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Costs of Franklin's ground squirrel (*Poliocitellus franklinii*) ectoparasitism reveal adaptive sex allocation.

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Pero, E.M., and Hare, J.F. Costs of Franklin's ground squirrel (*Poliocitellus franklinii*) ectoparasitism reveal adaptive sex allocation.

Abstract: Parasite infestation may impose direct costs of blood, nutrient, and energy depletion, along with indirect costs of increased immune response upon hosts. We investigated how ectoparasitism influences body mass and reproduction in a free-living population of Franklin's ground squirrels (*Poliocitellus franklinii* Sabine, 1822) located near Delta Marsh, Manitoba. We experimentally reduced ectoparasite burden by treating seven reproductive females with an insecticide following breeding, and contrasted body mass and reproductive performance of those individuals to seven sham-treated control females. Insecticide-treated dams did not differ from sham-treated dams in body mass, litter size, or juvenile mass, and thus, dam growth and reproduction were not compromised by ectoparasite defense at the infestation levels experienced in this study. Litter sex ratio differed significantly between insecticide-treated and control females, however, with a higher proportion of male offspring produced among females with reduced ectoparasite load. Our findings are thus consistent with the Trivers-Willard model for adaptive sex allocation, yet provide novel comparative insight into how sociality may modulate the expression of adaptive sex allocation among small mammals given the differential payoff associated with the production of high-quality female versus male offspring in more social versus less social species.

Key words: adaptive sex allocation, costs of parasitism, ectoparasites, Franklin's ground squirrels, *Poliocitellus franklinii*, Trivers-Willard model

Introduction

Life-history theory posits that organisms allocate finite resources among sometimes competing physiological systems to maximize fitness (Stearns 1976). Trade-offs between reproduction and immune function are apparent across taxa, as evidenced by negative associations between reproductive output and immune responses (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000). Decreased reproductive success sometimes follows elevated immune response (Rivera et al. 1998; Ilmonen et al. 2000; Cox et al. 2010; but see Williams et al. 1999), while depressed immune response may accompany increased reproduction (Deerenberg et al. 1997; Nordling et al. 1998; Cox et al. 2010; but see Scantlebury et al. 2010). Similar negative associations have been identified between immune defense and growth (Nestor et al. 1996; Bayyari et al. 1997; Ots et al. 2001), suggesting that factors that increase investment in immune function come at the expense of other life history components.

Increased immune activity in the face of parasitic infection, and the associated energetic, protein, and nutritional demands, are among the indirect costs of parasitism (Zuk and Stoehr 2002; Degen 2006; Bize et al. 2010). Costs associated with immune defense may include the costs of maintenance (i.e., keeping the system in a state of readiness to meet challenges), costs of use (i.e., mounting biochemical and physiological responses underlying recognition and attack), and associated nutritional constraints (i.e., anorexia; Lochmiller and Deerenberg 2000; Zuk and Stoehr 2002; Schmid-Hempel 2003). Beyond the indirect cost of resource allocation to elevated immune response, parasites may directly influence life-history traits by depleting an organism's blood, nutrient, and energy stores (Lehmann 1993; Sheldon and Verhulst 1996; Degen 2006). Both direct and indirect costs of parasitism may diminish energy that otherwise could be allocated to growth, maintenance, or reproduction.

Investigations of parasitic influences on sciurids generally reveal that parasitism negatively impacts reproduction, dam condition, or both. Correlative studies indicate that higher parasite loads are associated with reduced reproductive effort (e.g., yellow-bellied marmots, *Marmota flaviventris* Audubon and Bachman, 1841: Van Vuren 1996; American red squirrels, *Tamiasciurus hudsonicus* Erxleben, 1777: Gooderham and Schulte-Hostedde 2011), juvenile survival (e.g., Alpine marmots, *Marmota marmota* L., 1758: Arnold and Lichtenstein 1993), and adult growth (e.g., Alpine marmots: Arnold and Lichtenstein 1993; yellow-bellied marmots: Van Vuren 1996). Manipulative investigations reducing parasite load also indicate that parasitism may decrease reproductive success (e.g., Columbian ground squirrels, *Urocitellus columbianus* Ord, 1815: Neuhaus 2003; Cape ground squirrels, *Xerus inauris* Zimmerman, 1780: Hillegass et al. 2010; American red squirrels: Patterson et al. 2013) and adult condition (e.g., Columbian ground squirrels: Neuhaus 2003). Negative impacts of parasitism on reproduction or growth, however, are not ubiquitous across sciurid species or populations (e.g., Eastern gray squirrels, *Sciurus carolinensis* Gmelin, 1788: Scantlebury et al. 2010; Columbian ground squirrels: Raveh et al. 2015).

