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THE POTENTIAL ROLE OF *STRONGYLOIDES ROBUSTUS* ON PARASITE-MEDIATED COMPETITION BETWEEN TWO SPECIES OF FLYING SQUIRRELS (*GLAUCOMYS*)

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ABSTRACT: There is growing evidence that populations of the northern flying squirrel (*Glaucomys sabrinus*) are declining in the eastern United States, perhaps due to competition with the southern flying squirrel (*Glaucomys volans*). Potential causes include parasite-mediated or apparent competition from the shared intestinal nematode, *Strongyloides robustus*, which has been shown to detrimentally affect the northern flying squirrel but not the southern flying squirrel. To investigate this hypothesis, we conducted a preliminary study on the parasite community of both flying squirrel species from sites in Pennsylvania where the two species occur sympatrically and where *G. sabrinus* is now considered endangered at the state level. We compared these parasite communities with those from northern flying squirrels from northern New York where the southern flying squirrel is absent. We found eight species of gastrointestinal parasites (*Pterygodermatites peromysci*, *Lemuricola sciuri*, *Syphacia thompsoni*, *Syphacia* spp., *Capillaria* spp., *Citellinema bifurcatum*, *Strogyloides robustus*, and an unidentifiable cestode species) in both species of flying squirrels examined for our study. The parasite-mediated competition hypothesis was partially supported. For example, in Pennsylvania, *S. robustus* was overdispersed in southern flying squirrels, such that a small proportion of the hosts carried a large proportion of the worm population. In addition, we found *S. robustus* to be present in northern flying squirrels when the species were sympatric, but not where southern flying squirrels were absent in New York. However, there was no association between *S. robustus* and the body condition of flying squirrels. We detected a potential parasite community interaction, as *S. robustus* abundance was positively associated with *P. peromysci*.

Key words: Apparent competition, flying squirrels, *Glaucomys sabrinus*, *Glaucomys volans*, helminths, nematodes, Pennsylvania, sciurid, *Strongyloides robutus*, sympatry.

INTRODUCTION

North America is inhabited by two species of flying squirrel. The northern flying squirrel (*Glaucomys sabrinus*) is distributed across most of the northern states of the United States and much of Canada, where it is primarily found in coniferous forests. The northern flying squirrel is considered a state-endangered species in Pennsylvania, and a subspecies of the northern flying squirrel (*G. s. coloratus*) is federally endangered in the southern Appalachians. The southern flying squirrel (*Glaucomys volans*), in contrast, commonly occurs in all wooded habitats and has an eastern distribution south of the Great Lakes that extends up the east coast into southernmost Quebec (Dolan and Carter, 1977). In the midwestern and eastern United States, both species occur marginally sympatrically at a number of locations (Portage County, Wisconsin, Pauli et al., 2004; Ontario, Canada, Garroway et al., 2009), particularly in the Appalachians (North Carolina, Weigl, 2007; Pennsylvania, Mahan et al., 1999). At some locations, individuals of the two species have been known to show considerable overlap in habitat (Pauli et al., 2004; Weigl, 2007), share the same nesting sites, and even simultaneously share nest structures in extreme cold (G. Turner, Pennsylvania Game Commission, pers. obs., 2004). These observations, coupled with recent evidence that the southern flying squirrels may be moving northwards and replacing and hybridizing with the northern species (Garroway et al., 2009), suggest the possibility of competition between the two species.

One mechanistic hypothesis to explain the replacement of the northern species with the southern is parasite-mediated competition (Weigl, 1968; Hudson and Greenman, 1998; Pauli et al., 2004; Weigl, 2007), acting through the parasitic nematode *Strongyloides robustus*, whereby the southern flying squirrel acts as reservoir of infection. Studies on captive flying squirrels suggest that this nematode reduces survival and productivity of the northern flying squirrel while the southern flying squirrels are seemingly unaffected (Weigl, 1968). Transmission of *S. robustus* occurs when the free-living stages emerge from the feces and burrow through the skin of the host (Anderson, 2000), so that interspecific transmission can occur when one species defecates in a nest that is subsequently used by the second species when the infective stages are active. The interesting corollary of this transmission process is that the two host species need never come into close contact, but simply utilize the same space at different time periods. The larvae of *S. robustus* are susceptible to cold temperatures and, therefore, did not previously persist where northern flying squirrels were found in the wild (Pauli et al., 2004). Although parasite-mediated competition is not the only hypothesis that has been proposed to explain the recent decline in numbers of the northern species, factors such as climate change, habitat destruction, introduced insect pests, urban development, and pollution (Bayne and Hobson, 1998; Weigl, 2007) may act synergistically with *S. robustus* to influence the decline of the northern flying squirrel.

