Do habitat use and parasitism lead to reinforcement in a flying squirrel hybrid zone?

by

Paul Philip O’Brien

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Biological Sciences
University of Manitoba
Winnipeg

Do habitat use and parasitism lead to reinforcement in a flying squirrel hybrid zone?

Paul Philip O'Brien

Abstract

Northern and southern flying squirrels are sympatric in Ontario due to climate change. In this area of range overlap hybridization occurs. I investigated potential species barriers in this recent hybrid zone. First I examined whether divergence in microhabitat use through time would lead to reinforcement of reproductive isolation. I found microhabitat variables to be weak predictors of trap-level species presence and found no evidence of divergence between species over 18 years. I also found latitude to be the strongest predictor of species occurrence across sites. Second, I tested whether parasite-mediated competition via the parasite, *Strongyloides robustus*, could maintain species barriers. I found a weak negative effect of *S. robustus* on northern flying squirrels, but I found a low parasite prevalence in southern flying squirrels compared to northern flying squirrels. Further, I found no evidence that presence of *S. robustus* would lead to competitive exclusion of northern flying squirrels from woodlots through apparent competition with southern flying squirrels. Therefore, divergence in microhabitat use and parasite-mediated competition do not appear to contribute to reproductive isolation of flying squirrels in Ontario.

Keywords: *Glaucomys sabrinus; Glaucomys volans; reinforcement; secondary contact; microhabitat use; divergence; parasite-mediated competition; Strongyloides robustus; competitive exclusion; parasitism*

Acknowledgements

This project has been a collective effort with many people to thank. First I would like to acknowledge my supervisors Colin Garroway and Jeff Bowman. Thank you for all of your support, patience, knowledge, and input throughout the process. I also thank my committee members Jillian Detwiler and Saman Muthukumarana for your insight.

Thanks to my many field assistants Emily McNaughton, Hannah Hamblin, Janet Greenhorn, Maegwin Bonar, Rachel Dillon, Brian Hughes, Kirsten Solmundson, Jenn Baici, Lizzie Adey, Dan Switzer, MacKenzie Schmidt, Brenda O'Brien, Larry O'Brien, and Catherine O'Brien. I am especially indebted to Sasha Newar for her constant help in the field and willingness to lend a critical eye or ear whenever I needed it. Thanks to Chloe Schmidt, Riikka Kinnunen, and Kyle Lefort for their insightful comments on early drafts of my thesis. Thanks to Jamie Gorrell for providing parasite pictures.

This study could not have been completed without the financial support from Natural Sciences and Engineering Research Council of Canada (Discovery Grant to Jeff Bowman and Colin Garroway and Canada Graduate Scholarship to Masters research to me), University of Manitoba (Tri-Agency Supplemental Masters Award to me), and the OMNRF Wildlife Research and Monitoring Section. I thank Trent University, Ontario Parks, the City of Peterborough, and all the private landowners for allowing me to conduct research on their land.

Finally, a huge thank you to all my family and friends for the constant love and support throughout this endeavor. Without you I surely could not have managed.

Table of Contents

List of Tables

List of Figures

Chapter 1: General Introduction

Species ranges are limited by numerous factors that can be categorized as biotic, such as competition and predation, or as abiotic, such as suitable habitat and climate (i.e., temperature and precipitation; Sexton et al. 2009). In temperate regions, it is generally thought that northern species ranges tend to be constrained to the south by biotic factors, while abiotic factors tend to constrain southern species to the north (MacArthur 1972). Climate is a dominant driver of species range boundaries and alongside a rapidly changing climate, species may respond through expansion or contraction of their ranges to track these rapid environmental changes (Parmesan et al. 1999; Parmesan and Yohe 2003). Understanding the consequences of such range boundary shifts may be critically important, as species expand into previously uninhabited areas, both invaders and native species may be faced with new competitors, predators, or pathogens. Further, these range shifts may also bring recently diverged species into secondary contact (Parmesan 2006).

Reinforcement is the process by which natural selection drives reproductive isolation between related species to reduce maladaptive hybridization (Butlin 1987; Servedio and Noor 2003). Secondary contact between closely related species provides the opportunity to test if species barriers will be reinforced. When species diverge in allopatry, reproductive isolation through assortative mating is not able to evolve, thus secondary contact is required to complete the speciation process. Reinforcement is thought to 'finish' the speciation process (Coyne and Orr 2004) and if species barriers are not maintained through reinforcement then hybrid swarms can emerge (Rhymer and Simberloff 1996).

Two species of flying squirrel occur in eastern North America, the northern (*Glaucomys sabrinus*) and southern flying squirrel (*Glaucomys volans*) and phylogenetic analyses suggest the two diverged between 0.7 and 1.3 mya (Arbogast 2007). The distributions of these small, arboreal rodents are parapatric, although narrow regions of overlap exist throughout their distributions. In Ontario, contemporary climate change has led to increased overlap over a period of ~40 years, such that the two species occur in close, local sympatry throughout parts of the province (Bowman et al. 2005). In areas where the two species co-occur, low frequencies of hybridization have been documented (Garroway et al. 2010). While the impact of such hybridization on species diversity remains unclear, low frequencies of hybrids suggests the presence of imperfect species barriers. In Ontario, flying squirrel species occurrence (i.e.

allopatry vs. sympatry) varies from woodlot to woodlot. This system provides a natural setting to test potential mechanisms of reproductive isolation in areas of sympatry.

The geographic ranges of northern and southern flying squirrels closely follow that of the boreal forest and hardwood, deciduous forest of eastern North America, respectively (Dolan and Carter 1977; Wells-Gosling and Heaney 1984) and likely stems from the divergence of the two species in separate glacial refugia (Arbogast 2007). Further, their close associations with the boreal and hardwood forests have also led to unique ecological differences between the two. Northern flying squirrels primarily consume fungus and lichens (Maser, Maser, and Trappe 1985; Trapp, Smith, and Flaherty 2017), while southern flying squirrels consume hardwood mast crop (Weigl 1968; Dolan and Carter 1977). In terms of nesting preferences, northern flying squirrels exhibit flexibility in their nest choice, using cavities, external leaf nests, and ground nests (Carey et al. 1997; Trudeau et al. 2011). In contrast, southern flying squirrels appear to be obligate cavity nesters (Holloway and Malcolm 2007; Zweep et al. 2018). The exploitation of different resources (i.e., diet and habitat), as seen in northern and southern flying squirrels, could provide the basis for divergent selection to reinforce reproductive isolation (Hatfield and Schluter 1999; Rundle and Nosil 2005).