Beyond litter size and mass, the influence of parasitism on dam quality and reproduction may appear in the context of litter sex ratio. Significant deviations from equal investment in male and female offspring (Fisher 1930) are expected when fitness returns differ between sexes as influenced by local mate competition (Hamilton 1967), broader resource competition and enhancement (Clark 1978; Gowaty and Lennartz 1985), or maternal quality (Trivers and Willard 1973). Field research providing supplementary food for Virginia opossums (*Didelphis virginiana* Kerr, 1792) demonstrated that females are capable of manipulating offspring sex ratio in response to experimentally increased body condition (Austad and Sunkvist 1986). Both

laboratory and field studies have confirmed that females bias the allocation of resources to offspring sex according to dam quality (Labov et al. 1986; Meikle et al. 1993; Moses et al. 1995; Ryan et al. 2012, 2014). Inconsistencies in the direction of such biases, however, highlight the complexity of potential trade-offs among body condition, litter size and litter sex ratio in polytocous species. Consideration of litter sex ratio in investigations that affect maternal quality through parasite load manipulation remain rare (Kankova et al. 2007; Hillegass et al. 2010), yet invariably prove insightful.

We performed a manipulative field experiment to determine how ectoparasites influence life history traits and trade-offs in relatively asocial Franklin's ground squirrels (*Poliocitellus franklinii* Sabine, 1822). We experimentally reduced ectoparasite burdens on reproductive females with an insecticide following breeding, and contrasted body mass and reproductive performance (size, mass and sex ratio of juveniles produced) of those individuals to sham-treated control females. Specifically, we addressed the primary hypothesis that body condition and reproductive investment are affected by ectoparasite defense. We expected that in the face of decreased ectoparasite burden, insecticide-treated females would outperform sham-treated control females in both body condition and reproductive metrics. In that manipulative investigations of ectoparasite impacts on ground squirrels are rare, this study broadens the comparative picture on the potential costs of such infection to include the least social ground squirrel species investigated to date.

Materials and methods

Study area and animals

Field research on Franklin's ground squirrels was conducted from 27 April through 30 July 2014 at 50°9'N, 98°21'W near Delta Marsh, Manitoba, Canada. The study site consisted of an approximately 1 km² area comprised of hayfield, crop fields, mixed deciduous forest, marsh edge and rural roadsides (for details of the geology, biogeography, and ecology of the area see Love and Love 1954; Shay 1999).

Franklin's ground squirrels are obligate hibernators. In Manitoba, males emerge from hibernacula as early as the end of April, one to two weeks before females. Mating occurs from the time of female emergence in early May through early June (Sowls 1948; Iverson and Turner 1972; Pero and Hare 2017). Gestation lasts 28 d (Choromanski-Norris et al. 1986) and lactation lasts up to 31 d (Turner et al. 1976). Average litter size ranges from 6.3 (Pero and Hare 2017) to 9.4 (Iverson and Turner 1972), and juvenile males are roughly 5 g heavier than juvenile females at emergence (Pero and Hare 2017). Adult males immerse into hibernation as early as late July, followed by adult females in late August, and juveniles that remain active above ground as late as early October (Sowls 1948; Iverson and Turner 1972).

Franklin's ground squirrels are hosts to numerous ectoparasite species (Galloway and Christie 1990). The flea *Oropsylla (Opisocrostitic) bruneri* Baker, 1895 is primarily associated with Franklin's ground squirrels in Manitoba, with peak density of this species in early May around the time of adult emergence, and again in late August through early September (Reichardt and Galloway 1994). Franklin's ground squirrels are also hosts to at least seven other flea species, as well as accidental hosts to multiple flea species associated with other mammalian species (Galloway and Christie 1990).

