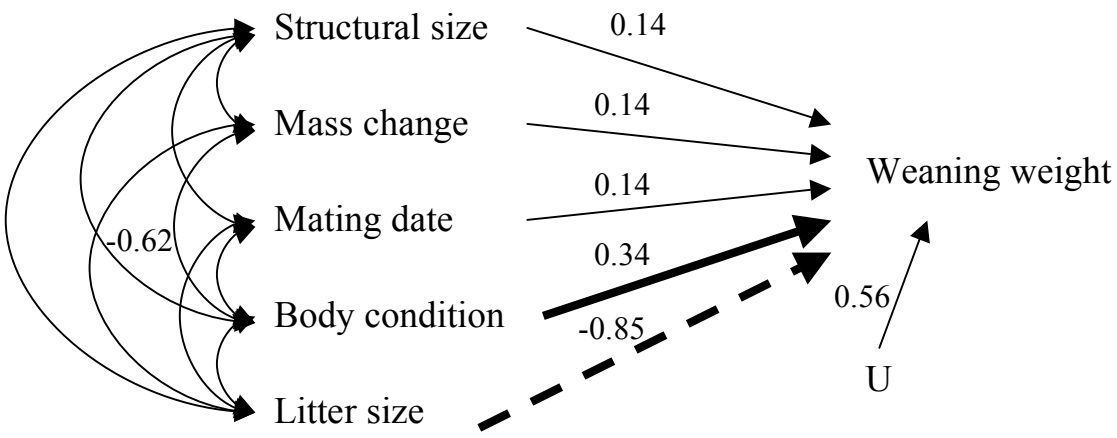


FIG. 1. Path model of the effects of maternal characteristics and litter size on offspring traits. Single headed-arrows represent paths from independent variables to the dependent variable. Double headed-arrows represent correlations between independent variables. Mass change is the difference between spring emergence mass and mass at litter emergence. Body condition was estimated from residuals of the regression of mass at spring emergence on zygomatic arch breadth. Offspring traits are average pup weaning weight, average pup growth rate, and average pup mass at spring emergence from hibernation. U is the path coefficient for unexplained variation in offspring traits and was calculated by the equation $\sqrt{1-r^2}$ (Li 1981)

A.



B.

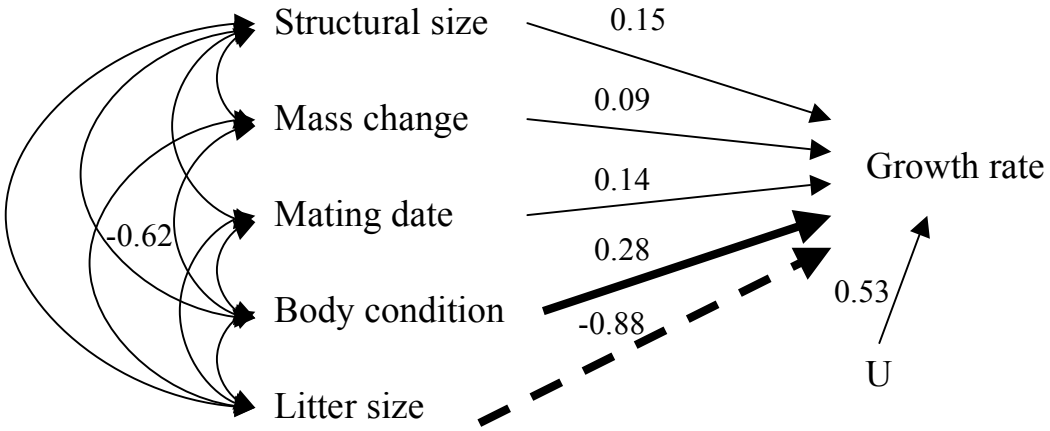


FIG. 2. Path models for the influence of maternal characteristics on (A) offspring weaning weight (n = 66) (B) offspring growth rate (n = 55). Mass change is the difference between spring emergence mass and mass at litter emergence, both adjusted for among year variation. Body condition was estimated from residuals of the regression of mass at spring emergence on zygomatic arch breadth. Structural size and mating date are adjusted for year effects. Growth rate is log transformed. Path coefficients are given

to the right of maternal characteristics. Mass change and body condition are significantly correlated and the correlation coefficient is located to the left of maternal characteristics. U is the path coefficient for unexplained variation in offspring traits and was calculated by the equation $\sqrt{1-r^2}$ (Li 1981). Solid lines are positive paths and dashed lines are negative paths. Bold arrows indicate significant path coefficients.