A second mechanism that has been hypothesized to contribute to reproductive isolation of northern and southern flying squirrels is parasite-mediated competition (Weigl 1968; Price, Westoby, and Rice 1988). This form of apparent competition occurs when an asymmetrical tolerance to a shared parasite exists between two hosts leading to competitive exclusion of the naïve host (Hudson and Greenman 1998). In flying squirrels, the intestinal nematode, *Strongyloides robustus,* can be fatal to northern flying squirrels, while southern flying squirrels are tolerant (Weigl 1968; Krichbaum et al. 2010). Direct contact in nest cavities with infected individuals or fecal matter is the most likely mode of transmission. Thus, in an area of sympatry where hybridization occurs, parasite-mediated competition could contribute to the reproductive isolation of northern and southern flying squirrels.

The objective of my Masters thesis was to test for evidence of reproductive barriers between the closely related northern and southern flying squirrels. In an area of recent sympatry, where hybridization has been documented, it is unclear whether reproductive isolation will be reinforced. To test this, I tested for evidence of two possible mechanisms of isolation: ecological isolation and parasite-mediated competition. In Chapter 2, I explored differences in fine-scale habitat use of flying squirrels and test for potential divergence through time. In Chapter 3, I tested the parasite-mediated competition hypothesis and examined the role of the intestinal nematode, *Strongyloides robustus,* in maintaining species barriers.

Chapter 2: Habitat use of northern and southern flying squirrels in a recent hybrid zone

Abstract

Secondary contact of closely related species may lead to hybridization if reproductive isolation is incomplete. If hybrid fitness is low, interspecific reproductive barriers might become reinforced due to selection against hybridization. We examined habitat use of northern and southern flying squirrels in an area of recent secondary contact in Ontario, Canada. Specifically, we looked at summer microhabitat use of both species within sites of local sympatry and allopatry to test for evidence of reinforcement of species barriers through diverging habitat use. We also examined differences in broad-scale habitat features at woodlots to determine predictors of species occurrence across sites. We used 18 years (2002 - 2019) of flying squirrel summer capture data from 6 sites along a north-south transect. Vegetation surveys were conducted at each trap location during summer 2010 to characterize the available microhabitats within sites. Site-level habitat variables were calculated using the collected microhabitat variables. We found microhabitat variables to be weak predictors of trap-level flying squirrel presence and we found no evidence of divergence in habitat use over the 18 years. Further, we found latitude and not broad-scale habitat was the strongest predictor of site-level flying squirrel occurrence. Overall, our findings suggests that microhabitat isolation is not being reinforced between northern and southern flying squirrels in Ontario, however, hybridization may be limited to areas where climate is suitable for both species.

Keywords: *divergence; Glaucomys sabrinus; Glaucomys volans; microhabitat; reinforcement; secondary contact*

Introduction

Reinforcement is the evolutionary process by which reproductive isolation between diverging species evolves via natural selection in response to maladaptive hybridization (Rundle and Schluter 1998; Noor 1999; Beysard, Krebs-Wheaton, and Heckel 2015). In areas of secondary contact interspecific breeding may occur and if pre-zygotic barriers are not in place, hybrids can be produced. If hybrids are less fit than parental types, then barriers to reproduction, and subsequently assortative mating, are expected to evolve (Matute 2010). Reproductive barriers can be pre-mating (e.g., conspecific mating preferences), post-mating/prezygotic (i.e., gametes do not form zygote), or post-zygotic (i.e., hybrid inviability/sterility; Matute 2010). While all three stages can result in barriers to reproduction, post-mating and post-zygotic barriers on their own still result in wasted reproductive effort (Butlin 1987). Thus, pre-mating isolation is required to complete speciation and maximize fitness. When diverging species are allopatric, they cannot produce hybrids and so there can be no direct selection for assortative mating and against interspecies breeding. Thus, areas of secondary contact between recently diverged species are required to study the evolution of reproductive barriers due to reinforcement.

Hybridization is ultimately a consequence of mate choice, specifically resulting from non-assortative mating among related species, but many factors influence the successful mating of two individuals. To successfully mate, individuals must 1) encounter one another, 2) send and receive a signal of mate recognition, 3) both be in their reproductive phase, and 4) have compatible reproductive organs. Pre-mating isolation can occur at any one of these steps and the reinforcement hypothesis predicts that species within an area of sympatry exhibit greater divergence of isolating traits than species in allopatry. Thus, selection against hybridization in areas of sympatry is strengthened by species' ability to recognize and discriminate between conspecifics and heterospecifics based on the divergence of the isolating trait.

Northern (*Glaucomys sabrinus*) and southern flying squirrels (*Glaucomys volans*) have recently diverged (i.e., early – mid Pleistocene; Arbogast 2007) and due to a more recent (i.e., ~40 years ago) and rapid range expansion of southern flying squirrels, have come into secondary contact in Ontario (Bowman et al. 2005). Within a zone of sympatry, hybrids have been discovered (Garroway et al. 2010). Secondary contact of flying squirrels within this hybrid zone provides the opportunity to test whether reinforcement will complete the speciation process. Behavioural isolation through mate choice plays an important role as a pre-mating barrier in hybrid zones of other species (Noor 1995; Smadja and Butlin 2009; Beysard, Krebs-Wheaton, and Heckel 2015), however testing this requires some prior knowledge of important cues for recognition of heterospecifics (i.e., visual, auditory, olfactory, etc.). Ecological isolation has also been shown to play a significant role in maintaining species barriers (Hatfield and Schluter 1999). In such cases, low hybrid fitness in parental habitats reinforces reproductive isolation between parental species. The distributions of northern and southern flying squirrels are closely associated with boreal and deciduous forests, respectively (Weigl 1968). Therefore, we suggest that reinforcement of ecological isolation between northern and southern flying squirrels is likely to contribute to species barriers in areas of sympatry.

