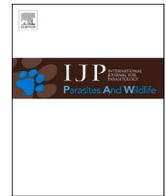


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Full Length Article

Testing the parasite-mediated competition hypothesis between sympatric northern and southern flying squirrels

Paul P. O'Brien^{a,*}, Jeff Bowman^{b,c}, Sasha L. Newar^b, Colin J. Garroway^a^a Department of Biological Sciences, University of Manitoba, Sifton Road, Winnipeg, Manitoba, R3T 2N2, Canada^b Environmental & Life Sciences Graduate Program, Trent University, 1600 West Bank Drive, Peterborough, Ontario, K9L 0G2, Canada^c Wildlife Research & Monitoring Section, Ontario Ministry of Northern Development, Mines, Natural Resources & Forestry, Trent University, 2140 East Bank Drive, Peterborough, Ontario, K9L 0G2, Canada

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ABSTRACT

Competition is a driving factor in shaping ecological communities and may act directly or indirectly through apparent competition. We examined a classic example of parasite-mediated competition between northern (*Glaucomyssabrinus*) and southern flying squirrels (*G. volans*) via the intestinal nematode, *Strongyloidesrobustus*, and tested whether it could act as a species barrier in a flying squirrel hybrid zone. We live-trapped flying squirrels (*G. sabrinus* and *G. volans*), grey squirrels (*Sciuruscarolinensis*), red squirrels (*Tamiasciurus hudsonicus*), and chipmunks (*Tamias striatus*) from June–September 2019 at 30 woodlots in Ontario, Canada. Fecal samples from squirrels were collected and analyzed for the presence of endoparasite eggs. For each individual, we calculated Scaled Mass Index (SMI) as a measure of body condition to assess the effect of *S. robustus* on squirrels. We found eggs of *S. robustus* in all species except chipmunks. Infection with *S. robustus* did not appear to affect body condition of southern flying squirrels and grey squirrels, but we did find a weak negative effect on northern flying squirrels and red squirrels. Despite a weak asymmetric effect of *S. robustus* on flying squirrels, we did not find any evidence that parasite-mediated competition could lead to competitive exclusion from woodlots. Furthermore, *S. robustus* eggs were common in feces of the red squirrel, a species largely sympatric with northern flying squirrel.

1. Introduction

There is much evidence that contemporary climate change has led to an increase in species range shifts as previous energetic constraints on expansion become lessened or previously suitable habitat becomes unsuitable (Hickling et al., 2006; Laliberte and Ripple, 2004; Parmesan, 2006). A potential consequence of these range expansions is that closely related species come into secondary contact, potentially leading to competition. While resources such as food or habitat are often considered the most important drivers of competition (i.e., direct competition), indirect or apparent competition via disease or parasites may play an equally or more important role (Hudson and Greenman, 1998). For example, the transmission of squirrelpox virus (SQPV) to red squirrels (*Sciurus vulgaris*) from introduced eastern grey squirrels (*Sciurus carolinensis*) is attributed as a major driver of mass declines of red squirrels and subsequent replacement with grey squirrels across the United Kingdom (Rushton et al., 2006; Tompkins et al., 2003). Although the

squirrelpox virus example is a result of species introduction, apparent competition mediated by novel parasites is also likely to occur in instances of range expansion, which may have important implications for the outcome of secondary contact.

Parasite-mediated competition is a form of apparent competition between host species driven by parasitism, such that asymmetrical tolerance of hosts can lead to competitive exclusion of the more susceptible host (Hudson and Greenman, 1998; Price et al., 1988). One well known example of parasite-mediated competition is the effects that meningeal brainworm (*Parelaphostrongylus tenuis*) in white-tailed deer (*Odocoileus virginianus*) can have on other cervids (Anderson, 1964). A similar scenario has been suggested involving the North American flying squirrels (Weigl, 1968). *Strongyloides robustus* is an intestinal nematode that infects a variety of sciurids, including northern (*Glaucomyssabrinus*) and southern flying squirrels (*G. volans*) and is transmitted via skin contact with infected feces and soil (Weigl, 2007; Wetzel and Weigl, 1994). Where these two squirrel species co-occur, transmission is likely

* Corresponding author.

E-mail address: obrienp1@myumanitoba.ca (P.P. O'Brien).<https://doi.org/10.1016/j.ijppaw.2021.11.001>

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to happen in tree cavities that are used consecutively by both species or when used at the same time (Olson et al., 2018). Southern flying squirrels appear to be more tolerant of *S. robustus* than northern flying squirrels for whom infection can be weakening (e.g., weight loss) or even fatal (Krichbaum et al., 2010; Pauli et al., 2004; Weigl, 1968). Data from captive animals suggest that *S. robustus* is more prevalent in southern flying squirrels than in northern flying squirrels (Wetzel and Weigl, 1994) and therefore may lead to apparent competition between the two flying squirrel species.

