Parental investment in the Columbian ground squirrel: empirical tests of sex allocation models

Thibaut Barra, Vincent Viblanc, Claire Saraux, Jan Murie, F. Stephen
Dobson

► To cite this version:

Thibaut Barra, Vincent Viblanc, Claire Saraux, Jan Murie, F. Stephen Dobson. Parental investment in the Columbian ground squirrel: empirical tests of sex allocation models. *Ecology*, 2021, 10.1002/ecy.3479 . hal-03299142

HAL Id: hal-03299142

<https://hal.science/hal-03299142>

Submitted on 26 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Parental investment in the Columbian ground squirrel: empirical tests of sex allocation models

Thibaut Barra^{1*}, Vincent A. Viblanc^{1*}, Claire Saraux¹, Jan O. Murie² & F. Stephen Dobson^{1,3φ}

* Equal contribution

¹University of Strasbourg, CNRS, Institut Pluridisciplinaire Hubert Curien, UMR 7178, 67000 Strasbourg, France

²Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9

³Department of Biological Sciences, Auburn University, 311 Funchess Hall, Auburn, AL 36849

^φCorresponding author: F Stephen Dobson, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9; fsdobson@msn.com

Running head: Sex allocation models in a wild mammal

ABSTRACT

Parental allocation of resources into male or female offspring and differences in the balance of offspring sexes in natural populations are central research topics in evolutionary ecology. Fisher (1930) identified frequency-dependent selection as the mechanism responsible for an equal investment in the sexes of offspring at the end of parental care. Three main theories are proposed for explaining departures from Fisherian sex ratios in light of variation in environmental (social) and individual (maternal condition) characteristics. The Trivers-Willard (1973) model of male-biased sex allocation by mothers in the best body condition is based on the competitive ability of male offspring for future access to mates and thus superior reproduction. The local resource competition model is based on competitive interactions in matriline, as occur in many mammal species, where producing sons reduces future intrasexual competition with daughters. A final model invokes advantages of maintaining matriline for philopatric females, despite any increased competition among females. We used 29 years of pedigree and demographic data to evaluate these hypotheses in the Colombian ground squirrel (*Urocyon columbianus*), a semi-social species characterized by strong female philopatry. Overall, male offspring were heavier than female offspring at birth and at weaning, suggesting a higher production cost. With more local kin present, mothers in the best condition biased their offspring sex ratio in favor of males, and mothers in poor condition biased offspring sex ratio in favor of females. Without co-breeding close kin, the pattern was reversed, with mothers in the best condition producing more daughters, and mothers in poor condition producing more sons. Our results do not provide strong support for any of the single-factor models of allocation to the sexes of offspring, but rather suggest combined influences of relative maternal condition and matriline dominance on offspring sex ratio.

Keywords: Kin selection, local resource competition, matriline, sex ratio allocation, Trivers-Willard hypothesis

INTRODUCTION

Life-histories are a central focus of both ecology and evolution (Stearns 1992; Roff 2001). The success of different life history strategies can be assessed through individual variations in fitness (characteristics of reproduction and survival), and the expression of traits contributing to higher reproductive and survival rates (e.g., Endler 1986; Kruuk and Hill 2008). The fitness differences of individuals that carry different trait forms, in turn, depend on the interactions of those traits with the ecological and social environments (e.g., Lande and Arnold 1983). Thus, our understanding of evolution rests on a foundation of ecological interactions that produce fitness differences (Hutchinson 1965). An aspect of reproduction that might result in fitness differences among individuals is the differential allocations of resources that parents make to male and female offspring. Such allocation differences might lead to variations in offspring sex ratio, if the fitness returns of producing males or females differ, depending on the environment.

In many animal species, offspring sex ratios are close to even, a generality that Darwin (1871) puzzled over. Fisher (1930) pointed out that it is not the numbers of male and female offspring that should be roughly equal in a population, but the amount of parental allocation of resources. Fitness returns of allocating resources to a male or female offspring should be equal in bisexual species, since every individual has a mother and father and in turn will potentially become a mother or father. In other words, the fitness returns of male and female offspring are equal. When the ratio of male to female offspring allocation varies from even, frequency-dependent selection should bring it back into balance. If both sexes are equally costly to produce, then an even sex ratio of offspring should occur (Fisher 1930, Charnov 1979).

Nonetheless, extremely biased offspring sex ratios have been found in nature (e.g., Hamilton 1967) and hypotheses for explaining adaptive deviations from so-called “Fisherian sex ratios” have been subsumed into a theory of how resources are allocated between the sexes

of offspring (Charnov 1982, Frank 1990, West 2009). In an early attempt at predicting biases from even sex allocations, Trivers and Willard (1973) suggested that mothers should vary in their body condition and ability to invest in offspring. If the future reproductive success of one sex is more variable than that of the other and can be influenced by augmented early investment, then mothers in better body condition should invest more in that sex. For instance, suppose that male future reproductive success is more variable than female future reproductive success, and is augmented by enhanced development early in life as in most polygynous and polygynandrous mammal species (Dobson 1982, Dobson et al. 2010). Then, mothers in relatively good body condition should produce more males, and mothers in relatively poor condition should produce more females. This hypothesis assumes that the relative cost of producing male and female offspring differs, that there is a positive association of maternal body condition and the production of males, that early investment in males should persist into adulthood, and that such males should have greater fitness as adults. Despite considerable empirical and theoretical study of offspring sex ratio in mammals (Clutton-Brock and Iason 1986; Festa-Bianchet 1996; Kojola 1998; Hewison and Gaillard 1999, Brown 2001; Cameron 2004; Cameron et al. 2008; Schindler et al. 2015; Toni et al. 2021), support for the prediction of a positive association of maternal condition and offspring sex ratio remains equivocal.

Clark (1978) developed the idea of local competition among relatives as an influence on offspring sex ratios that deviate from 50:50 to explain offspring sex ratios that typically vary between 60-75% male in species such as galagos (*Galago sp.*) and African wild dogs (*Lycaon pictus*). She noted that females are often limited in reproduction by competition over local resources, such as food, refuges from predation, or territories (reviewed by Emlen and Oring 1977). This competition should favor production of the dispersing sex. In polygynous and polygynandrous mammals, females are often highly philopatric and matrilineal, and the dispersing sex among juvenile and subadults is usually males (Greenwood 1980, Dobson

1982). When population density is already high, females should thus produce more males to prevent future local competition for resources. Several studies have provided empirical support for the local resource competition model, including primates (Clark 1978), marsupials (Cockburn et al. 1985; Schwanz and Robert 2014), and rodents (Wells and Van Vuren 2017).

Silk (1983) expanded on this model by suggesting different maternal allocations to male and female offspring according to maternal dominance status. Dominant, high-condition, mothers able to tolerate increased local resource competition may benefit from producing female offspring in greater proportion to ensure matriline dominance, especially in species where females exhibit cooperative breeding. In contrast, subordinate adult females should produce more dispersing sons to lower the costs of local competition. Support for this hypothesis has been found in social rodents (Armitage 1987a) and primates (Silk 1983; Simpson & Simpson 1982; Johnson 1988) (reviewed by Cockburn et al. 1985).

Biases in offspring sex ratios under the above hypotheses are expected to primarily result from differences in the fitness payoffs of producing or allocating resources to male or female offspring. The allocation of resources by parents might be best measured at the termination of parental care (Fisher 1930; Trivers and Willard 1973). However, in matrilineal species like social mammals, maternal investment can be extended throughout the lifespan, making estimation of maternal allocation of resources difficult. In addition, differential survival of offspring during the period of parental investment may cause a skew in allocation to the sexes (Austad 2015). Finally, the ecological and social influences on both allocation of resources to offspring and on offspring sex ratios are not mutually exclusive, so that more than one hypothesis might be supported in any particular species (e.g., Kruuk et al. 1999; Cockburn et al. 2002; Delean et al. 2009; Yeo & Hare 2021).