Northern flying squirrels are considered a boreal species with a strong affinity for coniferous and mixed-wood forests. Their strong association with the boreal forest is apparent from a diet largely comprised of lichens and hypogeous fungi (Maser, Maser, and Trappe 1985; Li et al. 1986). In contrast, the distribution of southern flying squirrels closely follows that of the deciduous, hardwood forests of eastern North America and as such they primarily consume hardwood mast. Both species of flying squirrel are considered to be secondary cavity nesters, relying on cavities for feeding sites, safety from predators and weather, and raising young (Bendel and Gates 1987; Weigl 1978). Southern flying squirrels, however, are more reliant on tree cavities, while northern flying squirrels are more flexible in their nest selection, using both external leaf nests and subterranean nests as well (Trudeau et al. 2011). Microhabitats have been defined by Morris (1987) as the environmental variables (i.e., physical and chemical) that influence how an individual allocates its time in space, namely their home range. For flying squirrels, variables related to daily activities (i.e., foraging and nesting), such as number of mast trees or cavity trees, probably make up important microhabitats influencing individual behaviour. Given the above differences in diet and nesting preferences of northern and southern flying squirrels, we expect that if reinforcement is acting against hybridization, then microhabitat use related to these differences should play a role.

The objective of this study was to test for evidence of divergence in summer habitat use by northern and southern flying squirrels in an area of recent sympatry. Within sites, we hypothesized that if reinforcement is acting against hybridization, then we should see divergence in microhabitat use by northern and southern flying squirrels at sympatric sites through time. We predict that the importance of microhabitat variables related to species-specific nest use and diet at sympatric sites will diverge through time, while no divergence will occur at allopatric sites. Across sites, we hypothesized that occurrence of flying squirrel species at a site will be predicted by broad-scale forest characteristics, such that areas of allopatry would reflect habitat preferences of each parent species, while areas of sympatry would be intermediate between the two. Specifically, we predicted that 1) at allopatric northern flying squirrel sites, the occurrence would be positively related to coniferous or coniferous-dominated forests; 2) at allopatric southern flying squirrel sites, occurrence would be positively related to hardwood or hardwood-dominated forests; and 3) sympatry at a site would be promoted by mixed woods.

Methods

Study Sites

Study sites were located along a north-south transect from Peterborough, Ontario (44.570°N) to Algonquin Provincial Park (45.584°N; Figure. 2.1). We trapped flying squirrels at 6 sites that represented areas of allopatric northern flying squirrels (*n* = 1), allopatric southern flying squirrels (*n* = 3), and sympatric sites (*n* = 2; Figure. 2.1). All sites used are part of long-term flying squirrel and small mammal research projects (Bowman et al. 2005).

Species Occurrence

We used 18 years (2002 – 2019) of flying squirrel capture data to estimate species occurrence at trapping sites. Trapping was carried out between June and August of each year for a range of 3 – 7 nights with 20 – 30 traps set at ~20m spacing. The same trap stations were repeatedly sampled throughout the study at each site. We trapped squirrels using Tomahawk model 102 live traps (Tomahawk, WI, USA) baited with sunflower hearts. Traps were mounted on wood platforms and secured ~2m in trees and were in place for the duration of the study. If platforms fell down they were remounted and in the rare event of a tree falling over, the platform was replaced on a neighbouring tree. An occurrence was recorded for a given trap after the 3 - 7 night period if a squirrel was ever captured at that trap, in that year. All captured individuals were identified to species, sexed, weighed, had age class assigned (adult or juvenile), and were marked with either a 1-g Monel ear tag (National Band and Tag Co., Newport, KY), or a 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 individual identification, depending on location.

Morphological features used to distinguish species included tail and hindfoot length, tail colour, and basal colour of fur on venter (Peterson 1966; Banfield 1987). All live-trapping and processing methods followed protocals approved by Trent University's Animal Care Committee.

Microhabitat Variables

Microhabitat surveys were conducted at each trapping site during the summer of 2016 by previous researchers. Within each site, a vegetation survey was conducted within a ~10m radius of each trap tree as a measure of the available microhabitats at a site. For each survey, species, diameter at breast height (DBH) and decay class (1 = healthy live tree; 9 = decayed stump; Thomas et al. 1979) of the trap tree were recorded. Researchers followed methodology outlined by Bowman, Forbes, and Dilworth (2001) for vegetation surveys. Within the 10m radius sample plot all trees ≥8cm DBH were counted, identified to species and had DBH recorded. Ground cover (i.e., grass, rocks, moss, lichens, leaf, needles, bareground, and shrubs) was measured using a scale of 0 - 5 (0 = 0%, 1 = <1%, 2 = <10%, 3 = 20%, 4 = <50%, 5 = ≥50%) to classify percent coverage. Mast trees, snags, and downed logs within the plot were also counted. In using vegetation surveys from a single year, we make the assumption that microhabitat is unchanging across years.

Site-level Habitat Variables

To estimate site-level habitat variables we calculated the site-level mean for all continuous microhabitat variables (i.e., DBH, canopy coverage, percent lichens, and number of hardwoods, softwoods, mast trees, and snags). For discrete variables (i.e., decay class), we first converted the variable to a binary scale, such that values ≤ 2 were classified as 1 (live) and values > 2 as 0 (dead). We then calculated the proportion of live trees within a site. To further reduce the number of variables, we combined mean number of hardwoods and softwoods by calculating the proportion of hardwood trees within a site, thus capturing an estimate for both in one variable.

Mixed Effects Models

To test for evidence of divergence in microhabitat use we ran Bayesian mixed-effects models. For the within-site models we used either northern flying squirrel or southern flying squirrel occurrence at a trap in a given year as the dependent variable. We selected a subset of microhabitat variables a priori from the total collected that we thought would be most important for flying squirrel habitat use. We chose these variables based on previous research of northern and southern flying squirrels (Holloway and Malcolm 2007; Diggins and Ford 2007; Bendel and Gates 1987; Meyer, Kelt, and North 2007). To further reduce the number of variables and collinearity, we ran Pearson's correlation tests. If any two variables had a correlation coefficient ≥ 0.5, we kept the variable we felt was most biologically relevant. The final suite of microhabitat variables we included were: DBH, tree decay, percent canopy, percent coverage of bryophytes, lichens, and logs, and number of softwoods, hardwoods, mast trees, and snags. We also included regional abundance of both species for each year as a covariate to account for variation in occurrence due to natural fluctuations in abundance. Yearly regional abundance was estimated as the total number of each species captured over 100 trap nights. Response variables for within-site models were nested, such that an observation represented the presence of a given species at a trap, within a site for a given year. To capture variation in species occurrence not accounted for by microhabitat or abundance, we included site, year, and trap ID as random intercepts for all models.