Trapping and handling of squirrels

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed as approved under Manitoba Conservation Wildlife Scientific Permit WB14952 and the University of Manitoba's Fort Garry Campus Animal Care Committee under Protocol F12-014. We trapped Franklin's ground squirrels using National and Tomahawk live traps (Tomahawk Live Trap, Tomahawk, WI) baited with peanut butter and rolled oats. To protect squirrels from overheating in intense sun, we covered all traps with corrugated plastic. Upon initial capture, we marked all squirrels with a unique metal ear tag (Monel #1, National Band & Tag Co., Newport, KY) for permanent individual identification and with a unique dye mark (Pearl Black #52, Clairol Hydrience™, Procter and Gamble Co., Stamford, CT) on their dorsal pelage for visual identification. We checked traps hourly to limit animal stress and re-applied dye marks as needed throughout the season. In the context of a concurrent study on nest relocation movements, we attached radio transmitter collars (model PD-2C, Holohil Systems Ltd., Carp, ON), which housed Passive Integrated Transponder (PIT) tags (14 mm Avid DNA Identification System, Avid Identification Systems, Inc., Norco, CA), to all adult female squirrels. Upon each capture, we weighed squirrels to the nearest 5 g (Pesola™ spring scale, Baar, Switzerland). Any ticks we encountered on squirrels were removed upon discovery during handling in that ticks aggregated on live traps on our site at high densities, and were not the focus of our ectoparasite quantification.

We assessed squirrels for reproductive status and assumed breeding date based on observation of genitalia as described by Murie and Harris (1982). We estimated parturition based on a 28 d gestation period (Choromanski-Norris et al. 1986) and observed weight loss > 40 g over a 24 hr period. We thereafter estimated juvenile emergence based on a 31 d lactation period (Turner et al. 1976), and began daily monitoring of nests within at least 2 d of estimated juvenile

emergence so as to trap young of the year as soon as possible. We sexed, marked, and weighed all juveniles upon capture, and completed full litter assessment within 5 d of initial emergence for all litters.

Measurement and manipulation of flea infestation

We systematically assigned female squirrels into control ($n = 7$ females) and insecticide-treatment ($n = 7$ females) groups, balancing the assignment of breeding females spatially across the study area, and avoiding any systematic bias in the mass of females comprising the two treatment groups. We sprayed squirrels with an anti-parasite product (FRONTLINE™ Spray, Merial Limited, Duluth, GA, fipronil 0.29%,) or with water (sham-control) early in the season post-conception but prior to estimated parturition (mean = 14 d prior to parturition, SD = 6 d), and again 21-30 d later.

Prior to applying the experimental or control treatment, we quantified relative flea abundance on all female squirrels. This quantification followed methods similar to those described by Patterson et al. (2013) with a search of the ventral pelage for 60 s using a metal flea comb, stroking the fur opposite to the direction of hair growth. We then spent an additional 60 s searching for fleas in the dorsal pelage in the same manner, paying particular attention to the shoulder, groin, and axillary regions, as fleas often accumulate in these areas (Patterson et al. 2013).

We removed any fleas encountered during quantification from squirrels in the insecticide-treatment group by combing fleas into a petri dish held under the squirrel containing isopropyl alcohol (70%). We did not identify flea species, however, *O. bruneri* is commonly associated with *P. franklinii* (Galloway and Christie 1990), with prevalence as high as 100% on Franklin's ground squirrels over the course of the active season elsewhere in Manitoba

(Reichardt and Galloway 1994). Thus, we are confident in the near-exclusive occurrence of this flea species on *P. franklinii* in our study area (T. Galloway, personal communication). We did not remove fleas from squirrels in the control group. Though it is possible that the same flea could have been counted twice on control squirrels, this was likely a rare occurrence, given both the rapid and systematic nature of the search. A single observer (EMP) conducted flea counts to reduce bias in flea abundance quantification (except in one instance during the field assistant training period).