Columbian ground squirrels (*Urocitellus columbianus*) are a good system for the study of offspring energy allocation and sex ratio. They are semi-social, hibernating sciurid rodents

that inhabit subalpine meadows in the Rocky Mountains of southwestern Canada (Boag and Murie 1981; Dobson et al. 1992). Within colonies, philopatric females overlap temporally and spatially with close kin, creating local matriline (King and Murie 1985; King 1989a, 1989b). Females with higher numbers of kin acquire direct and indirect fitness benefits from philopatry (Viblanco et al. 2010; Arnaud et al. 2012; Dobson et al. 2012). Males, on the other hand, contribute little or nothing to the production of offspring beyond sperm, during a short mating season (Manno and Dobson 2008; Raveh et al. 2010, 2011), and exhibit high variance in reproductive success (Raveh et al. 2010). Mothers are variable in size and body condition (Dobson 1992; Dobson et al. 1999; Rubach et al. 2016), and thus provide suitable variables for testing hypotheses about allocation of resources to offspring and variation in offspring sex ratios. The amount and quality of food resources are a major influence on allocations to offspring by mothers (Dobson and Kjelgaard 1985; Dobson and Muri 1987; Dobson 1988). Finally, year-to-year environmental variations are extreme (Lane et al. 2012, Dobson et al. 2016), thus providing ample variation in annual availability of resources.

The purpose of our study was five-fold. First, we tested the repeatability of offspring sex ratio over the lifetimes of adult females, to examine trait flexibility. Second, we evaluated the costs to mothers of producing male and female offspring. We examined the estimated costs of sons and daughters in terms of their mass at weaning, a value that should reflect the energetic costs of producing offspring. We also examined the fitness costs of producing sons and daughters for mothers, by testing for differences in maternal reproduction, survival and mass gain during the active season. Third, we tested the Trivers and Willard (1973) prediction that mothers in good condition should produce costlier sons. For this, we focused on female mass at the start of the breeding season, known to strongly influence subsequent reproductive success (Dobson et al. 1999; Broussard et al. 2005; Rubach et al. 2016). We tested if females in good condition biased maternal investment towards males. Fourth, we tested Clark's predictions

from the local resource competition hypothesis of male-biased offspring production when local density and competition for resources are high within matriline (Clark 1978). Fifth, we examined Silk's (1983) prediction that females in the best body condition and in large matriline should produce sex ratios biased towards daughters, since they can best bear costs associated with local resource competition and reap the benefits of augmented matriline (Armitage 1987a,b).

We used a 29-year long-term data set of known female reproductive allocations including litter size and offspring mass at birth and weaning, maternal mass at the start of the reproductive season, and matriline genealogies that reveal close kin relationships between Columbian ground squirrels, to empirically test models of allocation of resources to the sexes of offspring. The data set on offspring sex ratio from parturition to offspring weaning, uniquely allowed distinguishing between offspring sex ratio at birth and at about the time that the major period of maternal care ended, and differential maternal allocation to (and survival of) the sexes over the period of parental care.

MATERIALS AND METHODS

STUDY SITE AND LONG-TERM MONITORING

Data were collected from 1992 to 2020 at a 2.6 ha subalpine meadow in the Sheep River Wildlife Provincial Park, Alberta, Canada (50°38'10"N, 114°39'56"W, 1550m asl). Columbian ground squirrels were monitored yearly throughout the breeding season from emergence from hibernation (~ mid-April) to weaning of the offspring (~ early July). In each year of the study, all animals were trapped when first emerging from hibernation, using live traps (Tomahawk Live Trap Co., Hazelhurst, WI; 13 × 13 × 40 cm) baited with a small amount of peanut butter. Ground squirrels were weighed to the nearest 5 g using a Pesola® spring-slide scale, given a unique ear tag number (#1-Monel metal; National Band and Tag Company,

Newport, KY), and painted with a unique dorsal mark on the pelage for visual identification at a distance, using black human hair dye (Clairol, Stamford, CT).

Females typically estrous cycle within 3-5 days after emergence from hibernation, and are sexually receptive for a few hours on a single day (Murie and Harris 1982, Murie 1995, Raveh et al. 2010). We determined mating date for each female from behavioral observations, inspection of their genitalia, and the presence of copulatory plug material in or around the vulva (Murie and Harris 1982). Mated females were caught 22 days after mating, 2-3 days before expected parturition, and transported to a 1.5 km-distant field laboratory where they gave birth. Females were housed in polycarbonate microvent cages (47 x 27 x 20 cm; Allentown Caging Equipment Company, Allentown, NJ), and provided wood chip bedding and shredded newspaper for nesting material. Food, including grains (a molasses-enriched horse feed), fresh apples, fresh lettuce and water were provided *ad libitum* twice a day. At birth, mothers (nearest 5 g) and pups (nearest 0.01 g) were weighed. Pups were sexed and marked with a small tissue biopsy used for establishing paternities (Hare and Murie 1992). The biopsy created a partial claw loss on a rear foot, and thus pups could be identified at birth and later at weaning. Newborns and their mothers were released approximately one day after birth into their nest burrow, known from behavioral observations of females stocking them with dry grass prior to capture and marked with colored flags.

Entire litters and mothers were caught 27-28 days after birth, when young first emerged from nest burrows near the time of weaning. Young were then ear tagged for permanent identification and weighed to the nearest 1 g. Mothers were weighed again to the nearest 5 g. Since 1992, several generations of matrilineal genealogies are known from mother-offspring associations. Complete life histories (including age and pedigree relationships) are known for the vast majority of animals that lived on the study site. For each year of the study, we used the long-term data to determine: (1) offspring sex ratio and individual mass at birth and at weaning,

and sex ratio variation among adult females, (2) female condition and energy allocation to offspring in grams of body mass, and (3) the density of closely related kin.

OFFSPRING SEX RATIO

We calculated offspring sex ratio at birth and at weaning as the proportion of males produced in a litter: $SR = \frac{males}{(males+females)}$. Because some females did not give birth in the lab (especially in earlier years of the study, and after 2017), sample sizes differ between sex ratio at birth and at weaning. To characterize partial litter loss over the course of lactation, we further calculated the difference between sex ratio at weaning and sex ratio at birth for females having lost at least one offspring between birth and weaning: $\Delta SR = SR_{wean} - SR_{birth}$. ΔSR varied between -0.67 and 0.75 (mean $\pm$ s.d. = 0.00 ± 0.26 , $n = 102$ litters).

DATA ANALYSES

All analyses were conducted in the R statistical computing environment v. 4.0.2 (R Core Team 2020). Results are presented as means $\pm$ 1 S.E., unless otherwise noted. Independent variables were standardized prior to analyses, so that coefficients were directly comparable as effect sizes. Where appropriate, we examined model residuals for normality by visual inspection of density distributions, Q–Q plots, cumulative distribution functions and P–P plots using the “fitdistrplus” package in R (Delignette-Muller & Dutang 2015). We also insured that no substantial collinearity occurred between independent variables (all variance inflation factors < 3 ; suggested cut-off, see Zuur, Ieno & Elphick 2010). The goodness-of-fit of each model was evaluated by comparing the deviance of the model to the deviance of the null model (with the intercept only) and expressed as explained deviance $ED = \frac{deviance_{null} - deviance_{model}}{deviance_{null}}$, characterizing the percent-deviance explained by the model.

Repeatability of offspring sex ratio

For females that bred in multiple years, we estimated offspring sex ratio repeatability using the "rptR" package in R (Stoffel et al. 2017). Repeatability was calculated as $R = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_R}$, where V_G is the among-group variance, V_R is the residual variance, and $V_P = V_G + V_R$ is the total phenotypic variance in offspring sex ratio. V_G was either the among-individual variance or the among-year variance, depending on whether repeatability within females or within years was considered. Variance in offspring sex ratio was decomposed using separate generalized linear mixed-effects models (GLMM, 'lme4' package in R; Bates et al. 2015), with offspring sex ratio specified as a proportion of successes and failures for each mother as the dependent variable (count data; matrix of "success" and "failures" with male = "success", female = "failure"), and mother identity or year as a random factor. Confidence intervals around repeatability estimates were calculated by parametric bootstrapping (N = 1000 random data sets were generated from the distribution defined by the estimated parameters). As the amount of variance in offspring sex ratio explained by mother ID or year was virtually zero, we disregarded these factors as random variables in subsequent models on sex ratio.

