

Patterns of parasitism and body size in red squirrels (*Tamiasciurus hudsonicus*)

Jamieson C. Gorrell and Albrecht I. Schulte-Hostedde

Abstract: Parasites use their hosts for nutrition, shelter, and even dispersal; the latter can result in sex-biased parasite distribution. Host sex-biased parasitism has been well documented in vertebrates, including mammals, and males are often more parasitized than females. Male-biased parasitism is often attributed to sexual size dimorphism, with larger animals being more parasitized. Here, we used a natural population of red squirrels (*Tamiasciurus hudsonicus* (Erxleben, 1777)), a species without sexual size dimorphism, to test for sex-biased parasitism in ectoparasites and intestinal helminth parasites. We also tested for size-dependent parasitism to determine the importance of body size on parasitism. We predicted that males would be more parasitized and that larger individuals would be more parasitized. As well, we predicted a male-biased flea distribution on male squirrels. Parasitism fluctuated over the course of 4 months, with flea infection peaking in August and helminth infection peaking in June. We found evidence of male-biased parasitism in helminth and ectoparasite infections. Flea infection was weakly correlated with body size in females but not in males, while no correlation was found between body size and helminth infection. Lastly, fleas had a female-biased population; however, male fleas were more likely to be found on male squirrels, and this could be to maximize dispersal to avoid inbreeding.

Résumé : Les parasites utilisent leur hôtes pour leur alimentation, comme abri et même pour leur dispersion; ce dernier phénomène peut produire une répartition des parasites qui varie en fonction du sexe. Le parasitisme qui affecte de préférence les hôtes d'un des sexes a été bien étudié chez les vertébrés et, en particulier, chez les mammifères, chez lesquels les mâles sont souvent plus parasités que les femelles. On attribue souvent ce type de parasitisme au dimorphisme sexuel de la taille : les animaux de plus grande taille sont ainsi plus fortement parasités. Nous étudions ici une population naturelle d'écureuils roux (*Tamiasciurus hudsonicus* (Erxleben, 1777)), une espèce sans dimorphisme sexuel de la taille, afin de vérifier l'existence de parasitisme variant en fonction du sexe chez les ectoparasites et les helminthes parasites de l'intestin. Nous vérifions aussi le parasitisme en fonction de la taille, afin de déterminer l'importance de la taille corporelle sur le parasitisme. Nous avons prédit que les mâles seraient plus parasités et que les individus de plus grande taille seraient aussi plus parasités. De plus, nous avons prédit une répartition préférentielle des puces mâles sur les écureuils mâles. Le parasitisme a fluctué au cours des 4 mois, les infections de puces atteignant leur apogée en août et celles des helminthes en juin. Il y a des indications d'un parasitisme affectant préférentiellement les mâles, tant par les helminthes que par les ectoparasites. Il y a aussi une faible corrélation entre l'infection par les puces et la taille corporelle chez les femelles, mais non chez les mâles; en revanche, il n'existe aucune corrélation entre la taille corporelle et l'infection par les helminthes. Finalement, les populations de puces contiennent plus de femelles; cependant, les puces mâles ont plus de chances de se retrouver sur les écureuils mâles, ce qui pourrait maximiser leur dispersion et ainsi éviter les croisements consanguins.

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Introduction

By definition, a host provides shelter and nutrients to parasites without receiving any benefit. Parasites utilize host resources while increasing the hosts' energy expenditure associated with the immune response (Khokhlova et al. 2002; Martin et al. 2003). These energetic costs can be high, leading to a reduction in host reproductive success and survival (Zuk 1988; Forbes and Baker 1991; Fitze et al. 2004). The immune system is designed to minimize the neg-

ative effects of parasitism on fitness, yet the allocation of energy towards immune function often comes as a trade-off with less energy available for other processes such as growth and reproduction (Sheldon and Verhulst 1996).

Host sex-biased parasitism has been well documented (Zuk and McKean 1996; McCurdy et al. 1998; Isomursu et al. 2006) and males tend to be more parasitized than females across many vertebrate taxa, including mammals (Poulin 1996; Schalk and Forbes 1997; Moore and Wilson 2002; but see Christe et al. 2007). There are three main hypotheses that explain this bias. First, sex biases in parasitism may be linked to sexual size dimorphism, where the larger of the two sexes (usually male) is the more parasitized because a larger host is simply a bigger target (Moore and Wilson 2002). In addition, the well-fed host hypothesis predicts that larger animals will be more parasitized, as they represent a better nutritional resource (Christe et al. 2003; Hawlena et al. 2005). Second, male biases in parasitism may be the result of androgenic hormones such as testosterone suppressing the immune system, which leads to higher parasite

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J.C. Gorrell¹ and A.I. Schulte-Hostedde.² Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, Canada.

