

Testing Reproductive Tradeoffs and Fitness Measures in Female Columbian ground squirrels (*Urocitellus columbianus*)

By

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Abstract

Life history theory is a branch of ecology whose goal is to understand how animals optimize their survival and reproductive success. Under natural conditions, resources are finite and life history traits are subject to trade-offs and other types of constraints. The costs of reproduction are one of the most significant components underlying life-history trade-offs. In order to test for reproductive trade-offs we examined how female Columbian ground squirrels (*Urocitellus columbianus*) allocated resources to somatic and reproductive efforts. Furthermore, natural selection cannot maximize life history traits and thus fitness. Interpretation of selection of life history traits varies when using different measures of fitness as such, we examined the influence of age at reproductive maturity using two fitness measures, lambda and LRS (lifetime reproductive success). By studying life history traits (i.e age at reproductively maturity) through a variety of theoretical and empirical methods, a combination of traits that maximizes fitness can be determined and used to predict the evolution of major life history traits.

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**Chapter 1: Comparing Fitness Measures and the Influence of Age of Maturity in
Columbian Ground Squirrels**

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Abstract

Changes in the demography of populations may lead to adaptive expression of life history traits. Delaying age at first reproduction may result in reduced fitness (Cole's principle). This may not always be true depending on the study species and fitness measure. We presented three fitness measures unadjusted and adjusted lambda (for changes in population growth) via McGraw and Caswell's matrix method and LRS (lifetime reproductive success) to examine the influence of age at first successful reproduction on fitness. We also investigated whether the influence of age at maturity (age at first successful reproduction as defined by weaning a litter) on individual fitness changed with population growth. We also hypothesized that reproductive output would depend on changes in population growth. Using an early measure of fecundity (litter size at weaning), we found significant directional but not stabilizing selection for early maturity. Female Columbian ground squirrels that reproduced as yearlings had greater unadjusted and adjusted fitness than females who delayed first reproduction until 2 or 3+ years old. When a later fecundity measure (number of surviving juveniles) was used, there was no advantage for early reproduction, and no fitness difference among 1, 2, and 3+ year olds. Likewise, LRS did not differ significantly among females who matured at different ages. Reproductive output (litter size at weaning and number of surviving juveniles) did not depend on changes in the population nor did it significantly vary for females who matured at 1, 2, and 3+ years old. While LRS estimates fitness, it does not factor in the timing of reproduction (as lambda does) during the lifecycle, which can have a significant influence on fitness. More importantly, the measure of fecundity altered how age at first reproduction influenced fitness. As you increase the time from the immediate

act of reproduction, more environmental variation is introduced into fitness and stochastic variation has more time to intrude. Therefore, any fitness advantage that occurred over a short-term estimate may disappear when using a long-term estimate. Furthermore, the effect of the fitness pattern found at weaning could be minute or could be amplified in the right environment, which may not have been present in this study. Our results indicate that using an early measure of fecundity (i.e. litter size at weaning) adheres to Cole's principle, that age at reproductive maturity influences fitness, while measures taken at later in time do not. Important relationships between individuals and the growth of populations can be revealed through the examination of different fitness measure and the variation of life history traits.

Keywords: Lambda, lifetime reproductive success, Leslie Matrix, Population Growth

Introduction

The growth of populations is dependent on their demography; as such, life-history traits may change when populations grow or decline (Caswell 1989, 2001; Oli, Slade, & Dobson 2001; Dobson and Oli 2001, 2008). Changes in life history traits, such as age at first reproduction or greater longevity may lead to increases in fitness (Cole 1954). In long-lived species, such as iteroparous mammals, females may delay reproduction, potentially resulting in declines in fitness. Numerous factors could explain this deviation, one being resources available to population. A growing population is thought to have abundant resources and thus females can breed for the first time (termed "age of maturity") earlier in the life cycle (Stearns 1992). Contrarily, when the population is declining, individuals are assumed to have fewer resources, resulting in their devoting

more energy to survival and delaying maturity to a later age.

Theory suggests that as age of maturity is delayed, an individual's fitness may be reduced (Cole 1954; Bell 1976, 1980). Yet, this conclusion might depend on the study species and the choice of fitness measure. McGraw and Caswell (1996) developed a matrix analysis technique that they termed "individual fitness." Individual fitness encompasses an individual's life history parameters over its lifetime to produce a growth rate similar to that for population, λ , but under the assumption that a population of individuals similar in genotype would produce the same λ value as the individual under study. For example, individual fitness can be estimated for individuals that carry a specific life history trait (i.e. age of first reproduction). An individual λ value is estimated for each individual that expresses the form of the life history trait, and a mean and variance describes the fitness, over the lifespan.

Life-history traits that lead to higher fitness change with population size, thus the fitness of any trait is relative to the suite of traits present in the population. For instance, in years of population decline, delaying reproductive maturity and investing in survival may be necessary due to lack of resources (Bell 1976). Thus in a fluctuating population, it is beneficial to adjust λ for population growth during a female's lifetime. λ can be compared among individuals that express alternative trait forms (i.e. age at first successful reproduction), and used to comparatively evaluate the selective value of the traits.

Lifetime reproductive success, LRS, is another fitness measure that is widely used (e.g., Merilä and Sheldon 2000; Jensen et al. 2004; Descamps et al. 2006; McLoughlin et al. 2007; Holand, et al. 2015; Kleinteich et al. 2015; Zylberberg et al. 2015). LRS is the

total number of weaned offspring that an individual has over its lifetime (Grafen 1988). LRS does not factor in the timing of reproduction during the lifecycle, which can have a significant influence on the estimation of fitness (Cole 1954; Lewontin 1965; Roff 1992; Sterns 1992; Brommer et al. 2002). Interpretation of selection of life history traits differs when using LRS and lambda as measures of fitness (McGraw and Caswell 1996; Brommer et al. 2002).

Neuhaus et al. (2004) concluded that female Columbian ground squirrels (*Urocitellus columbianus*) match their reproductive output with environmental circumstances. With fewer individuals, resources were less limiting, leading to better body condition, greater survival and earlier reproduction. The study estimated fitness with LRS and made no adjustment for population changes or timing of reproduction. Using a matrix estimate of individual fitness, Oli and Armitage (2003) found that selection favored early maturity in Yellow-bellied marmots (*Marmota flaviventris*). However, they did not adjust their fitness estimates for fluctuations in population growth.

The main purpose of this study was to examine whether earlier age at maturity leads to increased fitness (“Cole’s prediction”, see Oli and Dobson 2003) and to compare individual fitness, unadjusted and adjusted for population growth, and LRS in order to evaluate which measure best describes the influence of age at maturity on fitness. We also examined whether the influence of age at maturity on individual fitness changed with population growth. We predicted that individual fitness would be greater for females that began reproducing earlier, particularly under increasing population growth, when resources should be abundant. Alternatively, under limited resources most likely associated with years of declining population growth, individual fitness for females who

reproduce earlier should decrease. We also hypothesized that reproductive output (i.e. litter size at weaning) will depend on changes in population growth (Cole and Batzli 1978, Pinter 1986, Morris 1989, Boutin et al. 2006). In populations with decreasing growth, lack of resources could lead to females investing more in somatic growth thus, diminishing from reproductive output. Alternatively, with abundant resources presumed under increased population growth, we predicted that weaning litter size would increase as reproductive females could then “afford” to allocate resources to both soma and reproduction.

Methods

STUDY SPECIES

We studied a population of Columbian ground squirrels in a montane meadow at the Sheep River Provincial Park, Alberta, Canada. Squirrels were caught with live traps (Tomahawk Live Trap Co., Tomahawk, WI, USA, 15x15x48 cm³ and 13x13x40 cm³) baited with peanut butter. All squirrels were trapped in the spring, within about three days of emergence from hibernation, and weighed to the nearest 5 g with a Pesola spring scale (Pesola Ag, Baar, Switzerland). Each squirrel was given a pair of uniquely numbered ear tags (National Band and Tag Company, Newport, KY, US; Monel metal tag # 1) and an individually distinctive black mark using black hair dye (Clairol Corporation, Stamford, CT, USA). Squirrels were observed from 3m tall wooden observation benches.

Columbian ground squirrels hibernate for approximately eight to nine months during the year (Dobson and Murie 1987; Dobson et al. 1992) and become active in mid-April and activity until late July or early August. Within a week of emergence from

hibernation, female ground squirrels copulate with males, usually in underground consortships (Raveh et al. 2010, 2011). If the mating date could not be determined, condition of the vulva and presence of copulatory material were used as indicators of successful mating (Murie and Harris 1982). After about 24 days of gestation and 27 days of lactation in nest burrows, pups emerge above ground for the first time (Murie and Harris 1982). Newly emerged pups are caught and uniquely marked, providing an accurate estimate of litter size at weaning. Mothers are trapped and examined at the same time weaned young are caught, and can be associated with litters through their overnight associations with young.

