



Original Investigation

Litter sizes of Daurian ground squirrels peak at intermediate body sizes

Xingrong Wan^a, Liang Liu^a, Guiming Wang^{b,*}, Yongwang Guo^c^a State Key Laboratory for Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, China^b Department of Wildlife, Fisheries and Aquaculture, Mail Stop 9690, Mississippi State University, Mississippi State, MS 39762, United States^c National Agro-tech Extensions and Service Center, Chaoyang District, Beijing 100125, China

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ABSTRACT

Litter size and body size of animals may coevolve under the concomitant pressures of sexual selection, fecundity selection, and viability selection. Studies have demonstrated the positive relationship between body size and litter size, consistent with Darwin's fecundity advantage of large body size. However, the counterbalancing selection hypothesis predicts fecundity selection would result in a positive correlation between litter size and body size initially, whereas the opposite pressure by viability selection would decrease litter size with further increasing body size beyond a threshold size. Moreover, the reproduction senescence hypothesis predicts that litter size of old, large females would decline with deteriorating body conditions. In this study, we tested the predictions of the counterbalancing selection hypothesis and the reproduction senescence hypothesis concerning the quadratic relationship between body size and litter size in Daurian ground squirrels (*Spermophilus dauricus*). Litter size increased initially with increasing carcass weight of females, and then decreased with further increases in carcass weight, supporting the prediction of the counterbalancing hypothesis. However, litter size was not related to body condition index of females, and body conditions improved with increasing body weight, suggesting that the reproduction senescence hypothesis alone cannot explain the observed quadratic relationship between litter size and carcass weight of female *S. dauricus*.

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Introduction

Fecundity, such as litter size and annual number of litters, is an important fitness component of animals (Dugdale et al., 2010). In addition to responses to changes in food availability and weather, litter sizes may vary with age, body size, and reproductive experience of reproducing females (Fokidis et al., 2007; Sharp and Clutton-Brock, 2010). Fecundity selection maximizes or increases the fitness of females by increasing female body size and subsequently enhancing the litter size of large-sized females (Blanckenhorn, 2000; Fokidis et al., 2007). Therefore, body size and litter size are two correlated quantitative traits, and previous studies have reported the positive relationship between body size and litter/clutch size in mammals, fish, and birds (Bünger et al., 2005). On the other hand, the cost of reproduction may result in trade-offs between current reproductive investment and future survival of breeding females (Roff, 2002). Thus, viability selection increases

fitness by enhancing survival and may select large body size with small litter size for improvement in survival (Blanckenhorn, 2000; Bonnet et al., 2000). Additional maintenance costs of large-sized individuals may reduce the energetic allocation to reproduction. The counter-balancing selection hypothesis posits that litter sizes of animals would initially increase with increasing body size owing to fecundity selection with the advantage of large body sizes and then would decline with further increases in body size because of viability selection for enhancements of survival (Blanckenhorn, 2000; Bonnet et al., 2000). The counter-balancing selection hypothesis predicts a quadratic relationship between female body size and litter size or the optimal litter size at intermediate body size.

Additionally, nutritional and body conditions of reproducing females influence gestation and the performance of post-parturition maternal care, including lactating, nursing, and protecting newborns, and may determine the number of offspring a female can nurse to weaning (Skibieli et al., 2013; Wauters and Dhondt, 1995). Reproductive senescence occurs in old individuals of mammals (Sharp and Clutton-Brock, 2010). The reproductive senescence hypothesis states that the deteriorating body conditions of old, large females would lead to reduction in litter size.

* Corresponding author. Tel.: +1 662 325 0414; fax: +1 662 325 8726.
E-mail address: gwang@cfr.msstate.edu (G. Wang).

Litter sizes may be limited by the body conditions of either young or old reproducing females. Thus, the reproductive senescence hypothesis also predicts that litter sizes peak at intermediate body sizes if body size represents ages of individuals accurately (Sharp and Clutton-Brock, 2010). The individual optimization hypothesis states that female mammals would optimize litter sizes to produce most offspring that survive to reproduction according to their body conditions (Lack, 1948). Therefore, we predict that litter sizes would be positively related to body condition indices of breeding females, with the assumption that young and old individuals have low body condition index values.