For each response (northern or southern flying squirrel occurrence) we fit models for 4 sets of predictor variables including: (1) a null model; (2) variables related to nesting (DBH, decay, canopy, number of hardwoods, number of softwoods, number of snags); (3) variables related to diet (number of logs, bryophyte coverage, lichen coverage, number of mast trees); and (4) a global model, for a total of 8 within-site models. We fit all models in a Bayesian framework with the *brms* package (Bürkner 2017) in R (Core Team 2020). We ran all models using default, uninformative priors, a warm-up of 5000, and 5000 sampling iterations. For each model we calculated marginal and conditional R^2 to evaluate and compare model fit using the *performance* package (Lüdecke et al. 2020). Marginal R^2 describes the variance explained by fixed effects, while conditional R^2 describes the variance explained by both fixed and random effects.

To test how broad-scale habitat influences site-level species occurrence, we also ran Bayesian mixed-effects models. For across-site models we used the proportion of captures as the dependent variable to capture both species in a single variable. We reduced variables further to include DBH, variance in DBH, percent canopy and lichen coverage, proportion of live trees, proportion hardwoods, and mean number of mast trees and snags. We were interested in testing for divergence in habitat use through time, thus we included year as a random intercept to allow the effect of year to vary between years. We fit two models for across-site hypotheses: one for habitat variables and the other only including latitude as an independent variable. Models were fit using the *brms* package and R^2 was calculated with the *performance* package. To help with model convergence, we used weakly informative, flat priors bounded at -10 and 10 to reduce the range of possible parameter estimates explored. As before, we specified 5000 warm-up and sampling iterations.

Results

Squirrel Captures

We surveyed 111 trap stations across 6 sites during 2002 to 2019, inclusive. Overall we had a total of 730 captures over 5511 trap nights. This included 147 captures of 116 northern flying squirrels and 582 captures of 423 southern flying squirrels.

Within-site Mixed Models

The relationship between most microhabitat variables and squirrel occurrence was not in the predicted direction and 95% credible intervals were overlapping 0 (Table 2.1). For northern flying squirrels, percent lichen coverage and northern flying squirrel abundance were strong predictors of squirrel occurrence for all models (Table 2.1). According to LOOIC, the diet model was most parsimonious for northern flying squirrels (Table 2.1). For southern flying squirrels, abundance was the strongest predictor of occurrence for all within-site models, but a weak relationship was found for DBH (Table 2.2). The nest model was the most parsimonious model for southern flying squirrels according to LOOIC (Table 2.2). For all within-site models, most variation was captured by the site level random effect (Table 2.1; Table 2.2). Site-level effects were in the expected directions for allopatric and sympatric sites (Figure. 2.2A and B), while random effect of year did not exhibit the expected pattern (Figure. 2.3A and B).

Across-site Mixed Models

For site-level models, no relationship was found between any habitat variables and proportion of captures (Table 2.3). Parameter estimates were not in the predicted direction and 95% credible intervals had wide ranges. The strong positive relationship between proportion of northern flying squirrel captures at a site and latitude was, however, in the predicted direction (Table 2.3).

Discussion

In an area of secondary contact between northern and southern flying squirrels and where hybridization has been documented (Garroway et al. 2010), we tested for evidence of divergence in summer microhabitat use. We did not find support for our hypothesis that within sympatric sites the microhabitats used by northern and southern flying squirrels would become more differentiated through time. We also did not find support for our hypothesis that the distribution of flying squirrel species across sites would be predicted by broad-scale forest characteristics, but rather that latitude was the strongest predictor. Overall, our findings do not provide evidence of reinforcement occurring between these two species.

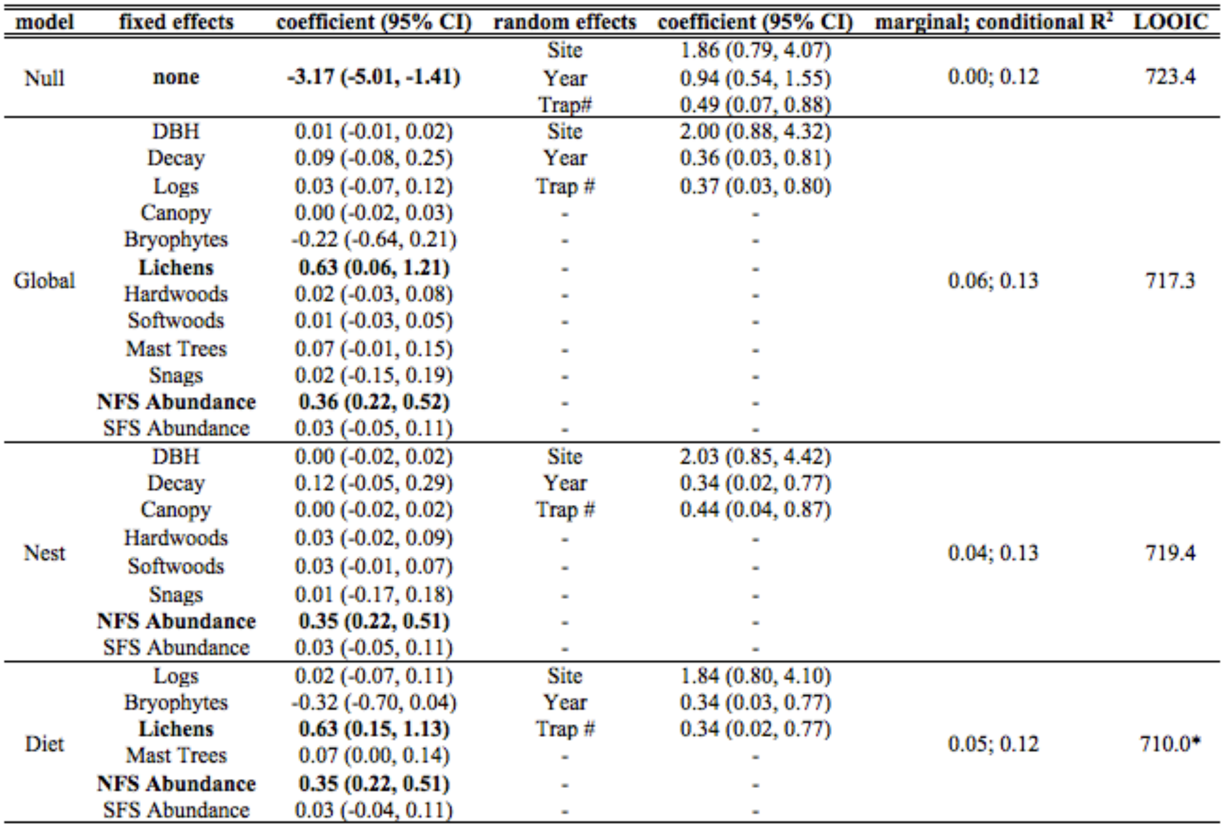
We found the relationship between species presence and most microhabitat variables to be weak or not as predicted. However, as predicted lichen coverage and regional abundance were the strongest predictors of trap-level occurrence of northern flying squirrels. As would be expected, we found a positive relationship between abundance and probability of northern flying squirrel capture. Lichens make up a significant part of the northern flying squirrel's diet in parts of their distribution (Maser, Maser, and Trappe 1985) and so it makes sense that it would be a significant predictor of their presence. Moreover, it may also be expected that a variable associated with diet would be significant considering the methods used. Live-trapping of flying squirrels occurs at night when squirrels are actively foraging and so where squirrels are captured may be reflective of important foraging areas, thus favouring variables related to diet over those related to nesting.