Statistical Analysis

LESLIE MATRIX

Using a long-term data set on female Columbian ground squirrels, we conducted a modified post-breeding census. In a post-breeding census, each individual is counted after annual reproduction. The Leslie matrix for a post-breeding census uses population age structure and annual breeding to derive an estimate of population growth (after McGraw and Caswell 1996) from the dominant eigenvalue of the matrix, or lambda (as previously defined). We estimated survival from emergence from one spring to the next, since spring populations are stable and reflect individuals truly resident in the population as reflected by having hibernated in the study site. The number of weaned young that emerged from nest burrows at the end of the lactation period was used to estimate reproduction effort.

Individual fitness was estimated using a Leslie (1945) matrix approach, as modified by McGraw and Caswell (1996). An example of the Leslie matrix for a post-breeding census is shown below:

$$\begin{array}{cccc}
 F_1 & F_2 & F_3 & 0 \\
 S_1 & 0 & 0 & 0 \\
 0 & S_2 & 0 & 0 \\
 0 & 0 & S_3 & 0
 \end{array}$$

Average fertility is across the top row of the matrix. The formula for fertility is as follows

$$F_x = S_x m_x$$

S_x is the survival rate, calculated as whether the female survives from the previous year to the current year. We calculated fecundity (m_x) two ways; using the size of litters at weaning and those young that survived to be 1 year old the subsequent year. We used both reproductive output measures to test for similarity in fitness patterns. The last column represents the year that the female died; hence it is comprised of zeros. Since, the Leslie matrix represents age-structured information about reproduction and survival for a population, lambda derived from this matrix can be considered adjusted for variations in population growth, (λ_{adj}).

In the individual fitness matrix, fitness is based on the reproduction and survival of a single individual. An example of this matrix is shown below:

$$\begin{array}{cccc}
 F_1 & F_2 & F_3 & 0 \\
 1 & 0 & 0 & 0
 \end{array}$$

$$\begin{matrix} 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{matrix}$$

The top row of the individual matrix was calculated by multiplying survival (0 or 1) by fecundity, m_x . These matrices represented individual females, so we assumed an equal offspring sex ratio and multiplied number of offspring by 0.5 to estimate the number of females in a litter.

$$F_x = S_x * 0.5 * m_x$$

Lambda, via the individual fitness matrix, represents individual fitness unadjusted for population growth (λ_{ind}).

Using an individual fitness matrix approach, (after McGraw & Caswell 1996; Oli and Armitage 2003), we examined longevity and reproductive success for 132 adult females. We constructed age specific individual matrices for females that varied from 1-12 years (average: 4.48 years, Viblanc et al. 2010; Dobson et al. 2012). Some females mated for the first time when they were 1 year old (i.e yearlings, N =10). Most females began mating at age 2 (N = 85) and occasionally started reproducing at 3 years or older (N = 37, Dobson and Murie 1987). Overall, 76.1% (283/372) of females that mated were successful at producing a litter, of those, 86.6% (245/283) were successful at weaning a litter. Thus, we used first successful reproduction, as evidenced by weaning a litter, as an estimate of age at maturity.

POPULATION DEMOGRAPHY

To account for fitness changes in relation to population growth, individual lambda

values for females were regressed on Leslie matrix estimates of population growth (during the individual females' lifetimes), and residuals were retained and used to estimate lambda values adjusted for population growth. These residuals were added to one to yield adjusted lambda (Viblanco et al. 2010, Dobson et al. 2012).

Lambda and LRS (total number of pups that a female weaned over a female's lifetime) were regressed on age at maturity to evaluate the direction and intensity of this trait (Lande and Arnold 1983). The sign of the coefficient (β) from linear regression indicates the degree of directional selection for age at reproductive maturity. We also used a quadratic regression in order to estimate stabilizing selection (γ) as evidenced by significant regression coefficients. Directional or stabilizing selection on age at maturity occurs when β and γ are significantly different from 0 (Lande and Arnold 1983; McGraw and Caswell 1996).

Population growth might influence fitness differently for different ages at maturity thereby causing an interaction. So, we regressed individual lambda on population growth for the age at which females became reproductively mature to determine if there was an interaction between age at maturity and population growth.

Matrices and lambda (scripts available on request) and generalized mixed models were calculated in R (version 0.98.1091) using the lme4 package (R Core Team 2013, Bates et al. 2014). Differences among age at maturity, lambda, and LRS were tested using two-way ANOVAS. When ANOVAS were significant, Tukey's test for multiple comparisons were used.

Results

POPULATION GROWTH

Between 1992 and 2013, female population size ≥ 1 year old fluctuated between 14 and 77, with a mean size of 37 individuals (Fig 1). There was not a significant interaction between age at maturity and population growth (Fig 2a, age at maturity, change in population, and interaction term of latter regressed on individual lambda, $R^2 = 0.37$, likelihood ratio test, d.f = 128, $F = 1.87$, $P = 0.17$). Individual lambda (using litter size at weaning as fecundity) in relation to population growth grew at a similar same rate for females maturing as yearlings ($N = 11$) and 2 year olds ($N = 88$, $\beta = 0.97$, 0.85 , respectively). 3+ year olds had the slowest rate of individual lambda increase per intrinsic population growth ($N = 36$, $\beta = 0.41$). There was not a significant interaction between individual lambda (using number of surviving juveniles as fecundity) and population growth (Fig 2b, age at maturity and change in population regressed on individual lambda, $R^2 = 0.36$, likelihood ratio test, d.f. = 127, $F = 0.37$, $P = 0.55$). Yearlings ($N = 8$) and 2 year olds ($N = 88$) exhibited similar patterns between individual lambda and population growth ($\beta = 1.23$, 1.36 , respectively). 3+ year olds ($N = 36$) had the lowest rate of change of individual lambda per intrinsic population growth ($\beta = 0.91$).

SHORT TERM-ESTIMATES OF FITNESS: LITTER SIZE AT WEANING

Linear regression of individual lambda on age at first reproduction suggested directional selection for early maturity (age at maturity and age at maturity squared regressed on unadjusted fitness, year of maturity and age random factors, $R^2 = 0.63$, $\beta \pm SE = -0.26 \pm 0.08$, likelihood ratio test, $N = 132$, $\chi^2 = 10.47$, $P < 0.001$). Stabilizing selection was not evident by quadratic regression (age at maturity and age at maturity squared regressed on unadjusted fitness, year of maturity and age random factors, $R^2 =$

0.63, $\gamma \pm SE = -0.002 \pm 0.01$, $\chi^2 = 0.04$, $P = 0.84$). Yearlings had a 39.2% higher individual fitness than 2 year olds (1.74 ± 0.15 , $N = 10$, 1.17 ± 0.03 , $N = 85$, Tukey's HSD test, $P < 0.001$) and 54.0% higher fitness than 3+ years old females. (1.74 ± 0.15 , $N = 10$, 1.00 ± 0.06 , $N = 37$, Tukey's HSD test, $P < 0.001$). 2 year olds had 11.3% higher lambda than 3+ years old females (1.12 ± 0.03 , $N = 85$, 1.00 ± 0.06 , $N = 37$, Tukey's HSD test, $P = 0.03$).

Adjusting for population change yielded similar directional and stabilizing selection for reproducing at an early age (age at maturity and age at maturity squared regressed on adjusted fitness, year of maturity and age random factors, $R^2 = 0.52$, $\beta \pm SE = -0.23 \pm 0.07$; $N = 132$, $\chi^2 = 9.61$, $P < 0.001$; $\gamma \pm SE = -0.004 \pm 0.01$, $N = 132$, $\chi^2 = 0.15$, $P = 0.70$). Yearlings also had 31.4% higher adjusted fitness than females that reproduced at 2 years of age (1.66 ± 0.09 , $N = 10$, 1.21 ± 0.03 , $N = 85$, Tukey's HSD test, $P < 0.001$) and a 44.1% higher fitness than 3+ year olds (1.06 ± 0.06 , $N = 37$, 1.66 ± 0.09 , $N = 10$, Tukey's HSD test, $P < 0.001$). 3+ year olds had 13.2% lower adjusted lambda than 2 year olds (1.06 ± 0.06 , $N = 37$, 1.21 ± 0.03 , $N = 85$, Tukey's HSD test, $P = 0.01$).