The Daurian ground squirrel (*Spermophilus dauricus*) is a grassland-dwelling sciurid and is the characteristic species of the mammalian fauna of the typical steppe on the Mongolian Plateau (Luo and Zhong, 1990). Daurian ground squirrels are omnivores, live solitarily, and have male-based sexual size dimorphism with body mass ranging from 55 g to 338 g (Liu et al., 1993; Luo and Zhong, 1990; Wang et al., 1994). Daurian ground squirrels hibernate during late autumn and winter from September to mid-March, emerge in early spring from later March to May, reproduce from April through May, and produce one litter a year with average litter size of 5–6 young (Luo and Zhong, 1990). The broad geographic distribution of *S. dauricus* on the Mongolian Plateau, where winter climate varies greatly over years and across space, makes *S. dauricus* an appropriate species to study the energy allocation strategies between reproduction and somatic growth in small mammals. However, no studies, to our knowledge, have investigated the relationship between litter sizes and body sizes or body conditions of *S. dauricus* in the typical steppe.

In this study, we tested the counter-balancing hypothesis that litter sizes of *S. dauricus* would initially increase with increasing body size owing to the advantage of large body sizes and then would decline with further increases in body size because of trade-off between survival and reproduction. We predict that litter sizes of *S. dauricus* would peak at intermediate body sizes. We also tested the prediction that litter size is positively related to body condition index.

Methods and materials

Study site

The study site was located in the typical steppe at Aquiwula, Hexiten Banner (county), Inner Mongolia, China (N 43.4377°, E 116.7651°; elevation 1244 m). The climate was semi-arid, with average annual precipitation being about 350 mm. Temperatures ranged from -40°C to 30°C . Winter was cold and long from October to early April, with snow cover lasting from November through March the following year (Zhong et al., 2007). The vegetation comprised *Leymus chinensis*, *Cleistogenes squarrosa*, *Artemisia frigida*, *Carex evergold*, *Potentilla tanacetifolia*, and *Potentilla biflora*. Grassland was grazed by sheep, cattle, and cows. Avian predators included *Accipiter gentilis*, *Falco cherrug*, and *Asio otus*, and major mammalian predators were *Meles meles*, *Mustela sibirica*, and *Vulpes corsac* on the study site.

Trapping *Spermophilus dauricus*

We trapped *S. dauricus* daily using snap traps (15 cm \times 7 cm) from May 10, 2006 to May 19, 2006. Snap traps were placed in two trap lines 50 m apart. Each trap line had 100 snap traps in 10 m intervals between traps. Traps were set at 0600 h and were checked four times a day. Two trap lines were displaced 2 m away from previous locations every two days to increase capture probabilities. Captured *S. dauricus* was assigned a unique identification

number, and their body mass was measured to the nearest 0.1 g using an electronic balance (Scout SE601F, Ohaus Corp., Parsippany, New Jersey, USA). All captured ground squirrels were transported to a laboratory for dissection. We sexed captured *S. dauricus* and counted fetuses in the uteri of reproducing females. The number of fetuses was used as the litter size of reproducing females. We measured body length as the distance from nose to anus to the nearest 1 mm with a ruler. We determined carcass mass of captured *S. dauricus* to the nearest 0.1 g after removing internal organs, including heart, lungs, intestines, kidneys, and reproductive organs, from all captured squirrels and fetuses from pregnant females. Therefore, the carcass was primarily made of fur, skin, muscle, skeleton, and body fat, which eliminates the confounding effects of different amounts of food in the stomach and intestines of captured *S. dauricus* and different numbers and sizes of fetuses in pregnant females when relating litter sizes to body weight. Our trapping and handling of *S. dauricus* were approved by the Institutional Animal Use and Care Committee (IACUC) of the Institute of Zoology, the Chinese Academy of Sciences.

Statistical analysis

We calculated body condition index using the formula: $\text{BCI} = \log_e(\text{BW})/\log_e(\text{BL})$, where BW is body mass (g) and BL is body length (mm), and \log_e is the natural logarithm (Huot et al., 1995). Labocha et al. (2014) have shown the ratio-based index at log scales performed better than other 16 indices as an index of body fat content in mice (*Mus domesticus*). We used carcass mass to calculate body condition index BCI.

We determined the 25% (Q25) and 75% (Q75) quantiles of the carcass mass distribution of captured female *S. dauricus* using the R function *quantile* (R Development Core Team, 2013). We divided female *S. dauricus* into three weight groups G1–G3 using Q25 and Q75 as criteria: G1 with carcass mass being less than Q25, G2 with carcass mass being greater than Q25 but less than Q75, and G3 with carcass mass being greater than Q75. We compared average log transformed litter sizes and average body condition indices (i.e., BCI) among carcass mass groups G1, G2, and G3 using analysis of variance. Multiple comparisons between the carcass mass groups were conducted with the Bonferroni correction of *t* tests (Zar, 1999). All tests were conducted at the significance level of 0.05.

