

EFFECTS OF RESOURCE AVAILABILITY ON SEX ALLOCATION IN
HERBIVORES

By

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EFFECTS OF RESOURCE AVAILABILITY ON SEX ALLOCATION OF HERBIVORES

Abstract

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For well over a century, ecologists have attempted to understand the evolutionary pressures shaping the allocation of resources to offspring of different sexes but support for competing theories remains inconclusive overall. Most empirical studies with mammals have been performed on free-ranging animals where researchers are unable to control environments or to trace the line of individuals over multiple years. At Washington State University and Starkey Experimental Forest and Range in Oregon, we set up nutritional trials to test the effects of body mass, body condition, and diet on the birth sex ratios of two species of polygynous mammals: mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*). Despite carefully controlling and measuring nutritional resources, we found little evidence that forage quality or body fat near breeding influences birth sex ratio (BSR) of female mule deer and elk, or the probability that a neonatal elk or mule deer would be certain sex. However, in our sample of 76 mule deer fawns, we found that digestible protein intake influenced the probability of having a male fawn. Similarly, although BSR of females treated with GC was absolutely higher than those treated with a placebo (0.71 vs. 0.50), BSR were not statistically different and corticosteroid treatment did not predict the sex of a fawn. In addition to analyzing the preexisting data, we used another herbivore, *Grammia nevadensis*, to test how growth rate, population density and diet affect sex ratio of offspring. We used high protein, low fiber kale and a low protein, high fiber pelleted diet to explore how these various diets affect other life-history traits of *G. nevadensis*. Despite

the benefits of using *G. nevadensis* as a model species to test sex allocation hypotheses, we found little evidence that forage quality, average number of days till pupation or average number of days to emergence affected population sex ratios. Forage quality did, however, affect growth rates and the average number of days till pupation or emergence. Our results illustrate the difficulties in testing evolutionary hypotheses such as TWH in wild ungulates and herbivores in general.

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Dedication

To the four most important males in my life.

My Past:

Dr. Tobias Oregon Yellin (1934-2005)

My Present:

Jason Neil Johnstone-Yellin

and

Cai Oregon Neil Johnstone-Yellin (born 10 February 2007)

And my Future:

Baby Boy #2 Johnstone-Yellin (due 14 May 2010)

USING MULE DEER (*ODOCOILEUS HEMIONUS*), ELK (*CERVUS ELAPHUS*) AND *G. NEVADENSIS* TO EXAMINE SEX ALLOCATION

Much scientific inquiry has focused on the evolution of sex ratios (i. e., how many males and females in a population) and sex allocation (i. e., how resources are allocated to each of the male and female functions) since Darwin published *The Descent of Man and Selection in Relation to Sex* in 1871. Today researchers continue to debate the models and look for evidence in species ranging from arthropods (Fernandes 1995) and annelids (Schäer et al. 2005), to birds (Sheldon et al. 1999, Polo et al. 2004), ungulates (Clutton-Brock et al. 2002) and primates (Silk 1983, Colas 1999), including human beings (Norberg 2004). Ecologists are particularly interested in the following questions about sex allocation, as outlined by Charnov (1982): (1) For a dioecious species, what equilibrium sex ratio does natural selection maintain? (2) What is the equilibrium sex order and time of sex change in sequential hermaphrodites? (3) What is the equilibrium of resource allocation to male and female function in a simultaneous hermaphrodite? (4) Under what conditions are the strategies of hermaphroditic and dioecious species evolutionarily stable? (5) Under what environmental or life history conditions does nature select for a change in an individual's allocation toward male versus female function? Exploring each of these questions increases our understanding of how natural selection favors genetic contributions towards male versus female function (Charnov 1982). Understanding these concepts may allow managers to manipulate sex ratios and population dynamics of declining or overabundant species.

Although Darwin considered the evolution of sex ratio briefly, he believed the problem was so intricate that it was “safer to leave its solution to the future” (Darwin 1871). Fisher (1930) was the first to propose the explanation of frequency dependent selection of offspring sex.

Large populations are expected to invest efforts in males and females equally because at the population level, males and females contribute genes equally to the next generations. In this case, the reproductive value of males and females is equal ($\frac{1}{2}$, Stearns 1987). Fisher proposed that if the reproductive values are not equal, populations would be biased towards one sex (1930). Consequently, the rarer sex would have more mates on average, and would have higher reproductive success. Offspring of this sex would improve the parent's chances of passing on its genes, thus creating a genetic tendency for parents to produce offspring of the rarer sex, which would subsequently even out the sex ratio. Fisher termed this the "frequency dependent reproductive advantage" of the rarer sex.

Trivers and Willard (1973) developed the condition dependent selection (CDS) variation of Fisher's principle (Fisher 1930), which models sex ratio at the individual level. Fisher's theory assumes investments and rewards are the same for both sexes resulting in linear investment and returns. The Trivers Willard Hypothesis (TWH, 1973), on the other hand, expanded Fisher's ideas to incorporate unequal investment and returns and to include mother condition in the model. If one sex benefits from extra parental investment more than the other, then mothers with more resources to contribute to offspring will bias allocation to that sex that yields greater rates of return. CDS assumes that the benefits from parental investment extend into adulthood and that condition affects male reproductive success more than females. Males in good physical condition will be able to compete successfully for mates and potentially sire multiple offspring. Females, on the other hand, can only have a set number of young per breeding season. For example, most ungulates have one litter of one or two offspring per year. Therefore, if a mother is in good condition and has sufficient access to resources, she should

invest in sons. Mothers in poor condition should invest in daughters to ensure they have at least one litter of grandchildren each breeding season (Trivers and Willard 1973).

Since it was first proposed, researchers have attempted to look for evidence to support the TWH. Some studies have shown that biased sex ratios do indeed occur in mammals and seem to correlate with parental condition and investment (Clutton-Brock 1984, Austad and Sunquist 1986, Johnstone-Yellin 2004). Still other studies show no significant relationship between sex ratios and mother condition (Setchell et al. 2002, Silk et al. 2005) or mothers in good condition produce more daughters (Altmann et al. 1988, see Clutton-Brock and Iason 1986 for more studies prior to 1984). With conflicting results such as these, condition dependent selection continues to elude researchers. In the following research, I used three herbivores to test applications of sex allocation.

In chapter two, I (along with Lisa A. Shipley, John G. Cook, Troy Tollefson and Erin Clancey) examined two species of polygynous mammals: mule deer (*Odocoileus hemionus*), a small-framed ungulate that typically produces twins of all sex combinations and elk (*Cervus elaphus*), a medium-framed ungulate that typically produces singletons. We tested the effects of diet quality, body condition and levels of corticosterone on birth sex ratios. Both mule deer and elk are sexually dimorphic species, thus bigger, stronger and dominant males get more breeding opportunities. Although both are classified as polygynous breeders, their life-history traits differ enough to warrant comparison. First, mule deer have a tending-bond where males tend to a single female until copulating and then moves on to another female (serial polygyny, Geist 1981). Elk, on the other hand, form true harems where bigger, stronger males attract groups of females and maintain that group until successfully breeding with every female (Geist 1982). Males expend energy fighting for these harems and if they are not the dominant male and harem

holder, they can still breed as ‘sneakers’ (Gibson and Guinness 1980, Clutton-Brock 1986). After males have filled their role, mule deer and elk females also have differing adaptations to gestation. Mule deer are able to conceive as yearlings if in adequate body condition but usually conceive for the first time during their second year (Nellis et al. 1976) and primarily produce twins. Most female elk will not conceive successfully until their second year (Flook 1970). In addition, mule deer will wean their young before breeding in November (Tollefson et al. in press) whereas elk will breed in October yet can nurse calves already at heel through February (Kittams 1953, Flook 1970). These differing life history-traits will affect BSR in addition to mother body condition and should be considered when examining reproductive success.

In chapter three, I used *G. nevadensis* as a model herbivore to test effects of resource availability on sex allocation further. *G. nevadensis* larvae can feed on more than 80 species and 50 taxa of flowering, herbaceous plants and can move between plant species within hours, even minutes (Singer 2000), presumably selecting an optimal diet. Diet not only affects growth rates of larvae but also, in turn, affects the size of adults at emergence and the time at which adults emerge in relation to other individuals within a population. In some insects, such as mosquitoes (*Aedes sierrensis*, Kleckner 1995) and grasshoppers (*Sphenarium purpurascens* Cuevo del Castillo and Núñez-Farfán 2002) female fecundity is size specific and the differing emergence times between sexes is adaptive (Wiklund et al. 1992, Nylin et al 1993). However, males and females undergo different selective pressures on emergence times. Protrandry, or the emergence of males from the pupal stage before females, is considered an evolutionary stable strategy and the mating rates of protandrous insects depend on sex ratios at emergence (Zonneveld and Metz 1991). Females emerge later to avoid early-emergence deaths before mating (Zonneveld and Metz 1991), and males emerge early to maximize the number of matings with the females that

emerge later (Bulmer 1983, Wiklund and Fagerström 1977). If females emerge after many males then female mating chances increase (Bulmer 1983, Thornhill and Alcock 1983, Zonneveld and Metz 1991). However, if sex ratios are skewed toward females and females no longer encounter males at a high probability, their risk of pre-reproductive deaths will increase. Thus a mechanism of differential sex allocation is in place in *G. nevadensis* and allowed me to manipulate that allocation by varying diet and population density.