For parasite-mediated competition to occur, two host species must share a parasite and its pool of infective stages. One of these hosts must be more tolerant of the parasite and, in turn, act as a reservoir host, resulting in transmission rates to the less-tolerant host being greater than would occur were the reservoir host absent (Hudson and Greenman, 1998). Thus, we predicted higher prevalence and abundance (over-dispersion) of *S. robustus* in the southern flying squirrels than in the northern flying squirrels when the two species occur sympatrically. Because past research suggests that northern flying squirrels are adversely affected by *S. robustus* (Weigl, 2007), we also predicted that northern flying squirrels would have reduced body condition, per worm, compared with the southern flying squirrels. Furthermore, we predicted that, in the absence of southern flying squirrels, we would not expect infection with *S. robustus* to be sustained by the northern flying squirrels. Infection with *S. robustus* could also be influenced by other parasites which could potentially increase susceptibility to *S. robustus* (e.g., Patrick, 1991a). Therefore, the objectives of our study were to: 1) determine if evidence exists for parasite-mediated competition between northern and southern flying squirrels by examining prevalence and abundance of *S. robustus* in both species where they do, and do not, occur symaptrically, and by comparing the body condition of flying squirrels in relation to parasite load; and 2) examine and describe the gastrointestinal helminth fauna of flying squirrels to determine potential interactions and associations among helminth species.

MATERIALS AND METHODS

In this study, we conducted necropsies of 31 flying squirrel specimens, 24 of which were collected at sites where the two species are sympatric in Pennsylvania (four northern and 20 southern flying squirrels). These sites were located in Carbon, Pike, and Warren Counties (41°01′43″N, 75°42′19″W) in northeastern Pennsylvania. The remaining seven specimens were northern flying squirrels collected from Huntington Wildlife Forest, Essex County (43°58′17″N, 74°09′39″W) in northern New

York, where no southern flying squirrels had been recorded prior to, or during, our study. Most specimens were found dead in nest boxes, or were accidentally killed during live-trapping procedures in a long-term study conducted by three of the authors. Specimens were identified as northern flying squirrels by their larger relative size and by their gray underbelly pelage (Dolan and Carter, 1977; Wells-Gosling and Heaney, 1984). Identification of all northern flying squirrels, and a sample of the southern flying squirrels, were confirmed with genetic analyses (Garroway et al., 2009).

Each specimen was stored frozen at −70 C until the time of necropsy. Upon defrosting, the body mass (g), body length (cm), hind foot length (mm), tail length (cm), tail width (mm), ear length (mm), and reproductive status (breeding, nonbreeding) were recorded. The gastrointestinal tract of each squirrel was dissected, and the spleen was removed and its mass (g) recorded. Spleen mass was used as a measure of body condition, as enlargement of the spleen can be symptomatic of pathology caused by parasitic infections (Ali and Behnke, 1985; Brown and Brown, 2002). Relative spleen mass (spleen mass/body mass) was used to normalize condition among individuals. The gastrointestinal tract was cut longitudinally and carefully examined under a dissecting scope. All helminth parasites were collected, preserved in 70% alcohol, photographed, and identified. Helminths were identified and catalogued by J. M. Kinsella, HelmWest Laboratory, Missoula, Montana and deposited in the US National Parasite Collection, Beltsville, Maryland.