LONG-TERM ESTIMATE OF FITNESS: NUMBER OF SURVIVING JUVENILES

Using number of surviving juveniles as fecundity, indicated no directional selection for reproducing at an earlier age (age at maturity and age at maturity squared regressed on unadjusted fitness, year of maturity and age random factors, $\beta \pm SE = 0.06 \pm 0.1$, $N = 102$, $\chi^2 = 0.34$, $P = 0.56$), nor was there stabilizing selection based on quadratic regression (age at maturity and age at maturity squared regressed on unadjusted fitness, year of maturity and age random factors, $\gamma \pm SE = -0.01 \pm 0.02$, $N = 102$, $\chi^2 = 0.55$, $P =$

0.45). Yearlings had 6.3% higher individual fitness compared to 2 year olds (1.3 ± 0.23 , 1.22 ± 0.04 , respectively) and 8.0% compared to 3+ year olds (1.2 ± 0.05 , 1.3 ± 0.23 , respectively). 2 year olds had 1.7% higher fitness than 3+ year olds (1.22 ± 0.04 , 1.2 ± 0.05 , respectively).

Similarly to unadjusted lambda, there was no directional selection for age at maturity when regressing on adjusted lambda (age at maturity and age at maturity squared regressed on adjusted fitness, year of maturity and age random factors, $\beta \pm SE = -0.008 \pm 0.09$; likelihood ratio test, $N = 101$, $\chi^2 < 0.001$, $P = 0.98$), nor stabilizing selection using quadratic regression either (age at maturity and age at maturity squared regressed on adjusted fitness, year of maturity and age random factors, $\gamma \pm SE$, 0.0003 ± 0.01 , likelihood ratio test, $N = 101$, $\chi^2 < 0.001$, $P = 0.99$). Among the three age classes, lambda was virtually the same (yearlings: 1.35 ± 0.19 , 2 year olds: 1.32 ± 0.03 , 3+ year olds: 1.33 ± 0.06).

LRS: LIFETIME REPRODUCTIVE SUCCESS

There was not an interaction trend between population growth and LRS (Fig 3, age at maturity, change in population, and interaction term of latter regressed on LRS, age and year of maturity random factors, likelihood ratio test, $d.f = 7$, $F = 1.34$, $P = 0.24$). 2 and 3+ year olds had the largest LRS increase per increase in intrinsic population growth ($\beta = 13.4$, 10.0 , respectively). Females who matured at 1 years old had the smallest LRS increase in relation to population growth ($\beta = 5.6$). There was no significant evidence of directional selection when fitness was estimated by LRS (age at maturity and age at maturity squared regressed on LRS, year at maturity and age random factors,

likelihood ratio test, $\beta \pm SE = -0.73 \pm 0.78$, d.f = 6, $\chi^2 = 0.86$, $P = 0.35$). Quadratic regression revealed significant stabilizing selection (age at maturity and age at maturity squared regressed on LRS, year at maturity and age random factors, likelihood ratio test, $\gamma \pm SE$, -0.26 ± 0.11 , d.f = 6, $\chi^2 = 5.3$, $P = 0.02$). 2 year olds had 25.7% higher individual fitness than yearlings (7.9 ± 0.59 , 6.1 ± 0.99 , Tukey's HSD test, $P = 0.59$). 3+ year old females had about 19.3% greater LRS than yearlings (Fig 4, 7.4 ± 0.97 , 6.1 ± 0.99 , Tukey's HSD test, $P = 0.79$). Females that first reproduced at 2 years old had about 6.5% greater LRS than older females (7.9 ± 0.59 , 7.4 ± 0.97 , Tukey's HSD test, $P = 0.89$).

REPRODUCTIVE OUTPUT

To evaluate reproductive output, we looked at litter size at weaning and the number of yearlings (one year olds) for each female in the year in which they became reproductively mature. The number of offspring that a female first successfully weaned was not dependent on changes in population (age at maturity, change in population, and the interaction term of the latter regressed on weaning litter size, year of maturity and age random factors, likelihood ratio test, d.f=7, $\chi^2 = 0.002$, $P = 0.96$). Litter size at weaning did not differ significantly for different ages of reproductive maturity (age at maturity regressed on litter size at weaning, year of maturity and age random factors, likelihood ratio test, d.f = 6, $\chi^2 = 0.46$, $P = 0.80$). Females who matured as yearlings had 2.4% smaller weaning litter sizes as females who matured at 2 year olds (2.50 ± 0.22 pups, 2.56 ± 0.09 pups, respectively). 3+ year olds had 1.2% larger weaning litter sizes than 2 year olds (2.62 ± 0.15 , 2.59 ± 0.09 , respectively). Yearlings had 2.7% smaller weaning litter

sizes than females who matured as 3+ year olds (2.50 ± 0.22 pups, 2.62 ± 0.15 pups, respectively).

The number of pups that survived to be yearlings for each female was not significantly influenced by age of maturation (age at maturity regressed on number of surviving yearlings, year of maturity and age random factors, likelihood ratio test, d.f = 6, $\chi^2 = 0.45$, $P = 0.80$). Likewise, population growth did not significantly influence the number of juveniles of females who matured at different ages (age at maturity regressed on number of surviving yearlings, year of maturity and age random factors, likelihood ratio test, d.f = 67 $\chi^2 = 0.04$, $P = 0.85$). Females who matured as yearlings, 2, and 3+ year olds had approximately the same number of pups survive to yearlings (1.10 ± 0.28 pups, 1.04 ± 0.11 pups, 1.05 ± 0.14 pups, respectively).

Discussion

Life history traits are an integral part of population demography, traits such as age at maturity, may change with increasing or decreasing population growth. As age of breeding is postponed, a female's fitness may be diminished (Bell 1976, 1980; McGraw and Caswell 1996).

The prediction that age at maturity has the largest relative influence on fitness (λ) was not conclusively supported (Cole 1954). In accordance with Cole's prediction and our predictions, using a short-term estimate of fitness (litter size at weaning) yielded that age at maturity did have a large relative influence on λ (Fig. 1). Females that reproduced as yearlings (1 year olds) had significantly greater individual and adjusted fitness (using litter size at weaning as fecundity) than compared to 2 and 3+

year olds. The former suggests that yearlings, which were still growing (Broussard et al. 2008), were able to procure enough resources to support developing soma as well as supplement reproduction. Rubach et al. (2015 manuscript) and Dobson et al. 1999 proposed that for female Columbian ground squirrels, a minimum body condition may be necessary to successfully initiate reproduction and rear offspring. Reproducing yearling females were still structurally growing as shown by a gain of 115.6g over their first year. This substantial mass gain may also have allowed those females to reach a body condition threshold that allowed them to complete structural growth as well as become reproductively successful. Females that reproduced for the first time as yearlings under positive population growth were able to sustain both biological functions, growth and reproduction. Thus, yearlings females could afford to breed for the first time earlier in their life cycle (Stearns 1992).

A later measure of fecundity (number of juveniles that survived to emerge from their first hibernation) did not produce estimates of fitness that increased with age at maturity. As more time passed after the initial reproductive event, more environmental and stochastic influences during the intervening period appeared to erode the differences in individual fitness among females that matured at different ages (Cole 1954, Bell 1980, McGraw and Caswell 1996). As you get farther from the immediate act of reproduction, there is more environmental variation introduced into fitness and more time for stochastic variation to intrude. Therefore, there could be a short-term reproductive advantage and any fitness pattern found at weaning could be subtle or any fitness benefits could disappear using a later time measure.

We found similar results for directional and stabilizing selection while using a late term estimate for early maturity in Columbian ground squirrel as Oli and Armitage (2003) found in Yellow-bellied marmots. Given that we found significant selection pressure for early age at maturity we would expect that younger (1 and 2 years old) age of maturity would exhibited the greatest frequency. In our study, 8.3% of females reproduced as 1 year olds and 64.3% as 2 year olds. The percent of females, who first reproduced as 3+ year olds, was lower than younger (1 and 2 year olds) females combined (28%) which is consistent with significant directional selection for early maturity. Thus, and in accordance with Cole (1954) and Oli and Armitage (2003), we found that early age of maturity was selected for in female Columbian ground squirrels.