By using two ungulate species and *G. nevadensis* as a model herbivore with a known sex allocation mechanism I attempted to weed out the most important life-history traits in facultative sex ratio adjustment.

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EFFECTS OF MATERNAL BODY CONDITION AND GLUCOCORTICOIDS ON BIRTH SEX RATIOS: A TEST OF THE TRIVERS AND WILLARD HYPOTHESIS

The Trivers and Willard Hypothesis (TWH, 1973) of condition dependent selection (CDS) predicts that mothers in better body condition will allocate resources toward male offspring because they have the potential for more than one mating per reproductive season and thus a higher reproductive success than female offspring. Despite theoretical and applied interest in whether and how mothers might bias sex ratios in their offspring to increase their own fitness, most empirical studies have been performed only with free-ranging animals in uncontrolled environments, in which researchers were unable to control for the availability or quality of resources nor were they able to measure body condition or hormone levels directly. They instead relied on indirect measures of resource availability such as timing of coat change (Clutton-Brock et al. 1984) and social dominance (Clutton-Brock 1986, Cassinello 1996, Byers 1997). Other studies used body mass as a measure of body condition (Kojola 1993, Schultz and Johnson 1995, Biggersson and Ekvall 1997), but body mass confounds energy stores with frame size, thus may not be as robust as other condition parameters. Finally, until recently, mechanisms of how mothers could facultatively adjust sex ratio of offspring could only be speculated.

With the technological advances of molecular biology, researchers have begun to test some of the mechanistic hypotheses of sex allocation. Possible mechanisms can be divided into three categories: post-parturition, pre-conception and post-conception. Post-parturition adjustment may include differential investment in rearing males versus females (Clutton-Brock et al. 1985, Brown 2001, Tollefson 2007, Koskela et al. 2009). Possible pre-conception mechanisms include Y-chromosome-biased male ejaculates (Deyoung et al. 2004), females as

the heterogametic sex controlling sex allocation (e.g., in birds, Rutkowska and Badyaev 2008), environmental conditions such as ambient temperature and wartime in human beings (Helle et al. 2009), or increased levels of testosterone in females (Grant 2007, Grant et al. 2008). Finally, post-conception hypotheses include resorption of the expensive sex when maternal reserves are limited (Krackow 1992, Owusu et al. 2010) and blood glucose levels at the time of implantation (Larson et al. 2001, Cameron 2004), a concept that we explore more thoroughly in this study.

Stored glucose is released into the bloodstream when glucocorticoids (GCs) stimulate gluconeogenesis. To kick off this response, corticotropin releasing hormone is released by the hypothalamus, which triggers the pituitary to release adrenocorticotropin releasing hormone (ACTH). This in turn spurs the adrenal glands to secrete GCs, specifically cortisol and corticosterone. The end result is more glucose circulating in the blood (Sapolsky et al. 2000). Usually this reaction is caused by stress from the environment and social dominance but can also be influenced by diet with higher fat diets causing higher levels of circulating glucose (Austad and Sunquist 1986, Rosenfeld et al. 2003). Variation in glucose levels just after conception allows females to differentially implant male and female blastocysts. The increase in glucose enhances growth of male blastocysts rather than females, increasing the chance of implantation of male embryos (Larson et al. 2001).

In our study we tested the TWH in polygynous ungulates by experimentally manipulating nutritional resources and glucocorticoid (GC) levels in controlled environments and measured nutritional condition and hormone levels directly. We focused our study specifically on the effects of maternal resources pre-conception and during the current breeding season, and controlled for any potential effects of the male on sex of offspring. To examine the effects of resource availability using two species of sexually dimorphic, polygynous cervids, mule deer

(*Odocoileus hemionus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*), in which larger, stronger males command more of the breeding opportunities. However, these cervids differ in key life history traits that might influence how resource availability might influence the fitness value of producing offspring of a specific sex. Mule deer are smaller, and tend to fall on the income side of the income-capital breeder continuum, whereas elk are larger and tend to fall towards the capital-breeder end (Stearns 1992). Further, elk typically produce singletons, thus their choice is limited to one male or one female offspring, whereas mule deer frequently produce twins of either the same or mixed sex, so have 5 different offspring combinations. Finally, mule deer males form tending bonds, thus are essentially serially monogamous, whereas elk are true harem breeders defending a group of females for exclusive breeding rights. Therefore, we expected female deer and elk receiving higher quality nutritional resources, those consuming more digestible energy or digestible protein per day, and those with greater fat and muscle stores to produce a higher BSR, that this affect would be the most prominent in elk with greater sexual dimorphism and larger harems, and that DEI and DPI would have a greater effect on sex ratio than body condition in the income-breeding mule deer.

Second, we also experimentally altered the amount of corticosterone to test whether artificially changing GC (and therefore glucose) levels in mule deer affects birth sex ratios. We expected mothers fed higher quality diets, achieving a greater digestible energy intake (DEI, MJ/d), and those with more body fat to produce a higher birth sex ratio (BSR, number of males/(number males + number of females)) than those consuming lower quality diets and with lower fat stores. Second, we expected that females exposed to higher levels of glucocorticoids would produce a greater BSR.

METHODS

Effect of forage quality, nutrient intake and body condition on Birth Sex Ratio.

To test the effects of diet and mother condition on BSR, 24 hand-raised, lactating mule deer housed at the Wild Ungulate Facility at Washington State University and 31 hand-raised, lactating elk housed in 1-ha pens near Kamela, Oregon were stratified by body mass, litter size, and fawn sex ratio and subjected to 1 of 3 nutritional treatments from mid-summer (early lactation) through late fall (just before breeding season), over two consecutive years. Complete details of these experiments are provided in Tollefson et al. in press, Tollefson 2007, and Cook 2004), but here we briefly describe the treatments and measures of condition and DEI. The 3 nutritional treatments consisted of either a completely-balanced custom herbivore pellet (deer) or a combination of a custom herbivore pellet and hay (elk) with digestible energy (kJ/g) values formulated to mimic natural declines in vegetation available to mule deer and elk in temperate North America at the end of summer (Van Soest 1996, Hodgman et al. 1996, Cook et al. 2004a). The nutritional quality of the diets fed to deer and elk began at 14.0 kJ/g DE and either was maintained (High DE diet) or declined to 11.9 kJ/g DE (medium DE diet) or 9.8 kJ/g DE by early fall. For the experiments with mule deer, the first year diet DE began to decline on 5 August 2004), whereas in the second year, diet DE began to decline 5 weeks earlier (29 June 2005) mimicking a drought year. For the experiments with elk DE began to decline on 1 July

The body fat composition and depth of the loin muscle of each animal was measured at the end of the nutritional experiments just before breeding season (i.e., first week of November for mule deer, and mid-October for elk). We anesthetized each mule deer with 0.4 mg/kg xylazine hydrochloride and reversed them with 0.3 mg/kg yohimbine hydrochloride, and each elk with 0.5 mg/kg xylazine hydrochloride and 0.1 mg/kg yohimbine hydrochloride. We

measured maximum subcutaneous rump fat thickness (MAXFAT, Stephenson et al. 1998, Cook et al. 2007) and thickness of the *longissimus dorsi* muscle (Herring et al. 1995) between the 12th and 13th rib, adjacent to the spine (hereafter called loin depth), using a portable ultrasonograph (Sonovet 600, Medison Corp. Universal Medical, Newbedford Hills, NY). We scored the rump body condition (rumpBCS) of each animal using palpation, validated for mule deer (Cook et al. 2007) and elk (Cook et al. 2001). We calculated percent body fat using the equations developed for mule deer in Cook et al. in press, and elk in Cook et al. (2001).

Elk were group-fed, so individual values of DE intake were not measured. Deer were housed together but fed individually using a Calan feeder system, thus daily DEI was calculated as the product of dry matter intake (DMI, g/d) and DE(kJ/g) content of the pellet, and DPI (g protein/d) was the product of DMI and the crude protein content (%) of the diet. DMI was the mass of pellets offered (g), less the mass refused (g), multiplied by the dry matter content (%) of the pellets. DE was calculated as the product of the gross energy concentration of the pellet (kJ/g) measured using bomb calorimetry and its dry matter digestibility (%) obtained from in vivo digestion trials with mule deer (Tollefson et al., in press). Crude protein was estimated as 6.25 the nitrogen content, as measured using the Kjeldahl procedure (Bradstreet 1965).

Following the nutritional experiments at the onset of breeding (i.e., late October- early November for mule deer, late September-early October for elk), males were placed in the pens with each treatment group. The following spring, fawns and calves were located and sexed. BSR was calculated as number of male neonates/total litter size for each animal that year. Elk were divided post hoc into early and late breeding treatments for the 1996 breeding season based on parturition dates in spring 1997.