¹Present address: Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.

²Corresponding author (e-mail: aschulte@laurentian.ca).

loads (Folstad and Karter 1992; Mougeot et al. 2006). A third and more classical hypothesis predicts males to be more parasitized because of their higher levels of activity and promiscuity, increasing their chances of becoming infected (Haas 1966; Brink-Lindroth 1968). Parasites often use their hosts to facilitate dispersal, and thus avoid inbreeding (Bursten et al. 1997; Walter and Proctor 1999). However, these dispersal patterns may differ between male and female parasites, as well as the sex of the host. Male parasites may prefer male hosts, as male hosts generally have larger home ranges and travel farther than females during natal dispersal and mate searching (Haas 1966; Bursten et al. 1997; Perrin and Mazalov 2000). Ectoparasites benefit greatly from dispersal, as they can readily change hosts to maximize reproductive success (Krasnov et al. 2004). Fleas, common ectoparasites of mammals, jump from one host to another, with sexual selection favouring more mobile males (Krasnov et al. 2003). Male fleas are capable of breeding with multiple females, which presumably makes them more active in mate searching (Rothschild and Clay 1952; Krasnov et al. 2003). Thus, male fleas may be more likely to parasitize the host sex that disperses farthest. Female fleas are generally larger and live longer than males, which typically results in female-biased populations (Rothschild and Clay 1952).

We investigated the effects of host sex and body size on ectoparasite and endoparasite abundances in North American red squirrels (*Tamiasciurus hudsonicus* (Erxleben, 1777)). Red squirrels have a wide variety of parasites, including both ectoparasites (fleas, mites) and endoparasites (nematodes) (Flyger and Gates 1982; Patrick and Wilson 1995). To rigorously examine whether male-biased parasitism is a result of large body size or immunosuppressant hormones, a model organism with no sexual dimorphism in size or ornaments should be used (Sheldon et al. 1998). Here, we test for differences in patterns of parasite load between male and female red squirrels. Because red squirrels are not sexually size dimorphic, any male-biased parasitism would not be a result of differences in body size. We also test the prediction that, within sexes, larger animals would be more parasitized than smaller animals. Lastly, we determine if flea sex is associated with host sex as a derivative of dispersal patterns. If fleas use their hosts for dispersal, then we expected to see male squirrels carrying relatively more male fleas compared with female squirrels.

Materials and methods

Field procedures

A population of red squirrels was sampled on a 23 ha grid of mixed deciduous–coniferous forest in Algonquin Provincial Park, Ontario (45°30'N, 78°40'W), from early May to late August 2005. Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were placed 40 m apart at a height of approximately 1.5–2 m from the ground on a platform constructed from a shelf bracket and a plank of wood (60 cm × 15 cm × 1.5 cm) attached perpendicular to a randomly assigned mature tree (diameter at breast height >30 cm). Traps were baited with a mixture of oats and peanut butter, as well as a slice of apple, for 3 days followed by a 3-day hiatus between sessions.

Each squirrel was removed from the trap with a cloth bag, marked with a numbered ear tag, age and sex were determined, and the squirrel was weighed with a Pesola® scale (± 0.1 g). We identified all young of the year based on body mass, and classified all other squirrels as adults. Calipers were used to measure skull length (distance from occipital crest to tip of nose; ± 0.1 mm) and skull width (zygomatic breadth; ± 0.1 mm), while a ruler was used to measure the right hind-foot length from the heel to the tip of the longest nail (± 1 mm). Skull length, skull width, and hind-foot length were measured upon each capture.

Upon first capture, squirrels were combed in the field for ectoparasites using a flea comb (teeth spacing <300 μ m or one-tenth the size of the smallest fleas; Burgham Ltd., Toronto, Ontario). While in a cloth restraint, each squirrel was placed in a clear plastic bag (38 cm × 46 cm) with a tea ball that contained a cotton ball soaked in the inhalant anaesthetic Aerrane® (isoflurane; Baxter Corporation, Mississauga, Ontario). Once anaesthetized, the squirrel was removed from the restraint and combed systematically inside the plastic bag. The flea comb was stroked 10 times down the back from the neck to the base of the tail, 10 times down the stomach from the sternum to the genitals, and 5 times dorsally and ventrally along the tail towards the distal end. All ectoparasites were combed into the plastic bag. The tea ball and flea comb were inspected thoroughly before tying the plastic bag, and the squirrels were released upon recovery from the anaesthesia (~5 min). Plastic bags were stored at -20 °C until later processing. Combing of the same individual were repeated with a minimum of 4-week interval to minimize the manipulative effects of parasite removal (Neuhaus 2003).