Directly following flea quantification, we applied a topical insecticide, fipronil (FRONTLINE™ Spray), to squirrels in the insecticide-treatment group. Fipronil is classified as a broad-spectrum phenylpyrazole insecticide and is used to control a wide range of insects by blocking chloride channels in the central nervous system (Fipronil Technical Fact Sheet, Merial Limited, Duluth, GA). We administered fipronil to squirrels by spraying 1.5 ml of FRONTLINE™ Spray on the dorsal pelage of squirrels as described by Hillegass et al. (2010). To control for a possible response to the spray manipulation itself, we sprayed control females with approximately 1.5 ml of ambient temperature tap water, thus ensuring squirrels in both the insecticide-treated and control groups were handled in the same manner.

Data analysis

We used the nonparametric Wilcoxon signed-rank test to compare relative flea abundance before and after initial treatment within the control and insecticide-treated groups, and the Mann-Whitney *U*-test to compare relative flea densities after initial treatment between groups. Comparisons between control and insecticide treatment groups for dam mass gain, average juvenile mass, total litter mass, and litter sex ratio (calculated as the proportion of males to total litter size at juvenile emergence) were tested using two-sample *t*-tests in that the parametric

assumptions of normality and homoscedasticity were met for those variables. We used the Mann-Whitney *U*-test to analyze comparisons of litter size, average male juvenile mass, and average female juvenile mass between groups in that those data failed to meet the parametric assumptions. Because our sample sizes for both the experimental and control groups were small, we conducted formal sensitivity power analyses for each independent variable examined. We calculated required effect sizes between our treatment groups based on reported sample sizes using G*Power 3 (Faul et al. 2007).

Dam reproductive mass gain was calculated by subtracting dam breeding mass from dam mass at the time of juvenile emergence. We also determined relative mass gain for each dam as the proportion of breeding mass at the time of juvenile emergence to account for scalar differences among individuals. Breeding mass was considered the mass measurement taken within 5 d of and closest to, but not before, the estimated breeding date. We considered mass at juvenile emergence as the mass measurement taken within 7 d of and closest to, but not after, juvenile emergence.

Data were assessed for normality and homoscedasticity using Shapiro-Wilk normality tests and Levene's tests (respectively), as well as by visual inspection. Summary values are reported as mean $\pm$ 1 SE. All statistics were performed using R software (version 3.0.2, R Development Core Team, 2013). We report actual significance levels from statistical tests, though differences were considered significant at $P \leq 0.05$.

Results

Flea manipulation and quantification

Relative flea abundance on insecticide-treated squirrels was successfully reduced from the time of initial treatment to second treatment ($V = 21$, $P = 0.015$; Figure 1). No significant reduction in relative flea abundance was observed among squirrels receiving the control treatment ($V = 4.5$, $P = 0.829$; Figure 1). Relative flea abundance was significantly greater on control squirrels than on insecticide-treated squirrels at the time of second treatment ($W = 44.5$, $P = 0.005$; Figure 1), but there was no difference in relative flea abundance between groups prior to initial treatments ($W = 33$, $P = 0.272$, Figure 1).

Statistical power of tests

Sensitivity power analyses were performed assuming relatively high power ($1 - \beta = 0.8$). These analyses revealed relatively high required effect sizes to detect statistically significant differences across all independent variables (Table 1).

Maternal mass and litter attributes

No differences in mass gain or relative mass gain were detected between the insecticide-treated and control groups over the reproductive period, despite a trend towards greater mass gain among insecticide-treated females (mass gain: $t_9 = 1.066$, $P = 0.157$, Figure 2a; relative mass gain: $t_9 = 0.833$, $P = 0.213$). Litters belonging to control and insecticide-treated females did not differ in average juvenile mass ($t_{12} = 0.420$, $P = 0.341$) or total litter mass at the time of juvenile emergence ($t_{12} = -0.124$, $P = 0.548$; Figure 2b). Likewise, there was no difference in litter size between females of insecticide-treated and control groups ($W = 18$, $P = 0.822$; Figure 2c). Litter sex ratio was, however, significantly more male biased among litters of insecticide-treated females than control females ($t_{12} = 3.974$, $P = 0.002$; Figure 2d), with all 7 insecticide-treated females producing litters with $\geq 50\%$ male offspring and all 7 control females producing litters with $\leq 50\%$ male offspring. Further, in contrast with the mean $\pm$ SE litter sex