Along with λ , LRS (lifetime reproductive success) has also been used as a measure of fitness (Merilä and Sheldon 2000; Jensen et al. 2004; Descamps et al. 2006; McLoughlin et al. 2007; Zylberberg et al. 2015; Kleinteich et al. 2015; Holand, et al. 2015). LRS is limited by the fact that it does not consider the timing of reproduction, which can have a significant influence on fitness (Cole 1954; Lewontin 1965; Roff 1992; Sterns 1992; Brommer et al. 2002). Variation in LRS is mainly due to differences in longevity rather than fertility, as such, large LRS values do not correlate to large values of lambda, which tend to have lower variance. Females who matured later might have an inflated LRS value (Brommer et al. 2002), which coincides with our results that were based on weaned offspring. LRS is usually measured after weaning of offspring, thus more stochastic variation can become detectable. LRS did not show any evidence of selection on age at maturity nor dependent on changes in population growth. These results reflect similar findings in European sparrowhawks (*Accipiter nisus*; McGraw and

Caswell 1996), Ural owls (*Strix uralensis*; Brommer et al. 1998), and wood ducks (*Aix sponsa*; Oli et al. 2002). Lambda on the other hand, incorporates timing of reproduction, which can have a significant influence on fitness (Cole 1954; Lewontin 1965; Bell 1976; Caswell and Hastings 1980; Bell 1980; Caswell 1982; McGraw and Caswell 1996). During periods of population growth, lack of resources could lead to females investing more resources into somatic growth and shift resources from reproduction. Since there were no costs to reproductive success in terms of weaning litter size and number of surviving yearlings, females are most likely able to energetically compensate for reproduction even at a young age. As reproductive output was similar among different ages at maturity, litter size at weaning and the number of juveniles that survive may be an inherent feature of female Columbian ground squirrel life history.

We also considered whether age at reproductive maturity changed with population growth or decline. Regardless of either short or long term estimates of fitness, when the rate of population growth was positive, all ages of first reproduction had higher average fitness. Yet, there were not significant differences in the rate of change in individual lambda per intrinsic population growth among females who matured at different ages (Fig 2a, 2b). These results indicated that individual lambda without adjustment for changes in population growth may be sufficient to explain the influence of age at maturity on fitness. Other factors that contribute to the matrix value, such as fecundity, may have a larger influence on lambda. Fecundity is a cumulative measure of survival and fertility. Since, litter size at weaning and number of surviving juveniles were comparable in all three age groups, it is not surprising that by using a short term estimate for fitness vs. a long-term estimate, yielded similar results. As such, an underlying

genetic or environmental basis may be present. Neuhaus et al. (2004) concluded that female Columbian ground squirrels reproduce as well as they could under environmental conditions. Lack of reproductive experience could explain the similarity in adjusted and individual lambda. Previous experience could increase the probability of a female successfully weaning offspring. Broussard et al. (2008) found that 2 and 3 year old females who had previous reproductive experience had slightly better body conditions and greater reproductive investments than inexperienced females. As previously stated, a minimum body mass (i.e. 'good' body condition) may be necessary to successfully reproduce, this may be especially true for yearlings that reproduce.

Females who reproduced at an early age had greater fitness overall than those who delayed reproduction. Population growth did not affect reproductive output of female Columbian ground squirrels, indicating that there is an underlying basis for successful reproduction. Applying McGraw and Caswell's (1998) matrix method as well as LRS, yielded different conclusions of age at reproduction's influence of fitness. Fitness via the matrix method was more concise since timing of reproduction was accounted for whereas LRS did not. Using different measures of fecundity did not consistently show support for Cole's prediction. An early measure of fecundity (litter size at weaning) corroborated that age at maturity heavily influences fitness while a later measure (number of juveniles surviving) did not support that conclusion. Thus, Cole's prediction can be upheld only under certain conditions.

Condition of offspring produced at different times can be influenced by environmental fluctuations (Lindström 1999). Thus, a longer time period to census offspring may be necessary to achieve a fitness value that minimizes environmental and

genetic noise (Clutton-Brock 1988; Gaillard et al. 2000). An appropriate time period to evaluate a female's fitness would be to census offspring when offspring are reproductively mature themselves. Future studies are needed to evaluate individual fitness patterns from long-term studies that include multiple generations would be valid for spatio-temporal environments and variation in offspring quality. By considering influences from age at reproductive maturity and other life history traits, important relationships can be revealed about population growth and its influence on life history traits.

Acknowledgements

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Fig. 1. Numbers of female Columbian ground squirrels (≥ 1 year old) at our study site in Sheep River Provincial Park, Alberta Canada from 1992-2013.

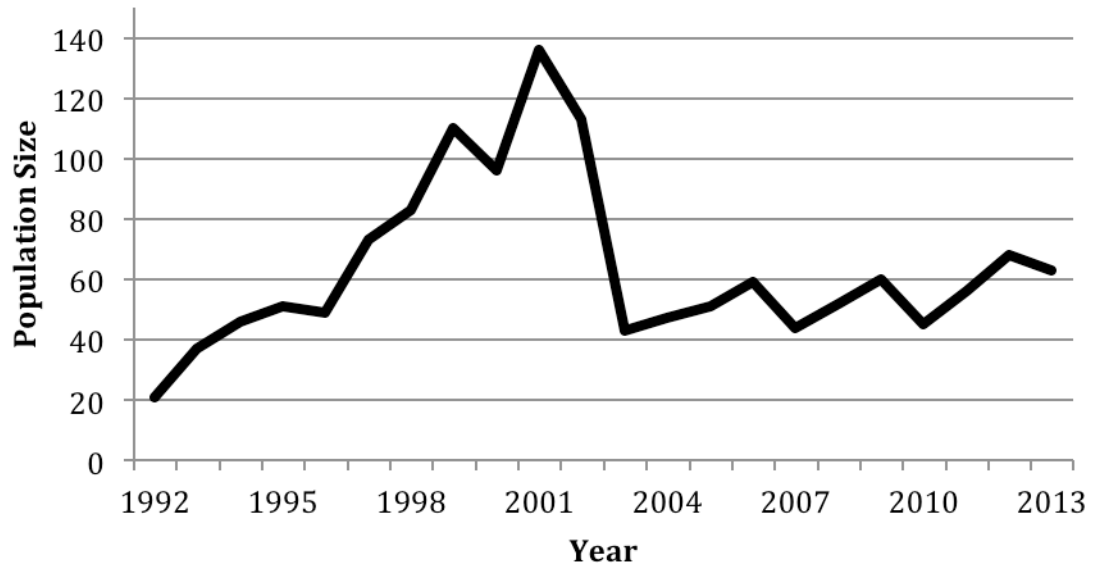


Fig. 2. Regression of individual lambda a) using a weaning litter size as fecundity), b) using number of surviving juveniles as fecundity, on finite population growth for three age groups at which females first reproduced (α).

Fig. 2a.

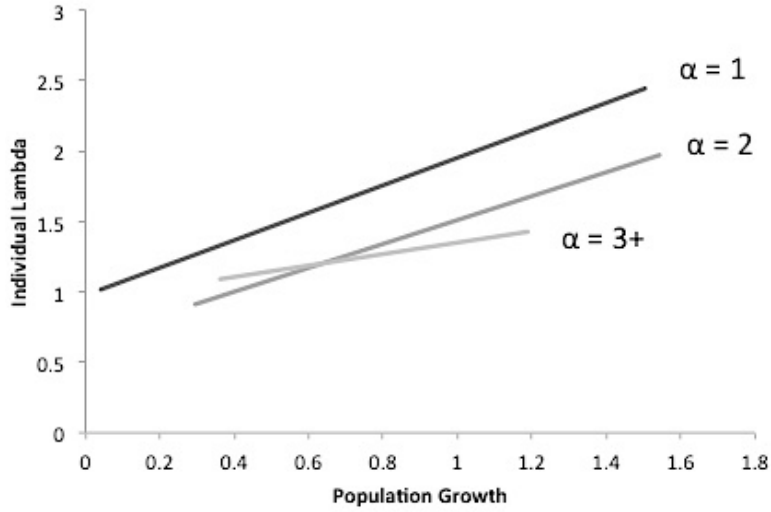


Fig. 2b.