We used generalized additive models (GAMs) to regress litter size on the smoothing term of carcass mass or body condition index with the quasi Poisson family (Wood, 2006). Because of correlations between carcass mass and BCI, we only built single-covariate GAMs to avoid multicollinearity. We conducted model selection with generalized cross-validation (GCV) score, R^2 value, *P* value of smoothing terms following Wood and Augustin (2002). The best fit model had the lowest GCV score, greatest R^2 value, and significant smoothing terms ($P < 0.05$). We also used generalized linear models (GLMs) to regress litter sizes on the linear and quadratic terms of carcass mass. If body sizes and litter sizes of female *S. dauricus* have a quadratic relationship in GAMs or GLMs, the prediction of counterbalancing hypothesis would be supported. Our data would support the prediction of the reproductive senescence hypothesis if average body condition index of group G3 is less than that of G2 and litter size is positively related to body condition index. If the quadratic relationship is significant, we solved the quadratic regression for carcass mass, at which litter size peaked. All means were presented with ± 1 standard deviation (SD).

Results

We captured 50 females and 26 males during 10 days of trapping. Average total body mass of females was 187.23 ± 22.94 g,

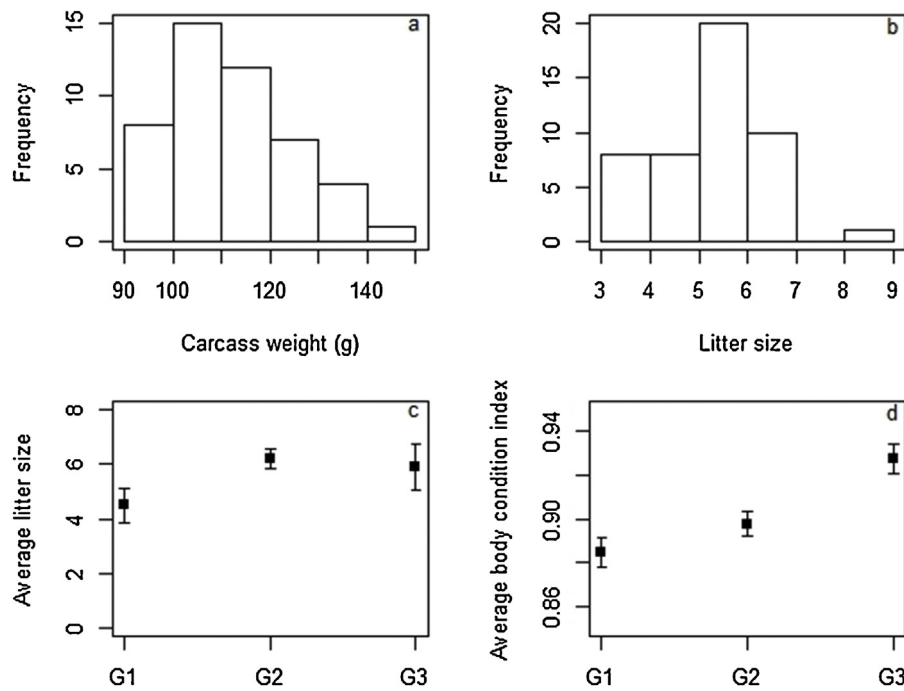


Fig. 1. Frequency distributions of (a) the carcass mass, (b) litter size (number of fetuses), (c) average litter sizes, and (d) average body condition indices of the carcass weight groups of female Daurian ground squirrels in Inner Mongolia, China. Symbols G1, G2, and G3 denote carcass mass groups divided by 25% quantile and 75% of quantile of the carcass mass distribution.

and that of males was 209.43 ± 27.74 g ($t = -3.72$, $df = 74$, $P \leq 0.01$). Average carcass mass of females was 117.09 ± 13.76 g, and that of males was 138.86 ± 19.39 g ($t = -7.50$, $df = 74$, $P \leq 0.01$). The distribution of the carcass mass of females was right skewed (Fig. 1a). The 25% quantile (Q25) was 101.60 g and the 75% quantile (Q75) was 120.5 g. Average carcass mass of the three groups G1, G2, and G3 of females differed, increasing from G1 to G2 to G3 (Table 1). Total body mass was positively related to carcass mass (regression slope = 0.52, $P \leq 0.01$).