Similarly, the strongest predictor of southern flying squirrel presence was regional abundance. However, in contrast to northern flying squirrels, we found a weak effect of tree diameter (DBH), which is likely explained by nesting preferences of southern flying squirrels and is an important structural feature in flying squirrel nest selection (Holloway and Malcolm 2007; Zweep et al. 2018). For both northern and southern flying squirrels, however, the effects of microhabitat variables were weak and most variation in capture probability was explained by random effects, particularly site-level effects. If the hypothesized microhabitat divergence were true, we would predict an increasingly positive year effect, however such a pattern was not observed. A review of small-mammal microhabitat studies by Jorgensen (2004) suggests that most studies fail to detect an effect of microhabitat partitioning among species as a result of inadequate study design. It is possible that we did not include enough microhabitat types in our study or that trapping effort was low, however, with 6 sites and 18 years of trapping data, we do not think unsubstantial trapping effort is an issue. We note, however, that this long-term study was not designed to test the current question. Moreover, Holloway and Malcolm (2007) observed little or no relationship between microhabitat variables and nest trees selected by flying squirrels in Algonquin Park. Thus, we suggest microhabitat use may be too fine-scale to detect divergence in habitat use within a site. The scale of effect for such processes may be more evident at the macrohabitat (i.e., home range) level rather than at the individual tree level.

Despite most variation of within site species presence being explained by site-level random effects, we found no site-level habitat variables to be important predictors of squirrel occurrence at sites. This may largely be a result of a low degree of variation in habitat variables among the included sites. Perhaps unsurprisingly, we found latitude to be the strongest predictor of squirrel occurrence among sites. With the majority of northern and southern flying squirrel's distributions occurring above and below the 45th parallel, respectively, and our study occurring along a north-south transect (~ 44.5 – 45.5°N), it makes sense that latitude would explain the greatest amount of variation in occurrence. Historically, the 45th parallel has been considered to be the northern range boundary for southern flying squirrels (Stabb 1988); however, Bowman et al. (2005) found the species range edge to fluctuate in relation to mean seasonal temperatures and mast crop success. Therefore, that latitude appears to be the strongest predictor of flying squirrel distributions among forest stands would suggest climate is the ultimate driver of site-level occurrence. For the most part, the forest composition of the woodlots used are fairly intermediate between the boreal forests to the north and hardwood forests farther south and so a landscape-level effect of habitat may be more evident at a larger scale where heterogeneity between woodlots would be higher.

From the current findings, it does not appear that northern and southern flying squirrels have diverged in summer habitat use through time at our study sites. We suggest that if reinforcement is acting in this area of sympatry, divergence in microhabitat use during the summer is not playing a role in reducing hybridization. Other recent research provides some weak evidence for divergence in winter nest selection (O'Brien et al., in review). Future research should focus more specifically on diet and nesting preferences of the two species and at a spatial scale larger than the microhabitat level. Further, research should also focus on the potential for divergence in winter nesting behaviour, since mating occurs in late winter. Finally, with a rising awareness of the value of long-term data, systems such as this should continue to be monitored to assess future changes over time.

Table 2.1. Within-site model summaries for northern flying squirrel (*Glaucomys sabrinus*) trap presence. Four models were fit for the binary response variable each with different sets of predictor variables, including 1) null model, 2) global model, 3) nest model, and 4) diet model. Significant variables are emboldened. Leave-one-out information criterion (LOOIC) indicates model parsimony, with the most parsimonious model indicated by an asterisk.