Some populations of northern flying squirrels in the US are listed as endangered due to declines in abundance. Declines are attributed to several factors including climate change, habitat loss, and infection with *Strongyloides robustus*. There appears to be evidence in support of parasite-mediated competition in other regions of the northern flying squirrel's range (Weigl 1968; Pauli et al., 2004; Krichbaum et al., 2010). Pauli et al. (2004) suggested that given the apparent differences in susceptibility of the two species of flying squirrel to *S. robustus* infection, the parasite could play an important role in the segregation of the two in areas of sympatry. It remains unclear, however, whether the hypothesis of parasite-mediated competition in flying squirrels is supported at higher latitudes, in areas where southern flying squirrels have expanded their range in response to a warming climate. There is some reason to expect that *Strongyloides robustus* is less common at higher latitudes due to its susceptibility to low temperatures (Bartlett, 1995; Wetzel and Weigl, 1994).

The ranges of northern and southern flying squirrels are parapatric, with a few areas of overlap where remnant glacial populations of northern flying squirrels are restricted to high elevations at the southern edge of their range (Arbogast, 2007). In Ontario, Canada, contemporary climate change has led to northward expansion of southern flying squirrels and increased overlap of these closely related species (Bowman et al., 2005). Increased sympatry has also resulted in hybridization between the two flying squirrel species (Garroway et al., 2010), however the long-term consequences of this hybridization remain uncertain. Secondary contact of closely related species provides the opportunity to test whether or not species barriers will be maintained. In general, the diets, habitats, and nest preferences of the two flying squirrels do not extensively overlap (Dolan and Carter, 1977; Wells-Gosling and Heaney, 1984), suggesting direct competition between these species is unlikely. Alternatively, competition may occur indirectly through the intestinal parasite, *Strongyloides robustus* (Weigl 1968). In such a case, the parasite could serve to reinforce reproductive barriers between the two flying squirrel species.

In Ontario, much remains unknown about the impact of *S. robustus* in sympatric populations of flying squirrels. In particular, *S. robustus* has been shown to have a low tolerance to below freezing temperatures (Wetzel and Weigl 1994) and parasites may lag behind their host during rapid range expansion (Phillips et al., 2010). Therefore, it is unclear what effect *S. robustus* has in this area of recent sympatry where winter temperatures regularly drop below the putative lower temperature that the parasite can tolerate. Further, to our knowledge, only one other study (Krichbaum et al., 2010) has quantitatively examined the effect of *S. robustus* on flying squirrel body condition (but see Weigl 1968 for qualitative example). Weigl (1968) suggested a deleterious effect of infection for northern flying squirrels, whereas Krichbaum et al. (2010) found no relationship between infection and body condition. Finally, while *S. robustus* is known to infect other sciurid species, past studies have not acknowledged the potential impact of other host reservoirs on parasite-mediated competition between flying squirrels. We therefore highlight several important knowledge gaps that are critical to our understanding of the role of *S. robustus* in sympatric populations of flying squirrels.

The purpose of the present study was to test for evidence of parasite-mediated competition in an area of sympatry between flying squirrel species. To test this we addressed the following three questions: (1) Is *S. robustus* present in northern squirrel populations? (2) Do other

sympatric squirrel species act as host reservoirs? (3) Does *S. robustus* negatively affect northern flying squirrel body condition? We hypothesized that if *S. robustus* is present, able to persist, and has an adverse effect on northern flying squirrels, then parasite-mediated competition could act as a pre-mating barrier between flying squirrel species. If an asymmetrical tolerance exists, we predict that northern flying squirrels infested with *S. robustus* will have lower body condition than those without, while southern flying squirrels and other squirrels will exhibit no difference in body condition when infested or not.

2. Methods

2.1. Trapping and sample collection

We trapped squirrels at 30 woodlots of varying patch size (1.1 to >1000 ha) in Peterborough, Ontario and the surrounding area (Fig. 1) during late summer and fall 2019. Most sites were selected based on re-sampling sites from a study 14 years prior (2006; Walpole and Bowman 2011). Given *S. robustus* has also been identified in eastern grey squirrels (*Sciurus carolinensis*), red squirrels (*Tamiasciurus hudsonicus*), and eastern chipmunks (*Tamias striatus*) (Espenshade and Stewart, 2013), we also sampled these species along with northern and southern flying squirrels. We trapped squirrels using Tomahawk model 102 live traps (Tomahawk, WI, USA) baited with sunflower hearts and mounted on wood platforms, secured ~2 m in trees. At each site, we set 20–30 traps with trap spacing of ~20–40 m apart. Each site was subject to 3 days and nights of consistent trapping (Walpole and Bowman 2011). Previous work in the same area has demonstrated that sciurids are well detected by these methods and this level of trapping effort (Walpole and Bowman

