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Effects of sex and body size on ectoparasite loads in the northern flying squirrel (*Glaucomys sabrinus*)

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Abstract: Ectoparasites can have profoundly negative fitness consequences for host organisms. Sex differences in parasite load have been documented in many mammals, and have been attributed either to the allocation of energy to growth rather than the immune system in mammals exhibiting male-biased sexual size dimorphism or to the immunosuppressive qualities of testosterone. In addition, ectoparasites can have negative effects on body size and condition, as energy is allocated to the immune system rather than to growth and maintenance. Here, we used the northern flying squirrel (*Glaucomys sabrinus* (Shaw, 1801)) and its ectoparasites to test two predictions: (1) males are more heavily parasitized than females and (2) individuals with high ectoparasite loads will be in poorer condition and be smaller than individuals with low ectoparasite loads. Males were significantly more parasitized than females, and there was a nonsignificant trend for small males to be more parasitized than large males. Because the northern flying squirrel is not sexually dimorphic, the immunosuppressive qualities of testosterone may explain the sex differences in ectoparasite load. Ectoparasites may also influence skeletal growth rates, and males that are more susceptible to ectoparasites may simply be unable to allocate as much energy to growth and are thus structurally smaller.

Résumé : Les parasites peuvent avoir des conséquences extrêmement négatives sur la fitness de leurs hôtes. Les différences de charges parasitaires entre les sexes ont été signalées chez de nombreux mammifères; on les explique par une allocation de l'énergie à la croissance plutôt qu'au système immunitaire chez les mammifères qui ont un dimorphisme sexuel de taille qui favorise les mâles, ou alors par les propriétés d'immunosuppression de la testostérone. De plus, les parasites peuvent avoir un effet négatif sur la taille et la condition corporelles puisque l'énergie est canalisée vers le système immunitaire plutôt que vers la croissance et le maintien. Nous avons utilisé le grand polatouche (*Glaucomys sabrinus* (Shaw, 1801)) et ses ectoparasites pour tester deux prédictions : (1) les mâles portent plus de parasites que les femelles et (2) les individus porteurs de charges parasitaires plus fortes sont en moins bonne condition et sont plus petits que ceux qui ont des charges moins importantes. Les mâles ont significativement plus de parasites que les femelles et il y a une tendance, bien que non significative, pour les petits mâles d'être plus parasités que les mâles plus grands. Parce que le grand polatouche n'a pas de dimorphisme sexuel, les propriétés d'immunosuppression de la testostérone peuvent peut-être expliquer les différences sexuelles de charge parasitaire. Les ectoparasites peuvent aussi influencer les taux de croissance squelettique; ainsi, les mâles qui sont plus vulnérables aux ectoparasites sont peut-être simplement incapables d'allouer autant d'énergie à la croissance et sont donc structurellement plus petits.

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Introduction

By its very definition, the word parasitism indicates that a host animal will experience a reduction in fitness when parasitized, whereas the parasite will reap the benefits of this one-sided association. Despite their small size, ectoparasites have the ability to negatively affect host fitness and life history (Khokhlova et al. 2002; Fitze et al. 2004). Immediate

effects include the removal of blood and nutrients, tissue damage caused by bites (Krantz 1975; Hoogland 1995), traumatic dermatitis, the injection of salivary toxins, and the transmission of pathogens that include endemic typhus and bubonic plague (Hazler and Ostfeld 1995; Arnett 2000; Khokhlova et al. 2002) and internal parasites (Krantz 1975). Ectoparasites are also capable of hindering many of their host's bodily functions, such as movement, copulation, feeding, and even breathing (Walter and Proctor 1999). The long-term effects of ectoparasitism are a result of the allocation of energy towards defence against the parasite. Defences include an increase in the activity of the immune system (Hoogland 1995; Khokhlova et al. 2002) and, when offspring are parasitized, an increase in parental effort (Fitze et al. 2004). In particular, increases in parental effort can result in a cost to future reproduction through reduced fecundity in females (Neuhaus 2003), reduced virility in males

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(Walter and Proctor 1999), nest abandonment (Fitze et al. 2004), reduced growth of affected juveniles (Neuhaus 2003), physical handicaps (Lott et al. 2004), and an overall reduction in life span (Walter and Proctor 1999).