Of the 50 females captured, 48 females were in reproductive condition with fetuses in different developmental stages. Litter sizes ranged from 3 to 9 and averaged 5.7 ± 1.23 , with the frequency distribution peaking at 6 (Fig. 1b). Log transformed litter size differed among three groups ($F_{2,44} = 11.77$, $P \leq 0.01$). Logged litter size of group G1 was smaller than that of G2 ($t = -5.5983$, $df = 33$, $P \leq 0.01$) and that of G3 ($t = -2.647$, $df = 22$, $P = 0.015$), whereas logged litter size of G2 did not differ from that of G3 ($t = 1.0553$, $df = 33$, $P = 0.299$; Fig. 1c).

Carcass mass was correlated with body condition index BCI of females (correlation = 0.86, $P \leq 0.01$). Body condition indices differed among three groups of carcass mass (Fig. 1d; $F_{2,44} = 41.88$, $P \leq 0.01$). Average BCI of group G1 was less than that of G2 ($t = -2.9664$, $df = 33$, $P = 0.006$) and that of G3 ($t = -9.6611$, $df = 22$, $P \leq 0.01$), whereas average BCI of G3 also was greater than that of G2 ($t = 6.8774$, $df = 33$, $P \leq 0.01$).

Litter sizes were not related to BCI ($P = 0.17$, $\text{adj. } R^2 = 0.13$) in GAMs, but were related to carcass mass nonlinearly in a convex

shape (Fig. 2a; $P \leq 0.01$, $\text{adj. } R^2 = 0.34$). The relationship between litter size and carcass mass was quadratic in GLMs (Fig. 2b; $P \leq 0.01$). Litter size peaked at the carcass mass of 119.16 g between the Q25 and Q75 (Fig. 2b).

Discussion

Body size coevolves with other life history traits, and thus influences variation in the fitness components of animal individuals, including litter size (Bünger et al., 2005; Roff, 2002). Positive correlation between litter size and body size has been well established in the literature, as predicted by Darwin's fecundity advantage (Bünger et al., 2005; Risch et al., 2007). In contrast, we found a non-linear, quadratic relationship between litter size and body carcass mass. Our findings support the prediction of the counterbalancing selection hypothesis that fecundity selection may increase litter size initially with increasing body size, also with improved body conditions (Fig. 1d); while body size exceeds a threshold, counterbalancing viability selection may outweigh fecundity selection to decrease litter size with further increases in body size. Moreover, the body condition of breeding female *S. dauricus* did not decline when growing large (Fig. 1d), suggesting that the senescence hypothesis alone cannot explain the quadratic relationship between body size and litter size of female *S. dauricus* (Fig. 2).

Daurian ground squirrels appear to be at the slow end of the slow-fast life history continuum (Dobson and Oli, 2007). Daurian ground squirrels produce one litter per year, with lesser multi-year fluctuations in abundance (Li et al., 1998; Luo and Zhong, 1990). From 1975 to 1994, densities of *S. dauricus* varied from 0.14 individuals ha^{-1} to 0.91 individuals ha^{-1} in Jilin (Li et al., 1998). Daurian ground squirrels behave like capital breeders, storing body fat during autumn for reproduction and likely adopting the risk avert strategy to allocate relatively small, stable amount of resources and energy to reproduction but invest variable amounts to somatic growth and body fattening (Jönsson, 1997). The proportion of breeding females is often greater than 0.9 in *S. dauricus* in

Table 1
Summaries and distributions of the carcass mass of Daurian ground squirrels in Inner Mongolia, China. Symbols G1, G2, and G3 denote carcass mass groups divided by 25% quantile and 75% of quantile of the carcass mass distribution.

Group	Sample size	Range	Mean \pm SD	<i>P</i> value of <i>t</i> test
G1	13	76.00–101.00	96.09 \pm 6.65	$P_{G1-G2} \leq 0.01$
G2	24	101.7–120.0	110.00 \pm 5.35	$P_{G2-G3} \leq 0.01$
G3	12	120.9–149.8	129.68 \pm 9.23	