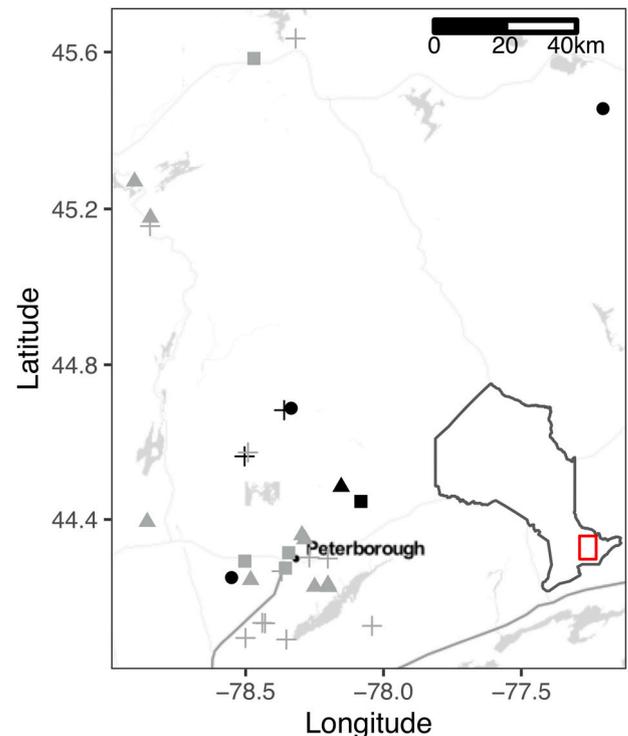


Fig. 1. Map of sites ($n = 30$) used to survey the presence of the intestinal parasite *Strongyloides robustus* in northern and southern flying squirrels in Ontario from June–September 2019. Symbol shape represents squirrel species detected at a site: square = northern flying squirrel, cross = southern flying squirrel, circle = both, and triangle = neither. The colour of symbol indicates whether *S. robustus* was detected at a site: grey = absent ($n = 23$) and black = present ($n = 7$). Inset shows the location of our study area in Ontario, Canada. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2011). To capture both nocturnal (flying squirrels) and diurnal (grey squirrels, red squirrels, and chipmunks) species, traps were left open the entire trapping period and were checked at dawn and dusk. Captured squirrels were identified to species, sexed, aged, weighed, and were given 1-g Monel ear tags (National Band and Tag Co., Newport, KY) for individual identification. At a subset of sites that were part of a long-term study, captured flying squirrels were injected with passive integrated transponder (PIT) tags (model TX1411SST, 12.50 mm × 2.07 mm, 134.2 kHz ISO, 0.1020 g; Biomark Inc., Boise, ID, USA) for identification and not ear tagged. Fecal samples were collected from trapped individuals to get an estimate of parasite prevalence within each species. Samples were placed directly into 10% buffered formalin for preservation until being processed (Waksmanski et al., 2017). When possible, fresh fecal samples were collected directly while handling squirrels to reduce the possibility of parasite eggs hatching and misidentification of species. When this was not possible, we collected the freshest samples from the trap platform, which was wiped clean after every squirrel capture (Gooderham and Schulte-Hostedde, 2011). All live-trapping and handling methods followed protocols approved by Trent University's Animal Care Committee (protocol nos. 08034 and 25668).

2.2. Habitat surveys

In addition to trapping, we also conducted habitat surveys to characterize the composition of forests at each site. The tenth trap location was selected as the starting point of each survey as this generally represented the middle of the trap grid. From the trap, a bearing (one of the four cardinal directions) and a distance between 1 and 10 m were randomly selected using a random number generator mobile phone app. The random bearing and distance were then used to determine the survey location. From this location, a 360° prism sweep was performed to identify trees characterizing the woodlot (Husch et al., 1972). Trees identified by the prism sweep were classified to species, had diameter at breast height measured, and were given a decay classification following the Maser scale (1 = healthy live tree; 9 = decayed stump; (Thomas et al., 1979). These habitat data were then included in our models to account for any variation in squirrel occurrence that may be the result of habitat differences between woodlots.

2.3. Fecal flotation and parasite identification

All fecal samples were processed using a modified McMaster technique (Coombs and Thesis, 2010). Fecal suspensions were prepared using pre-made sugar solution to ensure a consistent specific gravity of 1.27 (Sugar Sol, Partnar Animal Health, Ilderton, ON, Canada). Suspensions were then pipetted onto a McMaster slide and examined under a light microscope at 100× total magnification. Slides were viewed and photographed using an Infinity 1 Digital Kit (Media Cybernetics, Bethesda, MD) and any measurements were taken using Infinity Analyze (Teledyne Lumera, Ottawa, ON). An individual was classified as infected if parasite eggs were present in the fecal sample (Bush et al., 1997). All potential parasite eggs were identified using a parasite egg identification key (Patrick unpublished) and a photo of *Strongyloides robustus* eggs (Weigl, 1968). While use of egg counts for estimates of parasite prevalence commonly underestimates prevalence (de Vlas and Gryseels, 1992; Utzinger et al., 2000), we have chosen this technique to reduce invasiveness on our study species. For each species, parasite prevalence was calculated as follows:

$$\text{Prevalence} = \frac{n_{\text{infected}}}{n_{\text{total}}}$$

where n_{infected} = number of individuals within a species carrying a given parasite (i.e., *S. robustus*) and n_{total} = total capture number of a given species.