Sex differences in parasitism rates have been observed in a variety of mammalian taxa, with a general pattern of males carrying more parasites than females (Schalk and Forbes 1997; Moore and Wilson 2002). These differences are often attributed to the immunosuppressive effects of androgens such as testosterone (Folstad and Karter 1992). Testosterone weakens the immune system, causing a quantifiable decrease in health (Duffy et al. 2000). As testosterone increases, T-cell-mediated immunity decreases, rendering the animal more susceptible to infection and disease (Mougeot et al. 2004). Alternatively, this pattern has been attributed to male-biased sexual size dimorphism that is prevalent among mammals. Energy is allocated for growth rather than to immune defence, rendering males more susceptible to parasites than smaller conspecific females (Sheldon and Verhulst 1996). In addition, because of their size, males may simply be larger targets than females for parasites (Moore and Wilson 2002).

Body condition refers to the energy reserves that an individual animal possesses (Hayes and Shonkwiler 2001; Schulte-Hostedde et al. 2001), and the amount of energy reserves (fat and (or) protein) that an individual carries can have important fitness consequences. For example, individuals with larger fat reserves may have better fasting endurance and higher survival than individuals with smaller reserves (Millar and Hickling 1990). Parasites are expected to influence body condition (e.g., Neuhaus 2003; Whiteman and Parker 2004) because of the increased allocation of energy to immune function and reproductive effort when raising parasitized offspring (Sheldon and Verhulst 1996). Host energy reserves should thus be depleted when the host is parasitized.

Here we use the northern flying squirrel (*Glaucomys sabrinus* (Shaw, 1801)), a medium-sized nocturnal rodent common in the forests of central and northern Ontario, to examine several issues related to host-parasite relations. First, we document the predominant ectoparasites in our marked population of squirrels. Second, we test the hypothesis that males will have higher ectoparasite loads than females owing to the immunosuppressive effects of testosterone (the immunocompetence handicap; Folstad and Karter 1992). Northern flying squirrels do not exhibit male-biased sexual size dimorphism (Wells-Gosling and Heaney 1984), therefore any sex difference in parasite load should not be the result of sex differences in body size. Finally, we test the prediction that individuals with a high ectoparasite load will be in poorer body condition than those individuals with fewer parasites.

Methods

A northern flying squirrel population in Algonquin Provincial Park (45°30'N, 78°40'W) was sampled from mid-June to late August 2004. Nine locations were sampled for a total of 2900 trap-nights. Typically, 20–30 Tomahawk traps were placed 1.5–2 m high on trees approximately 30–40 m apart in coniferous and mixed coniferous forest. Traps were baited at dusk with a peanut butter and oats mixture and a

slice of apple, and checked at dawn. Each flying squirrel caught was sexed, aged as either an adult or juvenile, marked with a uniquely numbered ear tag, and weighed (± 0.1 g) using a Pesola® scale. Skull length (± 0.1 mm) was measured from the tip of the nose to the foramen magnum using dial callipers.

Each northern flying squirrel was held over a plastic shoe box lined with a plastic bag, containing a tea-ball with a tissue soaked in the inhalant anaesthetic Aerrane (isoflurane; Baxter Corporation, Mississauga, Ontario), to anaesthetize the ectoparasites. A flea comb was passed 15 times over the back towards the tail, and then 5 times towards the head. The belly was then combed 15 times towards the tail, and both the top and bottom parts of the tail were combed 10 times each towards the distal end. The comb was cleaned of hair and excess parasites as needed to prevent parasites from crawling back onto the animal. The comb was cleaned one final time and the flying squirrel was released. The ectoparasite sample was placed in a freezer for at least 12 h to immobilize the ectoparasites. Immobilized parasites were transferred to individual vials containing 70% EtOH. The numbers of fleas and mites were counted. Fleas and mites were sent to be identified by T. Galloway, University of Manitoba (fleas), and H. Proctor, University of Alberta (mites). There were negligible numbers (<2) of other ectoparasites (e.g., ticks, lice), so they were not considered further.

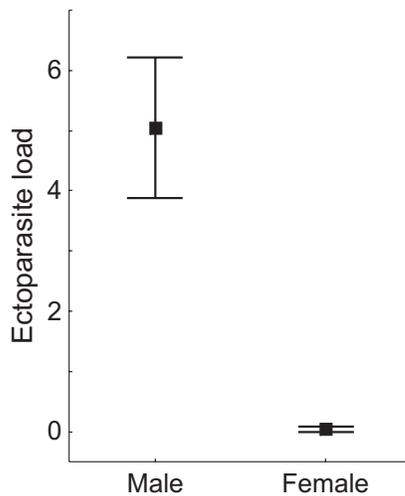
We estimated parasite load by counting the total number of individual fleas and mites collected. We used multiple regression to determine the relationship between the log-transformed mass and size (estimated by skull length) and the parasite load. We interpreted the partial correlation coefficient of body mass as “condition” because the coefficient represents the relation of body mass on parasite load after controlling for body length (skull length). This is analogous to using residuals from a regression of body mass on structural size as an index of condition and reflects variation in body composition: in small mammals, individuals in good condition have relatively more muscle mass and fat than individuals in poor condition (Schulte-Hostedde et al. 2001). When individuals were sampled more than once, we used the mean parasite load and mean mass and mean skull length for all analyses.