CONCLUSIONS

Maternal effects are ubiquitous in nature, occurring in many different species of both plants and animals. Maternal effects are a type of environmental influence that can modify offspring phenotypes and might occur through a variety of mechanisms, including directly through maternal traits, through transmission of antibodies or hormones, or through maternal behaviors, such as maternal care (Mather and Jinks 1971, Mousseau and Dingle 1991, Clark and Galef 1995). The current study addressed four questions regarding maternal effects in Columbian ground squirrels: 1) Are maternal effects responsible for variation in offspring phenotypes? 2) How do patterns of maternal investment influence offspring phenotypes? 3) Do influences of maternal characteristics on offspring phenotype dissipate over time? 4) Do maternal and offspring traits affect offspring survival to yearling age?

The first chapter attempted to determine if maternal effects contributed to variation in offspring growth rate and weaning weight. For approximately one month following birth, Columbian ground squirrel pups remain underground in natal burrows, where their only source of nutrition is through the mother's milk. This provides ample opportunities for maternal effects to occur. Therefore, we expected development of pups during the nursing period to be strongly influenced by maternal effects. A cross-fostering design was used to disentangle influences of direct genetic effects (i.e. heritability) from maternal effects on offspring development. In a cross-fostering experiment, litters are

paired at birth and pups are swapped between the litters. Thus, each litter consists of the mother's own pups and unrelated pups that were fostered into the litter.

Non-fostered pups explained 65% and 58% of the variation in weaning weight and growth rate of their unrelated littermates (e.g. fostered pups), respectively. However, some differentiation in weaning weight between unrelated littermates occurred because the slope of the estimated regression line was significantly different from 1.0. This suggested that there were either initial differences in weight between unrelated littermates at birth, before cross-fostering, or that mothers were favoring their own young. However, further analysis revealed that unrelated littermates were not different in birth weight and that mothers did not invest more in their own birth pups. In addition, there was no association between survival to yearling age and type of pup (*viz*., foster or non-fostered). Thus, the differentiation between weaning weights of unrelated littermates was likely an artifact of the type of regression used (Green 2001), rather than to differential investments by mothers to their own pups.

Genetic siblings that were raised in different litters were not similar in either growth rate or weaning weight, resulting in an estimate of heritability of 0. These results indicate that maternal effects were more important in contributing to variation in pup development than were heritable genetic effects. These results are consistent with a study of maternal effects on growth rate and weight in another sciurid species that found low heritability and high contribution of maternal effects (McAdam et al. 2002).

Chapter two sought to examine influences of specific maternal phenotypes, or traits, on offspring weaning weight and growth rate and the persistence of these effects.

Maternal traits investigated included timing of reproduction, body condition, structural size, and change in mass during reproduction. These maternal traits have been shown to influence offspring growth rate and weaning weight in other mammalian species (Zwank and Zeno 1986, Fairbanks 1993, Rieger 1996, Bowen et al. 2001, Côté and Festa-Bianchet 2001). In addition, empirical evidence suggests that both growth rate and weaning weight are important for survival, especially to yearling or reproductive age (Clutton-Brock et al. 1982, Murie and Boag 1984, Côté and Festa-Bianchet 2001).

We found that the only maternal trait to influence growth rate and weaning weight was a female's body condition at spring emergence from hibernation. This finding suggests that body condition is the only maternal trait important in the variation of pup growth rate and weaning weight. Litter size also had a significant influence on growth rate and weaning weight, which was not surprising given the abundance of species that exhibit tradeoffs between litter size and growth rate or weaning weight (Cameron 1973, Michener 1989, Rieger 1996). We also found that only growth rate was associated with survival of pups to yearling age, although weaning weight exhibited a positive, non-significant association with survival. Litter size at weaning and none of the maternal traits investigated were correlated with pup survival.

These results differ from findings of a similar analysis on Columbian ground squirrels inhabiting an area of lower elevation (Dobson et al. 1999). In that study, the maternal traits examined (which were the same as in this study) did not have an influence on offspring weight. However, in older females all maternal traits, except for timing of reproduction, had a positive influence on both litter size and total litter mass at weaning.

Dobson et al. (1999) also found that litter size was the best predictor of the number of surviving pups. In the present study, we found that growth rate had a significant positive effect on offspring survival.

Differences between the two studies on Columbian ground squirrels suggest that patterns of maternal investment may not be general to the whole species, but rather vary across populations and perhaps even among years. In fact, differences may be attributable to differences in elevations among habitats for different populations. Studies on Columbian ground squirrels indicate that differences in life histories occur among different populations at different elevations. Specifically populations occurring at higher elevations have later breeding dates, lower body mass and poorer body condition, smaller litters, higher survival, and females mature at a later age (Murie and Harris 1982, Dobson and Murie 1987, Dobson 1992). More research is necessary to determine if differences in investment patterns by mothers occur among the different elevations at which populations occur.

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