2.4. Body condition index

To determine if infection with *S. robustus* affects the body condition of squirrels, we calculated a body condition index. We chose to use the scaled mass index (SMI) outlined by Peig and Green (2009), as it was shown to correlate highly with relative body stores (i.e., fat, protein, water, etc.) and SMI values are standardized and thus comparable among individuals and populations. For each species, we chose which length measurement to use based on correlations between mass and each length variable on the log scale (Peig and Green, 2009). The length measurement most strongly correlated with mass was selected for calculation of SMI values for that species. SMI values were calculated for every individual for which we had a fecal sample and we compared index values within species for individuals infected to those not infected with *S. robustus*. We tested for differences in body condition using one-tailed Wilcoxon Rank Sum tests. We also tested for differences in body condition for all other parasites detected.

2.5. Generalized linear models

To test for evidence of competitive exclusion by parasite-mediated competition within woodlots, we fit logistic regression models using species presence (northern or southern flying squirrel) as the dependent variable. We fit a global model for each species response and included explanatory variables for: (1) co-occurring squirrel species (capture numbers of northern or southern flying squirrels, grey squirrels, red squirrels, and chipmunks); (2) endoparasite presence (presence/absence of *Strongyloides robustus* and *Capillaria*); and (3) woodlot characteristics (proportion of softwoods, DBH, decay class, and woodlot area). We fit all models in R (R Core Team 2020).

2.6. Ordinations

To examine squirrel community structure across our study area, we ran redundancy analyses (RDA). We used a matrix of raw species captures as our response for all analyses. Species captures were transformed using a Hellinger transformation to control for asymmetries in species captures. We examined squirrel community structure in relation to habitat variables, parasite community, and spatial variables. All RDAs were carried out using the *vegan* package (Oksanen et al., 2019) in R. We used the *varpart* function to partition the variance explained by each set of variables, any shared variation, and unexplained variation.

3. Results

3.1. Trapping

Overall we had 270 captures of 229 squirrels over 900 trap nights (TN). We captured 25 northern flying squirrels, 62 southern flying squirrels, 15 red squirrels, 69 grey squirrels, and 99 chipmunks (See Table S1 in Supplementary Material for breakdown of captures by woodlot). Of the 270 squirrels captured, we collected fecal samples from 182 individuals. A subset of the sites trapped in the current study ($n = 15$) were surveyed previously by Walpole and Bowman (2011). We found 10 of the 15 re-surveyed sites exhibited a transition in species occurrence over the 14 years as evidenced by the species of flying squirrel previously captured at a site compared to current captures (Table 1).

3.2. Fecal flotations

We detected 4 genera of endoparasites across all samples analyzed: *Strongyloides robustus*, *Eimeria* sp., *Capillaria* sp., and *Hymenolepis* sp. *Eimeria* oocysts were present in nearly all samples analyzed, while *Hymenolepis* (tapeworm) eggs were only detected in chipmunks. Eggs of *Strongyloides robustus* were present in all species except chipmunks.

Table 1

Species transitions between 2006 and 2019 at sites ($n = 10$) surveyed for northern (*Glaucomys sabrinus*) and southern flying squirrels (*Glaucomys volans*) around Peterborough, Ontario.

Transition	Frequency	Number of sites with <i>S. robustus</i>
Northern flying squirrel to neither	2	0
Northern flying squirrel to southern flying squirrel	2	0
Northern flying squirrel to sympatric	1	1
Southern flying squirrel to neither	1	1
Sympatric to southern flying squirrel	2	0
Neither to southern flying squirrel	2	0

Strongyloides robustus was detected at sites with both species of flying squirrel, only northern flying squirrels, only southern flying squirrels, and neither species (Fig. 1). Prevalence of *S. robustus* for northern flying squirrels, southern flying squirrels, red squirrels, and grey squirrels was 17.6% (95% CI: 3.8–43.4%), 4.9% (95% CI: 0.6–16.5%), 30.8% (95% CI: 9.09–61.4%), and 4.7% (95% CI: 0.6–16.2%), respectively (see Table S2 in Supplementary Material for details on other parasites). There was no correlation between the occurrence of *S. robustus* at a site and the transition of flying squirrel species at the site over 14 years (Table 1; Spearman's $\rho = 0$).

3.3. Body condition

Scaled mass index (SMI) for southern flying squirrels and grey

squirrels was not significantly different for squirrels infected with *S. robustus* versus those not (southern flying squirrels: $W = 44, P = 0.31$; grey squirrels: $W = 23, P = 0.59$; Fig. 2A and B). SMI was lower for northern flying squirrels and red squirrels infected with *S. robustus* than those not (northern flying squirrels: $W = 31, P = 0.07$; red squirrels: $W = 22, P = 0.03$; Fig. 2C and D). No differences were detected in body condition of any squirrel species infected with *Eimeria* or *Capillaria* (Figure S1 and S2), but chipmunks infected with *Hymenolepis* had lower body condition than those not (Figure S3).