ratio of 0.475 ± 0.042 for 42 litters ascribed unambiguously to their dams within this same population between 1998 and 2001 (Pero and Hare 2017), one-sample *t*-tests revealed that insecticide-treated females in 2014 produced significantly more male-biased litters (0.573 ± 0.031 ; $t_6 = 3.199$, $P = 0.019$), while the litter sex ratio of control females in 2014 tended to be more female-biased, but did not depart significantly from the long-term average (0.420 ± 0.024 ; $t_6 = 2.359$, $P = 0.056$). Neither male nor female juveniles of insecticide-treated females weighed more than juveniles of control females at emergence from the natal burrow (males: $W = 31.5$, $P = 0.203$; females: $W = 32.5$, $P = 0.169$).

Discussion

The significant difference in litter sex ratio between control and insecticide-treated females suggests that Franklin's ground squirrel reproduction is influenced by parasitism. Dams in the insecticide-treated group biased investment in offspring towards males, as predicted under the Trivers-Willard model (TWM) of adaptive sex allocation (Trivers and Willard 1973). Trivers and Willard (1973) posited that in polygynous species, where male offspring are generally costlier than female offspring, where males experience more variable reproductive success than females, and where fitness returns of producing high-quality male offspring exceed those of producing high-quality female offspring, dams in good condition will invest more heavily in male offspring, while dams in poorer condition will bias investment towards female offspring. Though litters of both control and insecticide-treated groups fall within the range of what can be considered litter sex ratio parity (Gedir and Michener 2014), the significant and consistent difference between the insecticide-treated and control litters in the present study, and significant male-bias in offspring production by insecticide-treated dams in this study relative to the

long-term average litter sex ratio for the population, support the notion of adaptive sex allocation.

Where post-conception adaptive sex allocation occurs, as would be the case with manipulation of ectoparasite abundance during gestation and lactation in the present study, we would expect a reduction in litter or clutch size as offspring of the non-preferred sex are aborted or otherwise neglected (Krackow 1995; Gedir and Michener 2014). No significant difference in the litter size of insecticide-treated versus control females was detected, however, despite the significant and consistent difference in litter sex ratios within the two treatment groups at juvenile emergence. It is possible, by chance alone, that with our small sample of dams in both the manipulated and control group, dams in the manipulated group commenced gestation with larger presumptive litter sizes than those in the control group, and reduced their litters to a similar number of offspring in response to the selective resorption of female blastocysts (Cameron and Linklater 2002; Ryan et al. 2012, 2014) or selective neglect of individuals of the non-preferred sex during lactation (Moses et al. 1995). We did not, however, sacrifice dams after young were weaned to count placental scars, as would be necessary to collect definitive data on gestational manipulation of offspring sex (though blastocysts resorbed prior to implantation would leave no detectable sign along a uterine horn), or excavate nest burrows for evidence of neglected neonates, out of concern for the persistence of our study population. Thus, the absence of any litter size difference in light of apparent adaptive sex allocation remains unexplained and requires additional study.

Further, despite a significant effect on litter sex ratio, increased ectoparasite abundance did not negatively affect maternal mass gain in this study. As noted above, however, sample sizes were small ($n = 7$), and thus trends must be interpreted cautiously. Among sciurid species studied

to date, female body mass is sometimes influenced by parasitism (Van Vuren 1996; Neuhaus 2003; Scantlebury et al. 2010), though not always (Hillegass et al. 2010; Raveh et al. 2015). Where dams maintain body condition in the face of increased parasite abundance, we might expect a decrease in investment in offspring (Patterson et al. 2013), though that trade-off was not evident in our data.