Intestinal nematodes release eggs in the hosts' feces, which can be quantified and used as an indication of the present worm burden (Wetzel 1992). Fecal egg counts are a noninvasive technique for estimating worm burden and have been used previously in other mammalian systems to quantify endoparasite load (Agevi 2003; Ferrari et al. 2004). Fecal samples were collected from the trap after releasing the animal and were stored in 70% ethanol.

Laboratory procedures

After a minimum of 24 h in a freezer at -20 °C, each ectoparasite bag was examined using a microscope with 10× power (Jena Bioscience, Jena, Germany), and all ectoparasites were collected and stored in 70% ethanol; samples were later examined at 40× power (Olympus America Inc., Center Valley, Pennsylvania, USA) for identification of parasites. Mites, ticks, and fleas were counted, and in addition, fleas were identified to species and sexed (Holland 1949). A large ectoparasite may consume more resources from its host than a small ectoparasite, and thus we measured total body length for each flea as an index of size. Flea body length was measured by placing fleas on their side between two glass slides to control positioning and pressure-induced measurement error (Tripet et al. 2002). Using a Sony XC-ST50 camera mounted on an Olympus SZ61 dissecting microscope and Image-Pro Express version 5.1 software (Media Cybernetics, Silver Spring, Maryland, USA), we calculated total body length as the mean of three measurements of the distance from the clypeal tubercle to the last abdomi-

Table 1. Physical size measurements of adult red squirrels (*Tamiasciurus hudsonicus*) from Algonquin Provincial Park, Ontario.

	No. of individuals	Skull length (mm)	Skull width (mm)	Hind-foot length (mm)	Body mass (g)
Male	21	49.7±0.3	26.7±0.2	47.2±0.3	172.8±2.5
Female	22	49.9±0.2	26.7±0.1	46.7±0.3	172.8±3.3

Note: Values denote means ± SE.

Table 2. Ectoparasite load, total flea size, and endoparasite load of male and female red squirrels (*Tamiasciurus hudsonicus*) from May to August.

	Squirrel	Ectoparasite load (no. of parasites)		Total flea size (µm)		Endoparasite load (1 × 10 ³ eggs/g)	
		N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
May	Male	5	0.40±0.40	5	1466.57±1466.57	1	6.80
	Female	5	1.00±0.55	5	4702.42±1921.11	9	186.67±94.41
June	Male	11	1.18±0.30	11	2993.38±1100.85	12	319.77±107.01
	Female	14	0.93±0.16	14	1543.63±507.79	18	338.94±84.20
July	Male	10	0.90±0.31	10	1029.31±534.01	8	129.71±34.71
	Female	15	1.27±0.47	15	3593.03±2032.80	12	265.38±99.05
August	Male	10	2.40±1.05	10	7795.21±3457.11	7	168.00±38.45
	Female	14	2.21±0.68	14	7506.45±2827.70	12	82.76±19.38

Note: Values are untransformed data. N, sample size.

nal segment for each flea (±1 µm). Ticks and mites were not measured because they occurred only rarely, and usually only a single acarus was noticed if at all.

Fecal egg counts were performed with 0.5 g of feces using a modified McMaster technique that floats helminth eggs on a grid-etched slide. The McMaster technique has been used with a variety of small mammals and is considered to be the most accurate method of estimating egg counts (Rossanigo and Gruner 1991; Ferrari et al. 2004; Pauli et al. 2004).

Statistical analyses

Several measures of parasite load were calculated including counts of ectoparasites (ectoparasite load), the sum of body lengths of all fleas (total flea size), and the number of nematode eggs per gram of feces (endoparasite load). Endoparasite load was log₁₀-transformed for normality, while ectoparasite load and total flea size were analyzed using nonparametric approaches because they were non-normal. Repeated sampling of individuals over time leads to pseudoreplication (Hurlbert 1984), and thus the data were partitioned by month and in some cases (when more than one set of measurements were taken from the same individual in a month) the mean within a month was calculated. ANOVAs were used to test for differences in parasite load across time and sex, while χ² analyses were used to test for differences in flea sex distribution across male and female squirrels.

Two separate estimates of host body size were calculated using both linear size and body mass measurements. Overall size was determined from the first principal component (PC1) of a principal component analysis (PCA) using skull length, skull width, and hind-foot length (all log-transformed). Surface area was determined by mass^{2/3}, which represents the intraspecific allometric relationship between body mass and

surface area (Heusner 1985). This technique has been previously used with small mammals (Hawlena et al. 2005; Hawlena et al. 2006). A parametric *t* test examined the difference in body size (PC scores derived from PC1) between adult male and female squirrels. ANCOVAs were used to investigate the relationship between endoparasite load and host body size, while nonparametric correlations were employed to determine the relationships between ectoparasite load and total flea size with host overall size and surface area. For repeated tests, we used a sequential Bonferroni correction to adjust the acceptable level of significance (Rice 1989). We used STATISTICA® version 6.0 (StatSoft, Inc., Tulsa, Oklahoma) for all statistical analyses.