Cost differences in male and female offspring

Differences in the energy invested in the production of male and female offspring

First, we tested for differential energy costs in the production of male/female offspring production by comparing male and female mass at birth and at weaning. Offspring mass at birth and at weaning were specified as dependent variables in separate linear mixed models (LMMs), and offspring sex was included as an independent factor. We controlled for litter size at birth (mean $\pm$ s.d. = 3.22 ± 0.87 , min = 1, max = 7) as a covariate in all models because of the known trade-off between number and mass of offspring in our species (e.g., Dobson et al. 1999; Skibieli et al. 2009). Mother ID and litter ID were included as random variables in the

models to account for repeated measures over the years, and the non-independence of offspring born from the same mother and raised within the same litter. We also tested if female and male offspring differed in their survival from birth to weaning using a binomial GLMM. Offspring survival was included as the dependent variable (binary; survived = 1, died = 0) and offspring sex as the independent variable of interest. We further accounted for litter size and offspring mass at birth in the model as known influences on survival. As above, mother ID and litter ID were originally included as random variables in the models to account for repeated measures over the years, and the non-independence of offspring born from the same mother and raised within the same litter. However, mother ID explained virtually no variance in pup survival, and was removed from the final model.

Fitness costs to mothers

Second, we tested if maternal fitness costs could be detected for mothers that invested more into male or female-biased litters. We considered the effects of litter sex ratio at birth, or at weaning, on 3 proxies of maternal fitness: (1) reproductive output over the season (litter size at weaning; count data, mean $\pm$ s.d. = 2.54 ± 1.28 offspring, min = 0, max = 7; Poisson GLMM), (2) maternal mass gain (or loss) over the breeding season (continuous data, mean $\pm$ s.d. = 108.7 ± 46.8 g, min = -50 g, max = 280 g; LMM), and (3) mother survival (binary, 0/1; binomial GLMM) to the next year. We initially included mother ID, age and year as random factors in the models to account for variance in fitness parameters, but removed these when the variance explained was virtually null.

Testing Trivers and Willard's model of sex allocation

Maternal condition effects on litter sex ratio

We used a 3-step analysis to test for maternal condition effects on sex ratio.

First, to test whether mothers that raised a litter, on average, biased the offspring sex towards males in particularly favorable years, we calculated the average mass of breeding females at emergence from hibernation (mean $\pm$ s.d. = 419.72 g $\pm$ 20.70 g, min = 376.6 g, max = 460.55 g), and the average sex ratio (see Fig 1) at weaning for the population, for 28 years of the 29 year-study (no data on maternal mass in 2020 due to COVID-19). We then regressed mean offspring sex ratio at weaning on mean maternal mass at emergence (LM; N = 28).

Second, we used the entire data set to test for individual effects of maternal body mass at emergence of hibernation on offspring sex ratio at weaning. For this, we used a generalized linear model (GLM) specifying offspring sex ratio as a proportion of successes and failures for each mother and as the dependent variable, and maternal mass at the start of the season as the independent variable. Further, considering only females having lost at least one offspring during lactation, we tested the effects of maternal body mass at emergence from hibernation on the change in offspring sex ratio from birth to weaning (Δ SR) by regressing Δ SR on maternal mass at emergence. We also tested if the proportion of offspring (dependent variable, binomial proportion GLM) lost during lactation for individual mothers, controlling for litter size at birth, depended on maternal body mass at emergence.

Third, to test whether mothers biased the sex ratio of their litters towards males in years where they were in particularly good condition compared to their lifetime average, we calculated relative maternal mass at emergence within a year, i.e. female mass compared to her mean mass over the years she weaned a litter. This annual relative condition index (mean $\pm$ s.d. = 0.00 g $\pm$ 30.60, min = -133.00 g, max = 106.43 g) was calculated as:

$$\text{annual relative condition}_{i,y} = \text{mass}_{i,y} - \mu_i ,$$

where i is mother ID and y is year. A mother with a condition index > 0 (or < 0) in a given year, thus was in better (or worse) condition than her lifetime average. We then used a GLM

specifying offspring sex ratio as a proportion of males and females for each mother and as the dependent variable, and annual relative condition as the independent variable.

Maternal condition effects on male and female offspring mass gain

We tested whether the gain in offspring mass over the course of lactation for males (mean $\pm$ s.d. = 97.34 g $\pm$ 22.14, min = 45.04 g, max = 198.06 g) and females (92.12 g $\pm$ 19.57, min = 35.82 g, max = 169.61 g) was differently affected by maternal condition at the start of the breeding season. We used a linear model (LM) with offspring mass gain specified as the dependent variable, and maternal mass at emergence from hibernation, offspring sex and the interaction between offspring sex and maternal mass at emergence specified as independent variables. In addition, we controlled for litter size at birth, offspring mass at birth, and lactation duration (mean $\pm$ s.d. = 26.27 days $\pm$ 1.12, min = 23 days, max = 31 days) as covariates in the model, because they were possible influences on offspring mass gain.

Testing local resource competition models of sex allocation

Clark's model of local resource competition

To test Clark's (1978) prediction that mothers should bias offspring sex ratio towards the dispersing (male) sex when local kin competition for resources is high, we performed a two-step analysis. First, we tested if offspring sex ratio was biased towards males (the dispersing sex) in years of high population density. For this, we calculated the average sex ratio (see Fig 1) at weaning for the population, and regressed it on overall population density (mean $\pm$ s.d. = 62.69 individuals $\pm$ 22.87, min = 32 days, max = 120 days; N = 29 years). Second, we used the long-term matrilineal genealogies to determine the total number of kin females (mothers, daughters, and littermate sisters) in each year of the study (mean $\pm$ s.d. = 0.89 $\pm$ 0.72, min = 0, max = 4). We restricted our analyses to reproductive females only, defined as females that

mated and were potentially able to hold a territory at some point during the breeding season (Festa-Bianchet and Boag 1982, Murie and Harris 1988). We subsequently examined litter sex ratio at weaning and changes in offspring sex ratio from birth to weaning (LM; Δ SR) in relation to the numbers of kin present in the population (independent variables). We also tested if the proportion of offspring (dependent variable, binomial proportion GLM) lost during lactation by individual mothers, controlling for litter size at birth in the model, depended on the numbers of kin present in the population.

Silk's model of matriline dominance

To test Silk's (1983) prediction that dominant females in relatively good body condition (i.e., who can best incur the costs of local resource competition with kin) should produce sex ratios biased towards daughters, we further examined the interaction between maternal condition relative to other females and kin numbers. Here, we calculated relative condition as the differential between the mass of a mother and the mean mass of all females in the population at the start of the breeding season in a given year. Lactating females are socially dominant over other ground squirrels (Murie and Harris 1988), females in better body condition produce larger and heavier litters (Dobson et al. 1999), and mothers with the greatest reproductive investments are socially dominant (Viblanc et al. 2016). Condition relative to other females (mean $\pm$ s.d. = 0.00 g $\pm$ 49.51, range = -191.25 g to 174.23 g) was calculated as:

$$relative\ condition_{i,y} = mass_{i,y} - \mu_y ,$$

where i is mother ID and y is year. A relative condition > 0 (or < 0) would indicate that a female was in relatively better (or worse) condition than other females of the population in a given year. Relative condition increased in a quadratic fashion with age, so that, on average, it was < 0 for females until 4 years, increased up until 6-7 years old, before decreasing afterwards (see Appendix S1), consistent with previous quadratic relations found for dominance-related

aggression and age in this species (Viblanco et al. 2016). We subsequently examined litter sex ratio at weaning (GLM, binomial) and changes in offspring sex ratio from birth to weaning (LM; Δ SR) in relation to a female's relative condition, the number of kin present in the population, and the interaction between both variables (independent variables).