We examined the effect of diet quality, body condition, and nutrient intake during breeding season on the BSR of females the following spring using PROC GLM (SAS software, Version 9.1.3, SAS System for Microsoft Windows, Cary, NC, USA). We also tested the probability that a particular neonate would be male using PROC LOGISTIC with stepwise regression and an $\alpha = 0.15$ for inclusion into the model. In the mule deer models, we included the independent variables diet treatment, experimental year, body fat, loin depth, DEI and DPI, although DEI and DPI were not included in the same model because they were significantly correlated ($N = 47$, $R = 0.81$, $p < 0.001$). BSR was weighted for litter size (i.e., 1 or 2). In the elk models, we included the independent variables of diet treatment, experimental year and body fat.

Effect of Corticosterone on Birth Sex Ratio.

Females were stratified into two groups based on temperament, any known dominance interactions and sex ratios of previous offspring. On 25 October 2007, we anesthetized (0.3 mg/kg xylazine hydrochloride) and implanted 21-day release pellets of corticosterone (Innovative Research of America, Sarasota, Florida, USA) in a biodegradable matrix (up to 600 mg) under the skin of 12 females. The other 12 females were implanted with pellets made from matrix only (Institutional Animal Care and Use Protocol # 3718). Steroid implants were 21-day release pellets made of 200 mg corticosterone in a matrix of cholesterol, cellulose, lactose, phosphates and stearates. Five steroid pellets, three on one side of the neck, two on the other, were surgically placed subcutaneously between the skin and muscle. Only one placebo pellet was implanted subcutaneously on the neck of each control animal.

We obtained blood and fecal samples from all 24 does while immobilized for surgery. All animals were reversed with 0.2 mg/kg yohimbine hydrochloride. Females were immobilized

again on 8 November to obtain another blood and fecal sample, remove surgical staples and assess body condition using ultrasonography (Cook et al, 2007). We obtained fecal samples from all does twice a week from 28 October to 9 December and once on 6 March. Samples were stored at -20 degrees in preparation for GC analysis. The male mule deer was released into the pen with the females on 31 October and breeding behavior was observed at dawn and dusk (3 hours each) until 1 December using a remote camera.

We freeze-dried fecal samples and sent them to the WSU Center for Reproductive Biology Core Lab where they were assayed for GCs using the immunoassay described in Creel et al. (2002). GCs were measured using an ImmuChem Double Antibody 125 I-corticosterone Kit (MP Biomedicals, Solon, Ohio, USA).

Within 6 hr of birth, we sexed neonates and calculated BSR of each female as described previously

First, to test whether the corticosterone pellets influenced systemic, and thus fecal GC levels, we compared corticosterone levels in fecal pellets between treatment groups just before treatment (25 October), averaged for the rest of October, all of November, all of December and one value on 6 March using PROC GLM. Next, we examined the effects of treatment group and body condition on BSR using PROC GLM weighted for litter size, and the probability that a particular fawn would be a male using PROC LOGISTIC. We then repeated these analyses using actual fecal corticosterone levels rather than treatment groups.

RESULTS

Effect of forage quality, nutrient intake and body condition on Birth Sex Ratio.

Our nutritional treatments that varied 1.4-fold in DE content, mimicking common extremes in nutritional quality experienced by wild ungulates, resulted in female mule deer that ranged from 17.04 – 21.13 MJ/g in DEI and 2.1 – 20.5% in body fat, and female elk that ranged from 3.4 – 21.3% in body fat. During the 2-year experiments, 45 mule deer females (two females died before the end of the 2005 trial and one female died before the end of the 2006 trial) produced 40 males and 35 females, 11 singletons and 64 twins, and 71 elk females produced 15 males and 29 females, all singletons (Table 1). Neither nutritional treatment (N = 43, F = 0.09, p = 0.91), decline experiment (N = 43, F = 1.72, p = 0.20) body fat at breeding (N = 43, F = 1.91, p = 0.17), nor DEI (N = 43, F = 2.50, p = .12) influenced BSR weighted for litter size of female mule deer. However, female mule deer with a higher average DPI during the month before breeding (October) produced a lower BSR when weighted for litter size (N = 43, F = 4.53, p = .04). Likewise, a fawn's sex was not predicted from its mothers body fat near conception (N = 75, $\chi^2 = 2.57$, p = 0.11), nutritional treatment (N = 75, $\chi^2 = 0.25$, p = 0.88), decline experiment (N = 75, $\chi^2 = 2.26$, p = 0.13), or loin muscle depth (N = 75, $\chi^2 = 1.36$, p = 0.24). However, fawns whose mothers had higher average DEI (N = 75, $\chi^2 = 3.35$, p = 0.07) and DPI (N = 75, $\chi^2 = 5.85$, p = 0.02) during the month before conception had a higher probability of being males. The best model for predicting sex of a fawn included DPI only:

$$P = \frac{e^{2.84+0.0173*DPI}}{1 + e^{2.84+0.0173*DPI}}$$

(N = 75, $\chi^2 = 5.85$, p = 0.02).

In elk, neither nutritional treatment (N = 44, F = 0.18, p = 0.84), timing of breeding (N = 23, F = 1.0, p = 0.32), nor body fat at breeding (N = 44, F = 0.10, p = 0.76) influenced BSR of

females. Because elk only have one neonate, the sex of fawn was likewise not predictable from treatment ($N = 44$, $\chi^2 = 0.39$, $p = 0.82$), timing of breeding ($N = 23$, $\chi^2 = 0.17$, $p = 0.68$), and its mother's body fat composition ($N = 44$, $\chi^2 = 0.10$, $p = 0.75$).

Effect of Corticosterone on Birth Sex Ratio.

Twenty-four females ranging in body fat from 7.7 to 20.9%, produced 45 fawns (29 males, 16 females, 42 twins, 3 singletons). Three females died (from causes unrelated to the experiment) before parturition (two before March data collection and one after) but fetus sex was determined via post-mortem necropsies and sex ratio was included in all analyses. All 24 mule deer females had similar fecal corticosterone levels in October before treatment (placebo: $\bar{X} = 2002.08$, $SE = 395.68$, treatment: $\bar{X} = 1994.61$, $SE = 288.46$; $N = 24$, $F = 0.00$, $p = .99$) and five months later in March (placebo: $\bar{X} = 1942.86$, $SE = 296.46$, treatment: $\bar{X} = 2096.35$, $SE = 305.43$; $N = 22$, $F = 0.13$, $p = 0.73$). However, deer treated with corticosterone subcutaneously had about 50% higher fecal corticosterone in the two months post-treatment (October, $\bar{X} = 3811.49$, $SE = 313.31$, November $\bar{X} = 3753.22$, $SE = 302.52$, and December $\bar{X} = 2830.15$, $SE = 282.64$) than those with a placebo (October, $\bar{X} = 1686.44$, $SE = 113.99$, $N = 24$, $F = 40.63$, $p < 0.0001$; November $\bar{X} = 1885.00$, $SE = 163.65$, $N = 24$, $F = 29.50$, $p < 0.0001$; and December $\bar{X} = 1942.86$, $SE = 296.46$, $N = 22$, $F = 6.79$, $p = .02$). However, BSR ($\bar{X} = .71$, $SE = .097$) weighted for litter size for deer treated with corticosterone did not differ from those treated with placebo ($\bar{X} = .50$, $SE = .11$, $N = 24$, $F = 2.11$, $p = 0.16$). BSR weighted for litter size also was not influenced by body fat of females in November ($N = 24$, $F = 0.55$, $p = 0.47$) or March ($N = 22$, $F = 0.01$, $p = 0.92$), or average corticosterone levels in October ($N = 24$, $F = 1.33$, $p = 0.26$), November ($N = 24$, $F = 0.91$, $p = 0.35$), December ($N = 24$, $F = 0.00$, $p = 0.96$) or March ($N = 22$, $F = 2.96$, $p = 0.10$). Finally, treatment ($N = 45$, $\chi^2 = 1.85$, $p = 0.17$), mother

body fat before breeding ($N = 45$, $\chi^2 = 0.51$, $p = 0.47$) and corticosterone levels at any time (October, $N = 45$, $\chi^2 = 1.24$, $p = 0.27$, November, $N = 45$, $\chi^2 = 0.86$, $p = 0.35$, $N = 45$, December, $\chi^2 = 0.02$, $p = 0.88$, March, $N = 41$, $\chi^2 = 2.45$, $p = 0.12$) did not predict the sex of each neonate.

DISCUSSION

Despite carefully controlling and measuring nutritional resources, we found little evidence that forage quality or body condition near breeding consistently influences BSR of mule deer and elk, or the probability that a neonatal elk or mule deer would be a certain sex. However, in our sample of 75 mule deer fawns, we found that DPI influenced the probability of having a male fawn. Similarly, although BSR of adult females treated with GC was absolutely higher than those treated with a placebo (0.71 vs. 0.50), BSR were not statistically different and corticosteroid treatment did not predict the sex of a fawn. Our results illustrate the complexity involved in testing evolutionary hypotheses such as TWH in wild ungulates.