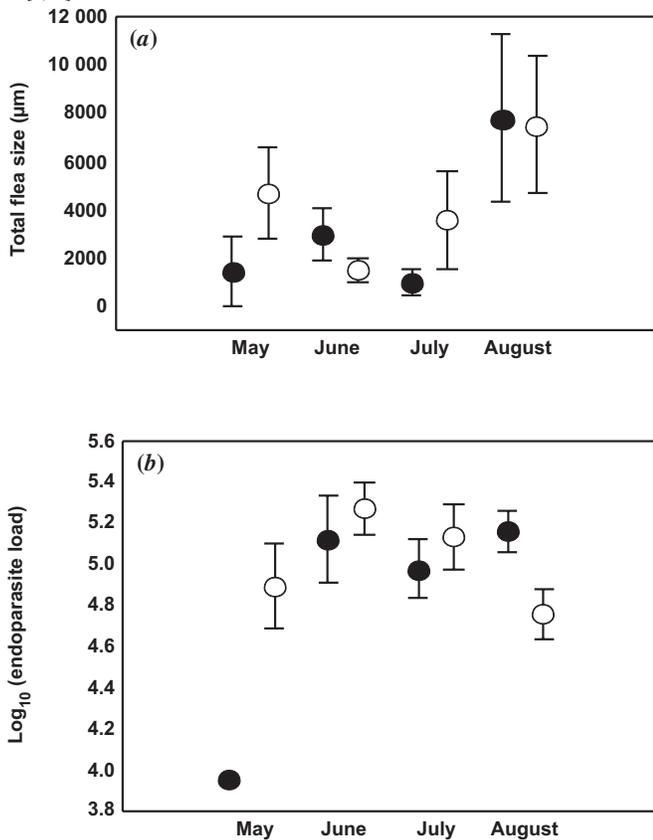
Results

Over the course of the field season, 21 adult males and 22 adult females were measured for physical size. Mean values for male and female squirrels are presented in Table 1 as a reference for squirrels from this region. In addition, we combed ectoparasites off 43 adults and examined fecal samples for eggs from 37 adult squirrels (15 males and 22 females) over 4 months. See Table 2 for means and SE of all measured data. The mean recapture rate for all squirrels was 7.7 captures per individual over the 4 months.

Ectoparasite load

Collected fleas were separated by sex and identified into two distinct species based on morphological characteristics: *Opisodasys pseudarctomys* (Baker, 1904) and *Orchopeas caedens* (Jordan, 1925). Comparing flea body length using a two-way ANOVA, we found that female fleas (4110.9 µm) were bigger than male fleas (3163.5 µm; *F*_[1,214] = 89.21, *p* < 0.0000), and that *O. pseudarctomys* (4049.0 µm) were bigger than *O. caedens* (3685.8 µm; *F*_[1,214] = 13.48, *p* <

Fig. 1. (a) Monthly variation (mean \pm SE) in total flea size of male (●) and female (○) fleas (*Opisodasys pseudarctomys* and *Orchopeas caedens*) found on red squirrels (*Tamiasciurus hudsonicus*). Male total flea size did not vary across time ($H_{[3,36]} = 3.39$, $p = 0.34$), whereas female total flea size varied marginally ($H_{[3,48]} = 7.28$, $p = 0.06$) when total flea size in August was higher than in July ($Z = 2.27$, $p = 0.02$, $N = 20$). (b) Monthly variation (mean \pm SE) in endoparasite load. Egg counts in June were higher than in May and August ($F_{[3,39]} = 3.84$, $p = 0.02$), and male squirrels (●) had higher egg counts than female squirrels (○) in August ($F_{[1,18]} = 4.23$, $p = 0.06$). There was only one male squirrel in May.