Results

Our trapping efforts yielded 26 captures of 18 individual adult northern flying squirrels (10 males, 8 females). All individuals were sampled once except for 3 males and 3 females, which were sampled twice. Females (mass (mean \pm SD) = 110.08 ± 7.79 g) were significantly heavier than males (91.56 ± 4.06 g; $t_{[16]} = 6.63$, $P < 0.001$), although skull length did not differ between the sexes (female: 39.59 ± 1.85 mm; male: 39.74 ± 1.30 mm; $t_{[16]} = 0.22$, $P = 0.83$).

The average number of ectoparasites found per individual ($n = 18$) was 3.83 ± 5.08 . Interestingly, only 1 of 8 females were found with ectoparasites, whereas 9 of 10 males were parasitized ($\chi^2_{[1]} = 10.81$, $P = 0.01$). Thus, there was a significant difference in total ectoparasite load between the sexes (Mann-Whitney U test, $U = 4.50$, $P < 0.001$; Fig. 1). These differences occurred both with respect to fleas ($U = 13.5$,

Fig. 1. Mean (\pm SE) ectoparasite load (number of parasites/individual squirrel) for male and female northern flying squirrels (*Glaucomys sabrinus*). Male squirrels ($n = 10$) had significantly higher ectoparasite loads than female squirrels (Mann–Whitney U test, $U = 4.50$, $P < 0.001$, $n = 8$).



$P = 0.009$) and mites ($U = 16.0$, $P = 0.011$). The morphology of the two flea species found on the northern flying squirrels was consistent with that of *Opisodasys pseudarctomys* (Baker, 1904) and *Orchopeas caedens* (Jordan, 1925). The morphology of the mite species found corresponded to *Haemogamasus reidi* Ewing, 1925, a parasitic mite from the family Laelapidae.

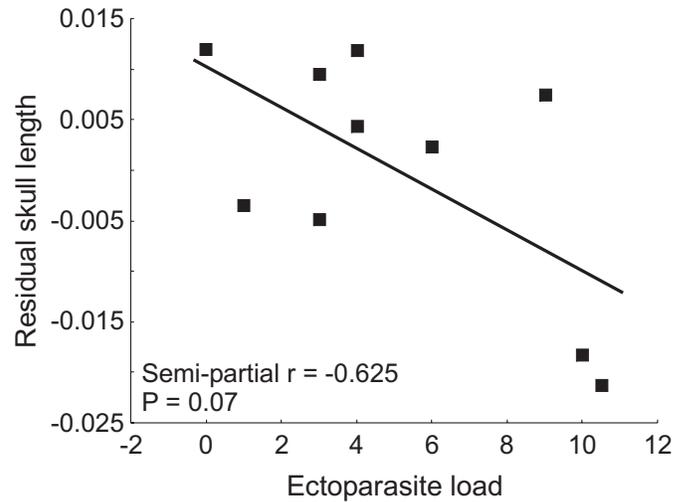
Multiple regression of male body mass and skull length on total parasite load ($F_{[2,7]} = 2.31$, $r^2 = 0.397$, $P = 0.16$) yielded no effect of total parasite load on condition (mass: partial $r = 0.33$, $P = 0.40$); however, there was a nonsignificant trend toward smaller males being more heavily parasitized than larger males (skull length: partial $r = -0.627$, $P = 0.07$; Fig. 2). This trend was found with mites ($F_{[2,7]} = 1.76$, $r^2 = 0.334$, $P = 0.24$; skull length partial $r = -0.578$, $P = 0.1$) but not with fleas ($F_{[2,7]} = 0.11$, $r^2 = 0.03$, $P = 0.89$; skull length partial $r = -0.056$, $P = 0.88$).

Discussion

Two flea species were found on the northern flying squirrels. *Opisodasys pseudarctomys* is a true parasite of the northern flying squirrel and *Orchopeas caedens* is common in tree squirrels (Holland 1949). Although *Orchopeas caedens* has been previously found on northern flying squirrels, it is not a known parasite of the subspecies *Glaucomys sabrinus macrotis* (Mearns, 1898) (the subspecies found in Algonquin Park). The presence of *Orchopeas caedens* on the northern flying squirrel may represent the most southern range of this flea species in Ontario (Holland 1949).