First, testing the TWH ultimately requires a trade-off between sample size and the ability to control and accurately measure resources. Our goal was to improve upon previous studies examining sex allocation by experimentally controlling resources and GC's, and directly measuring relevant measures of nutritional resources (e.g., diet quality, body fat, DEI) rather than indirect measures such as visual scores (Clutton-Brock et al. 1984) and social structure (Clutton-Brock 1986, Cassinello 1996, Byers 1997). However, because of the difficulty of conducting these types of controlled experiments with many wild animals over multiple years, this approach necessarily precludes very large data sets that may be required to detect small differences in BSR based on maternal resources. Even still, large data sets can confound or

swamp parameters influencing BSR of individuals, making powerful tests of TWH generally infeasible (Krackow 2002). Further, even if large data sets were available to detect small differences in sex ratios in relation to maternal resources as has been done in some studies with humans, the biological relevance of these slight differences may be slight.

Second, our results highlight that a clear understanding of the mechanism of sex allocation adjustment is required to measure resources available to females that might be expected to affect BSR. In our study, we accurately measured diet quality, DEI, DPI, body fat, and loin muscle depth in each female, each of which has been shown to affect reproductive output in mule deer (Tollefson et al. in press, Tollefson 2007), and elk (Cook et al. 2004). However, each of these indices of resource availability affected the sex of our mule deer fawns in a different way. Male fawns were more likely to have mothers that had a higher nutrient intake (DEI and DPI) near breeding. At the same time, neither body fat nor food quality influenced the sex of the fawn. Differences in how a female allocates these resources to offspring may be caused by the fact that DEI and body fat influence the physiology of estrus, ovulation and conception differently (Wettemann et al. 2003).

Life history strategies, even among closely-related species, likely also influence how resource availability affects the extent to which a female might bias the sex of her offspring, and the mechanism by which she might achieve it. Mule deer are more similar to the small, polytocous roe deer (Andersen et al. 2000), which fall on the income side of the capital-income breeder continuum, than larger monotocous ungulates such as elk, which are considered capital breeders. This means mule deer rely more on consumed energy to support reproduction than on stored energy, or body fat (Stearns 1992). In mule deer DEI would be immediate energy income available for reproductive needs, whereas the stored capital, body fat, is held in reserve for

survival (Anderson et al. 1972). TWH predicts that an income breeder with excess energy income would be better equipped to rear males than mothers that have lower DEI, as we found in this study. Inadequate energy intake can disrupt release gonadotropin-releasing hormone (GnRH), which affects lutenizing hormone (LH), and interferes with ovulation (Randel 1990, Robinson 1990, Schillo 1992, Mani et al. 1996, Wade et al. 1996, Chilliard et al. 1998, Senger 1999, Tanaka et al. 2003). Studies suggest that energy intake, and its effects on blood glucose and insulin (Ingvarsten and Boisclair 2001), rather than body fat itself is the causal mechanism for changes LH (Randels 1990, Barb 2005). In addition, food restriction can decrease the production of progesterone receptors (Sosa et al. 2004), decreasing the probability of implantation and embryonic survival (Senger 1999) even if ovulation is not impaired. Even though body fat has been linked to estrus and reproduction in ungulates (Adamczewski et al. 1998, Testa and Ward 1998, Keech et al. 2000, Cook et al. 2004), we did not see a relationship between body fat and pregnancy rates (Tollefson et al. in press) nor the end result of reproduction, the birth sex ratio.

Similarly, the production of GCs, a proposed mechanism for facultatively adjusting sex ratios, also relies on pathways of energy metabolism and synthesis in the body. In our controlled, but relatively small experiment, increasing corticosterone in the body 1.5 to 2.5 times did not statistically change the BSR or the probability of producing a male fawn. However, we saw an absolute difference in BSR, 0.50 v. 0.71, that suggests that GC has the potential to influence BSR in mule deer. However, the effect of GC may be more important for testosterone production rather than blastocyst development (Powell et al. 2002). Females are more receptive to y-bearing spermatozoa if testosterone levels are higher in the female (Grant 2003, 2007, Grant et al. 2008). Corticosterone pellets may not have been strong enough to raise circulating glucose

concentrations to levels that would affect blastocyst development or hormone levels to affect gamete receptivity. Perhaps the 21-day, slow release pellets were countered by a feedback loop, such as an insulin response to raised glucose levels or suppression of gonadotropin releasing hormone in response to increased testosterone, before it could elicit a pre- or post-conception response. Had we used pellets containing cortisol, another GC, which is five times more potent than corticosterone, the pellets may have superseded any feedback response.

Testing the TWH in cervids is further complicated by variation in litter size among ungulates and the fitness value of producing males. In monotoxic species like elk, the evolutionary decision in relation to food resources or GC of the animal is relatively simple – produce a male, a female, or no offspring, each having a relatively clear affect on potential fitness. However, a polytocous species like mule deer has 6 choices -- produce a male, a female, 2 males, 2 females, 1 male and 1 female, or no offspring, each with a potentially different fitness outcome relative to resources and GC, but 2 ways to achieve each BSR (e.g., one male and two males both yield a BSR of 1). Furthermore, if maternal resources influence the sex of her offspring, then the sex of twins is likely not independent (Gaillard et al. 1998), which may explain why we found no relationship between nutrient intake and body condition on BSR per female, but nutrient intake and loin depth predicted the sex of each fawn.

In ungulates that commonly produce a litter, producing two sons may not be the best strategy even for females in excellent physical condition and are able to raise them to weaning. Instead, having one male and one female may be more valuable to future fitness to reduce the risk of having no grandchildren at all. If males are more expensive to raise (Clutton-Brock 1991, Clutton-Brock et al. 1985, Cassinello and Gomendio 1996, Tollefson 2007) and have higher mortality rates before weaning (Johnstone-Yellin et al. 2009) than mothers, having one of each

sex, may optimize their future genetic output. This would also be true at the population level where a female may not 'know' what other females in the population are producing and therefore would not know in which direction to skew their investment. On the other hand, for ungulates like elk that commonly produce only one offspring per season, the benefit to producing a male may depend on the trade-offs between the larger investment needed to raise a male and the resources available to future offspring (Clutton-Brock 1991). For example, red deer raising males were less likely to survive the following year or to raise subsequent offspring successfully (Clutton-Brock et al. 1982, 1983).

Finally, fitness returns for a large ungulate with a polygynous mating system may not actually be improved by producing sons than daughters as assumed in TWH. Data supporting this assumption of TWH alone is absent in the literature (Hewison and Gaillard 1999). Even studies that have supported the first two hypotheses (maternal investment affects quality of offspring at weaning and benefits of maternal investment extend into adulthood) have little or no data on the third assumption that the improved quality in adulthood actually translates into a great reproductive success of sons (Hewison and Gaillard 1999). On the one hand, males are expected to monopolize maternal investment until weaning more than females because of higher growth rates and great nutritional demands (Clutton-Brock et al. 1985, Tollefson 2007), but when they disperse, maternal investment comes to a definitive end. Female offspring often remain with the resident herd and perhaps benefit from maternal social rank and access to resources (daughter advantage hypothesis, Hiraiwa-Hasegawa 1993) over a long period of time and thus, maternal investment continues, albeit indirectly (Hewison and Gaillard 1999). In addition, daughters, although only able to produce one litter per year, are more likely to become pregnant each year starting at 1.5 – 2.5 years old, whereas males may still be establishing

themselves in a herd several years after weaning (Hewison and Gaillard 1999). This aspect of the social system of ungulates could delay reproductive success of male offspring and therefore fitness returns to the mother fitness returns, especially in elk where males form harems.

Therefore, producing males rather than females might not automatically increase fitness for female ungulates, even when they can obtain adequate resources.

In sum, despite carefully controlled experiments and relevant measures of female resources, the ambiguity of our results is not surprising. Even Trivers himself stated that, “even if I’m wrong, it will take them years to find out” (Hrdy 1987). Although TWH makes intuitive sense, making sense of how females should allocate resources to sons versus daughters and whether individually adjusting BSR is adaptive requires a more complex model of the relationship among more life history traits than just maternal body condition than what the TWH proposes (Figure 1). Because life-history traits vary from ungulate to ungulate, even among those with similar breeding strategies, we cannot expect to find a relationship using maternal body condition alone.

For example, whereas mule deer and elk are both classified as polygynous breeders, their life-history traits differ enough to warrant separate models of sex allocation. First, mule deer have a tending-bond where males tend to a single female until copulating and then moves on to another female (serial polygyny, Geist 1981, Figure 2B). Elk, on the other hand, form true harems where bigger, stronger males attract groups of females and maintain that group until successfully breeding with every female (Geist 1982). Males expend energy fighting for these harems and if they are not the dominant male and harem holder, they can still breed as ‘sneakers’ (Gibson and Guinness 1980, Clutton-Brock 1986, Figure 3B). Once males have filled their role, mule deer and elk females also have differing adaptations to gestation. Mule deer usually

conceive for the first time during their second year (Nellis et al. 1976, Figure 2A), with breeding occurring primarily in November (Mueller and Sadleir, 1979) and primarily producing twins (Figure 2C). Female elk are able to conceive as yearlings if in adequate body condition (Hudson et al. 1991, Figure 2A) but most will not conceive successfully until their second year (Flook 1970). These differing life history-traits will affect BSR in addition to mother body condition and should be considered when examining reproductive success.