We examined the frequency distribution of parasites, including *S. robustus*, in flying squirrels using the k statistic to test for a binomial distribution. We compared abundance and prevalence of parasites between flying squirrel species using generalized linear models (GLM; Nelder and Wedderburn, 1972). To determine the number of flying squirrels needed to obtain a significant, two-tailed difference in *S. robustus* abundance and prevalence, we conducted a power analysis (power=0.8; $P<0.05$; two-tailed). We chose our power and alpha levels based on conventional values for determining effect size (in our case, squirrel species) on *S. robustus* abundance (Cohen, 1988; Nakagawa, 2004). Finally, we examined correlation between the presence of parasite species and body condition (e.g., body size, relative spleen mass [spleen mass divided by body mass]) using GLM with negative binomial errors and Pearson correlation. Statistical analyses were undertaken using both the softwares R and S-plus (Version 2.8.0 and 8.0, respectively Insightful Corporation, Palo Alto, California, USA [2005]).

RESULTS

Strongyloides robustus were over-dispersed in the southern flying squirrels such that a small proportion of the hosts carried a large proportion of the worm population (Fig 1a; mean=13.9, SE=5.51, k=0.33). *S. robustus* was recorded in 45% of the southern flying squirrels collected, with a mean abundance of 14 worms, and was found in three out of four (75%) of the sample of northern flying squirrels coexisting with southern flying squirrels, with a mean abundance of 5.5 worms (Table 1). There was no significant difference in abundance of *S. robustus* between the group of northern and southern flying squirrels that lived sympatrically in Pennsylvania (F=35.77, P=0.53). Our power analysis showed that a minimum sample size of 15 southern flying squirrels and three northern flying squirrels would be needed to obtain a significant, two-tailed difference, with the northern species having significantly greater *S. robustus* abundance (power=0.8, $P\leq0.05$). To detect a difference in prevalence would have required 305 southern flying squirrels and 61 northern flying squirrels (power=0.8, $P\leq0.05$). There were no *S. robustus* nematodes found in the sample of northern flying squirrels (n=7) taken from New York, where the southern flying squirrel is absent (Table 1).

Along with *S. robustus*, we also recorded a number of other gastrointestinal helminths in both squirrel species: *Pterygodermatites peromysci*, *Lemuricola scuiri*, *Syphacia thompsoni*, *Syphacia* spp., *Capillaria* spp., *Citellinema bifurcatum*, and an unidentified cestode species (Table 1). *Strongyloides robustus* was found in the anterior part of the small intestine (duodenal muscosa), and all other helminths were posterior to it in the gastrointestinal tract.