3.4. Generalized linear models

For both models, the relationships between northern and southern flying squirrels were negative with standard errors not overlapping zero (Table 2). Standard errors were overlapping zero for the relationship between southern flying squirrels and presence of *S. robustus* at a site, however a positive relationship with northern flying squirrels was inconsistent with the parasite-mediated competition hypothesis (Table 2). Of the remaining predictor variables, some of the estimated coefficients were in the predicted direction, while others were not, and standard errors in most cases overlapped zero (Table 2).

3.5. Ordinations

For the first ordination, species occurrence was not significantly related to habitat variables ($F = 1.34, P = 0.32$). The proportion of variation explained by this RDA was 0.15. The biplot of species and

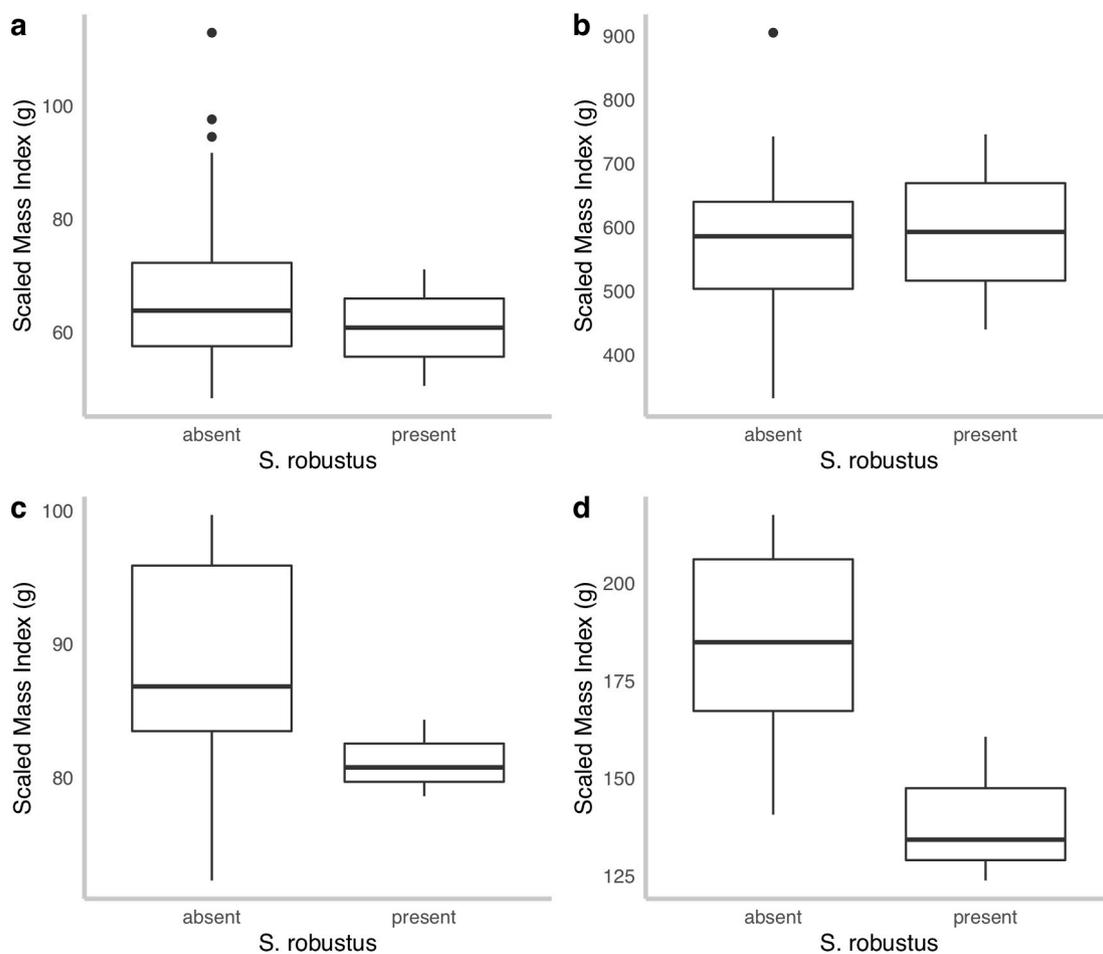


Fig. 2. Body condition of A) southern flying squirrels ($n = 41$), B) grey squirrels ($n = 42$), C) northern flying squirrels ($n = 17$), and D) red squirrels ($n = 13$) infested with *Strongyloides robustus* compared to those not infested. Body condition was calculated for squirrels captured near Peterborough, Ontario between June–September 2019.