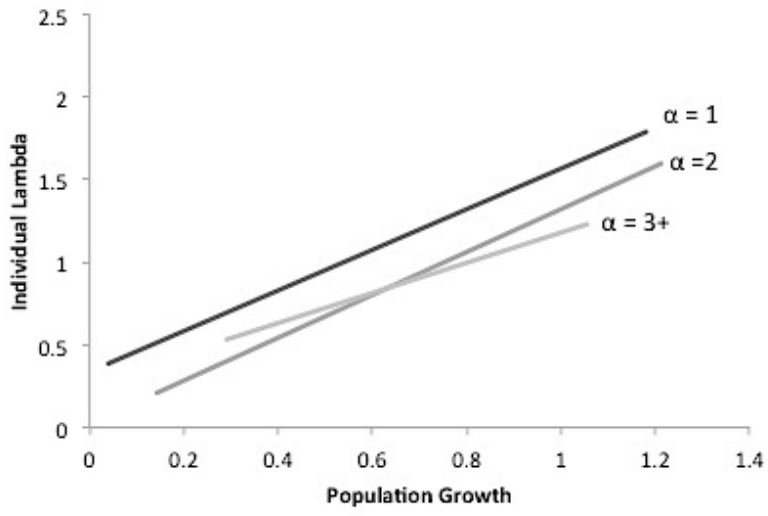
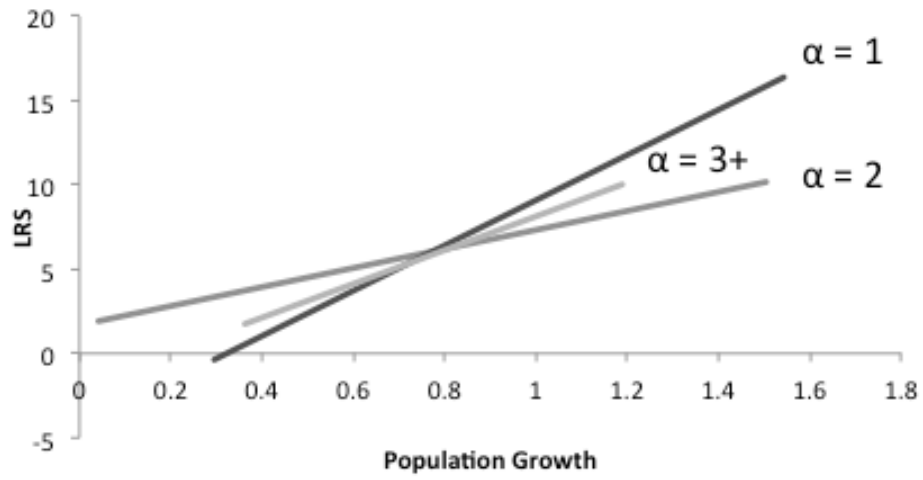


Fig. 3. Regression of LRS (lifetime reproductive success) on finite population growth for three age groups at which females first reproduced (α).



Chapter 2: Testing Reproductive and Somatic Tradeoffs in Female Columbian Ground Squirrels

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Summary

1. Energetic tradeoffs in resource allocation form the basis of life-history theory, which predicts that reproductive allocation in a given season, should negatively affect future reproduction or individual survival.
2. We examined how allocations of resources differed between successful and unsuccessful breeding female Columbian ground squirrels to discern any effects of resource allocation on reproductive and somatic efforts.
3. We compared the survival rates and mass gain of successful breeders (females that successfully weaned young) and unsuccessful breeders (females that failed to give birth or wean young), and investigated “carry-over” effects to the next year.
4. Starting capital appeared an important factor influencing whether successful reproduction was initiated or not, as females with the lowest spring emergence masses did not give birth to a litter in that year. Whereas successful and unsuccessful breeding females showed no difference in over winter survival, females that failed to wean a litter gained additional mass during the season when they failed. The next year, those females had increased energy “capital” in the spring, leading to larger litter sizes.

5. Columbian ground squirrels appear to act as income breeders that also rely on stored capital to increase their propensity for future reproduction. Failed breeders in one year “prepare” for future reproduction by accumulating additional mass, which is “carried over” to the subsequent reproductive season.

Key-words: Capital breeding, Columbian ground squirrels, Energy allocation, Income breeding, Reproductive allocation

Introduction

In order to survive and reproduce, animals must acquire energy from the environment, and successfully allocate it to various metabolic needs (Brown et al. 2004). However, because energy resources are usually limited under natural conditions, life history traits are seldom maximally expressed (Fisher 1930; Reznick 1985; Kunz & Orrell 2004). Resources allocated to one biological function often reduce availability for allocation to other biological function, a type of life-history tradeoff (Stearns 1992).

An assumption of life-history theory are the existence of “energy costs” associated with trade-offs with respect to survival and reproduction (Lack 1966; Williams 1966; Hirshfield and Tinkle 1975; Bell 1980; Stearns 1992). In iteroparous species (i.e., those that reproduce more than once), annual resource allocation can be divided into two primary biological functions: somatic and reproductive efforts (Hirshfield and Tinkle, 1975). Resources can be allocated either to individual soma in the form of growth, personal maintenance, and survival, or to reproduction in the form of immediate offspring production and parental care. When environmental resources are acquired annually or

when the annual energy budget is fixed, these two categories add up to the total energy resources available for allocation. If current reproduction requires a high level of allocation, females must reallocate energy from growth and somatic maintenance to accommodate this need, giving rise to the idea that reproduction might entail costs, as somatic development (maintenance) and/or future reproduction may be reduced (Williams 1966; Partridge & Harvey 1985; Reznick 1985; Partridge 1992).

One way to study somatic and reproductive allocations is to focus on the ways in which resources are accumulated (Jönsson 1997, Houston et al. 2007). In instances where stored resources are devoted to reproduction, a species is considered a “capital breeder” under the analogy that saved capital is “funding” reproduction. In contrast, when reproduction relies on the daily accumulation of resources, the species is considered an “income breeder” (Jönsson 1997). In the latter case, females faced with increased reproductive demands may either reallocate somatic resources to reproduction or augment their energy income by increased foraging (Van Noordwijk and DeJong 1986). In ground squirrels, females in good body condition (high body mass) allocate more resources to reproduction in the form of larger or heavier litters than females in poor condition (Murie & Dobson 1987; Michener 1989; Dobson, Risch & Murie 1999; Risch, Michener & Dobson 2007). Females in good condition may also show improved survival and concurrent subsequent breeding through increased somatic effort. Changes in resource allocation might involve costs in terms of future survival or reproduction by the parent (Williams 1966; Fisher & Bloomberg 2011), or costs may be defrayed onto offspring if the increasing demands on the parent are not met elsewhere (Morris 1986).

Columbian ground squirrels (*Urocitellus columbianus*) are predominantly income breeders with a short active season of 3-4 months in which major breeding events (mating, gestation, and lactation) occur within a brief time period coinciding with seasonal environmental changes (Murie & Harris 1982; Dobson, Badry & Geddes 1992; Broussard, Dobson & Murie 2005). Virtually all females attempt breeding during their prime reproductive years (ages 3 to 7); and while some are successful at weaning litters, others are not (Dobson & Kjelgaard 1985; Dobson & Murie 1987; Dobson 1988; Broussard et al. 2003, Broussard, Dobson & Murie 2008). Although stored capital in the form of body mass has a significant influence on ground squirrel reproduction, energy allocated to young during gestation and lactation comes primarily from daily resource acquisition (Risch, Dobson & Murie 1995; Dobson, Risch & Murie 1999; Broussard, Dobson & Murie 2005). Therefore, the balance of resources between somatic and reproductive allocations might be especially important for adult females in this species.

Comparisons between females that successfully breed and females that are not successful at breeding can be used to test for energy allocation shifts between reproduction and somatic maintenance. Murie & Dobson (1987) and Neuhaus (2000) found that survival did not vary for mothers with different levels of reproductive effort, as reflected by litter size. These authors suggested that an association of body condition and reproduction might explain the apparent lack of phenotypic costs of reproduction, so that costs were masked by differences in resource accumulations among mothers. A similar mechanism might explain the lack of phenotypic costs in experimental studies of litter manipulation. For instance, neither experimental increases nor reductions in litter size affected maternal survival or future reproduction (Hare & Murie 1992, Skibieli et al.

2013). Both types of manipulation, however, affected mothers' body mass at subsequent spring emergence such that females with lower levels of reproductive allocation were heavier.

If reproductive costs exist, they are likely to be most evident in a comparison of individuals of different reproductive status. Females that do not produce a litter have little or no current reproductive effort, and should thus provide an excellent model for examining the ramifications of reproduction. If they carry stored resources over into the following year, any additional body mass might have a positive influence on reproductive success the subsequent year. Spring body mass at emergence from hibernation has a significant positive influence on the production of offspring, both in terms of numbers and quality (Risch, Dobson & Murie 1995; Dobson, Risch & Murie 1999; Skibieli, Dobson & Murie 2009). Thus, improved body condition associated with failed breeding is expected to increase a female's capacity for future reproduction.

The purpose of our study was to examine how allocations of resources differed between successful and unsuccessful female Columbian ground squirrel, allowing us to discern the effects of resource allocation to reproductive and somatic efforts. We hypothesized that reproduction should have negative influences on adult females' future survival and reproduction, as a result of higher resource allocation to offspring rather than soma in a given year. Thus, we expected successful reproduction, as evidenced by weaning a litter, to have negative associations with subsequent survival and reproduction, when compared to females that did not reproduce. Alternatively, if breeding females were able to compensate by increasing their daily intake of resources, we would expect them to show no "cost of reproduction" in terms of survival or reproduction in the following year.