RESULTS

REPEATABILITY OF OFFSPRING SEX RATIO

At termination of parental care (weaning), offspring sex ratio in our population was on average 51.3 % male ($n = 1581$ offspring, $N = 195$ mothers), ranging from 36.2% in 2020 to 74.2% in 2009 (Fig. 1). The estimated repeatability of offspring sex ratio was virtually nil both when considering inter-individual variation (binomial GLMM; $R = 0.000 \pm 0.006$, $CI_{95} = [0.000 - 0.022]$, $n = 622$ litters, $N = 203$ mothers), and when considering inter-annual variation ($R = 0.007 \pm 0.006$, $CI_{95} = [0.000 - 0.021]$, $n = 622$ litters, $N = 29$ years).

COST DIFFERENCES BETWEEN MALE AND FEMALE OFFSPRING

Differences in the energy invested in the production of male and female offspring

At birth, controlling for litter size, male offspring were 4.15% heavier than female offspring (Fig 2A; Appendix S2: Table S1A). Similarly, at weaning and when controlling for litter size, male offspring were 3.61 % heavier than female offspring (Fig 2B; Appendix S2: Table S2B). Controlling for litter size and offspring mass at birth, male and female offspring did not differ significantly in their survival rates (75% for males, 76.8% for females; Appendix S2: Table S2) from birth to weaning. Offspring survival was positively associated with offspring mass at birth (z -mass odds ratio = $+1.86 \pm 0.16$, Appendix S2: Table S2), but not significantly with litter size at birth (z -litter size odds ratio = $+1.20 \pm 0.25$, Appendix S2: Table S2).

Fitness costs to mothers

When testing for costs to mothers, maternal reproduction (litter size weaned) was not significantly related to litter sex ratio at birth (GLMM, with Poisson error term; odds ratio = 0.87 ± 0.09 , $CI_{95} = [0.71, 1.07]$, $z = -1.34$, $P = 0.18$, $n = 415$) or at weaning (0.94 ± 0.07 , $CI_{95} = [0.81 - 1.10]$, $t = -0.76$, $P = 0.45$, $n = 559$). Maternal survival to the next year was neither significantly influenced by litter sex ratio at birth (GLMM, with binomial error term; odds ratio = 0.98 ± 0.39 , $CI_{95} = [0.45 - 2.13]$, $z = -0.05$, $P = 0.96$, $n = 415$) or at weaning (odds ratio = 1.08 ± 0.35 , $CI_{95} = [0.57 - 2.05]$, $z = 0.24$, $P = 0.81$, $n = 536$). Maternal mass gain over the season was positively, but not significantly, related to litter sex ratio at birth (LMM; 8.44 ± 7.83 , $CI_{95} = [-6.90 - 23.78]$, $t = 1.08$, $P = 0.28$, $n = 350$), and tended to be positively associated with litter sex ratio at weaning (9.81 ± 5.39 , $CI_{95} = [-0.75 - 20.37]$, $t = 1.82$, $P = 0.07$, $n = 508$). Thus, for a 1%-point increase in litter sex ratio at weaning (e.g. from 50 to 51% male), maternal mass gain over the course of reproduction increased by 0.098 grams, on average.

TESTING TRIVERS AND WILLARD'S MODEL OF SEX ALLOCATION

Maternal condition effects on sex ratio

Over 28 years, the mean sex ratio at weaning of offspring in the population (Fig 1) was not significantly associated with mean maternal mass at the start of the breeding season (LM; z -mean female mass = -0.01 ± 0.02 , $t = -0.41$, $CI_{95} = [-0.04, 0.03]$, $P = 0.69$, $N = 28$ years).

Maternal body mass at the start of the breeding season did not significantly affect offspring sex ratio at weaning (GLM; binomial; z -mass odds ratio = 0.88 ± 0.09 , $CI_{95} = [0.74, 1.04]$, $z = -1.46$; $P = 0.15$, $n = 1499$, $ED = 0.29\%$). Similarly, for females that lost at least one offspring over lactation, maternal body mass did not significantly affect changes in offspring sex ratio from birth to weaning (LM; z -mass = $+0.03 \pm 0.03$, $CI_{95} = [-0.02, 0.08]$, $t = 1.11$, $P = 0.27$, $n = 102$, $ED = 1.22\%$). Overall, controlling for litter size at birth (all VIFs < 1.2), the

proportion of offspring lost during lactation was not significantly related to maternal mass at emergence (GLM binomial; odds ratio: z -emergence mass = 0.96 ± 0.07 , $CI_{95} = [0.84 - 1.10]$, $P = 0.57$, $n = 1336$, $ED = 0.03\%$).

When considering maternal yearly condition index (maternal yearly mass relative to her lifetime average), mothers did not seem to bias litter sex ratio differently in years when they were in relatively better or worse condition compared to their lifetime average (GLM; z -yearly condition odds ratio = 0.96 ± 0.05 , $CI_{95} = [0.86, 1.06]$, $z = -0.84$, $P = 0.40$, $n = 1499$, $ED = 0.1\%$). Maternal yearly condition also did not seem to significantly affect changes in offspring sex ratio from birth to weaning, for those mothers that lost young during lactation (LM; z -yearly condition = 0.01 ± 0.03 , $CI_{95} = [-0.04, 0.07]$, $t = 0.54$, $P = 0.590$, $n = 102$, $ED = 0.29\%$).

Maternal condition effects on mass gain of male and female offspring

Regardless of sex (non-significant interaction, Appendix S2: Table S3A), offspring mass gain over lactation was positively related to maternal mass at the start of the breeding season (z -maternal mass = $+3.42 \pm 1.28$), to offspring mass at birth (z -offspring mass birth = $+4.40 \pm 0.44$), to lactation duration (z -lactation duration = $+4.48 \pm 0.66$), and negatively related to litter size at birth (z -litter size birth = -7.58 ± 1.08) (Fig 3; Appendix S2: Table S3B). Controlling for these effects in the model (Appendix S2: Table S3B) male offspring gained 1.68 % more mass than females over lactation (Fig. 3A). The absence of an interaction between maternal body mass and offspring sex suggests no differential allocation to males and females according to maternal condition. Taken together, the results do not provide strong support for Trivers and Willard's model of sex allocation in Columbian ground squirrels.

LOCAL RESOURCE COMPETITION MODELS OF SEX ALLOCATION

Clark's model of local resource competition

Offspring sex ratio at weaning was not significantly associated with population size over the course of our study (LM; z -population density = -0.02 ± 0.02 , $t = -1.16$, $CI_{95} = [-0.05, 0.02]$, $P = 0.25$, $N = 29$ years) (Appendix S2: Figure S1). Offspring sex ratio at weaning was positively and significantly, though weakly, associated with a female's kin numbers in a given year (GLM; z -kin odds ratio = $+1.10 \pm 0.05$, $CI_{95} = [1.00, 1.21]$, $z = 2.05$, $P = 0.040$, $n = 1371$, $ED = 0.6\%$). This effect appeared to be mediated through differential mortality of male and female offspring during lactation, since offspring sex ratio at birth was not significantly related to a female's kin numbers in a given year (GLM; z -kin odds ratio = $+1.02 \pm 0.05$, $CI_{95} = [0.92, 1.11]$, $z = 0.30$, $P = 0.764$, $n = 1243$, $ED = 0.02\%$). Indeed, for females that lost at least one offspring over lactation, kin numbers were positively, though weakly, associated with changes in offspring sex ratio from birth to weaning (LM; z -kin = $+0.05 \pm 0.03$, $CI_{95} = [-0.00, 0.10]$, $t = 1.87$, $P = 0.065$, $n = 97$, $ED = 3.5\%$). Controlling for litter size at birth (all VIFs < 1.01), the overall proportion of offspring lost during lactation was not significantly related to kin numbers (GLM binomial; odds ratio: z -kin = 1.08 ± 0.07 , $CI_{95} = [0.95 - 1.23]$, $P = 0.22$, $n = 1246$, $ED = 0.18\%$). Taken together, these results appear to provide some support for local resource competition as an influence on sex ratio in Columbian ground squirrels.