Table 2

Model summaries for northern (*Glaucomys sabrinus*; n = 30) and southern flying squirrel (*G. volans*; n = 30) woodlot presence. Two global models were fit for each binary species response with predictor variables for 1) co-occurring squirrel species, 2) parasite presence, and 3) habitat variables.

response	fixed effect	coefficient	standard error	p-value
Northern flying squirrel	Southern flying squirrel	-0.38	0.26	0.14
	Grey squirrel	0.29	0.26	0.28
	Red squirrel	0.79	0.95	0.40
	Chipmunk	0.08	0.23	0.73
	<i>Strongyloides robustus</i>	1.63	1.55	0.29
	<i>Capillaria</i> sp.	1.72	1.61	0.29
	Mean DBH	-0.03	0.03	0.38
	Mean decay	1.73	2.62	0.51
	Proportion softwood	-1.13	4.11	0.78
	Area	0.0005	0.002	0.80
Southern flying squirrel	Northern flying squirrel	-2.65	1.82	0.15
	Grey squirrel	0.09	0.27	0.72
	Red squirrel	0.49	1.48	0.74
	Chipmunk	-0.67	0.50	0.18
	<i>Strongyloides robustus</i>	-0.03	2.52	0.99
	<i>Capillaria</i> sp.	4.68	4.06	0.25
	Mean DBH	-0.04	0.03	0.25
	Mean decay	-2.04	2.66	0.44
	Proportion softwood	2.74	5.26	0.60
	Area	0.002	0.003	0.39

habitat showed an association between northern flying squirrels and mean tree decay and between red squirrels and mean tree dbh (Fig. 3A). A weak association was also shown between southern flying squirrels and proportion of hardwood trees. The RDA for species constrained by parasites showed a significant relationship between squirrel species occurrence and parasite presence ($F = 2.40, P = 0.001$). The proportion of variation explained by this RDA was 0.28. The biplot for the relationship between squirrel species and parasites showed northern flying squirrels and red squirrels grouping with *S. robustus* and chipmunks grouping with *Capillaria* sp. (Fig. 3B). Southern flying squirrels and grey squirrels were grouped on their own with no relationships to parasites. The final ordination of species by spatial location was not significant ($F = 1.47, P = 0.16$) and proportion of variation explained by this RDA was 0.1. The biplot for species and spatial variables showed no association between any species with spatial variables (Fig. 3C). Variance partitioning showed unique variation explained by habitat, parasite, and spatial variables as 0%, 15%, and 0.02%, respectively. Variation shared between all three suites of variables was shown to be 0% and

unexplained variation was 81%.

4. Discussion

The presence of *Strongyloides robustus* at 7 of our 30 study sites and within 4 of 5 squirrel species indicated that the parasite can persist at northern latitudes and can be maintained by a variety of host species. Further, lower body condition of northern flying squirrels, but not southern flying squirrels, infected with *S. robustus*, supported the parasite-mediated competition hypothesis between flying squirrels. The presence of the parasite at sites where both species occurred and at sites where only northern flying squirrels occurred lent some uncertainty to the magnitude of the effect.

We found a prevalence of ~5% for *S. robustus* in southern flying squirrels, which is higher than was previously reported for the area (0% Coombs and Thesis, 2010), but the lowest reported for any other study (30% Espenshade and Stewart 2013; 45% Krichbaum et al., 2010; 52% Pauli et al., 2004; 100% Patrick 1991). Prevalence of *Strongyloides robustus* in the northern US has been found to be lower compared to southern populations and so may be expected to be even lower at the northern edge of the southern flying squirrel’s range, especially in the case of a range expansion when parasites can lag behind their hosts or may even be lost (Phillips et al., 2010; Romeo et al., 2014). As expected, prevalence in northern flying squirrels was lower compared to studies farther south (50% Pedder et al. 2009; 75% Krichbaum et al., 2010), but higher than Pauli et al. (2004) who report a prevalence of 11% farther north in Wisconsin. *Strongyloides robustus* may itself be limited at its northern range edge by cold temperatures (Wetzel and Weigl, 1994), although the geographic range of the species is somewhat uncertain based on literature reports.

In contrast to our expectations, we found the highest prevalence of *S. robustus* in red squirrels, followed by northern flying squirrels (~30% and 17%, respectively). This relationship is illustrated in our ordination biplots, which suggest a positive association between occurrence of these species with *S. robustus* (Fig. 3B). In their examination of helminth parasites of squirrels in Saskatchewan, McGee (1980) reported first observations of *S. robustus* in Canada for 3 of 7 species including Richardson’s ground squirrels (*Urocitellus richardsonii*), thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), and red squirrels, but not in sympatric northern flying squirrels. Later, Bartlett (1995) detected *S. robustus* in 93% of red squirrels from their study in Nova Scotia. Both studies indicated that these observations were farther north than have been reported for this helminth species and beyond the suggested geographic range. These findings, taken together with the results of the current study suggest that the geographic range of *S. robustus* is farther north than has been apparent from some literature (Krichbaum et al., 2010; Pauli et al., 2004; Wetzel and Weigl, 1994) and that temperature limitations of this species may not be as significant as suggested.