Two hypotheses might explain the energy fate of unsuccessful breeding. First, females that did not successfully breed may have allocated more resources into somatic development. We would then expect unsuccessful females to exhibit greater increase in body mass and higher survival rates the subsequent year compared to co-occurring breeding females (e.g., Clutton-Brock 1984; Festa-Bianchet, Gaillard & Jorgenson 1998; Fisher & Bloomberg 2011; Rughetti et al. 2015). Alternatively, if little or no difference in body mass and survival to the following year were found between successful and unsuccessful breeders, this would suggest that unsuccessful females are not shifting resources to somatic effort (e.g., Millar, Derrickson & Sharpe 1992), but rather decreasing their overall resource income. Thus, there might be no benefit to somatic allocation that would offset the lack of reproductive allocation in the current year. In this case, failure to reproduce would not enhance future reproductive value, and females may be using a “best of a bad job” strategy, “waiting” until the next year to attempt reproduction once again.

Material and methods

GENERAL METHODS

Columbian ground squirrels are semifossorial hibernating rodents that live in subalpine and alpine meadows in the Rocky Mountains of the northwestern US and southwestern Canada. We studied them from 1992-2015 in the Sheep River Provincial Park, Alberta, Canada, (50° 39' 7" N, 114° 37' 27" W; 1550m elevation). These ground squirrels emerge from hibernation in late-April to early May and have an active season that extends into late July and early August. We trapped ground squirrels as they emerged

from hibernation in the spring, using live traps (13x13x40 cm, Tomahawk, WI, USA) baited with peanut butter. At the time of trapping, each squirrel was weighed to the nearest 5g using a Pesola spring scale (Pesola Ag, Baar, Switzerland). The ground squirrel was then given a unique ear tag number (#1-Monel metal, National Band and Tag Company, Newport, KY, USA) and a unique mark for visual identification with black hair dye (Clairol, Stamford, CT, USA). The young first emerged from nest burrows at the time of weaning in mid June to early July. At that time, adult females were caught with their litters and the young were ear tagged and given unique dye markings.

Behavioral observations were taken daily from 3m tall wooden stands. Mating dates for females were determined from the occurrence of above and below ground consortships with males (Raveh et al. 2010, 2011). From these dates, parturition and weaning dates for litters could be estimated. When mating date of a female was undetected, the condition of the vulva and presence of copulatory plug material in the vulva, sperm, and copulatory plug material on the fur were used as indicators of successful mating (Murie & Harris 1982). Following methods developed by Hare and Murie (1992), we trapped females two to three days before their expected parturition date, and about 22 days after mating, (Shaw 1925; Murie & Harris 1982; Murie 1992), brought the females into an on-site laboratory and housed then in polycarbonate microvent rat cages (267 x 483 x 20 mm; Allentown Caging Equipment Company, Allentown, New Jersey). They were given wood shavings and newspaper as nesting material and apple, lettuce, and horse feed (EQuisine sweet show horse ration, Unifeed, Okotoks, Alberta, Canada) *ad libitum*. At parturition, mothers (nearest 5 g) and pups (nearest 0.01g) were weighed. Pups were sexed and marked with a small tissue biopsy by clipping a toenail

bud as previously described by Hare & Murie (1992). Mothers and neonatal young were released approximately a day after birth into nest burrows. These nest burrows were previously known from observations of females entering them with loads of nest material (natural dry grass from the meadow). If a female did not give birth in the laboratory after approximately 7-10 days, she was examined for the presence of mammary tissue and released at her original capture location.

In the 24-year data set, 1992-2015, we recorded life histories of 125 females that lived to be at least 2 years old, the most likely age at which they become reproductively mature (Dobson and Murie 1987). Few females breed as 1 year olds (N=11), since yearling females are still growing and are of relatively low body mass (Dobson and Murie 1987; Dobson 1992). Two-year-old females that failed to reach reproductive maturity were also still growing (Broussard, Dobson & Murie 2008), and thus may have exhibited different patterns of somatic allocation from fully-grown adults. We thus restricted our analyses to females that were ≥ 3 years old, all of whom mated and had the opportunity to reproduce. When older than 9 years of age, females (N = 6) exhibited evidence of senescence (losses in maternal body mass or extremely low litter sizes) and we excluded these cases from analyses.

Females can breed successfully in some years but not in others, thus the reported sample sizes are cumulative for each breeding status. Females that produced a litter and weaned pups were considered reproductively successful and classified as breeders (N = 321). Reproductively unsuccessful females (“failed to wean offspring”, N = 99) were females that mated but either did not give birth in the laboratory (“failed during gestation”, N = 34) or gave birth but were unsuccessful at weaning young (“failed during

lactation”, N = 41). Because we did not know when some mothers failed at reproduction (N = 24), we pooled failed breeders for some analyses. Lactation is a highly demanding period in terms of energy expenditure (Clutton-Brock et al. 1989; Robbins 1993; Speakman 2008; in ground squirrels, Skibiél et al. 2013). Thus comparison of successful breeders (viz., those that weaned young), females that failed to birth, and females that failed to wean offspring allowed us to examine how resources were allocated between successful females and those that did not allocate resources to offspring during, at least part of, the lactation period.

SOMATIC AND REPRODUCTIVE ALLOCATIONS

Somatic allocation was estimated by measuring female body mass at two different times during the active season, at emergence from hibernation and at weaning. When young first emerge from natal nest burrows, lactation is essentially completed (Murie and Harris 1982) and the resource commitment to offspring has virtually ceased (Mattingly & McClure 1982; Kenagy, Sharbaugh & Nagy 1989; Michener 1989). Body mass of unsuccessful breeding females was also measured at the time that they would have weaned a litter had they been successful (i.e., about 52 days after mating, Murie and Dobson 1987). Mass at emergence the subsequent year was used to determine whether females that failed started the next year with more capital, and whether they had a greater likelihood to successfully reproduce that year than successful females (Broussard, Dobson & Murie 2005). Because samples were limited, we also examined female body mass dynamics (mass gain or loss) between spring emergence in a given year to spring emergence in the next. Reproductive allocation was estimated from the presence or

absence of a litter in the present year, and “carry-over” effects were investigated by considering the size at birth of the subsequent litter, the year *after* a female was successful or not.

STATISTICS

We tested for the effects of female breeding status and mass at weaning (independent variables) on her survival (dependent variable). Multivariate Cox regression models were used to analyze the time between when females entered our study and death (binomial factor, 0 = present in study, 1= death). Breeding status (successful and unsuccessful) and weaning mass were included as predictor variables, with age as a time-varying covariate and year as a random intercept factor (Cox and Oakes 1984). A maximum likelihood fit of the model was obtained via simultaneous maximization of the integrated partial likelihood (Ripatti and Palmgren 2000) over the fixed effects and the random effect covariance parameters. We further examined survival (binomial factor, 0 = death, 1= surviving) with GLMM's by regressing mass of females when they weaned litters or would have done so (for females that failed during lactation) on when females failed in their reproductive cycle (i.e. successful, failed during gestation or lactation). Year and female identity were included as random intercept factors. Female Columbian ground squirrels are highly philopatric, and emigration by adults is rare (Wiggett & Boag 1992; Neuhaus 2006; Arnaud et al. 2012), thus any disappearance from the population was most likely due to death.

When analyzing somatic and reproduction allocation, generalized mixed models were implemented in R (version 0.98.1091) using the lme4 package (R Core Team 2013,

Bates et al. 2013). We included individual identification number as a random intercept factor to account for repeated measures on the same individual in multiple years. Further, as resources and population density may fluctuate among years, we also included year as a random intercept variable in our models. Emergence mass of adult females (in the current and subsequent year), weaning mass, and litter size at birth were normally distributed as evident by visual inspections of histograms of residuals, so we used Gaussian distributions for modeling these variables. Coefficients of determination (conditional R^2 values) were calculated following Nakagawa and Schielzeth (2013).

The influence of breeding status in one year on breeding status in the next year was examined using Markov transition mixed models (Diggle et al. 2002) in which breeding status in the second year was regressed on breeding status in the first year, with spring body mass in the second year as a covariate and year as a random variable, and applying a binomial error term. Future litter size (in the next year) was compared among females of different breeding status using linear models and Tukey post-hoc tests. Finally, the relationship between litter size and body mass was examined in mixed models where female identity was a random variable, and conditional coefficients of determination were used to estimate effect sizes.