Silk's model of matriline dominance

Adding maternal body mass relative to female conspecifics in a given year and its interaction with kin numbers to the above models revealed a significant interaction between maternal relative condition and kin numbers affecting sex ratio at weaning (Fig 4; z -maternal relative condition $\times z$ -kin odds ratio = $+1.18 \pm 0.06$, $CI_{95} = [1.06, 1.33]$, $t = 2.88$, $P = 0.004$, $n = 466$; see Appendix S2: Table S4A; $ED = 2.1\%$). The proportion of males in weaned litters increased with maternal condition relative to other females within a year, but only when kin numbers

were high (with 2 or more co-breeding female kin). It was only when kin were absent that mothers in relatively good condition produced more daughters (Fig 4). Changes in offspring sex ratio from birth to weaning, in contrast, did not seem to be significantly affected by the interaction between maternal relative condition and kin (Appendix S2: Table S4B). Removing the interaction did not reveal any effect of maternal relative condition (z -maternal relative condition = $+0.00 \pm 0.00$, $CI_{95} = [-0.00, 0.00]$, $t = 1.26$, $P = 0.21$, $n = 97$), but indicated a subtle effect of kin numbers (z -kin = $+0.04 \pm 0.02$, $CI_{95} = [-0.00, 0.08]$, $t = 1.93$, $P = 0.056$, $n = 97$, $ED = 5.1\%$) on changes in sex ratio from birth to weaning (ΔSR). These results do not seem to support Silk's model of matriline dominance in Columbian ground squirrels.

DISCUSSION

As predicted by Fisher (1930), and over 29 years of study, offspring sex ratio at the end of parental care in Columbian ground squirrels was roughly even at 51.3 % male. However, sex ratio varied markedly, from 36.2% to 74.2% male (repeatability was virtually null) among years, suggesting scope for trait responses to variations in maternal, social, and ecological conditions. We tested three hypotheses proposed to explain deviations from Fisherian sex ratios in the animal realm from an adaptive perspective: the Trivers and Willard (1973), Clark (1978), and Silk (1983) models of maternal allocation to male and female offspring. These hypotheses are not mutually exclusive, but make different predictions about parental allocations to male and female offspring that can influence offspring sex ratio. Trivers and Willard (1973) focused on female energy allocation. For Columbian ground squirrels, where increased maternal condition is related to increased reproductive output (Dobson and Kjelgaard 1985; Dobson and Murie 1987; Dobson 1988; Risch et al. 1995; Dobson et al. 1999; Skibieli et al. 2009), they predict a positive association between maternal body condition and male offspring production. In contrast, Clark (1978) focused on local resource competition, predicting an increased

production of the dispersing sex (males) when local competition is high. Finally, Silk (1983) made the opposite prediction of Trivers and Willard (1973), in that behaviorally dominant females in good condition (in our case, indexed by higher body mass relative to other females) should produce females to enhance the benefits of matriline.

The Trivers and Willard (1973) hypothesis was not well supported by our results for Columbian ground squirrels (but see Schindler et al. 2015), although the conditions for this hypothesis were generally met. Offspring sex ratio was close to Fisher's (1930) even expectation at about 51%. Males seemed slightly more energetically expensive to produce, because they were about 4% heavier than females. However, they incurred no detectable fitness costs to mothers. Part of this mass difference was accounted for by differential growth during lactation, when young are under ground and solely dependent on the mother for sustenance. This condition likely persists into adulthood, since males are both larger and heavier than females at every age (Dobson 1992). Heavier males obtain advantageous matings and produce more offspring (Raveh et al. 2010; Balmer et al. 2019). Thus, we could examine the prediction that more should be invested in males by mothers with the greatest breeding resources. Female ground squirrels breed primarily from daily resource consumption, but their resources at the start of the spring breeding season have a significant influence on reproduction (Dobson et al. 1999; Broussard et al. 2005; Rubach et al. 2016).

Adult female condition at near the time of conception is most likely to influence offspring sex ratio (Cameron et al. 1999; Cameron 2004; Sheldon and West 2004; Cameron and Linklater 2007). However, we found no significant pattern for male-biased litters in years where the mean condition of females in the population was higher at the beginning of the spring breeding season. Also, there was no significant association between individual maternal body mass at the beginning of the spring breeding season and either sex ratio of young at weaning, or change in sex ratio of young between birth and weaning for mothers that lost young during

lactation. Finally, individual mothers did not produce male-biased litters in years when they were in better condition compared to their lifetime average, as the Trivers-Willard (1973) hypothesis would predict. The Trivers-Willard hypothesis appears well supported in some taxa and weakly or not supported in others (Cameron 2004; Robert and Schwanz 2011; Schindler et al. 2015; Douhard et al. 2016; Hamel et al. 2016; Douhard 2017). Identification of the underlying reasons for such variation (e.g., phylogeny, social structure, food availability, environmental constraints) might be evident in a meta-analysis of published results on sex ratio and the Trivers-Willard hypothesis.

We found a weak, but significant, association between kin numbers and sex-ratio at weaning. The proportion of males in a litter increased by a factor 1.15 (unstandardized odds ratio) for a 1 unit increase in kin numbers in the population. In other words, for one extra breeding kin in the population, sex ratio would increase by 15%, for instance from 50% male to 57.5% male. This observation is consistent with Clark's (1978) model of local kin competition. Clark's hypothesis was devised to explain a strong overall male bias in the offspring sex ratios of species such as galagos and African wild dogs. To avoid competition with close relatives, Clark (1978) suggested that breeding females in such systems might produce more sons, if males were the predominant dispersing sex. For species without a strong overall sex bias in numbers of offspring, however, the hypothesis still makes predictions about the preferential production of the sex of offspring by mothers with respect to kinship, when resources are in short supply. In Columbian ground squirrels, females with co-breeding close relatives, like mothers, daughters, and sisters, live in close proximity in matriline (Arnaud et al. 2012; Dobson et al. 2012), whereas subadult males are predominant dispersers (Wiggett and Boag 1992; Neuhaus 2006). Closely related adult females have the potential for competition with respect to using environmental resources, but the presence of close co-breeding female kin clearly benefits reproduction and fitness (Viblanco et al. 2010; Dobson et al. 2012).

Silk (1983) expanded on Clark's original model by considering the importance of kin neighbors on offspring sex ratio, but argued that mothers in good condition should produce more daughters to maintain and promote advantages associated with the matriline of closely related kin, while mothers in poor condition should produce more males to avoid additional competition. Silk's (1983) hypothesis was that dominance matters in primate species, such that dominant females produced more daughters and subordinate females produced more sons that dispersed from their natal groups. In Columbian ground squirrels, adult females are individually territorial, but show both material and fitness benefits to living close to co-breeding relatives, despite the possibility of reproductive competition. Material benefits include lowered aggression, and closer proximity of more easily-defendable territories (Harris and Murie 1984; King and Murie 1985; King 1989a, 1989b; Arnaud et al. 2012). Fitness benefits of proximity to close relatives include improved individual reproductive success (Viblanco et al. 2010) and augmented inclusive fitness via a substantial indirect fitness component (about 40%; Dobson et al. 2012). Thus, it is reasonable to predict that adult females in the best condition and social circumstances (viz., surrounded by close kin) should produce daughters.

When considering maternal body mass relative to other females in a given year, we found that the presence of female kin was associated with offspring sex ratio bias. Mothers in better body condition produced slightly more sons, but only when more co-breeding close kin were present. The opposite pattern occurred for mothers that started the season in relatively poor condition. This would seem to contradict Silk's (1983) ideas about promotion of the matriline through the birth of daughters to adult females in good condition, but support Clark's (1978) idea that mothers in matrilineal groups that suffer from resource competition should produce more of the dispersing sex among their offspring. Further support for Clark's (1978) hypothesis of the influence of local resource competition was somewhat present in adult females that

suffered a partial litter loss. These females tended to produce more males as the number of co-breeding female close kin increased (with an associated trend towards greater juvenile male survival from birth to weaning), as might be expected if they invested more in males to avoid future resource competition. Silk (1983) suggested that competitive mothers reduce daughter production of subordinate females via harassment. In Columbian ground squirrels, lactating mothers in better body condition produce larger and heavier litters (Dobson et al. 1999), and are known to be socially dominant (Murie and Harris 1988; Viblanc et al. 2016). On the other hand, harassment as a result of aggression and infanticide is biased towards non-kin in this species (King 1989a, Stevens 1998, Viblanc et al. 2016), and whether the selective mortality of daughters we observed with increasing kin numbers during lactation resulted from increased female harassment towards subordinate kin remains unclear.