The mites found on northern flying squirrels were of the species *H. reidi*. This mite has been previously found on northern flying squirrels (Wells-Gosling and Heaney 1984). Previous studies have shown that these mites prefer the female of the host species (Redington and Jachowski 1972). This is contrary to our findings, as female northern flying squirrels had negligible amounts of ectoparasites.

Fig. 2. Semi-partial correlation between ectoparasite load and residual skull length (corrected for body mass) for male northern flying squirrels ($n = 10$). Small males tended to have more parasites than large males.



Male flying squirrels generally had more ectoparasites than females. This difference is consistent with previous meta-analyses that have reported similar patterns across mammals (Schalk and Forbes 1997; Moore and Wilson 2002). Two classes of hypotheses have been employed to explain sex differences in parasite load (Moore and Wilson 2002). The first has been applied to mammals with male-biased sexual size dimorphism. It suggests that body size is important in explaining sex-biased parasitism because energy is limiting, and males must partition energy for growth at the expense of energy for the immune system, making them susceptible to parasites (Sheldon and Verhulst 1996). Thus, because mammals tend to exhibit male-biased sexual size dimorphism, males are more likely to be parasitized (Moore and Wilson 2002). The second hypothesis suggests that males may be more susceptible to parasites because of the immunocompetence handicap associated with testosterone (Folstad and Karter 1992). Testosterone is required for the development of sexually selected traits, yet it has immunosuppressive side effects; therefore, males suffer from a handicap with respect to the immune system. The northern flying squirrel is not dimorphic with respect to size; therefore, the immunocompetence handicap associated with testosterone may be a likely explanation for the sex differences in ectoparasite load. Testosterone may make male flying squirrels more susceptible to ectoparasites and their effects, such as loss of nutrients and an increase of secondary infections caused by lacerations to the skin. Testosterone has been linked to disease in Iberian red deer (*Cervus elaphus hispanicus* (Helzheimer, 1909)) (Vicente et al. 2004), sand lizards (*Lacerta agilis* L., 1758) (Olsson et al. 2000), and house sparrows (*Passer domesticus* (L., 1758)) (Poiani et al. 2000).

The sex difference in ectoparasite load may be caused by the ectoparasite itself, instead of the host. Most ectoparasites are known to favour the host den (Holland 1949). In fact, the only time that some ectoparasites leave the nest is when they are on the animal when the animal is outside during the mat-

ing season. Because male northern flying squirrels appear to relocate to different nests and move greater distances than females (Wells-Gosling and Heaney 1984), it is possible that ectoparasites may favour males to maximize dispersal distances and minimize inbreeding (Walter and Proctor 1999).

Finally, sex differences in ectoparasite load may be the result of sex differences in grooming rate. It is well established that in ungulates males groom less than females, leading to the males being more heavily parasitized than the females (Mooring et al. 2002, 2004). Males may trade off time used for grooming and removal of parasites for time for finding or defending mates, thus leading to higher parasitism rates. These differences in grooming rate may be modulated by testosterone levels (Mooring et al. 1998), thus providing a potential mechanism by which testosterone leads to increases in parasite loads.

There was a nonsignificant trend for small males to be more parasitized than large males, and there was no evidence that highly parasitized males were in poorer condition than males with fewer parasites. This pattern suggests two things. First, ectoparasites may influence skeletal growth rates, and males that are more susceptible to ectoparasites may simply be unable to allocate enough energy to growth and are thus smaller. This pattern has been shown in great tits (*Parus major* L., 1758), where male nestlings that were raised in nests infested with ectoparasites were smaller than males raised in uninfested nests (Tschirren et al. 2003). Second, ectoparasite loads do not seem to affect body condition, suggesting that the immediate costs of ectoparasites may not be significant. There are studies that show that ectoparasites negatively affect host body condition (Polak 1998; Neuhaus 2003; Fitze et al. 2004), but nonsignificant results have also been reported (e.g., Pacejka et al. 1998; Khokhlova et al. 2002). The energetic costs of ectoparasitism may be minimal on adult northern flying squirrels, thus not having an impact on energy reserves.

Our findings suggest two new areas of inquiry. First, sex differences in ectoparasite load may be the result of the immunosuppressive qualities of testosterone, and our next step will be to examine the relationship between testosterone and both ecto- and endo-parasite loads. Second, growth rates of offspring are predicted to be lower in individuals that are heavily parasitized. In a future study, we will examine the growth rates of offspring with variable parasite loads.

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