model	fixed effects	coefficient (95% CI)	random effects	coefficient (95% CI)	marginal; conditional R^2	LOOIC
Null	**none**	**-3.17 (-5.01, -1.41)**	Site	1.86 (0.79, 4.07)	0.00; 0.12	723.4
			Year	0.94 (0.54, 1.55)		
			Trap#	0.49 (0.07, 0.88)		
Global	DBH	0.01 (-0.01, 0.02)	Site	2.00 (0.88, 4.32)	0.06; 0.13	717.3
	Decay	0.09 (-0.08, 0.25)	Year	0.36 (0.03, 0.81)		
	Logs	0.03 (-0.07, 0.12)	Trap #	0.37 (0.03, 0.80)		
	Canopy	0.00 (-0.02, 0.03)	-	-		
	Bryophytes	-0.22 (-0.64, 0.21)	-	-		
	Lichens	**0.63 (0.06, 1.21)**	-	-		
	Hardwoods	0.02 (-0.03, 0.08)	-	-		
	Softwoods	0.01 (-0.03, 0.05)	-	-		
	Mast Trees	0.07 (-0.01, 0.15)	-	-		
	Snags	0.02 (-0.15, 0.19)	-	-		
	NFS Abundance	**0.36 (0.22, 0.52)**	-	-		
	SFS Abundance	0.03 (-0.05, 0.11)	-	-		
Nest	DBH	0.00 (-0.02, 0.02)	Site	2.03 (0.85, 4.42)	0.04; 0.13	719.4
	Decay	0.12 (-0.05, 0.29)	Year	0.34 (0.02, 0.77)		
	Canopy	0.00 (-0.02, 0.02)	Trap #	0.44 (0.04, 0.87)		
	Hardwoods	0.03 (-0.02, 0.09)	-	-		
	Softwoods	0.03 (-0.01, 0.07)	-	-		
	Snags	0.01 (-0.17, 0.18)	-	-		
	NFS Abundance	**0.35 (0.22, 0.51)**	-	-		
	SFS Abundance	0.03 (-0.05, 0.11)	-	-		
Diet	Logs	0.02 (-0.07, 0.11)	Site	1.84 (0.80, 4.10)	0.05; 0.12	710.0*
	Bryophytes	-0.32 (-0.70, 0.04)	Year	0.34 (0.03, 0.77)		
	Lichens	**0.63 (0.15, 1.13)**	Trap #	0.34 (0.02, 0.77)		
	Mast Trees	0.07 (0.00, 0.14)	-	-		
	NFS Abundance	**0.35 (0.22, 0.51)**	-	-		
	SFS Abundance	0.03 (-0.04, 0.11)	-	-		

Table 2.2. Within-site model summaries for southern flying squirrel (*Glaucomys volans*) trap presence. Four models were fit for the binary response variable each with different sets of predictor variables, including 1) null model, 2) global model, 3) nest model, and 4) diet model. Significant variables are emboldened. Leave-one-out information criterion (LOOIC) indicates model parsimony, with the most parsimonious model indicated by an asterisk.

model	fixed effects	coefficient (95% CI)	random effects	coefficient (95% CI)	marginal; conditional R^2	LOOIC
Null	**none**	**-1.34 (-2.42, -0.28)**	Site	1.13 (0.51, 2.44)	0.00; 0.16	1530.9
			Year	0.78 (0.51, 1.16)		
			Trap #	0.52 (0.32, 0.73)		
Global	**DBH**	**0.02 (0.01, 0.03)**	Site	1.04 (0.48, 2.23)	0.09; 0.17	1518.6
	Decay	-0.09 (-0.22, 0.04)	Year	0.41 (0.20, 0.71)		
	Logs	-0.01 (-0.07, 0.06)	Trap #	0.42 (0.11, 0.66)		
	Canopy	0.01 (0.00, 0.03)	-	-		
	Bryophytes	0.07 (-0.33, 0.37)	-	-		
	Lichens	0.10 (-0.34, 0.52)	-	-		
	Hardwoods	-0.01 (-0.04, 0.02)	-	-		
	Softwoods	-0.01 (-0.04, 0.02)	-	-		
	Mast Trees	-0.01 (-0.06, 0.04)	-	-		
	Snags	0.06 (-0.05, 0.17)	-	-		
	NFS Abundance	-0.10 (-0.22, 0.02)	-	-		
	SFS Abundance	**0.14 (0.07, 0.21)**	-	-		
Nest	**DBH**	**0.02 (0.01, 0.03)**	Site	1.07 (0.49, 2.27)	0.08; 0.17	1513.1*
	Decay	-0.10 (-0.22, 0.03)	Year	0.40 (0.21, 0.68)		
	Canopy	0.01 (0.00, 0.02)	Trap #	0.38 (0.08, 0.62)		
	Hardwoods	-0.01 (-0.04, 0.02)	-	-		
	Softwoods	-0.01 (-0.04, 0.01)	-	-		
	Snags	0.07 (-0.03, 0.17)	-	-		
	NFS Abundance	-0.10 (-0.22, 0.03)	-	-		
	SFS Abundance	**0.14 (0.07, 0.21)**	-	-		
Diet	Logs	-0.02 (-0.09, 0.05)	Site	1.14 (0.51, 2.48)	0.06; 0.17	1521.1
	Bryophytes	0.08 (-0.22, 0.39)	Year	0.41 (0.22, 0.69)		
	Lichens	-0.19 (-0.64, 0.24)	Trap #	0.55 (0.36, 0.77)		
	Mast Trees	-0.02 (-0.08, 0.04)	-	-		
	NFS Abundance	-0.09 (-0.21, 0.03)	-	-		
	SFS Abundance	0.14 (0.07, 0.21)	-	-		

Table 2.3. Across-site model summaries for proportion of northern flying squirrels (*Glaucomys sabrinus*) captured at a site. Two models were fit for the response variable each with different sets of predictor variables, including 1) a habitat model and 2) a spatial model. Significant variables are emboldened.

Model	**fixed effects**	**coefficient (95% CI)**	**random effects**	**coefficient (95% CI)**	**marginal; conditional R^2**
Habitat	DBH	0.00 (-1.19, 1.09)	Year	0.37 (0.01, 1.01)	0.07; 0.08
	Variance of DBH	-0.01 (-0.19, 0.20)			
	Canopy	-0.12 (-1.41, 1.35)			
	Lichen	-0.55 (-9.62, 9.39)			
	Proportion Hardwood	-0.10 (-9.52, 9.70)			
	Mast Trees	-0.07 (-3.32, 2.76)			
	Snags	-0.53 (-9.22, 9.21)			
	Proportion Live	-0.78 (-9.71, 9.39)			
Spatial	**Latitude**	**1.51 (0.43, 2.60)**	Year	0.38 (0.01, 0.99)	0.02; 0.03