Even if we incorporate these additional life history traits into a new model of sex allocation, we still need to turn from models that examine birth sex ratios and move towards those that investigate mother fitness (Figures 2 and 3). A mule deer with twins and an elk with a singleton can both have a BSR of one even though the elk produces only one male and the mule deer has two males. Further, the birth sex ratio only indicates what each female has produced at parturition of a given year. Fitness, on the other hand, incorporates not only the number and sex of offspring produced, but also the survival of those offspring and their reproductive success. Fitness, not BSR, is the true measure of the adaptive value of producing offspring of each sex.

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Table 1. Mean (\pm SE) characteristics of 24 lactating mule deer (*Odocoileus hemionus*) and 31 lactating elk (*Cervus elaphus*) assigned three nutritional treatments (high, medium and low digestible energy) before breeding and the resulting birth sex ratio. Nutritional treatments were assigned over two consecutive years: mule deer, 2005 and 2007 at Washington State University, Pullman, WA and elk, 1996 and 1997 in pens near Kamela Oregon.

Species	Parameter	High DE	Med DE	Low DE
Mule deer	Sex ratio (M:F)	0.50 (13:13)	0.57 (17:13)	0.53 (10:9)
	Body fat (%)	8.5 \pm 0.80	7.8 \pm 1.2	7.3 \pm 0.90
	Loin depth (cm)	3.58 \pm 0.072	3.43 \pm 0.12	3.37 \pm .085
	DEI (MJ/day)	21.13 \pm 1.21	17.29 \pm 0.84	17.04 \pm 0.92
	DPI (g N/day)	168.3 \pm 9.6	166.7 \pm 8.2	186.6 \pm 9.9
Elk	Sex ratio	0.32 (7:15)	0.39 (7:11)	0.25 (1:3)
	Body fat (%)	15.5 \pm 0.44	9.8 \pm 0.33	5.9 \pm 0.34

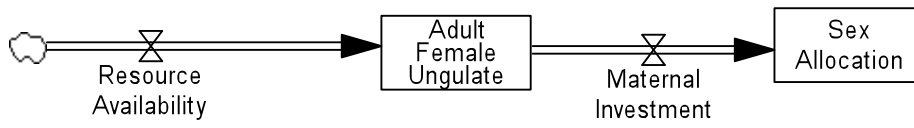
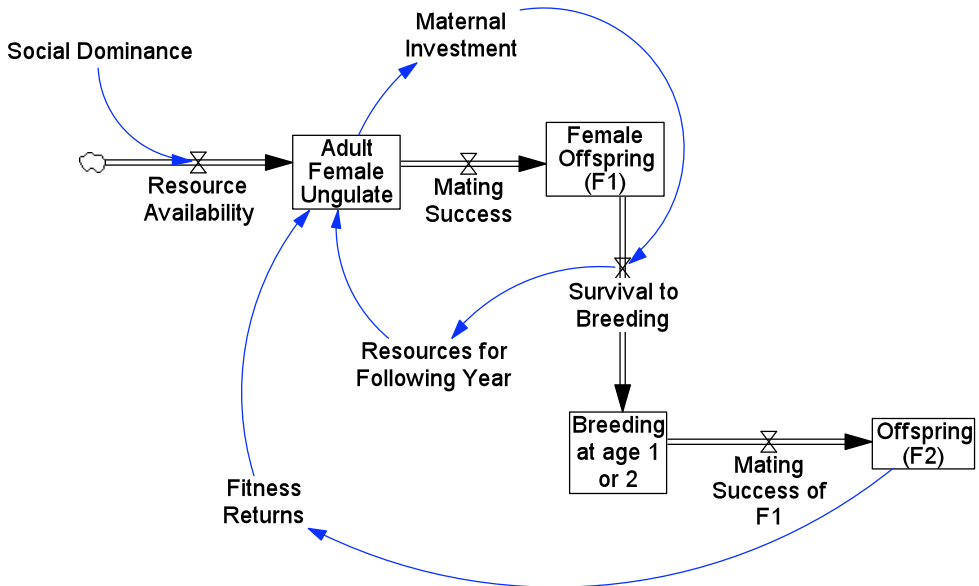
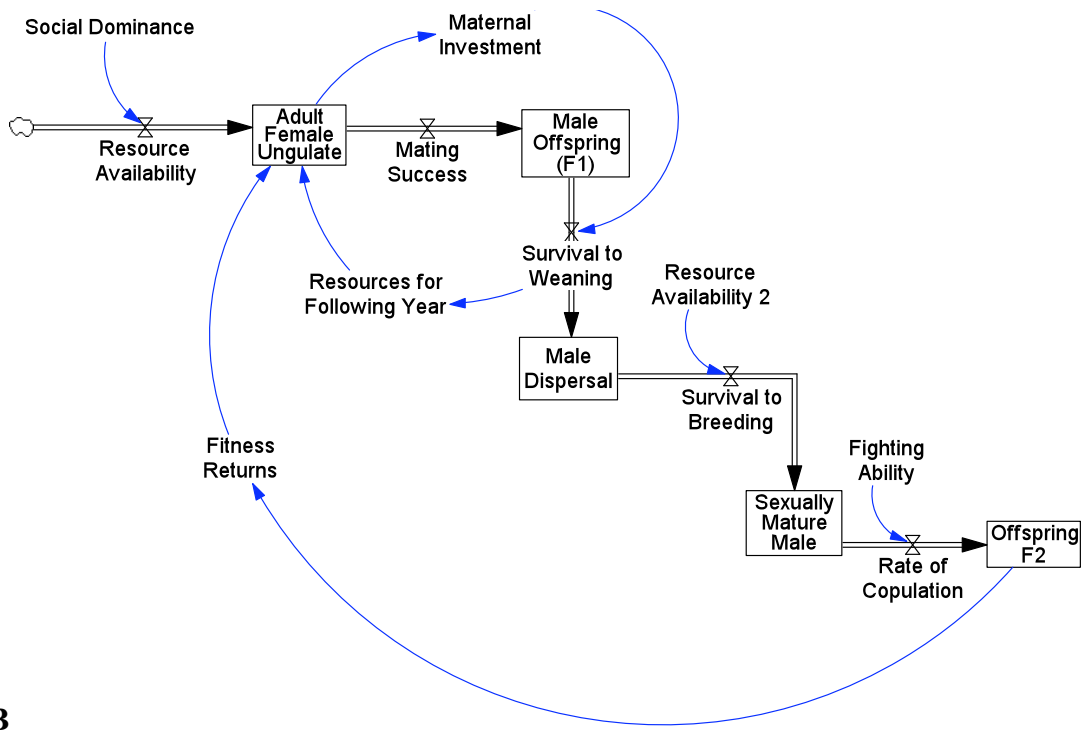


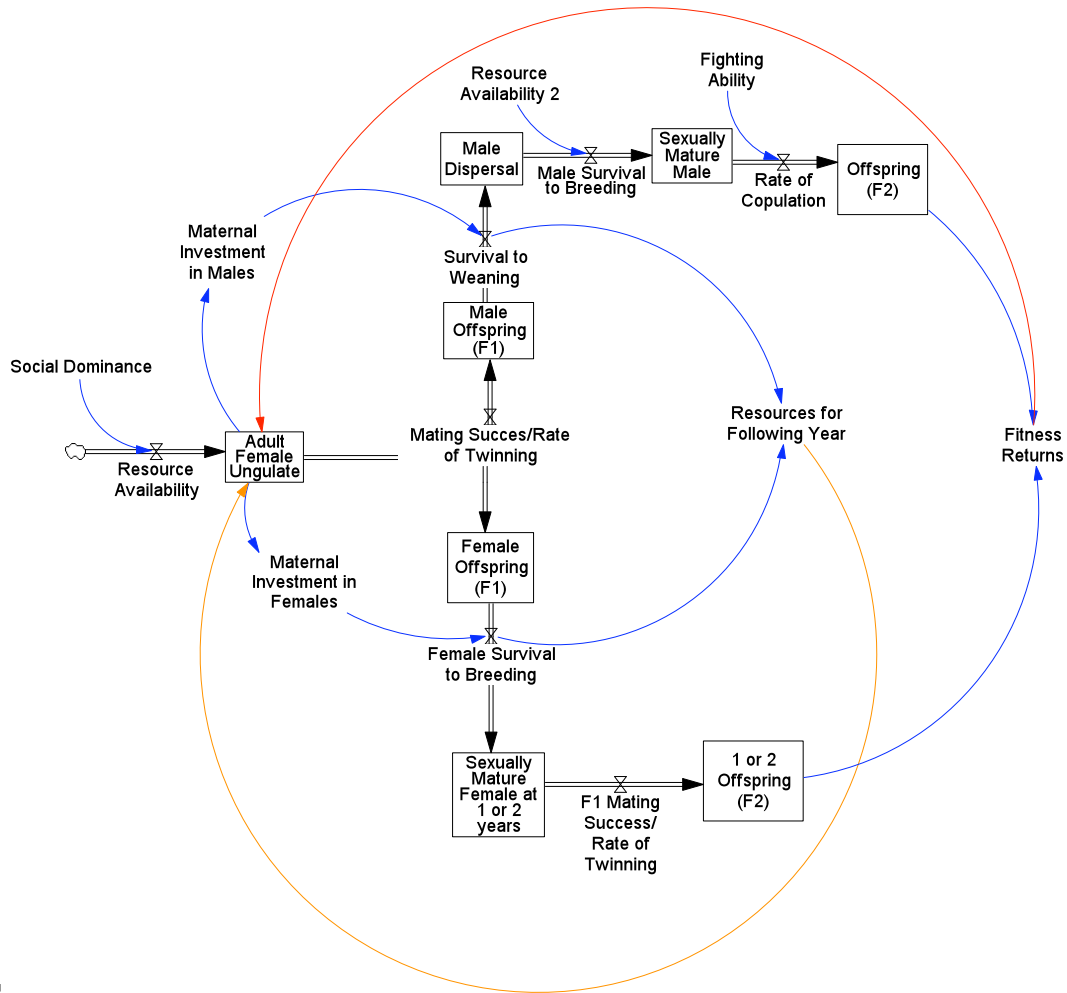
Figure 1. Schematic representing the Trivers and Willard Hypothesis (1973).