Examining offspring sex ratios at weaning, Wells and Van Vuren (2018) found that golden mantled ground squirrels (*Callospermophilus lateralis*) had increasing male-bias at weaning with increased numbers of nearby close kin, though older females surrounded by many kin had offspring sex-ratios biased towards females. Armitage (1987a), working with yellow-bellied marmots (*Marmota flaviventris*), found that young breeding females in matriline produced more daughters, and females without kin nearby produced more males. Michener (1980) found that female Richardson's ground squirrels (*Urocitellus richardsonii*) that produced more females maintained matriline over generations, whereas those producing fewer females did not. More recently, Yao & Hare (2021) suggested that litter sex ratio in this species varied with litter size and female experience (first-time, second or third litters produced) (but see Gedir & Michener 2014), indicating possible complex adjustments related to resource competition and matriline maintenance. In alpine marmots (*Marmota marmota*), females without kin helpers produce significantly more of that sex (male) in their litters (Allaine et al. 2000; Allaine 2004). The presence or absence of close kin clearly has an

influence on the sex ratio of offspring in this group of rodents (viz., marmotines), but the influences and effects appear to vary from species to species.

Given the variation among species in likely influences on sex ratios and allocation of resources to the sexes of offspring, multiple influences in Columbian ground squirrels should not be surprising. In addition, skewed sex ratios could also occur because of differential susceptibility of male and female fetuses to *in utero* environments and stress (Vandenbergh & Huggett 1994; Schacht et al. 2019; Firman 2020). In our study, two factors appeared most influential, the relative body condition of mothers when breeding commenced (a strong influence on reproductive success; Dobson et al. 1999; Broussard 2005), and how many co-breeding close kin these females shared (another major influence on reproduction and fitness; Viblanc et al. 2010; Dobson et al. 2012). Mothers in superior body condition in a strong matriline produced more sons. Mothers in good condition but little or no matriline advantages produced more female offspring, perhaps favoring a stronger future matriline advantage. Mothers in relatively poor condition but with strong networks of close kin produced more daughters, while those with no or few co-breeding kin produced more sons. Thus, variation in sex ratios among offspring are perhaps best explained by a combination of factors with demonstrated influences on reproduction.

ACKNOWLEDGMENTS

We are grateful to Alberta Parks, and Alberta Environment & Parks (Fish and Wildlife division) for their continued support in long-term monitoring programs. We are especially grateful to the Biogeoscience Institute (especially E. Johnson, Director; A. Cunnings, Station Manager; K. Ruckstuhl, Responsible Faculty), University of Calgary, for providing us with field camp and laboratory facilities. A large number of field assistants over the years made our data collection possible. We thank Y. Handrich and M.-A. Forin-Wiart for previous discussions

and suggestions on this study, and are especially grateful to B. Kotler and two anonymous reviewers for their critical insights on the paper. T.B. was supported by a scholarship from the Institut Pluridisciplinaire Hubert Curien during the data analyses and paper writing. The research was funded by a U.S.A. National Science Foundation grant (DEB-0089473), to F.S.D., a research grant from Natural Sciences and Engineering Research Council of Canada to J.O.M., a CNRS Projet International de Coopération Scientifique grant (PICS- 07143) to VAV, and a research grant from the Fondation Fyssen to V.A.V. F.S.D thanks the Institute of Advanced Studies of the University of Strasbourg for financial support through an USIAS fellowship, and the Région Grand Est and the Eurométropole de Strasbourg for the award of a Gutenberg Excellence Chair.

AUTHOR CONTRIBUTION

TB analyzed the data, collected data in the field, and co-wrote the manuscript; VAV co-designed the study, analyzed the data, collected data in the field, and co-wrote the manuscript; CS co-designed the study, analyzed the data, collected data in the field, and commented on the manuscript; JOM originated the long-term study, collected data in the field and commented on the manuscript; FSD co-designed the study, collected data in the field and co-wrote the manuscript.

REFERENCES

- Allaine, D., F. Brondex, L. Graziani, J. Coulon, and I. Till-Bottraud. 2000. Male-biased sex ratio in litters of alpine marmots supports the helper repayment hypothesis. *Behavioral Ecology* 11:507–514.
- Allaine, D. 2004. Sex ratio variation in the cooperatively breeding alpine marmot *Marmota marmota*. *Behavioral Ecology* 15:997-1002.

624 Armitage, K. B. 1987a. Do female yellow-bellied marmots adjust the sex ratio of their
625 offspring ? *The American Naturalist* 129:501-519.

626 Armitage, K. B. 1987b. Social dynamics of mammals: reproductive success, kinship and
627 individual fitness. *Trends in Ecology and Evolution* 2:279–284.

628 Arnaud, C. M., F. S. Dobson, and J. O. Murie. 2012. Philopatry and within-colony
629 movements in Columbian ground squirrels. *Molecular Ecology* 21:493–504.

630 Austad, S. N. 2015. The human prenatal sex ratio: A major surprise. *Proceedings of the*
631 *National Academy of Sciences of the United States of America* 112:4839–40.

632 Balmer, A., B. Zinner, J. C. Gorrell, D. W. Coltman, S. Raveh, and F. S. Dobson. 2019.
633 Alternative reproductive tactics and lifetime reproductive success in a
634 polygynandrous mammal. *Behavioral Ecology* 30:474-482.

635 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models
636 using lme4. *Journal of Statistical Software* 67:1–48. doi: [10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01).

637 Boag, D. A., and J. O. Murie. 1981. Population ecology of Columbian ground squirrels in
638 southwestern Alberta. *Canadian Journal of Zoology* 59:2230–2240.

639 Broussard, D. R., F. S. Dobson, and J. O. Murie. 2005. The effects of capital on an income
640 breeder: evidence from female Columbian ground squirrels. *Canadian Journal of*
641 *Zoology* 83:546–552.

642 Brown, G. R. 2001. Sex-biased investment in nonhuman primates: can Trivers & Willard’s
643 theory be tested? *Animal Behaviour* 61:683–694.

644 Cameron, E. Z., W. L. Linklater, K. J. Stafford, and C.J. Veltman. 1999. Birth sex ratios
645 relate to mare condition at conception in Kaimanawa horses. *Behavioral Ecology*
646 10:472-475.

647 Cameron, E. Z. 2004. Facultative adjustment of mammalian sex ratios in support of the
648 Trivers-Willard hypothesis: evidence for a mechanism. *Proceedings. Biological sciences*

649 271:1723–8.

650 Cameron, E. Z., and W. L. Linklater. 2007. Extreme sex ratio variation in relation to change
651 in condition around conception. *Biology Letters* 3:395–397.

652 Cameron, E. Z., P. R. Lemons, P. W. Bateman, and N. C. Bennett. 2008. Experimental
653 alteration of litter sex ratios in a mammal. *Proceedings. Biological sciences* 275:323–7.

654 Charnov, E. L. 1979. *The Genetical Evolution of Patterns of Sexuality: Darwinian Fitness*.
655 *The American Naturalist* 113:465–480.

656 Charnov, E. L. 1982. *The theory of sex allocation*. Princeton University Press, Princeton.

657 Clark, A. B. 1978. Sex ratio and local resource competition in a prosimian primate. *Science*
658 201:163–5.

659 Clutton-Brock, T. H., and G. R. Iason. 1986. Sex Ratio Variation in Mammals. *The Quarterly*
660 *Review of Biology* 61:339–374.