0.0003), although the interaction was not significant ($F_{[1,214]} = 0.02$, $p > 0.88$). To account for this bias in size across species and sex, we calculated total flea size by summing all the body lengths of fleas from the same sample as an additional index of flea burden to the host. A Kruskal–Wallis nonparametric ANOVA showed that total flea size varied across months (Fig. 1a; $H_{[3,84]} = 7.69$, $p = 0.05$) and Wilcoxon dependent t tests revealed that total flea size in August was higher than in July ($Z = 2.27$, $p = 0.02$, $N = 20$). When analyzing monthly variation within each host sex separately, we found that only female total flea size were marginally higher in August than in July ($H_{[3,48]} = 7.28$, $p = 0.06$; $Z = 1.89$, $p = 0.06$, $N = 12$), while male total flea size showed no variation across time ($H_{[3,36]} = 3.39$, $p = 0.34$). Male flea ($H_{[3,36]} = 2.99$, $p = 0.39$), female flea ($H_{[3,48]} = 3.12$, $p = 0.37$), and pooled ectoparasite ($H_{[3,84]} = 5.02$, $p = 0.17$) loads did not significantly vary over time.

To examine biased sex ratios across host sex, we first determined the population-wide flea sex ratio within each flea species by examining all fleas collected throughout the

Table 3. Nonparametric ANOVAs of ectoparasite load and total flea size of *Opisodasys pseudarctomys* and *Orchopeas caedens* with respect to sex of the red squirrels (*Tamiasciurus hudsonicus*).

	Ectoparasite load			Total flea size		
	H	df	p	H	df	p
May	1.13	1, 9	0.29	2.34	1, 9	0.13
June	0.39	1, 24	0.53	0.61	1, 24	0.43
July	0.01	1, 24	0.93	0.03	1, 24	0.86
August	0.21	1, 23	0.65	0.13	1, 23	0.72

Note: Results were not significantly different between the squirrel sexes between months.

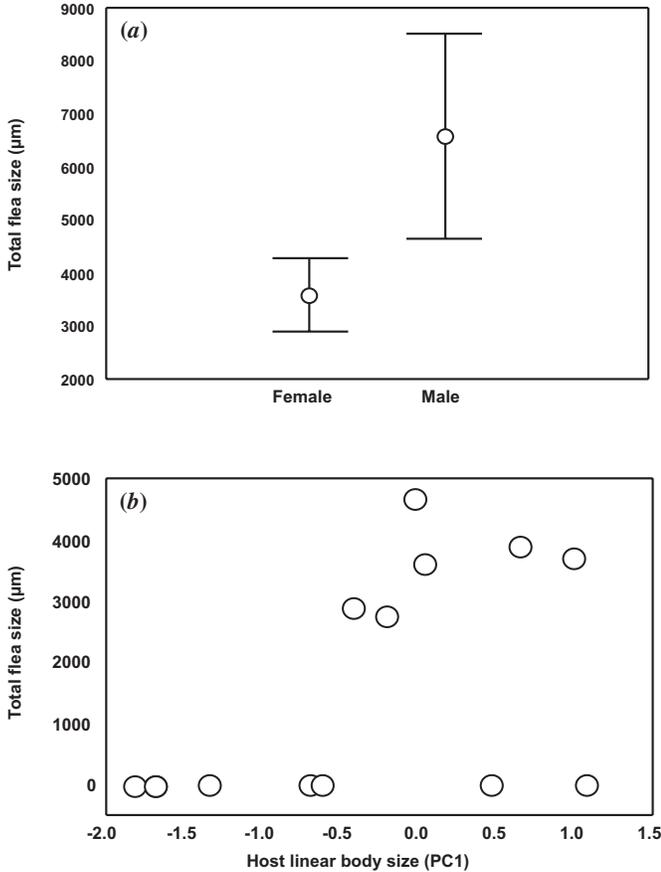
summer regardless of host sex. Chi-square analysis revealed a female-biased sex ratio, in both species, that was significantly different from the expected 1:1 ratio (*O. pseudarctomys*: $\chi^2_{[1]} = 29.45$, $p < 0.001$; *O. caedens*: $\chi^2_{[1]} = 13.07$, $p < 0.001$). Using the observed sex ratio for each species (*O. pseudarctomys* 2.7:1, *O. caedens* 4.3:1) as the new expected ratio, we then tested the sex ratio of fleas on male and female squirrels separately during August (the only month with >5 fleas of each sex). *Orchopeas caedens* had a male-biased distribution on male squirrels ($\chi^2_{[1]} = 4.70$, $p = 0.03$), although there was no biased distribution on female squirrels ($\chi^2_{[1]} = 0.62$, $p = 0.45$). *Opisodasys pseudarctomys* did not have a biased distribution on either male squirrels ($\chi^2_{[1]} = 0.21$, $p = 0.65$) or female squirrels ($\chi^2_{[1]} = 2.89$, $p = 0.09$). Using nonparametric ANOVAs, we tested for bias in ectoparasite loads between squirrel sexes within each month. Neither ectoparasite load ($p > 0.29$) nor total flea size ($p > 0.13$) differed between the sexes across the duration of the study (Table 3). However when the analyses included only infected individuals, males had higher total flea size (but not ectoparasite load) in June (Fig. 2a; $H_{[1,11]} = 4.03$, $p < 0.05$), although significance was lost after correcting for multiple tests.