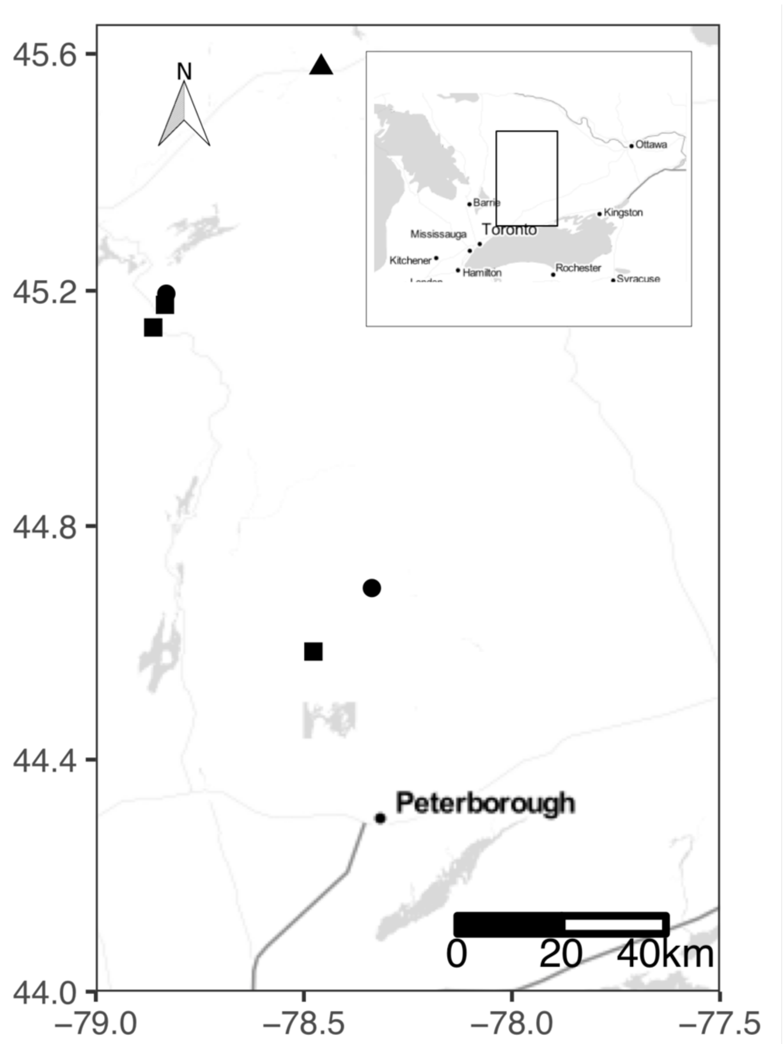


Figure 2.1. Locations of long-term flying squirrel trapping sites in Ontario, Canada. The 6 sites encompass areas of southern flying squirrel allopatry (*n* = 3), northern flying squirrel allopatry (*n* = 1), and sympatry (*n* = 2). Shape represents squirrel species detected at a site: square = southern flying squirrel, triangle = northern flying squirrel, circle = both.

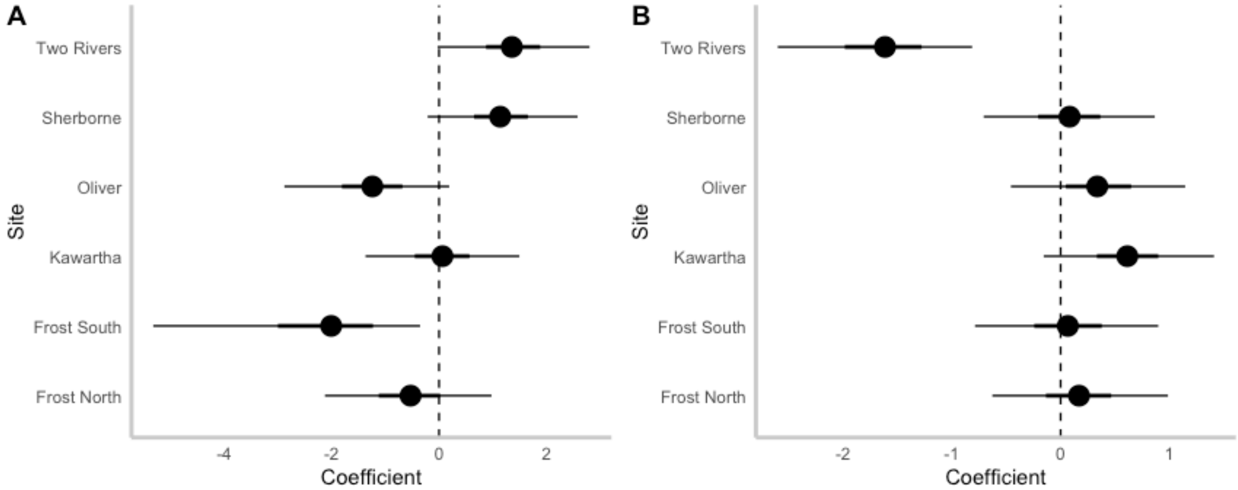


Figure 2.2. Site-specific parameter estimates for the effect of **A)** diet-related microhabitat variables on within-site northern flying squirrel (*Glaucomys sabrinus*) presence; and **B)** nest-related microhabitat variables on within-site southern flying squirrel (*Glaucomys volans*) presence. Ranges are 90% (thick line) and 95% (thin line) credible intervals.