A

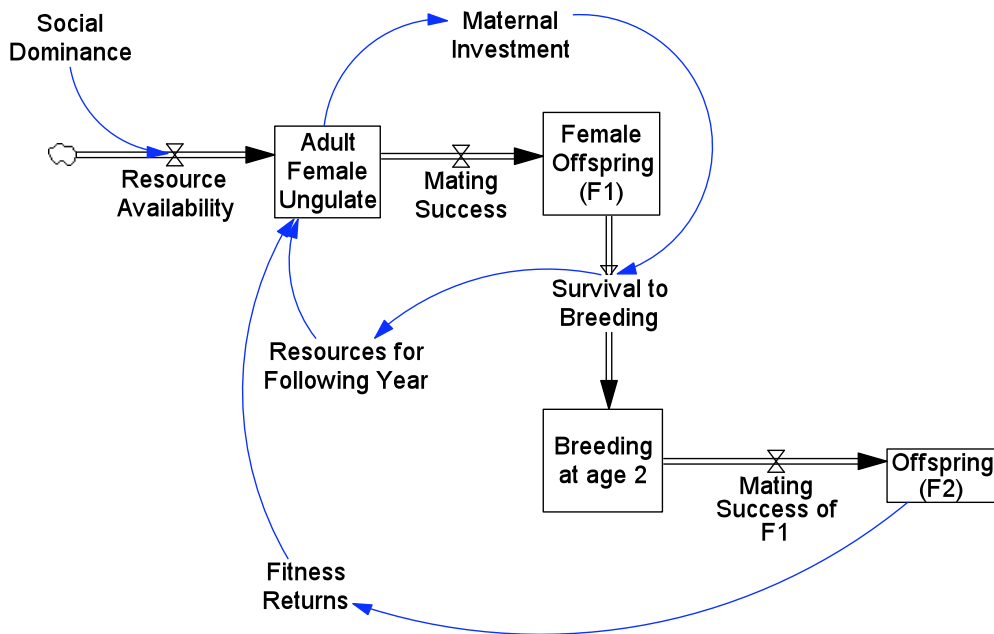


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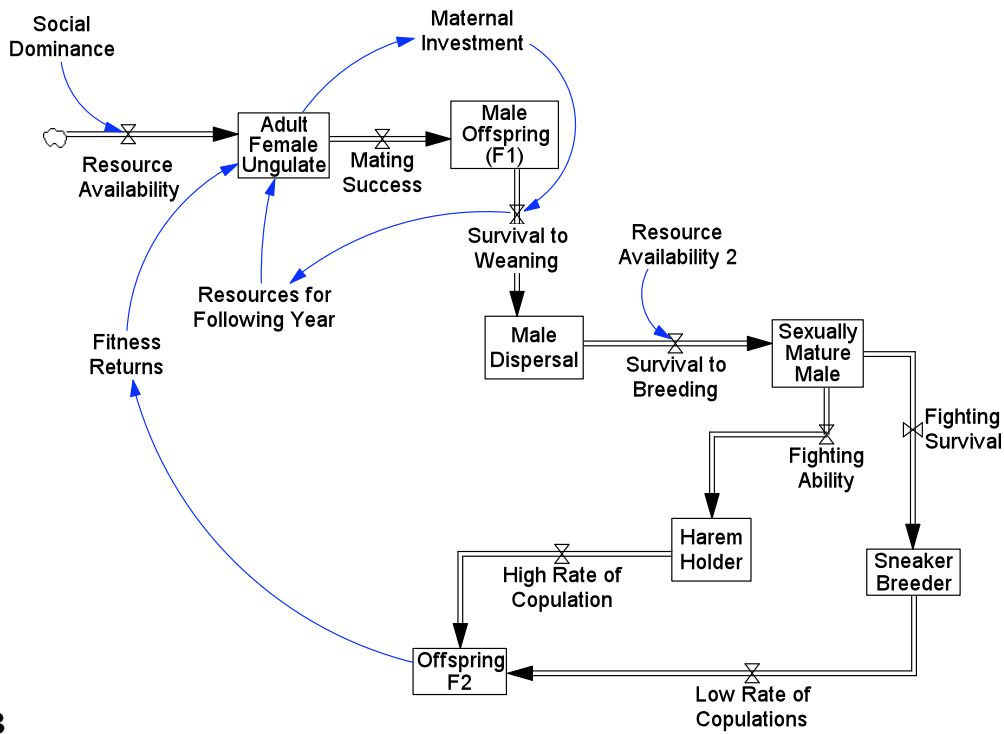


C

Figure 2. Schematic of a proposed fitness model incorporating multiple life-history traits of a female mule deer (*Odocoileus hemionus*) when producing a A) female offspring, B) male offspring, and C) one male and one female offspring.



A



B

Figure 3. Schematic of a proposed fitness model incorporating multiple life-history traits of a female elk (*Cervus elaphus*) when producing a A) female offspring and B) male offspring.

USING GRAMMIA NEVADENSIS TO TEST THE EFFECTS OF RESOURCE
ALLOCATION ON GROWTH AND BIRTH SEX RATIOS

The physiological efficiency hypothesis predicts that a true generalist feeding strategy would allow larvae like *G. nevadensis* to select foods to create a balanced, nutrient-rich diet (Rapport 1980) while avoiding high doses of secondary plant chemicals (Freeland and Janzen 1974) and therefore, optimize growth. However, tests of this prediction have yielded mixed results in generalists (Freeland and Jansen 1974, Bernays and Minkenberg 1997). *G. nevadensis* larvae, in particular, can feed on more than 80 species and 50 taxa of flowering, herbaceous plants and can move between plant species within hours, even minutes (Singer 2000), presumably selecting an optimal diet. Singer (2001), however, found that growth rates of *G. nevadensis* varied based on the type of diet (mixed- or single-plant) on which they subsisted. Single-plant diets actually yielded a higher growth rate than the mixed diet (Singer 2001), opposite of what the physiological efficiency hypothesis predicts. Other studies found support the hypothesis in grasshoppers (Bernays and Bright 1993) and suggest it is the nitrogen and water content (along with secondary metabolite content) that are of primary importance to physiological efficiency of food utilization (Slansky and Sciber 1985, Bernays and Chapman 1994) rather than mere diet mixing, which may not always be effective in optimizing growth.

Diet not only affects growth rates of larvae but also, in turn, affects the size of adults at emergence and the time at which adults emerge in relation to other individuals within a population. In some insects, such as mosquitoes (*Aedes sierrensis*, Kleckner 1995) and grasshoppers (*Sphenarium purpurascens*, Cuevo del Castillo and Núñez-Farfán 2002) female fecundity is size specific and the differing emergence times between sexes is adaptive (Wiklund

et al. 1992, Nylin et al 1993). However, males and females undergo different selective pressures on emergence times. Protrandry, or the emergence of males from the pupal stage before females, is considered an evolutionary stable strategy and the mating rates of protandrous insects depend on sex ratios at emergence (Zonneveld and Metz 1991). Females emerge later to avoid early-emergence deaths before mating (1982, Zonneveld and Metz 1991), and males emerge early to maximize the number of matings with the females that emerge later (Bulmer 1983, Wiklund and Fagerström 1977). If females emerge after many males then female mating chances increase (Bulmer 1983, Thornhill and Alcock 1983, Zonneveld and Metz 1991). However, if sex ratios are skewed toward females and females no longer encounter males at a high probability, their risk of pre-reproductive deaths will increase. Cueva del Castillo and Núñez-Farfán (2002) found that the proportion of female grasshoppers that mated was related positively to the sex ratio, but not population density; females that matured at the population's maximal sex ratios were more likely to mate.