Skull length, skull width, and hind-foot length of squirrels were \log_{10} -transformed and used in a PCA to determine linear size (PC1 loading coefficients were 0.72, 0.71, and 0.67, respectively, for skull length, skull width, and hind-foot length). The first factor accounted for 49.1% of the variance (eigenvalue = 1.47), which was suitable to account for variation in physical size (Schulte-Hostedde et al. 2001). A parametric t test revealed no difference in overall size (PC1) ($t_{[45]} = 0.19$, $p = 0.85$) or surface area (mass^{2/3}) ($t_{[45]} = 0.49$, $p = 0.63$) between male and female squirrels. Using correlations, we investigated the relationship between body size and parasite load. A summary of all correlations is given in Table 4. Female overall size and surface area were positively correlated with total flea size in June (Fig. 2b; Spearman $R = 0.53$, $p = 0.05$ for both); however, these relationships were no longer statistically significant after correcting for multiple tests. We did not find any correlation between female body size and ectoparasite load or male body size and ectoparasite load or total flea size; we also did not find any difference after excluding noninfected individuals from the analysis.

Endoparasite load

All eggs were identified as *Strongyloides* sp., and was

Fig. 2. (a) Differences (mean \pm SD) in total flea size of *Opisodasys pseudarctomys* and *Orchopeas caedens* in June between the sexes of red squirrels (*Tamiasciurus hudsonicus*). Male squirrels had more flea burden than female squirrels ($H_{[1,11]} = 4.03, p < 0.05$). (b) Spearman correlation of total flea size and female host body size in June. As female body size increases, flea load increases ($R = 0.53, p = 0.05$).



presumed to be *Strongyloides robustus* Chandler, 1942 because it is the only known *Strongyloides* to infect red squirrels (Flyger and Gates 1982). We used a nested ANOVA to test for variation across months in endoparasite load. Squirrel identification was included as a random effect and was nested inside sex. We found that endoparasite load changed over time (Fig. 1b; $F_{[3,39]} = 4.03, p = 0.01$) and Fisher post hoc tests revealed that the load was higher in June than in May ($p = 0.005$) and August ($p = 0.01$) but not in July ($p = 0.22$). There was no difference between male and female squirrels among the 4 months ($F_{[1,39]} = 0.18, p = 0.68$); but, we did find a sex difference when using ANOVA within each month with males having marginally higher egg counts than females in August ($F_{[1,18]} = 4.23, p = 0.06$). However, this trend between the sexes was no longer statistically significant after correcting for multiple tests. There was no difference between the sexes in May ($F_{[1,8]} = 2.12, p = 0.18$), June ($F_{[1,28]} = 0.30, p = 0.59$), or July ($F_{[1,17]} = 0.43, p = 0.52$). To examine the relationship between endoparasite load and body size, we first tested for differences in slopes between male and female squirrels. Because the slopes were not significantly different, we used an ANCOVA, with sex as a covariate, and found that endoparasite load was not cor-

related with overall size or mass of squirrels in May ($F_{[1,7]} = 0.05, p = 0.83$ and $F_{[1,7]} = 0.14, p = 0.72$, respectively), June ($F_{[1,27]} = 0.25, p = 0.62$ and $F_{[1,27]} = 0.18, p = 0.67$, respectively), July ($F_{[1,17]} = 0.25, p = 0.62$ and $F_{[1,17]} = 0.04, p = 0.62$, respectively), or August ($F_{[1,17]} = 1.19, p = 0.29$ and $F_{[1,17]} = 0.09, p = 0.77$, respectively) (for details see Table 5).

Discussion

Effects of host sex and body size on parasite load of a natural population of red squirrels were investigated over 4 months. We predicted that male squirrels would be more parasitized than females, and that larger animals would be more parasitized than smaller animals. Our predictions were not clearly supported, although we did find weak evidence of male-biased parasitism and a positive association between parasitism and body size.

We found that female fleas were larger than male fleas as expected, likely because females are usually larger and longer lived than males (Holland 1949). As well, *O. caedens* was bigger than *O. pseudarctomys*, which is a known difference between the species. To account for variation in flea size and therefore potential variation in parasitic burden, total flea size was calculated in addition to counting the absolute number of fleas on each host because parasitic burden may be better described by biomass than intensity.