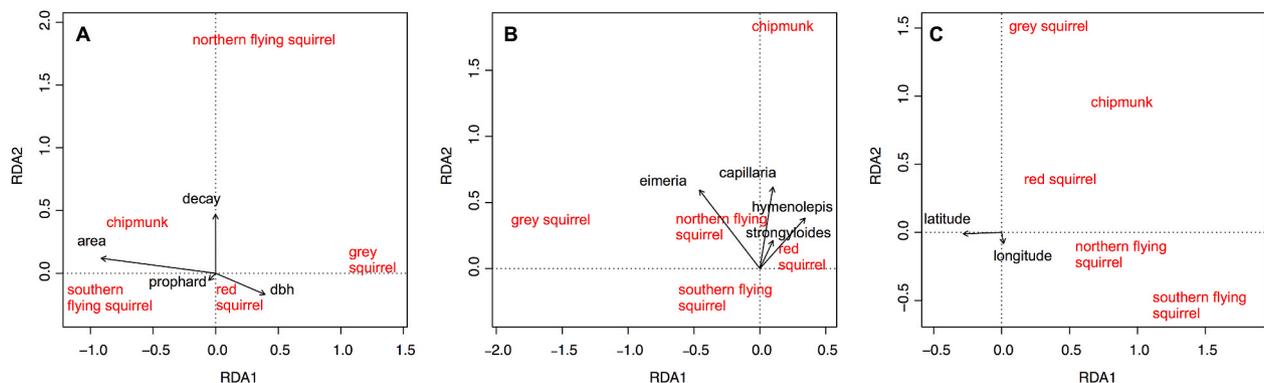


Fig. 3. Ordination biplots for presence of squirrel species at woodlots based on live-trapping surveys conducted near Peterborough, Ontario during the summer of 2019. Biplots show A) the species and habitat matrices, B) the species and parasite matrices, and C) the species and spatial matrices.

The presence of *S. robustus* in several hosts, including both red and grey squirrels, suggests that an understanding of the ecological role of this parasite may benefit from assessing the full squirrel community, which is not typically considered in studies of apparent competition in flying squirrels. For example, northern flying squirrels may experience a more severe impact of infection with *S. robustus* in the presence of these additional host species. Thus, if parasite-mediated competition takes place, interactions within the entire squirrel community could be relevant and not just interactions between northern and southern flying squirrels.

Inconsistent with the parasite-mediated competition hypothesis is the low prevalence of *S. robustus* we detected in southern flying squirrels and higher prevalence in northern flying squirrels. It is possible that the low prevalence of *S. robustus* found in southern flying squirrels may be associated with the recent northward range expansion; *S. robustus* may be lagging behind or have been lost due to stochastic founder effects (Phillips et al., 2010; Romeo et al., 2014). An alternative explanation is that *S. robustus* has a higher prevalence in southern flying squirrels than we detected, but given the inconsistency of egg detection in feces due to sporadic egg shedding, we underestimated the prevalence. While prevalence was likely underestimated for all species, we believe the overall lower prevalence in southern flying squirrels to be accurate given the higher capture number of this species compared to other species and still low detection observed. In addition, this finding is corroborated by previously low reporting by Coombs and Thesis (2010). Further, the high prevalence in northern flying squirrels and red squirrels may be expected given the high degree of sympatry between the two species. Given this long-standing sympatry, it is possible that the parasite has long been present in northern populations of these squirrel species and thus they may not be naïve hosts. The detection of *S. robustus* in sympatric populations of red squirrels and northern flying squirrels in Saskatchewan (McGee, 1980) provide further support against the naïve host hypothesis; however, our findings on body condition lend some uncertainty to this idea.

If parasite-mediated competition via *Strongyloides robustus* contributes to competitive exclusion of northern flying squirrels, an asymmetrical tolerance to the parasite must exist between hosts. We found evidence of a weak effect of *S. robustus* on body condition, such that northern flying squirrels and red squirrels infected with the parasite exhibited lower body condition compared to those without, while no such difference was found in southern flying squirrels or grey squirrels. In comparison, Weigl (1968) suggested a strong effect (i.e., death) in captive flying squirrels, while Krichbaum et al. (2010) found no correlation between parasite presence and body condition of wild squirrels. Our findings suggest there is a weak negative effect of *S. robustus* on northern flying squirrels body conditions, but not the acute pathology that has been suggested by previous literature and thus not likely strong enough to lead to competitive exclusion. This finding is corroborated by studies from our research group of wild caught squirrels observed in a captive setting. Northern and southern flying squirrels have been housed together (e.g., Olson et al. 2018) and subsequently monitored upon release with no evidence of pathology. Some of our sample sites have also exhibited long-term (e.g., >18 years) sympatry and hybridization (e.g., Garroway et al., 2010). Pathological effects might require higher parasite prevalence or more intense infections than occur at the northern latitudes of our study area, however we do not have the data to support the latter. An alternative explanation of our findings is that squirrels with poorer body condition may be more susceptible to infection. Moreover, Sparks Jr. (2005) suggested that northern flying squirrel populations suffering from inbreeding may experience stronger effects of infection with *S. robustus*, however, we do not have any reason to suspect northern flying squirrels in our study area suffer from inbreeding.