I explored the relationship between diet, growth rate and sex ratios using a high protein, low fiber plant diet (Maris Kestrel Kale, *Brassica oleracea*) and a pelleted diet that mimicked a high fiber, moderate protein mixed plant diet. I expanded on Singer's findings by performing more trials with the two diet and also tested the effects of competition on the growth rates and how these diets affect other life-history traits of *G. nevadensis*. In particular, I looked at how resource availability (quantified as neutral detergent fiber (NDF%) and percent crude protein (CP%) in the diet) and growth rates affected pupation times and adult emergence times and how these factors affect sex ratios.

First, I expected that resource availability would affect growth rates. I expected that the larvae would grow faster on the higher nutrient kale diet than the more fibrous pelleted diets, and

that *G. nevadensis* larvae housed in individually would be expected to grow faster than those housed in groups regardless of diet because individuals are not competing for food. In turn, faster growth rates caused by food quality and animal density would then affect other life-history traits. Like the polyphagous grasshopper, I predicted *G. nevadensis* will yield sex ratios based on resource availability, growth rates and emergence time. I expected the faster-growing larvae to pupate sooner than slower growing larvae and larvae that pupate sooner to be smaller at emergence. Growth rates should also affect trade-offs in sex allocation. Faster- growing larva should pupate earlier and larvae that pupate earlier should emerge earlier. Males should grow faster than females to emerge before females to increase chances of mating. I also hypothesized competition will affect sex allocation. Populations of competing larvae will adjust growth rates, and therefore sex, to maintain a 50:50 sex ratio.

METHODS

Animals and Diets

To determine whether nutritional resources affect the growth rate of *G. nevadensis* larvae I fed larvae two diets that varied in nutritional. Late instar *G. nevadensis* larvae were originally collected August 2008 in the foothills of the Santa Rita Mountains in southern Arizona, under US Department of Agriculture Animal and Plant Health and Inspection Service permit #P526P-08-02893. Eggs, larva, pupae and adults, raised in growth chambers set at 28:25 C for 12:12 hours of light and darkness, were housed at the E. H. Steffen Center at Washington State University, Pullman, WA, USA (46°44', 117°10' W, elevation 777m). Larvae for each trial came from a large source population reared from the larvae collected in Arizona and reared in a plastic container with dimensions of 61 x 40.6 x 12.7 cm. The source populations were given a

maintenance diet of kale (*Brassica oleracea* var. *acephalaxe*) and allowed to grow, pupate, emerge and mate without intervention. To avoid using single familial lines, all subsequent groups and individuals were randomly selected from the current generation of source larvae shortly after they hatched. Adults were allowed to mate freely in a container with paper to cling to and eggs were collected for a subsequent source population.

I selected the two diets to maximize differences in nutrient quality while ensuring the larvae would eat the diet. I chose kale as the high quality diet because in preliminary trials, larvae preferred and grew better on kale over other herbaceous plants I tried (e.g., *Taraxacum officinale*, *Cichorium intybus*, *Trifolium repens*, *Lactuca sativa*). Because it was difficult to get the larvae to consume high fiber plants, I selected a custom-made completely balanced grain-alfalfa herbivore pellet for the low quality diet. Maris Kestrel Kale (British Seed Houses, Bristol, UK) was grown in Steffen Center greenhouses and only leaves at 13 to 17 on the BBCH scale (Zadoks et al. 1974) were fed to larvae. The grain-alfalfa pelleted diet was formulated at the Animal Science Preparation Laboratory at WSU (Pullman, WA, USA) and consisted primarily of alfalfa meal, oats, wheat middlings, soybean meal and rice hulls (Table 1). The pelleted diet was prepared for feeding to the larvae in 250 mL batches as follows: pellets were ground and mixed with water to form a paste and methyl paraben and sorbic acid (Bio-serv, Frenchtown, NJ, USA) were added at 2.5 g per liter of prepared diet as mold inhibitors. I stored batches in the refrigerator to maintain freshness and moisture until use. The fiber content (%NDF) of kale and pelleted diets were analyzed in the Wildlife Habitat Lab at WSU using sequential detergent analysis (Goering and Van Soest 1970) in an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY, USA). I determined the nitrogen content of the diets using a

carbon-nitrogen analyzer (TruSpec, LECO Corporation, St. Joseph, MI), and multiplied the nitrogen content by 6.25 to estimate crude protein.

Testing Effects of Diet and Competition on Growth Rate and Sex Ratio

To compare growth rates and sex ratios of larvae raised on kale and the pelleted diet, I placed newly hatched larvae in commercial salad containers (25.4 x 15.2 x 10.2 cm) in groups of 50 and fed them *ad libitum* every 2-3 days. Number of larvae surviving were counted and weighed to the nearest ten thousandth of a gram as a group each week. Group weight was divided by number of surviving larvae to determine average individual growth rates within each group. After larvae reached an average individual weight of approximately 0.1000 g, I added tissue paper to each group for cocoon building. After cocoons were built and dried, I weighed and noted the date of pupation before moving them to a separate container for emergence. Adults were weighed and sexed after emergence and the date noted. Once weighed, each moth was marked with a small amount of non-toxic hair dye (to distinguish them from newly emerged moths) and returned to the container for mating.

To compare growth rates and sex ratios of larvae housed in groups to those housed individually, I placed newly hatched larva in individual deli containers and also fed them kale or the pelleted diet *ad libitum* every 2-3 days. I weighed individual larvae weekly to the nearest ten thousandth of a gram. After most of the individuals weighed ≥ 0.1000 g, tissue paper was added to all containers for cocoon building. Date of pupation was noted and pupae were weighed and returned to their containers. After emergence, weight, sex and date of emergence were recorded.

I calculated sex ratio as the number of males emerged divided by total adults emerged. This was calculated for each trial group and for all larvae housed individually and fed a particular diet.

Statistical Analysis.

To test the effects of diet on larvae grown in groups, I compared average growth rates, average days to pupation and average days to emergence on the kale and pelleted diets using PROC GLM (SAS software, Version 9.1.3, SAS System for Microsoft Windows, Cary, NC, USA). I also use PROC GLM to test the effects of diet, growth rate, average days to pupation and average days to emergence on sex ratio, weighting for the number of larvae that survived to adulthood. PROC LOGISTIC was used to determine whether diet predicted sex of any one larva. Finally, I used a one-sample t-test to test whether average sex ratios from kale or pelleted diets varied significantly from 0.50 (or 1:1). Larvae reared in individual containers on kale and pelleted diets were also examined using PROC GLM as follows. Within the kale treatment alone I tested the effects of growth rate on days to pupation and emergence and whether days to pupation or emergence or growth rate predicted sex of individual larvae. I also tested whether growth rates of larvae were different for larvae fed kale and those fed the pelleted diet. I compared sex, growth rate, days to pupation and days to emergence on larvae fed kale in groups versus those fed individually. Finally, I used a two-sample t-test to compare the average sex ratio of larvae housed in groups to the sex ratio of all larvae housed as individuals.

RESULTS

Diet influenced the growth rates, percent survival to adulthood and days to life stages in *G. nevadensis* larvae housed in groups of 50. Larvae on the kale diet (21.2 – 22.7% NDF, 17.0 – 40.4% protein), which was half as fibrous and had up to 2.8 times the protein content at the pelleted diet (55.0% NDF, 14.4% protein), grew almost twice as fast as larvae on the pelleted diet (N = 23, F = 9.12, p = 0.007, Table 2). Likewise, larvae fed kale pupated on average 26.7

days earlier ($N = 153$, $F = 27.52$, $p < 0.0001$) and emerged 20.3 days earlier ($N = 129$, $F = 6.82$, $p < 0.01$), than those on the pelleted diet (Table 2). However, average survival to adulthood of larvae did not differ between the diets (Table 2, $N = 23$, $F = 0.98$, $p = 0.33$).

Despite differences in growth and maturity between diets, diet did not predict the sex of larvae housed in groups ($N = 129$, $\chi^2 = 0.91$, $p = 0.34$), nor did the average sex ratio of groups differ significantly between diets when weighted for the number surviving to adulthood ($N = 23$, $F = 0.07$, $p = 0.8$, Table 2). Days to emergence of males ($N = 20$, $F = 0.8$, $p = 0.1$) and females ($N = 21$, $F = 2.4$, $p = 0.2$) were not predicted by diet. The average sex ratio of adults on kale diet and pelleted diet were not significantly different from each other ($N = 23$, $t = 1.3$, $p = 0.2$, $df = 21$). The average sex ratio of groups fed the pelleted diet (0.69, Table 2) were greater than 0.50 (or 1:1 male to female ratio, $N = 8$, $t = 2.3$, $p = 0.06$) but the average sex ratio for groups fed kale did not differ from 0.50 ($N = 15$, $t = 0.53$, $p = 0.61$). Finally, neither average days to pupation ($N = 23$, $F = 0.20$, $p = 0.66$), average days to emergence ($N = 23$, $F = 0.09$, $p = 0.8$), nor growth rate ($N = 23$, $F = 0.62$, $p = 0.61$) influenced sex ratios.