Because male-biased parasitism is prevalent in other mammalian systems (Schalk and Forbes 1997; Moore and Wilson 2002; but see Christe et al. 2007), we predicted that male red squirrels would be more parasitized than female red squirrels. Surprisingly, we found only weak evidence of a difference between the sexes. During the month of June, males had higher total flea size than females, but not necessarily more fleas. Our results are similar to Bursten et al. (1997) and Krasnov et al. (2005) who found no difference in the number of fleas between sexes in 9 of 10 different species of rodents. This contrasts with results from similar work on northern flying squirrels (*Glaucomys sabrinus* (Shaw, 1801)) at the same location where males had more ectoparasites than females (Perez-Orella and Schulte-Hostedde 2005). The mean number of ectoparasites on red squirrels was lower than that on the flying squirrels (1.5 vs. 3.8), which suggests that the ectoparasite abundance may have been too low on red squirrels to find a difference between the sexes. The difference in total flea size suggests that fleas might have higher growth rates on male squirrels or that larger fleas select males, possibly because male squirrels have a slightly weaker immune defense. Also, we found faint evidence that male squirrels had higher fecal egg counts of the endoparasite *S. robustus* than female squirrels. *Strongyloides robustus* is a common species of nematode infecting red squirrels, which can cause severe hemorrhagic enteritis (Lichtenfels and Haley 1968; Davidson 1976). Egg counts are indicative of the number of worms present in the intestine (Wetzel 1992), as only parthenogenetic females of *Strongyloides* are known to parasitize vertebrates (Chandler 1955; Eckerlin 1974), eliminating the confusion of cryptic males parasitizing an animal without releasing eggs. Male-biased parasitism of *S. robustus* has also been noted in gray squirrels

Table 4. Nonparametric correlations of ectoparasite load and total flea size of *Opisodasys pseudarctomys* and *Orchopeas caedens* with the body size (linear size PC1 and surface area) of adult male and female red squirrels (*Tamiasciurus hudsonicus*).

	Squirrel	N	Linear size (PC1)				Surface area (mass ^{2/3})			
			Ectoparasite load		Total flea size		Ectoparasite load		Total flea size	
			R	p	R	p	R	p	R	p
May	Male	5	0.00	1.00	0.00	1.00	0.35	0.56	0.35	0.56
	Female	5	0.21	0.73	0.05	0.93	0.53	0.36	0.67	0.22
June	Male	11	0.04	0.91	0.05	0.88	0.00	0.99	0.12	0.73
	Female	14	0.50	0.07	0.53	0.05*	0.31	0.28	0.53	0.05*
July	Male	9	0.21	0.58	0.47	0.21	0.56	0.12	0.15	0.70
	Female	15	0.04	0.90	0.23	0.41	0.42	0.12	0.28	0.30
August	Male	10	0.51	0.13	0.48	0.16	0.34	0.34	0.19	0.60
	Female	14	0.09	0.76	0.01	0.97	0.03	0.93	0.08	0.77

*Total flea size increases with increasing female body size in June ($p < 0.05$).

Table 5. ANCOVA results of relationship between endoparasite load and body size (linear size PC1 and mass) of red squirrels (*Tamiasciurus hudsonicus*).

		Linear size (PC1)			Mass		
		F	df	p	F	df	p
May	Endoparasite	0.05	1, 7	0.83	0.14	1, 7	0.72
	Sex	1.69	1, 7	0.24	2.19	1, 7	0.18
	Interaction	—	—	—	—	—	—
June	Endoparasite	0.25	1, 27	0.62	0.18	1, 27	0.67
	Sex	0.44	1, 27	0.51	0.52	1, 27	0.48
	Interaction	3.62	1, 26	0.07	0.24	1, 26	0.63
July	Endoparasite	0.25	1, 17	0.62	0.04	1, 17	0.84
	Sex	0.52	1, 17	0.48	0.55	1, 17	0.47
	Interaction	0.77	1, 16	0.39	0.35	1, 16	0.56
August	Endoparasite	1.19	1, 17	0.29	0.09	1, 17	0.77
	Sex	4.61	1, 17	0.05	3.87	1, 17	0.07
	Interaction	0.03	1, 16	0.87	0.30	1, 16	0.59

Note: The interaction term tested for differences between the slopes of the sexes (covariate), but could not be calculated in May because there was only one male squirrel.

(*Sciurus carolinensis* Gmelin, 1788; Parker 1971). Although the sex difference in egg release found in our study was only apparent during August, this may be an indication of immunosuppression. Reproductive investment is known to be a trade-off with parasitism, as energy is invested in gametogenesis instead of self-maintenance (Sheldon and Verhulst 1996; Pelletier et al. 2005). In August, circulating levels of testosterone in these squirrels were higher than in July (Gorrell 2006), and males were scrotal for the second time that season, indicating that they had allocated energy towards spermatogenesis. Also, squirrels had higher flea loads in August, perhaps adding more stress to the animal. This pattern excludes the hypothesis of male-biased parasitism as a result of sexual size dimorphism because red squirrels in our study are monomorphic. Thus, alternative hypotheses, such as the role of testosterone as an immunosuppressant, should be explored.