661 Cockburn, A., M. P. Scott, and C. R. Dickman. 1985. Sex ratio and intrasexual kin
662 competition in mammals. *Oecologia* 66:427–429.

663 Cockburn A., S. Legge, and M. C. Double. 2002. Sex ratios in birds and mammals: can the
664 hypotheses be disentangled? In: Hardy, I. C. W., editor. *Sex ratios: concepts and*
665 *research methods*. Cambridge (UK): Cambridge University Press. p. 266–286.

666 Darwin, C. 1871. *The Descent of man and selection in relation to sex*. Modern Library, New
667 York.

668 Delean, S., G. De’ath, and H. Marsh. 2009. Climate and maternal effects modify sex ratios in
669 a weakly dimorphic marsupial. *Behavioral Ecology and Sociobiology* 64:265–277.

670 Delignette-Muller, M. L., and C. Dutang. 2015. *fitdistrplus: an R package for fitting*
671 *distributions*. *Journal of Statistical Software* 64(4):1-34.

672 Dobson, F. S. 1982. Competition for mates and predominant juvenile male dispersal in
673 mammals. *Animal Behaviour* 30:1183–1192.

674 Dobson, F. S., and J. D. Kjelgaard. 1985. The influence of food resources on life history in
 675 Columbian ground squirrels. *Canadian Journal of Zoology* 63:2105–2109.

676 Dobson, F. S., and J. O. Murie. 1987. Interpretation of intraspecific life history patterns:
 677 evidence from Columbian ground squirrels. *American Naturalist* 129:382-397.

678 Dobson, F. S. 1988. The limits of phenotypic plasticity in life histories of Columbian ground
 679 squirrels. Pages 193-210 in M. S. Boyce, ed. *Evolution of life histories of mammals*.
 680 Yale University Press, New Haven, Conn.

681 Dobson, F. S. 1992. Body mass, structural size, and life-history patterns of the columbian
 682 ground squirrel. *The American naturalist* 140:109–25.

683 Dobson, F. S., M. J. Badry, and C. Geddes. 1992. Seasonal activity and body mass of
 684 Columbian ground squirrels. *Canadian Journal of Zoology* 70:1364–1368.

685 Dobson, F. S., T. S. Risch, and J. O. Murie. 1999. Increasing returns in the life history of
 686 Columbian ground squirrels. *Journal of Animal Ecology* 68:73-86.

687 Dobson, F. S., B. M. Way, and C. Baudoin. 2010. Spatial dynamics and the evolution of
 688 social monogamy in mammals. *Behavioral Ecology* 21:747–752.

689 Dobson, F. S., V. A. Viblanc, C. M. Arnaud, and J. O. Murie. 2012. Kin selection in
 690 Columbian ground squirrels: direct and indirect fitness benefits. *Molecular Ecology*
 691 21:524–531.

692 Dobson, F. S., J. E. Lane, M. Low, and J. O. Murie. 2016. Fitness implications of seasonal
 693 climate variation in Columbian ground squirrels. *Ecology and Evolution* 6:5614–5622.

694 Douhard, M., M. Festa-Bianchet, and F. Pelletier. 2016. Maternal condition and previous
 695 reproduction interact to affect offspring sex in a wild mammal. *Biology Letters*
 696 12:20160510.

697 Douhard, M. 2017. Offspring sex ratio in mammals and the Trivers-Willard hypothesis: in
 698 pursuit of unambiguous evidence. *BioEssays* 39:1700043.

699 Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating
700 systems. *Science* 197:215–223.

701 Endler, J. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton.

702 Festa-Bianchet, M., and D. A. Boag. 1982. Territoriality in adult female Columbian ground
703 squirrels. *Canadian Journal of Zoology* 60:1060–1066.

704 Festa-Bianchet, M. 1996. Offspring sex ratio studies of mammals: Does publication depend
705 upon the quality of the research or the direction of the results? *Écoscience* 3:42–44.

706 Firman, R. C. 2020. Exposure to high male density causes maternal stress and female-biased
707 sex ratios in a mammal. *Proceedings of the Royal Society B* 287:20192909.

708 Fisher, R. A. 1930. *The genetical theory of natural selection*. Clarendon Press, Oxford.

709 Frank, S. A. 1990. Sex Allocation Theory for Birds and Mammals. *Annual Review of*
710 *Ecology and Systematics* 21:13–55.

711 Gedir, J. V., and G. R. Michener. 2014. Litter sex ratios in Richardson’s ground squirrels:
712 long-term data support random sex allocation and homeostasis. *Oecologia* 174:1225–
713 1239.

714 Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals.
715 *Animal Behaviour* 28:1140–1162.

716 Hamel, S., M. Festa-Bianchet, and S. D. Cote. 2016. Offspring sex in mountain goat varies
717 with adult sex ratio but only for mothers in good condition. *Behavioral Ecology and*
718 *Sociobiology* 70:123–132.

719 Hamilton, W. D. 1967. Extraordinary Sex Ratios. *Science* 156:477–488.

720 Hare, J. F., and J. O. Murie. 1992. Manipulation of Litter Size Reveals No Cost of
721 Reproduction in Columbian Ground Squirrels. *Journal of Mammalogy* 73:449–454.

722 Harris, M. A., and J. O. Murie. 1984. Inheritance of nest sites in female Columbian ground
723 squirrels. *Behavioral Ecology and Sociobiology* 15:97–102.

724 Hewison, A. J. M., and J.-M. Gaillard. 1999. Successful sons or advantaged daughters? The
 725 Trivers–Willard model and sex-biased maternal investment in ungulates. *Trends in*
 726 *Ecology & Evolution* 14:229–234.

727 Hutchinson, G. E. 1965. *The Ecological Theater and the Evolutionary Play*. Yale University
 728 Press, New Haven.

729 Johnson, C. N. 1988. Dispersal and the sex ratio at birth in primates, 332: 726–728.

730 King, W. J. 1989a. Kin-differential behaviour of adult female Columbian ground squirrels.
 731 *Animal Behaviour* 38:354–356.

732 King, W. J. 1989b. Spacing of female kin in Columbian ground squirrels (*Spermophilus*
 733 *columbianus*). *Canadian Journal of Zoology* 67:91–95.

734 King, W. J., and J. O. Murie. 1985. Temporal overlap of female kin in Columbian ground
 735 squirrels (*Spermophilus columbianus*). *Behavioral Ecology and Sociobiology* 16:337–
 736 341.

737 Kojola, I. 1998. Sex Ratio and Maternal Investment in Ungulates. *Oikos* 83:567.

738 Kruuk, L. E., T. H. Clutton-Brock, S. D. Albon, J. M. Pemberton, and F. E. Guinness. 1999.
 739 Population density affects sex ratio variation in red deer. *Nature* 399:459–461.

740 Kruuk, L. E. B., and W. G. Hill. 2008. Introduction. Evolutionary dynamics of wild
 741 populations: the use of long-term pedigree data. *Proceedings of the Royal Society B*
 742 275:593–596

743 Lande, R., and S. Arnold. 1983. The measurement of selection on correlated characters.
 744 *Evolution* 37:1210–1226.

745 Lane, J. E., L. E. B. Kruuk, A. Charmantier, J. O. Murie, and F. S. Dobson. 2012. Delayed
 746 phenology and reduced fitness associated with climate change in a wild hibernator.
 747 *Nature* 489:554–557.

748 Manno, T. G., and F. S. Dobson. 2008. Why are Male Columbian Ground Squirrels

749 Territorial? *Ethology* 114:1049–1060.

750 Michener, G. R. 1980. Differential reproduction among female Richardson's ground squirrels
751 and its relation to sex ratio. *Behavioral Ecology and Sociobiology* 7:173–178.

752 Murie, J. O., and M. A. Harris. 1982. Annual Variation of Spring Emergence and Breeding in
753 Columbian Ground Squirrels (*Spermophilus columbianus*). *Journal of Mammalogy*
754 63:431–439.