Diet also affected the growth rates of larvae housed individually ($N = 52$, $F = 30.8$, $p < 0.001$). Individual larva fed kale grew 3 times faster than those fed the pelleted diet (Table 2). Individuals on the kale diet that had a higher growth rate pupated ($N = 45$, $F = 23.8$, $p < 0.001$) and emerged earlier ($N = 41$, $F = 15.93$, $p = 0.0003$). In addition, larvae that grew faster tended to be female ($N = 41$, $\chi^2 = 3.63$, $p = 0.06$). However, days to pupation ($N = 41$, $\chi^2 = 0.17$, $p = 0.68$) and days to emergence ($N = 41$, $\chi^2 = 0.017$, $p = 0.90$) did not predict sex of larvae.

Finally, larvae fed kale housed individually grew faster ($N = 60$, $F = 52.72$, $p < .0001$), pupated sooner ($N = 121$, $F = 208.65$, $p < 0.001$) and emerged sooner ($N = 76$, $F = 1.84$, $p =$

0.18) than those housed in groups. However, I found no evidence that sex of each larvae fed kale was affected by the size of group in which it was reared ($N = 117$, $\chi^2 = 0.14$, $p = 0.71$).

DISCUSSION

Although growth, pupation, and emergence were accelerated on the higher quality kale diet, neither diet quality, rate of growth, rate of pupation, nor rate of emergence affected population sex ratios of *G. nevadensis*. As predicted, larvae growing on the kale diet, with high protein and low fiber, grew and matured more quickly than those on the high fiber and lower protein pelleted diet. This accelerated growth rate occurred even though previous studies have suggested ground plant cells found in pelleted diets are assimilated more efficiently by larvae than are whole plants, which are snipped by caterpillars (Chown and Nicolson 2004). For example, caterpillars fed pelleted diets had higher fat content than those fed leaves (Ojeda-Avila et al. 2003), but also had smaller heads and chewing musculature because pelleted diets are softer than plant material (Bernays 1986) possibly affecting their overall growth and size. This suggests leaves only need to be porous, not necessarily crushed and ground, for caterpillars to use the nutrients (Barbehenn 1992). Further, proteins of plants and pelleted diets differ in passage rate with plant fragments going farther down the midgut (Woods and Kingsolver 1999) for more complete absorption.

Despite strong differences in growth rate and maturation between diets in *G. nevadensis*, the effects of growth and maturation did not consistently or predictably affect sex ratios. Sex ratios of larvae reared in groups did not differ significantly between diets, although the sex ratio of groups fed the pelleted diet was skewed towards males (0.69). However, when larvae were fed individually, females grew faster than males. Our results contrasted with hypotheses about

the role of protandry in insects, which suggests that males should grow faster and pupate sooner so they can emerge sooner and increase chances of mating (Wiklund et al. 1991 Zonneveld and Metz 1991, Fischer and Fiedler 2000). Further, although the differences in population size during rearing affected growth rate, they did not, in turn, affect the sex ratio in the expected manner. It was the more-slowly growing population fed the pelleted diet that tended to produce a sex ratio biased towards males. Perhaps it is not the growth rate of the larvae but the rate at which larvae reach developmental milestones (larval instars, pupation and emergence) and instantaneous growth rate (Tammaru et al. 2010) that may provide greater insight on the proximate causes of sex allocation.

By using *G. nevadensis* as a fast-growing model species with a continuous flow digestive system (and thus eliminating the complications of a complex digestive system and varying passage rates as seen in ruminants), I isolated variables of diet and growth rate to examine hypotheses about how resources available to animals might affect reproductive strategies and sex allocation. However, even in a controlled environment, sex ratio was inconsistently and unpredictably related to nutritional resources, suggesting that the adaptive advantage of sex allocation still remains embedded in the life-history traits of this herbivore.

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Table 1. Composition of the pelleted diet fed to *G. nevadensis* larvae at Washington State University, Pullman, WA.

Ingredients	% Dry Mass
Rice hulls	29.4
Alfalfa meal	28.7
Oats	15
Wheat middlings	12.1
Soybean meal	11
Molasses	2.5
DiCalcium Phosphorus	1
Selenium premix	0.2
Vitamin E-50 (500 KIU/kg)	0.08
Vitamin A premix (30 KIU/g)	0.03
Vitamin D premix (9 MIU/kg)	0.02
Calcium	0.01
Magnesium	0.01
Iron	0.01
Zinc	0.01
Copper	0.001
Iodine	0.00003

Table 2. Mean (\pm SE) characteristics of *G. nevadensis* fed two diets (*Brassica oleracea*, a plant diet, and a pelleted diet) and housed in groups of 50 or individually in a growth chamber at 28:25 C for 6:18 hours of light and darkness, at Washington State University, Pullman, WA.

Treatment	Parameter	Kale	Artificial
Groups	Sex Ratio	0.54 \pm 0.073	0.69 \pm 0.082
	% Survived to Adulthood	10.7 \pm 1.2	13.6 \pm 3.4
	Growth Rate	0.046 \pm 0.0047	0.024 \pm 0.0050
	Days to Pupation	141.7 \pm 7.9	168.5 \pm 18.1
	Days to Emergence	162.5 \pm 8.4	182.8 \pm 16.8
	Males	170.7 \pm 6.8	185.1 \pm 17.2
	Females	165.5 \pm 9.7	192.9 \pm 13.6
	Individuals	Sex Ratio (M:F)	0.46 (19:22)
% Survived to Adulthood		78.8	--
Growth Rate		0.13 \pm 0.0069	0.04 \pm 0.008
Days to Pupation		61.7 \pm 2.4	--
Days to Emergence		81.3 \pm 2.5	--
Males		81.7 \pm 3.5	--
Females		81.0 \pm 3.5	--

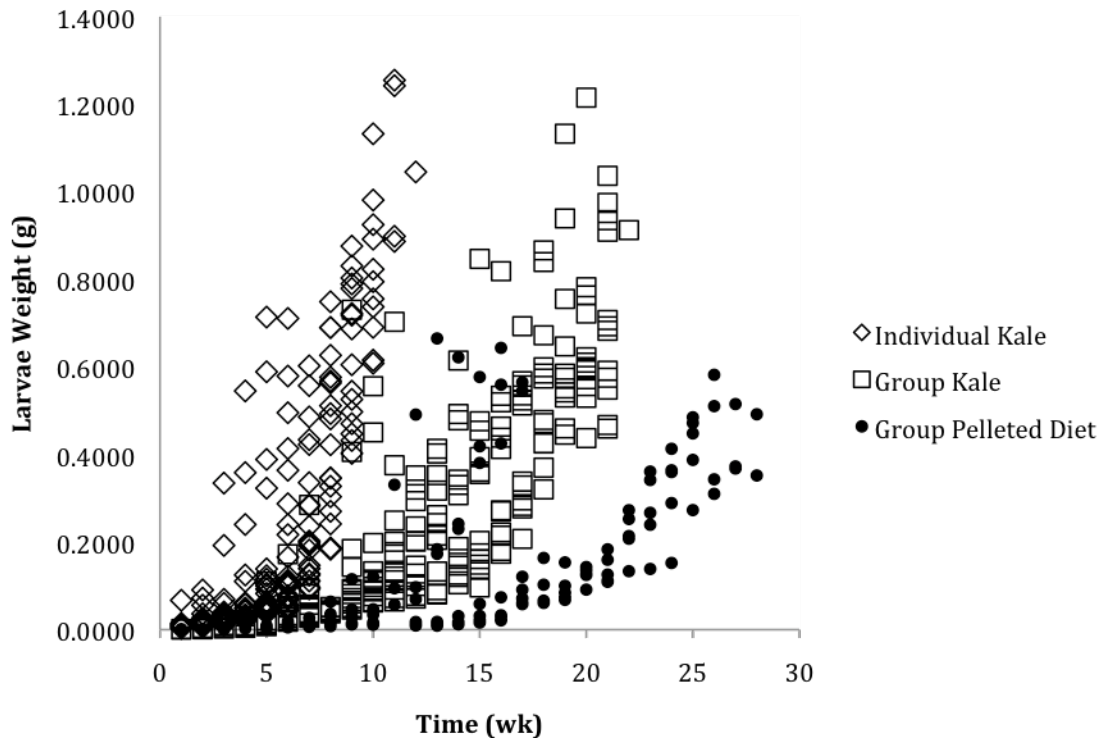


Figure 1. Growth rate of *G. nevadensis* fed two diets (*Brassica oleracea*, a plant diet, and a pelleted diet) and housed in groups of 50 or individually in a growth chamber at 28:25 C for 12:12 hours of light and darkness, at Washington State University, Pullman, WA.