We examined the relationship between body size and parasite load of squirrels with the expectation that larger animals would be more parasitized because of their larger surface area and increased mass, making them more desirable hosts. This is presumably more important to ectoparasites than endoparasites, as surface area predicts how many

fleas the host is able to sustain (Hawlena et al. 2006). Our hypothesis was only supported for female squirrels in June, when total flea size was positively correlated with both indices of host body size. It is interesting, but not surprising, that these two indices of body size were correlated with flea load because mass is size-dependant (Schulte-Hostedde et al. 2001); these results suggest that both methods are suitable estimates of body size. Although male host body size did not correlate with total flea size, parasitism could be more important in females because they may be more sensitive to parasite-induced mortality (Bize et al. 2005). The lack of such a relationship during the other months is less clear, and may be a result of rising flea density during the course of the summer while body size remained constant. As flea density increases over the warmer months, selectivity for hosts may decrease as parasites disperse evenly to avoid aggregations on certain individuals. Tripet and Richner (1999) found that flea reproductive success declined as flea density increased on blue tits, *Parus caeruleus* (= *Cyanistes caeruleus* (L., 1758)).

Ectoparasite and endoparasite loads did not correlate with either measure of host body size. Dawson and Bortolotti

(1997) found no correlation between ectoparasites and physical size of nestling American kestrels (*Falco sparverius* L., 1758), while Pelletier et al. (2005) found no correlation between fecal lungworm larvae counts and body mass of big-horn sheep (*Ovis canadensis* Shaw, 1804). Our study does not replicate the negative trend found in an overlapping study using a population of northern flying squirrels infected with ectoparasites (Perez-Orella and Schulte-Hostedde 2005). Krasnov et al. (2005) and Morand et al. (2004) found no evidence of sex-biased parasitism to be the result of sexual size dimorphism in several species of rodents and small mammals, indicating that fleas do not always prefer a larger host. This study does not support size-dependent parasitism or the well-fed host hypothesis, but indicates rather that parasites may choose their host not because of size but for other factors. We found a significant female-bias in both species of fleas combed off red squirrels. Mallory et al. (2006) found female-biased sex ratios of two species of lice on northern fulmars (*Fulmarus glacialis* (L., 1761)). Female fleas are generally larger and live longer than males, which typically results in female-biased populations (Holland 1949; Rothschild and Clay 1952). Yet, after correcting for this biased sex ratio, we found that male squirrels had more male fleas (*O. caedens*) than female squirrels, which could be a mechanism of flea dispersal to reduce inbreeding (Bursten et al. 1997). Activity and promiscuity have been found to increase adult male levels of parasitism (Haas 1966). Red squirrels have male-biased dispersal, and male red squirrels actively search for females during the breeding season that could lead to increased parasitism (Rusch and Reeder 1978; Berteaux and Boutin 2000). Alternatively, female squirrels lacked a significantly different sex-biased flea distribution as a result of being selected by female fleas. Female fleas lay their eggs in the host's nest (Holland 1949), and a female squirrel nest with juvenile squirrels would have more nutritional value than a solitary male nest. If female fleas are actively selecting their hosts to increase fitness, male fleas may passively select the less desirable host as a result of density-dependence (Tripet and Richner 1999). These two flea species have been found before in Algonquin Park on northern flying squirrels (Perez-Orella and Schulte-Hostedde 2005), which suggest that their distribution is influenced by more than one host. In the study area, red squirrels and flying squirrels are sympatric and may exchange parasites.

Current theory predicts that in dimorphic species the larger sex will be more heavily parasitized because they are larger targets (Moore and Wilson 2002). An alternative theory suggests that male-biased parasitism is hormonally driven, because estrogen stimulates immune activity while testosterone decreases it (Moore and Wilson 2002). Because red squirrels are a monomorphic species, any difference in parasitism between the sexes could be explained by hormonal processes. We did find limited evidence of male-biased parasitism, which suggests that testosterone may be suppressing immunity and is the cause of the sex difference in parasitism, although future work is recommended. However, we found no strong evidence to support our prediction that larger individuals are more parasitized than smaller individuals. Overall, this study does not support the size-dependant hypothesis to explain sex-biased parasitism, but rather indi-

cates that alternatives such as the hormone-dependant hypothesis should be explored.

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