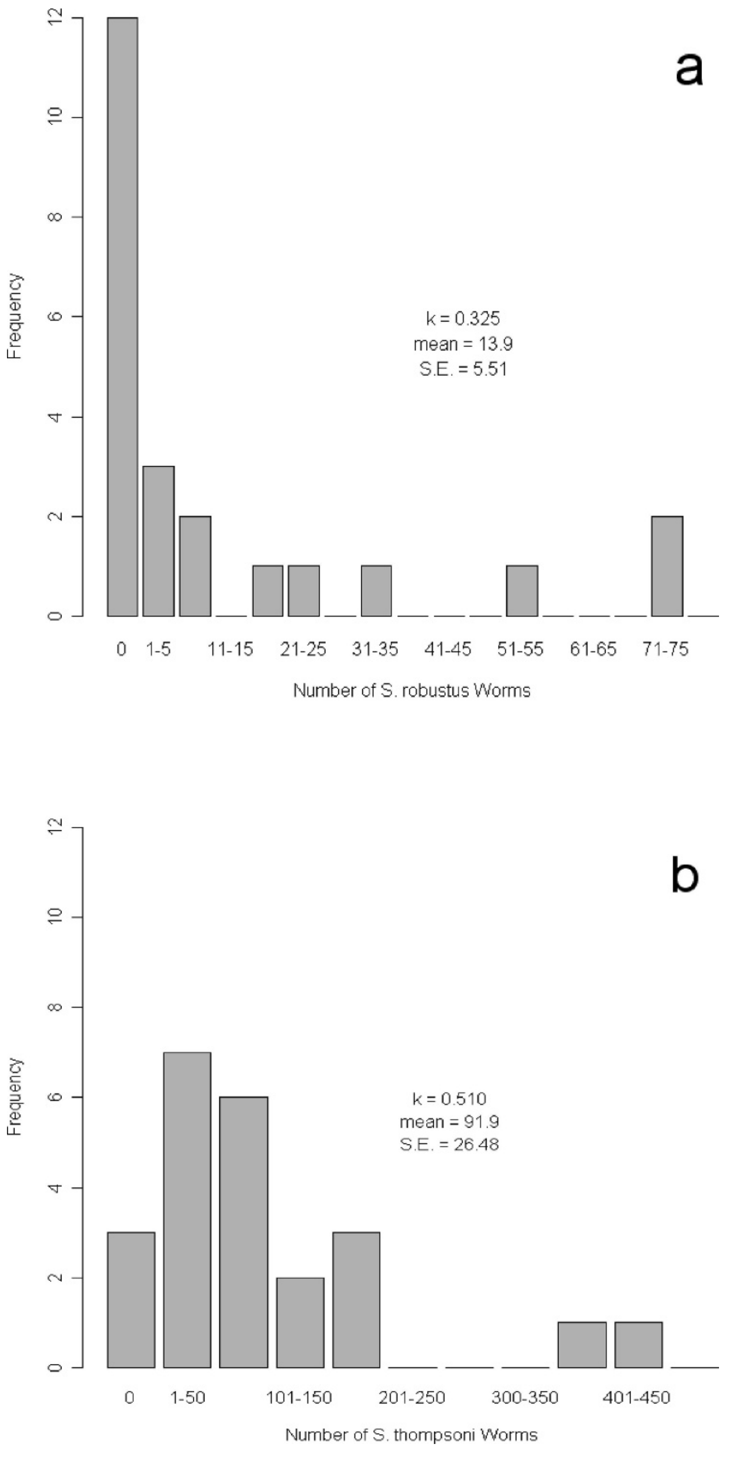

FIGURE 1. Frequency distributions of two nematodes found within the southern flying squirrels; (a) *Strongyloides robustus*; (b) *Syphacia thompsoni*.

TABLE 1. Prevalence, mean abundance, range of abundance, and standard error of the mean of the intestinal parasites found in the southern (*Glaucomys volans*) and northern (*G. sabrinus*) flying squirrels co-existing in Pennsylvania, as well as the group of northern flying squirrels from New York living in the absence of their southern competitor. No significant difference ($P>0.05$) in prevalence or abundance of parasites was detected between flying squirrel species in our study.

Intestinal parasite	Southern flying squirrel ($n=20$)			Pennsylvania northern flying squirrel ($n=4$)			New York northern flying squirrel ($n=7$)		
	Prevalence	Mean abundance (range)	±1 Standard error	Prevalence	Mean abundance (range)	±1 Standard error	Prevalence	Mean abundance (range)	±1 Standard error
Pterygodermatites peromysci	0.10	0.25 (2–3)	0	0.33	0.67 (2)	0.26	0	0	0
Syphacia spp.	0.05	0.05 (1)	0	0	0	0	0	0	0
Syphacia thompsoni	0.90	91.9 (1–433)	20.35	0.75	48 (2–190)	47.366	0.286	5.857 (2–190)	0.202
Capillaria spp.	0.10	0.15 (1–2)	0	0	0	0	0	0	0
Lemuricola sciuri	0	0	0	0.33	13.33 (40)	5.16	0	0	0
Tapeworm (unidentified)	0.05	0.20 (4)	0	0.50	4.25 (1–10)	2.529	0.571	0.571 (1)	0.202
Strongyloides robustus	0.45	13.90 (1–75)	0.22	0.75	5.5 (6–10)	2.062	0	0	0
Citellinema bifurcatum	0	0	0	0	0	0	0.429	0.571 (1–2)	0.297

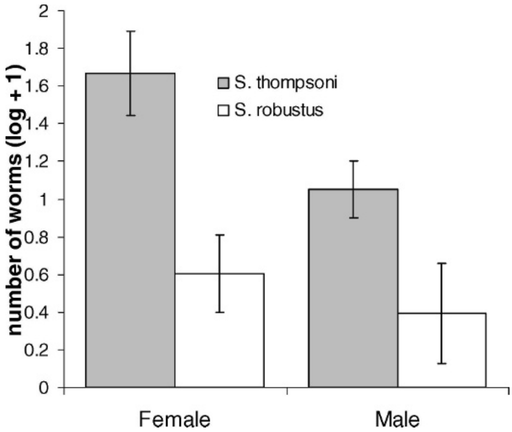

FIGURE 2. Sex bias in abundance of *Syphacia thompsoni* in southern flying squirrels ($P=0.02$). There is no evidence for sex bias in the intensities of the nematode of particular interest in this disease system, *Strongyloides robustus*.