Our failure to detect any effect of ectoparasitism on maternal mass or current reproductive output may reflect relatively minor costs of ectoparasitism, or costs that only become significant at parasite levels higher than those incurred by dams in our study. Franklin's ground squirrels may compensate for any slight costs of ectoparasitism during lactation and gestation with an influx of resources available during the active season (Schmid-Hempel 2003; Raveh et al. 2015), or sufficiently combat ectoparasite burdens behaviorally (behavioral parasite avoidance through nest relocation, Hart 1994; Pero and Hare unpublished data). Møller et al.'s (2001) comparative analysis of bird species in the family Hirundinidae revealed that social species invested more heavily into immune function. As Franklin's ground squirrels are relatively asocial (Hare 2004) compared to other ground squirrel species in which parasite effects were apparent (Arnold and Lichtenstein 1993; Van Vuren 1996; Neuhaus 2003; Hillegass et al. 2010), it is possible that Franklin's ground squirrels have evolved fewer mechanisms for allocating resources to immune function. Thus, any life history trade-offs with immune function, as evidenced by mass gain or reproductive effort, may not be as pronounced.

It is also possible that ectoparasite burden exerted negative effects on untreated Franklin's ground squirrels apart from influencing offspring sex, but that such effects escaped detection within season. Effects on maternal mass may be delayed to the subsequent season (Hare and Murie 1992; Skibieli et al. 2013), or, because Franklin's ground squirrels are an iteroparous

species, effects of parasitism may be observed in subsequent litters (Kristan 2002). Additionally, though body mass can provide an acceptable indicator of body fat in sciurid species (Becker et al. 1998; Humphries and Boutin 1996), a more robust estimation of body condition may have revealed an effect of insecticide treatment (Schulte-Hostedde et al. 2001). Indeed, costs of parasitism may not be discernible by external metrics given potential disparate effects on different internal systems, or may be detectable only as they accumulate over the long term (Zuk and Stoehr 2002; Kristan 2004; Scantlebury et al. 2010). As mass is not the sole measure of juvenile quality, juvenile survival may provide a more informative measure of the influence of parasitism on dam fitness (Arnold and Lichtenstein 1993; Van Vuren 1996; Patterson et al. 2013).

Empirical support for adaptive sex ratio allocation, particularly the TWM, has been extensively reviewed and investigated in a broad range of taxa (Charnov 1982; Clutton-Brock and Iason 1986; Frank 1990; Hardy 1997; Cameron and Linklater 2002). There has been mixed support for the TWM from research involving ground squirrels (Ryan et al. 2012, 2014; Gedir and Michener 2014). Compared to more social Marmotini, relatively asocial Franklin's ground squirrels are more likely to conform to the cardinal predictions of the TWM. More social species tend to be associated with tighter matrilineal units (Armitage 1981; Michener 1983, 1984; Rayor and Armitage 1991; Hare and Murie 2007), wherein territory or rank may be inherited along maternal lines (Harris and Murie 1984). Within these species, if high quality dams pass on higher quality territory or rank to daughters, those daughters may then produce higher quality progeny ("advantaged matriline", Leimar 1996; Hewison and Gaillard 1999; Ryan et al. 2012). That is, investment in high quality daughters may actually lead to equal or greater fitness than investing in high quality males, thus failing an assumption of the TWM in its cardinal form. This

interpretation is consistent with the findings of Ryan et al. (2012; 2014) who reported that stressed dams produced smaller, more male-biased litters than dams that were subject to lower stress levels, as measured by fecal glucocorticoid concentrations (Hare et al. 2014). In more social species, where space use by dams and daughters overlaps significantly (Rayor and Armitage 1991), post-weaning investment in daughters may be great enough to balance the higher pre-weaning investment required for sons (Gedir and Michener 2014), again failing cardinal TWM assumptions.

In less social species, where maternal transmission of rank or territory is not as prevalent or complete, the fitness benefits of investing in high quality males presumably outweigh those of investing in high quality females, as the TWM assumes. While the per capita litter sex ratio of a more social ground squirrel species may be unaffected by parasitism (Cape ground squirrels: Hillegass et al. 2010), the more male-biased sex ratio of insecticide-treated Franklin's ground squirrels in this study is consistent with the original formulation of the Trivers-Willard hypothesis. Kankova et al. (2007) likewise provided evidence for a more female-biased litter sex ratio in parasitized laboratory mice as compared to unparasitized controls. More comprehensive investigation of the spatial and social organization of Franklin's ground squirrels and other comparable species are necessary to empirically address how sociality may influence the expression of adaptive sex allocation.