Results

SURVIVAL

Females that failed during gestation suffered poorer survival to the next spring compared to females that failed to wean a litter and those that weaned litters (by 11.2% and 12.2%, respectively; Table 1). These differences were not significant, perhaps due to

more limited samples of females that failed during gestation and lactation (N = 34 and 41, respectively, N = 321 for breeders; mixed model with random year and female identity, $R^2_{\text{conditional}} = 0.232$, likelihood ratio test, $\chi^2 = 2.41$, $P = 0.49$). Reproductive status (successful vs. unsuccessful) and mass at weaning in a given year did not significantly influence female survival to the next year (Cox regression with binomial error, random year, $R^2 = 0.324$; breeders N = 321, failed breeders N = 75; likelihood ratio test, $\chi^2 = 1.71$, $P = 0.19$). Including mass gained from 1st year emergence to either subsequent weaning time or 2nd year emergence had trivial effects on the model.

BREEDING SUCCESS AND BODY MASS

At emergence from hibernation in the spring, body mass varied with the reproductive success of adult females (Fig. 1; mixed model, year and female identity random variables, breeding status fixed factor; $R^2_{\text{conditional}} = 0.646$; likelihood ratio test, $\chi^2 = 14.7$, $P = 0.002$, N = 392). Females that subsequently failed during gestation were 7.0% lighter than females that successfully produced weaned offspring ($406.9 \pm 6.9\text{g}$, N = 34, and $437.5 \pm 2.7\text{g}$, N = 319, respectively; Tukey difference = -30.60g , 95% CI = -52.26g to -8.94g , $P = 0.002$). At the same time, females that subsequently failed during lactation were only 2.0% lighter in body mass to those that later successfully weaned offspring ($429.0 \pm 6.7\text{g}$, N = 39, and $437.5 \pm 2.7\text{g}$, N = 319, respectively; Tukey difference: = -8.53g , 95% CI = -28.90g to 11.83g , $P = 0.70$).

For analyses of body mass at weaning, due to low sample size, we pooled females failing during gestation and lactation. Unsuccessful females were 8.5% heavier than successfully breeding females (Fig. 1; mixed model, year and females identity random

variables, breeding status fixed factor; $R^2_{\text{conditional}} = 0.565$; likelihood ratio test, $\chi^2 = 13.7$, $P = 0.0002$, $N = 328$; mean mass = $551.9\text{g} \pm 12.4\text{g}$, $N = 15$, and $534.4\text{g} \pm 2.9\text{g}$, $N = 313$, respectively). The former gained 23.5% more body mass over the reproductive season than mothers that successfully weaned offspring (mixed model, year and females identity random variables, breeding status and spring emergence mass fixed factors; $R^2_{\text{conditional}} = 0.511$; likelihood ratio test for breeding status, $\chi^2 = 16.8$, $P < 0.0001$, $N = 327$; mean mass gain = $120.9\text{g} \pm 13.5\text{g}$, $N = 14$, and $97.9\text{g} \pm 2.9\text{g}$, $N = 313$, respectively).

At emergence from hibernation in the following spring, successful breeders were 3.7% lighter than females that failed during gestation (Fig. 1, $435.6\text{g} \pm 2.9\text{g}$, $N = 218$, $452.3\text{g} \pm 12.7\text{g}$, $N = 20$, respectively, Tukey difference = 16.63, 95% CI = -11.2g to 44.4g, $P = 0.41$). Females that failed during lactation were 1.3% heavier than those who failed during gestation ($458.0\text{g} \pm 11.0\text{g}$, $N = 27$, $452.3\text{g} \pm 12.7\text{g}$, $N = 20$, respectively, Tukey difference = 5.7, 95% CI = -29.4g to 40.8g, $P = 0.97$). Emergence mass next year for females that failed during lactation were 5.0% heavier and approached significance compared to successful breeders ($458.0\text{g} \pm 11.0\text{g}$, $N = 27$, $435.6\text{g} \pm 2.9\text{g}$, $N = 218$, respectively, Tukey difference = 22.3, 95% CI = -1.9g to 46.6g, $P = 0.08$). Due to low sample sizes and to examine this difference further, we pooled females that had failed during gestation or lactation. Those that were successful at weaning litters were significantly lighter in body mass compared to those that failed either at gestation or lactation (mixed model, year and female identity random variables, breeding status fixed factor; $R^2_{\text{conditional}} = 0.728$; likelihood ratio test, $\chi^2 = 37.6$, $P < 0.0001$, $N = 265$).

Between the time of offspring weaning in a given season and the following spring, females that failed to wean lost significantly more body mass than females successfully

raising a litter, (mixed model, year and females identity random variables, breeding status and body mass at the time of weaning of litters fixed factors; $R^2_{\text{conditional}} = 0.598$; likelihood ratio test for breeding status, $\chi^2 = 4.2$, $P = 0.04$, $N = 222$; mean mass loss = $108.9\text{g} \pm 19.0\text{g}$, $N = 9$, and $97.9\text{g} \pm 3.6\text{g}$, $N = 213$, respectively).

Females failing to wean offspring in a given year exhibited 11.1% increase in body mass the following spring, whereas successful breeders lost 0.4% (mixed model, year and females identity random variables, breeding status and previous spring emergence mass fixed factors; $R^2_{\text{conditional}} = 0.520$; likelihood ratio test for breeding status, $\chi^2 = 16.8$, $P < 0.0001$, $N = 243$; mean mass gain = $28.5\text{g} \pm 6.7\text{g}$, $N = 26$, and $-2.0\text{g} \pm 2.7\text{g}$, $N = 217$, respectively).

REPRODUCTIVE SUCCESS AND CARRY- OVER EFFECTS

Successful breeding in a given year did not depend on the previous breeding outcome (Table 2). The proportion of females from each reproductive status that successfully bred in the next year did not significantly differ (Fisher exact test, $N = 283$, $P = 0.49$). Litter size at birth in the succeeding year was not significantly different for reproductively successful and unsuccessful females (3.5 ± 0.08 pups, $N = 139$; 3.3 ± 0.2 pups, $N = 40$; respectively; Tukey difference = -0.19 , CI -0.72 to 0.35 , $P = 0.81$). Similarly, females that failed during gestation had nearly the same reproductive success at birth in the succeeding year as females that breed successfully the previous year (3.3 ± 0.2 pups, $N = 19$, 3.5 ± 0.08 pups, $N = 139$, Tukey difference = 0.003 , CI -0.56 to 0.56 , $P = 0.99$). Finally, time of reproductive failure in the former year did not significantly influence her reproductive success at birth in the following year (failed during gestation

= 3.5 ± 0.2 , N = 19, failed during lactation = 3.3 ± 0.2 , N = 21, Tukey difference = -0.19, CI -0.91 to 0.54, $P = 0.91$).

To examine whether success or failure in one year influenced the probability of successful breeding in the next year, we examined the predicted breeding probability for females of different emergence mass in the same year (Fig. 2). Changes in emergence mass the year subsequent to successfully breeding or failing, significantly influenced whether previously successful breeders maintained their breeding status or became failed breeders in the subsequent year (mixed model, emergence mass the next year fixed factor, random variable year and female id, binomial error distribution, $R^2_{\text{conditional}} = 0.273$, N = 218, $\chi^2 = 8.19$, $P = 0.0004$). For females that failed to wean offspring in the previous year, success in the subsequent year did not depend on emergence body mass (mixed model, data subset with only failed breeders [N = 38], emergence mass in the subsequent year fixed factor, random year, binomial family, $R^2_{\text{conditional}} = 0.047$, likelihood ratio test, $\chi^2 = 0.33$, $P = 0.56$). Litter size at birth and weaning in the year subsequent to successfully breeding or failing to do so were significantly associated with spring body mass in that year (mixed model, random female identity; $R^2_{\text{conditional}} = 0.384$, N = 180, likelihood ratio test, $\chi^2 = 21.4$, $P < 0.0001$; $R^2_{\text{conditional}} = 0.111$, N = 222, likelihood ratio test, $\chi^2 = 13.0$, $P < 0.0001$; respectively). Litter size at birth and weaning in the year subsequent to successfully breeding or failing to do so were also significantly associated with change in body mass from one spring to the next (mixed model, random female identity; $R^2_{\text{conditional}} = 0.375$, N = 180, likelihood ratio test, $\chi^2 = 21.4$, $P < 0.0001$; $R^2_{\text{conditional}} = 0.117$, N = 222, likelihood ratio test, $\chi^2 = 13.0$, $P < 0.0001$; respectively). These patterns did not differ significantly between females that were successful or that

failed in reproduction in the previous year, nor was there a significant interaction between breeding status and spring mass in the subsequent year; analyses not shown.

Discussion

In this study, we tested for potential costs of reproduction in Columbian ground squirrels by comparing the survival and future reproduction of females that bred successfully or not in given years. Our results provide little evidence of substantial “costs” to reproduction for females in traditional fitness measures.