Syphacia thompsoni was the most prevalent parasite found in both *Glaucomys* spp. and, like *S. robustus*, was highly aggregated (Fig. 1b; mean=91.9, SE=26.48, $k=0.51$) in the southern species. *Syphacia thompsoni* was found in 67% of the southern species, with a mean abundance of 92 worms (SE±26.48); in one of two northern flying squirrels from Pennsylvania with a mean abundance of 48 worms; and in three of seven northern flying squirrels from New York with a mean abundance of five worms (Table 1). There was no difference in abundance of this parasite between the two host species from Pennsylvania ($P=0.708$), but there was a significant effect of sex within the southern flying squirrel species, with females being 11% more heavily infected than were males ($P=0.02$; Fig. 2). *Lemuricola scuiri* was only found in the northern species of flying squirrel from Pennsylvania, where 40 individuals were found in a single infected female squirrel (Table 1). Two *P. peromysci* were found in a one of four northern flying squirrel from Pennsylvania (25%) and in two southern flying squirrels (10%), with a mean abundance of 2.5 worms (Table 1). A *Capillaria* sp. was found in two female southern flying squirrels, with a mean abundance of 1.5 worms, as well as a single immature female *Capillaria* sp. in one northern flying squirrel from New York (Table 1).

A comparison of the abundance of each parasite species in southern flying squirrels showed that the abundance of *S. robustus* was significantly greater than that of *P. peromysci* (Pearson correlation $t=3.43$, df=18, $P=0.003$ cor=0.63). We found no relationship between parasite abundance and host condition in any flying squirrels examined based on our GLMs (spleen mass, $P=0.72$; relative spleen mass, $P=0.72$; body mass, $P=0.54$; sex, $P=0.40$).

DISCUSSION

The lack of *S. robustus* in northern flying squirrels from New York, where southern flying squirrels are absent, and the occurrence of *S. robustus* in three of four northern flying squirrels from Pennsylvanaia, where the two species occur sympatrically, are consistent with the predictions from the parasite-mediated competition hypothesis (Weigl, 1968; Hudson and Greenman, 1998; Pauli et al., 2004; Weigl, 2007). Though far from conclusive, these data indicate parasite-mediated competition is worthy of further investigation in this system. In addition, the over-aggregation of *S. robustus* in southern flying squirrels may indicate some tolerance to the parasite and facilitate its role as a host. However, the lack of correlation between parasite abundance and body condition does not demonstrate that parasites are negatively affecting the condition of flying squirrels.

There is a problem with testing the parasite-mediated hypothesis in the field because it predicts that infected northern flying squirrels die at a faster rate than do southern flying squirrels. Therefore, northern flying squirrels may be difficult to capture at sympatric sites. Further investigation may depend on laboratory studies, rather than the field, to show that apparent competition may be mediated by parasite infection. A further limitation is that northern flying squirrels are protected in the southern portion of their range, and increased care is needed not to exacerbate their decline. One constructive way forward would be to work out a way of treating the southern flying squirrels to remove infection, in which case the hypothesis would predict reduced rates of mortality in the northern flying squirrels.

We found a significant correlation between the prevalence of *S. robustus* with *P. peromysci*, indicating that competition among nematode species in the gut may play a role in the organization of the parasite community and the location and effects of *S. robustus* (Patrick, 1991a, 1991b). The correlations could exist due to facilitation of one species by another; for example, one species could alter the immune system, allowing the second parasite to enter where otherwise it could not. Alternatively, this correlation could be a reflection of both parasites being abundant in the same geographic areas.

There are no records prior to 2006 of southern flying squirrels existing in the area of New York where our sample of isolated northern flying squirrels was collected. However, after our study was completed, the first southern flying squirrel was recorded at our study area. It would further support our parasite-competition hypothesis if northern flying squirrels, collected after the appearance of southern flying squirrels, are infected by *S. robustus* and if populations of northern flying squirrels become limited or replaced by southern flying squirrels.

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