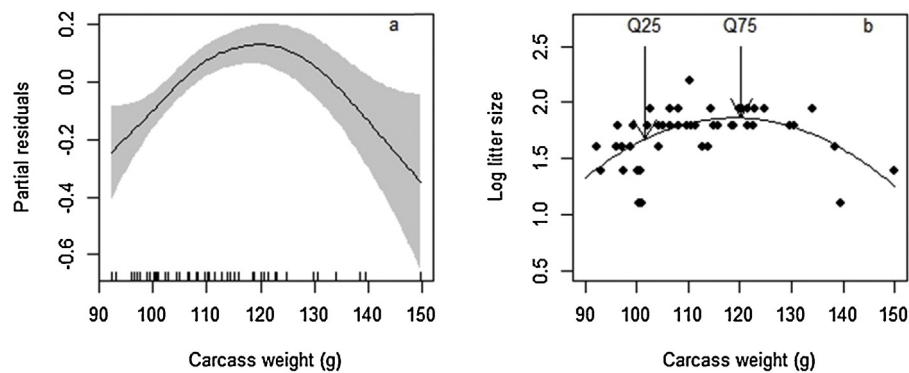


Fig. 2. The relationship between litter size and body (carcass) mass of Daurian ground squirrels in Inner Mongolia, China in (a) generalized additive models and (b) generalized linear models.

May (this study; Luo and Zhong, 1990). Females rarely reproduce in June, and thus have about three months (mid-June, July, August and early September) of foraging time to fatten their bodies before hibernation for over-winter hibernation and early spring reproduction after emergence (Luo and Zhong, 1990). Daurian ground squirrels are primarily herbivorous during summer, with green plants constituting about 90% of the summer diet and insects 10% (Wang et al., 1994). The standing biomass of Inner Mongolian grasslands peaks in August (Wang et al., 1999). Daurian ground squirrels may acquire sufficient food during summer to reach the body condition required for spring reproduction if they survive through severe winters. The life history strategies of *S. dauricus* suggest the importance of body size to both reproductive success and over-winter survival of the species.

The evolution of body size can be under fecundity selection, viability selection, and (or) sexual selection (Blanckenhorn, 2000; Bonnet et al., 2000; Schulte-Hostedde et al., 2004). Sexual selection may enhance female fitness by increasing female body sizes and litter sizes and resulting in the female-biased sexual size dimorphism. The quadratic relationship between body size and litter size of female *S. dauricus* is unlikely to be caused by sexual selection because *S. dauricus* has the male-biased sexual size dimorphism, with the carcass of males being 19% heavier than that of females (this study; Liu et al., 1993). Optimal body size for litter size may be the outcome of the opposite selective forces on fecundity and survival (Blanckenhorn, 2000). In addition to the fecundity advantage of large body sizes resulting from fecundity selection, larger-sized bodies physically allow *S. dauricus* to store more body fat for over-winter hibernation and spring reproduction. The average body conditions of heavier breeding *S. dauricus* were better than those of lighter groups (Fig. 1d). The body condition index used in this study is correlated with body fat amount in mice (Labocha et al., 2014). On the other hand, the number of fetuses represents current reproductive investment of females. The larger the litter size, the more the current reproductive investment (Ung et al., 2014). Large-sized individuals also require additional foraging time and energy for maintenance, which may limit or diminish the allocation of resources to reproduction when body size exceeds a threshold and subsequently decrease litter size (Blanckenhorn, 2000; Shine, 1988). In this sense, the modal litter size is optimal between reproduction and somatic growth (Roff, 2002).

The reproductive senescence or the cost of reproduction hypothesis predicts that old, heavy females may produce fewer offspring than they did at younger age due to the physiological senescence (Sharp and Clutton-Brock, 2010). The litter size of the meerkat (*Suricata suricatta*) varies quadratically with increasing ages (Sharp and Clutton-Brock, 2010). However, we did not find a positive relationship between body conditions and litter sizes. Heavier *Spermophilus dauricus* had better body conditions from G1

to G2 to G3 (Fig. 1d). Carcass mass may not represent the age of *S. dauricus* accurately. For example, old, senescent individuals may lose body mass although body mass increases with increases in age in general, and heavier groups such as G3 may consist of individuals of various ages. Without determining the ages of captured *S. dauricus*, we cannot conclude that heavier individuals were older ones with the reproductive senescence in our study. Liu et al. (1993) did not observe declines in litter size in old *S. dauricus*. Our study only had 50 females captured during one year. Nevertheless, larger sample sizes than ours and long-term monitoring with capture–recapture methods are needed to better discern the effects of the reproductive senescence definitively. The findings of this study on the optimal or peak litter size at the intermediate body size of *S. dauricus* suggest that the litter size and body size of small mammals may coevolve under joint pressures of fecundity selection, and viability selection, but also indicate that body size or body mass may serve as a state variable to predict individual variability in survival and reproduction for better understanding how variability in resource availability influences the population dynamics of small mammals.

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