Whereas we expected future survival to be higher for females not reproducing in a given year, we found that survival was actually similar between successful and unsuccessful females. Females that failed to give birth had poorer survival (though not significantly) than successfully reproducing females. Those results confirm previous findings having failed to detect a long-term survival cost to experimental manipulations of female reproductive effort via litter size manipulation (Hare & Murie 1992; Skibieli, Speakman & Hood 2013, but see contrasting results over shorter time periods in Neuhaus 2000). Our results were also similar to those of females of other Sciurid species (North American red squirrels (*Tamiasciurus hudsonicus*), Descamps et al. 2009, Fletcher et al. 2015; yellow ground squirrels (*Spermophilus fulvus*), Vasilieva & Tchabovsky 2014).

We also predicted that, when compared to unsuccessful breeders, successful females in a given year should experience poorer reproduction in the next year. However, we found little difference in the likelihood of future breeding in the next year for females that successfully reproduced or failed to wean young in the previous year, though the latter group was slightly more likely to be successful at future reproduction. There were

also no clear difference in litter size during the following year for females that were previously successfully reproductive versus those that previously failed. These results confirm comparative and experimental studies that reveal little or no influence of litter size in one year with litter size in the next year, whether the initial litter size was artificially manipulated or not (Murie & Dobson 1987; Hare & Murie 1992; Neuhaus 2000; Skibieli, Speakman & Hood 2013).

Despite no apparent negative fitness cost to breeding, allocation of resources clearly differed between females of different breeding status. First, females that failed during gestation were significantly lighter in body mass than successful females, suggesting they lacked sufficient capital reserves to produce offspring. Stored capital at spring emergence from hibernation is known to strongly influence subsequent reproductive success in Columbian ground squirrels (Dobson, Risch & Murie 1999; Broussard, Dobson & Murie 2003). By the time of weaning unsuccessful females (those that failed during gestation and lactation) had gained significantly more body mass than successful females. While this difference was slightly reduced by the following spring emergence from hibernation, unsuccessful females gained close to 25% in body mass from the previous spring, while the mass of successfully reproducing females changed only slightly. Thus, while there appeared to be no fitness costs to breeding in these ground squirrels (see also Murie & Dobson 1987; Hare & Murie 1992; Skibieli, Speakman & Hood 2013), there were consequences for body mass dynamics.

For females that successfully breed, the likelihood of success in the subsequent year depended strongly on energy stores at spring emergence from hibernation, with lighter females more likely failing to repeat as successful breeders. Experimental results

suggest advantages in body mass for females that forego reproduction (Hare & Murie 1992; Neuhaus 2000; Skibieli, Speakman & Hood 2013). This pattern however was not evident, for females that had failed to wean litters, as they were only slightly more likely to breed successfully in the subsequent year. Thus, the success or repeat failure of previously non-breeding females must depend on factors other than body mass. Other factors that might influence reproductive success include daily resource income, variation in the richness of the habitat, predation, and weather patterns (e.g., Dobson & Murie 1987; Karels et al. 2000; Lane et al. 2012).

In the spring following a breeding failure, females gained significantly more body mass from the previous year than reproductively successful females. While this did not improve their subsequent success, it resulted in these females being heavier at spring emergence, as compared to the previous year. This might explain why females that had previously failed at breeding did not subsequently show a dependency of reproductive success on body mass in the next year (Fig. 2), as the females that reproduced successfully did (Fig. 2). All females, however, showed a dependence of litter size on body mass in the subsequent year, such that heavier females had larger litters, as also found by Risch, Dobson & Murie (1995) in an earlier study. Since previously failed breeders gained more body mass on average from the previous year and were heavier in the next spring (Fig. 1), they were in a better position to produce larger litters. Thus, the body mass “carry over” effect after failure to reproduce may have augmented subsequent reproduction; not to a greater level than continuously reproductive females, but to a commensurate level with them. The only advantage to current failure was a chance to

recoup the condition necessary to be reproductively successful in terms of producing a litter.

In conclusion, our results fail to support the hypothesis of short-term costs to reproduction for fully-grown female ground squirrels, but demonstrate that there were consequences to successful breeding in terms of a lack of gain in body mass and perhaps body condition. Columbian ground squirrels are income breeders that use “capital” (stored resources) to increase their likelihood of future reproduction (Broussard, Dobson & Murie 2005). Females with a heavier weaning mass proceeded to a heavier emergence mass in the next year, and a higher chance of producing greater numbers of offspring. To some extent, failing to breed in one year allowed females to prepare for reproduction in the next year by accumulating extra energy reserves that, provided “carry-over” benefits in terms of body mass, likely augmented fitness.

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Table 1: Survival of reproductively mature (≥ 3 years old) female Columbian ground squirrels belonging to breeder or non-breeder classes. Average survival listed with standard errors. Significance based on Tukey post hoc test

Breeding Status	Average survival	N	<i>P</i>
Breeders	76.9 \pm 2.4%	321	
Failed at Birth	64.7 \pm 8.3%	34	0.39 ¹
Failed at Weaning	75.7 \pm 6.8%	41	0.99 ² 0.70 ³

¹comparison with breeders.

²comparison with breeders.

³comparison of failed at birth and failed at weaning.

Table 2: Probability of reproductively mature (≥ 3 years old) female Columbian ground squirrels belonging to certain reproductive groups from current year to the next year.

Year	Next		Failed	
	Breeders	N	Breeders	N
Current Year				
Breeders	78.9%	172	21.1%	46
Failed Breeders	82.0%	50	18.0%	11

Fig. 1. a) Spring emergence mass in the current year (Breeders, N = 319, No Birth, N = 34, No Wean, N = 39) b) weaning mass of females in the current year (Breeders, N = 313, No Wean, N = 15, as No Birth was 1 we combined No Birth and No Wean), and c) spring emergence mass the next year, of breeders (Breeders, N = 218, No Birth, N = 20, No Wean, N = 27). Significance determined with Tukey's HSD test and indicated by different letters.

Fig. 1.

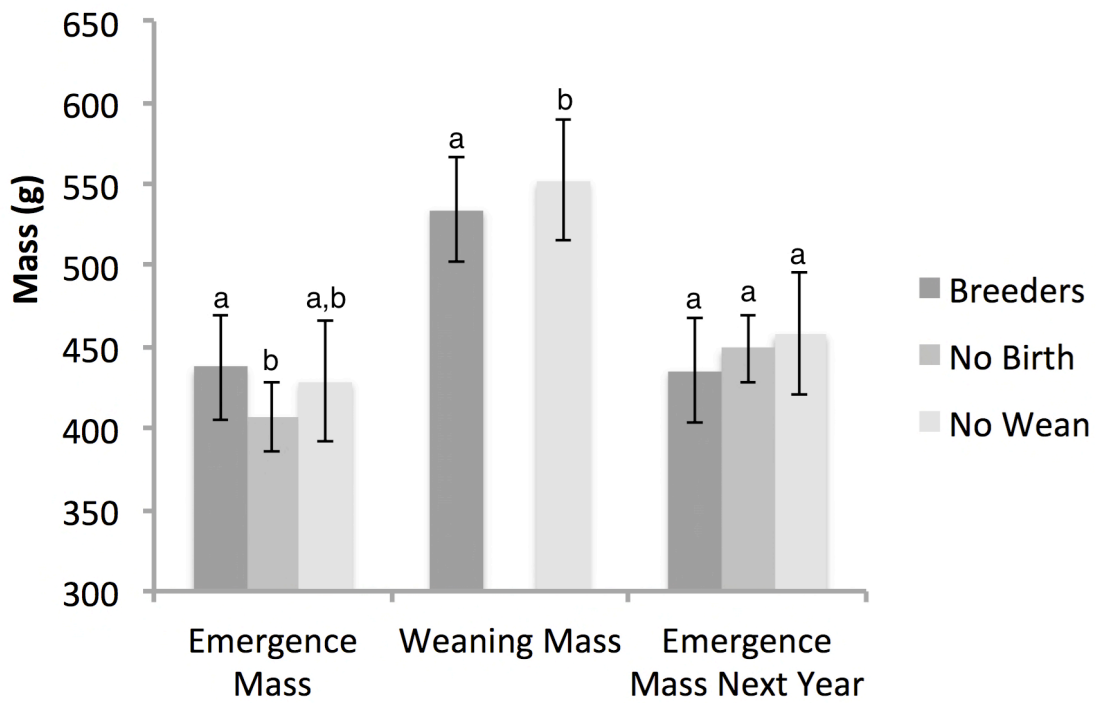


Fig. 2. Predicted probability by year (light grey lines, each dot represents an individual) of transitioning into breeder class from previous year's breeding status (left = Failed breeders, N = 99, Right = Breeders, N = 321) based on emergence mass in the next year. Solid lines are average breeding probability with 95% confidence interval dotted lines.

Fig. 2.