755 Murie, J. O., and M. A. Harris. 1988. Social interactions and dominance relationships
756 between female and male Columbian ground squirrels. *Canadian Journal of Zoology*
757 66:1414–1420.

758 Murie, J. O. 1995. Mating behavior of Columbian ground squirrels. I. Multiple mating by
759 females and multiple paternity. *Canadian Journal of Zoology* 73:1819–1826.

760 Neuhaus, P. 2006. Causes and consequences of sex-biased dispersal in Columbian ground
761 squirrel, *Spermophilus columbianus*. *Behaviour* 143:1013–1031.

762 R Core Team. 2020. R: A language for statistical computing. R Foundation for Statistical
763 Computing, Vienna, Austria.

764 Raveh, S., D. Heg, F. S. Dobson, D. W. Coltman, J. C. Gorrell, A. Balmer, and P. Neuhaus.
765 2010. Mating order and reproductive success in male Columbian ground squirrels
766 (*Urocitellus columbianus*). *Behavioral Ecology* 21:537–547.

767 Raveh, S., D. Heg, V. A. Viblanc, D. W. Coltman, J. C. Gorrell, F. S. Dobson, A. Balmer,
768 and P. Neuhaus. 2011. Male reproductive tactics to increase paternity in the
769 polygynandrous Columbian ground squirrel (*Urocitellus columbianus*). *Behavioral*
770 *Ecology and Sociobiology* 65:695–706.

771 Risch, T. S., F. S. Dobson, and J. O. Murie. 1995. Is mean litter size the most productive? A
772 test in Columbian ground squirrels. *Ecology* 76:1643–1654.

773 Robert, K. A., and L. E. Schwanz. 2011. Emerging sex allocation research in mammals:
 774 marsupials and the pouch advantage. *Mammal Review* 41:1–22 .
 775 Roff, D. A. 2001. *Life History Evolution*. Oxford University Press, Oxford.
 776 Rubach, K., M. Wu, A. Abebe, F. S. Dobson, J. O. Murie, and V. A. Viblanc. 2016. Testing
 777 the reproductive and somatic trade-off in female Columbian ground squirrels. *Ecology*
 778 and *Evolution* 6:7586–7595.
 779 Schacht, R., D. Tharp, and K. R. Smith. 2019. Sex ratios at birth vary with environmental
 780 harshness but not maternal condition. *Scientific reports* 9: 1-7.
 781 Schindler, S., J. Gaillard, A. Grüning, P. Neuhaus, L. W. Traill, S. Tuljapurkar, and T.
 782 Coulson. 2015. Sex-specific demography and generalization of the Trivers–Willard
 783 theory. *Nature* 526:249–252.
 784 Schwanz, L. E., and K. A. Robert. 2014. Proximate and ultimate explanations of mammalian
 785 sex allocation in a marsupial model. *Behavioral Ecology and Sociobiology* 68:1085-
 786 1096.
 787 Sheldon, B. C., and S. A. West. 2004. Maternal dominance, maternal condition, and offspring
 788 sex ratio in ungulate mammals. *American Naturalist* 163:40–54.
 789 Silk, J. B. 1983. Local Resource Competition and Facultative Adjustment of Sex Ratios in
 790 Relation to Competitive Abilities. *The American Naturalist* 121:56–66.
 791 Simpson, M. J. A., and A. E. Simpson. 1982. Birth sex ratios and social rank in rhesus
 792 monkey mothers. *Nature* 300:440-441.
 793 Skibiell, A. L., F. S. Dobson, and J. O. Murie. 2009. Maternal influences on reproduction in
 794 two populations of Columbian ground squirrels. *Ecological Monographs* 79:325-341.
 795 Stearns, S. C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.

796 Steven, S. D. 1998. High incidence of infanticide by lactating females in a population of
797 Columbian ground squirrels (*Spermophilus columbianus*). *Canadian Journal of Zoology*
798 76:1183-1187.

799 Stoffel, M. A., S. Nakagawa, and H. Schielzeth. 2017. rptR: repeatability estimation and
800 variance decomposition by generalized linear mixed-effects models. *Methods in Ecology*
801 and *Evolution* 8:1639–1644.

802 Toni, P., D. Forsyth, and M. Festa-Bianchet. 2021. Determinants of offspring sex in
803 kangaroos: a test of multiple hypotheses *Behavioral Ecology*, in press.

804 Trivers, R., and D. Willard. 1973. Natural selection of parental ability to vary the sex ratio of
805 offspring. *Science* 179:90–92.

806 Vandenberg, J. G., and C. L. Huggett. 1994. Mother's prior intrauterine position affects the
807 sex ratio of her offspring in house mice. *Proceedings of the National Academy of*
808 *Sciences USA*, 91: 11055-11059.

809 Viblanc, V. A., C. M. Arnaud, F. S. Dobson, and J. O. Murie. 2010. Kin selection in
810 Columbian ground squirrels (*Urocitellus columbianus*): littermate kin provide
811 individual fitness benefits. *Proceedings of the Royal Society B: Biological Sciences*
812 277:989–994.

813 Viblanc, V. A., C. Pasquaretta, C. Sueur, R. Boonstra, and F. S. Dobson. 2016. Aggression in
814 Columbian ground squirrels: relationships with age, kinship, energy allocation, and
815 fitness. *Behavioral Ecology* 27:1716-1725.

816 Wells, C. P., and D. H. Van Vuren. 2017. Female kin density affects offspring sex ratio in an
817 asocial mammal, the golden-mantled ground squirrel, *Callospermophilus lateralis*.
818 *Animal Behaviour* 134:71–77.

819 West, S. A. 2009. Sex allocation. Princeton University Press, Princeton.

820 Wiggett, D. R., and D. A. Boag. 1992. Natal dispersal in Columbian ground squirrels: is body

821 mass the proximate stimulus? Canadian Journal of Zoology 70:649–653.

822 Yeo, A. R., and J. F. Hare. 2021. Richardson’s ground squirrel litter size-sex ratio trade-off

823 reveals conditional adaptive sex allocation 195: 915-925.

824 Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid

825 common statistical problems. Methods in Ecology and Evolution 1:3-14.

826

827

828

Figure captions

Fig 1. Annual offspring sex ratio (% males) at weaning in the Columbian ground squirrel (*Uroditellus columbianus*) from 1992 to 2020. The black line represents the mean litter sex ratio for mothers (mean of individual mother values = 51.3%) in the population each year with associated 95%CI obtained by bootstrap (1000 simulations, 50% of the litters resampled each time). The blue dotted line represents the sex ratio of all offspring at weaning in the entire population (total number of weaned males / (total number of weaned males + total number of weaned females)). A sex ratio above 50% (solid black horizontal line) is biased towards males, and below, towards females.

Fig 2. Offspring mass (in grams) at (A) birth and (B) weaning according to sex in the Columbian ground squirrel. Boxplots present the median, 25 and 75 percentiles of the distribution. Red dots and lines present the marginal estimated means and 95% CI for female and male offspring mass, accounting for litter size at birth and weaning, respectively (see Models in text).

Fig 3. Offspring mass gain (g) over the lactation period as a function of (A) offspring sex, (B) offspring mass at birth (standardized), (C) maternal mass at the start of the breeding season (standardized), (D) litter size at birth (standardized), and (E) lactation duration (in days, standardized) in Columbian ground squirrels. Boxplots present the median, 25 and 75 percentiles of the distribution. Red dots and lines present the marginal estimated means and 95% CI for female and male offspring mass, accounting for all other effects in the model. Linear relationships show the raw data points and the predicted effect with 95% confidence intervals, also accounting for the other effects in the model (see text).

854

855 **Fig 4.** Interaction plot representing the effects of maternal relative condition within years
856 (standardized, see Methods) on litter sex ratio at weaning as a function of number of kin
857 present. The line and 95% confidence intervals were computed from a Generalized Linear
858 Model that examined litter sex ratio at weaning as a matrix of count data, i.e. matrix of
859 “success” and “failures” with male = “success”, female = “failure.”

860

Fig 1.

Fig 2.

Fig 3.

Fig 4.