While cautious interpretation is warranted due to small sample sizes and potentially spurious results, the results of the present study provide only marginal support for the hypothesis that dam growth and reproduction trade-off with ectoparasite defense. However, the parasite-host system examined is more complex than reported here, as type and intensity of immune response, and ensuing costs, may differ among species of parasites (Degen 2006; Hillegass et al. 2008;

Manjerovic and Waterman 2012; Waterman et al. 2014). Little is known about what endoparasites Franklin's ground squirrels harbor (Tobon et al. 1976; Durham et al. 1988; Jardine et al. 2005), let alone their possible influence on the immune system and other life-history traits, thus presenting a promising avenue for future study. Taken at face value, the present findings suggest that dams partially compensate for any costs of immune response associated with ectoparasite defense with elevated resource intake, or adequately manage infestation levels through non-immune defense (i.e., behavioral parasite avoidance; Pero 2015). A statistically significant difference in litter sex ratio between insecticide-treated and control females suggests, however, that female Franklin's ground squirrels are influenced by ectoparasite pressure and manipulate litter sex in accord with the predictions of the Trivers-Willard model for adaptive sex allocation.

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Table 1. Required effect sizes resulting from post-hoc sensitivity power analyses assuming relatively high power ($1 - \beta = 0.8$) for all mean comparisons (G*Power 3; Faul et al. 2007)

Response variable	Required effect size (Cohen's d)	Required unstandardized effect size*	Observed unstandardized effect size
Dam mass gain	1.633	41.61g	16.33g
Litter size	1.449	2.83 pups	0.429 pups
Litter mass	1.411	146.67g	7.143g
Average juvenile mass	1.411	16.79g	2.761g
Litter sex ratio	1.411	0.149	0.154
* Unstandardized required effect size was determined by multiplying required effect size (Cohen's d) by the pooled SD of treatment and control groups for each response variable			

Figure Captions

Fig. 1. Mann-Whitney U -tests reveal no difference in mean flea counts between control and insecticide-treated groups before treatment, but do reveal a difference after initial treatment. A Wilcoxon signed-rank test reveals no change in mean relative flea abundance within control dams before and after initial treatment, but does reveal a decrease in relative flea abundance within insecticide-treated dams. Error bars represent SE.

Fig. 2. (a) Student's two-sample t -test reveals no difference between control and insecticide-treated groups in dam mass gain (g) over the course of reproduction (see Methods for calculation), (b) Student's two-sample t -test reveals no difference between groups in total litter mass (g) at the time of juvenile emergence, (c) Mann-Whitney U -test reveals no difference between groups in litter size at the time of juvenile emergence, and (d) Student's two-sample t -test reveals that insecticide treated females produced litters that were significantly more male-biased than control females at the time of juvenile emergence. Error bars represent SE.

Fig. 1. Mann-Whitney *U*-tests reveal no difference in mean flea counts between control and insecticide-treated groups before treatment, but do reveal a difference after initial treatment. A Wilcoxon signed-rank test reveals no change in mean relative flea abundance within control dams before and after initial treatment, but does reveal a decrease in relative flea abundance within insecticide-treated dams. Error bars represent SE.

175x116mm (240 x 240 DPI)

Fig. 2. (a) Student's two-sample *t*-test reveals no difference between control and insecticide treated groups in dam mass gain (g) over the course of reproduction (see Methods for calculation), (b) Student's two-sample *t*-test reveals no difference between groups in total litter mass (g) at the time of juvenile emergence, (c) Mann-Whitney *U*-test reveals no difference between groups in litter size at the time of juvenile emergence, and (d) Student's two-sample *t*-test reveals that insecticide treated females produced litters that were significantly more male biased than control females at the time of juvenile emergence. Error bars represent SE.

228x179mm (240 x 240 DPI)