Consistent with the low likelihood of competitive exclusion, we found no relationship between the occurrence of flying squirrels at woodlots and parasite presence. The parasite-mediated competition

hypothesis would predict that if present, a shared parasite should lead to exclusion of one host over the other. Such a pattern is supported in wild populations of a variety of taxa including squirrels (Tompkins et al., 2003), pheasants (Tompkins et al., 2000), and lizards (Schall, 1992). It has been suggested that parasite-mediated competition plays an important role in mediating interactions between northern and southern flying squirrels (Weigl 1968; Pauli et al., 2004; Krichbaum et al., 2010), however, our findings do not support this hypothesis. Despite the presence of *S. robustus* in our study area and evidence of a negative effect on body condition, we found no evidence of a relationship between parasite presence and occurrence of flying squirrels. We detected *S. robustus* at sites where both northern and southern flying squirrels occurred and those with only one or the other. This lends further support for the ability of other species of squirrels to act as reservoir hosts, but suggests that any negative effect of *S. robustus* on northern flying squirrels is too weak to lead to exclusion. We recognize, however, that a caveat of our study is that it was a single year, which may only provide a snapshot of the complex interaction between hosts and parasite. In a different system, Romeo et al. (2021) did not find a negative effect of *S. robustus* infection on body mass of native Eurasian red squirrels (*Sciurus vulgaris*), but found infection intensity reduced long-term survival. Therefore, long-term monitoring of *S. robustus* in our study area may be necessary to understand how this parasite may mediate competition between northern and southern flying squirrels as well as other sympatric sciurids. In fact, there has been a slight increase in estimated prevalence of *S. robustus* in our region during the last decade, from ~0% (Coombs and Thesis, 2010) to ~5% (the present study).

Despite a lack of support for parasite-mediated competition between sympatric northern and southern flying squirrels, an apparent transition of species occupancy at woodlots appears to have occurred. A subset of the woodlots used in the current study ($n = 15$) were sampled ~14 years prior (Walpole and Bowman, 2011). Of this subset, the majority of sites appear to have experienced one of the following transitions in species occurrence: 1) northern flying squirrel occupied to southern flying squirrel occupied; 2) northern flying squirrel occupied to sympatric; 3) neither species to southern flying squirrel occupied; or 4) sympatric to southern flying squirrel occupied. No relationship was evident between presence of *S. robustus* and transition of a woodlot, providing further support against competitive exclusion via parasite-mediated competition. An alternative explanation for the observed transitions is that as southern flying squirrels diffuse through the landscape, large, well-connected woodlots are the first to be colonized, while smaller, fragmented woodlots that may have been strongholds for northern flying squirrels are slower to be colonized. As southern flying squirrels reach these insular woodlots, unoccupied ones are easily colonized and those too small to support both species shift to the more abundant southern species. The larger of these, however, may be able to support both species. While northern and southern flying squirrels generally exploit different food and habitat resources, it is important to consider that competitive exclusion is not necessarily the result of direct or indirect competition alone, but rather may be the result of a complex interaction between the two (see Tompkins et al., 2003). Thus, further research is required to understand this pattern; however, our findings to date suggest *S. robustus* is not likely playing a significant role in the exclusion of northern flying squirrels from woodlots.

Parasite-mediated competition via the intestinal nematode, *S. robustus*, has been proposed to be an important contributor to isolation in sympatric populations of northern and southern flying squirrels. In an area of sympatry where hybridization between the two species is known to occur, we tested whether parasite-mediated competition could serve as a barrier to reproduction between the two. Our study highlights that *S. robustus* can persist at northern latitudes and has an asymmetric effect on flying squirrel hosts. These findings only partially support the parasite-mediated competition hypothesis, however, lack of evidence of parasite presence leading to exclusion of northern flying squirrels, suggests the effect is too weak to be significant. Therefore, we conclude that

weak support of the parasite-mediated competition hypothesis in our study area suggests it is unlikely to contribute to reproductive isolation between these sympatric populations of flying squirrels. However, we note that our study would be strengthened by a larger sample size and data collected over a longer temporal scale. As such, we suggest that our results be interpreted with these caveats in mind. Nonetheless, we think our findings may help to explain the occurrence of hybridization between northern and southern flying squirrels at high latitudes (Garroway et al., 2010), since parasites do not appear to be a mechanism enforcing reproductive isolation. It may be that reinforcement of the species barrier will occur over time through low hybrid fitness (O'Brien et al., 2021). We will continue to monitor parasites in squirrel populations given their ability to influence interspecific interactions and ecological communities and to better understand any long-term effects of *S. robustus* in an area of secondary contact between closely related species. While the current study focused on the effects of *S. robustus*, we suggest future research should explore the complex interactions of parasite communities within hosts, particularly regarding the effects of parasite coinfection (Veitch et al., 2020), given the diversity of parasites we detected across squirrel species in our study area.

Author agreement

All authors have reviewed the manuscript and have approved its submission. The manuscript is not under consideration elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2021.11.001>.

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