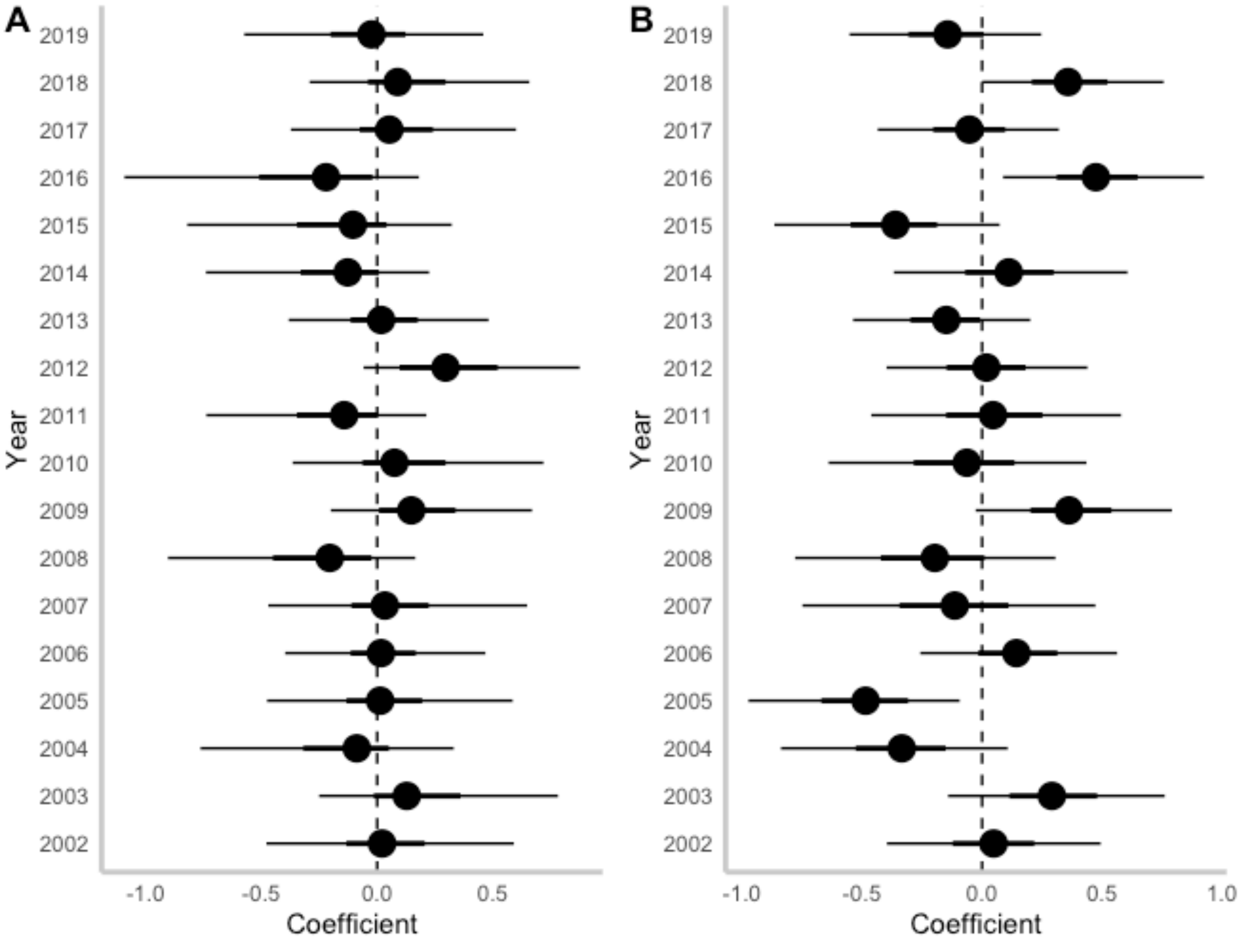


Figure 2.3. Year-specific parameter estimates for the effect of **A)** diet-related microhabitat variables on within-site northern flying squirrel (*Glaucomys sabrinus*) presence; and **B)** nest-related microhabitat variables on within-site southern flying squirrel (*Glaucomys volans*) presence. Ranges are 90% (thick line) and 95% (thin line) credible intervals.

Chapter 3: Testing the parasite-mediated competition hypothesis between sympatric northern and southern flying squirrels

Abstract

Competition is a driving factor in shaping ecological communities and may act directly or indirectly through apparent competition. We examined whether parasite-mediated competition via the intestinal nematode, *Strongyloides robustus,* could act as a species barrier in a flying squirrel hybrid zone in Ontario. We live-trapped flying squirrels (*Glaucomys sabrinus* and *G. volans*), grey squirrels (*Sciurus carolinensis*), red squirrels (*Tamiasciurus hudsonicus*), and chipmunks (*Tamias striata*) from June - September 2019 at 30 woodlots around Peterborough, Ontario. Fecal samples from squirrels were collected and analyzed for the presence of endoparasitic eggs. For each individual, we calculated Scaled Mass Index (SMI) as a measure of body condition to determine the effect of *S. robustus* on squirrels. We found eggs of *S. robustus* in all species except chipmunks. Infestation 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 possible asymmetrical effect of *S. robustus* on flying squirrels, we did not find any evidence that parasite-mediated competition could lead to competitive exclusion from woodlots.

Keywords: *apparent competition; competitive exclusion; Glaucomys sabrinus; Glaucomys volans; parasitism; secondary contact; Strongyloides robustus*

Introduction

It is well evidenced that contemporary climate change has led to an increase in species range shifts as previous energetic constraints of expansion become lessened or previously suitable habitat becomes unsuitable (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 equal or more important role. For example, the transmission of squirrelpox virus (SQPV) to red squirrels (*Tamisciurus vulgaris*) from introduced eastern grey squirrels (*Sciurus carolinensis*) is attributed as the cause of mass declines of red squirrels and subsequent replacement with grey squirrels across the United Kingdom (Tompkins, White, and Boots 2003; Rushton et al. 2006). 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 expansions, which may have important implications for biodiversity.

The ranges of northern (*Glaucomys sabrinus*) and southern flying squirrels (*G. volans*) are for the most part 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, 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 (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 squirrels do not 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*. 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 susceptible host (Wetzel and Weigl 1994; Pauli et al. 2004).

Strongyloides robustus is an intestinal nematode that infects a variety of sciurids, including northern and southern flying squirrels and is transmitted via skin contact with infected feces and soil (Wetzel and Weigl 1994; Weigl 2007). Where these two species co-occur, transmission is likely to happen in tree cavities that are used consecutively by both species or even at the same time (Olson, Bowman, and Burness 2018). Southern flying squirrels are more tolerant of *S. robustus* than northern flying squirrels for whom infection can be weakening (i.e., weight loss) or even fatal (Weigl 1968; Pauli et al. 2004; Krichbaum et al. 2010). 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 act as a barrier to reproduction between the two.

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 (Pauli et al. 2004; Krichbaum et al. 2010). It is unclear, however, whether this hypothesis is supported at higher latitudes in areas where southern flying squirrels have expanded their range in response to a warming climate. Pauli et al. (2004) suggest that given the 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.

In Ontario, where the two species occur in sympatry as a result of recent northward expansion of southern flying squirrels (Bowman et al. 2005), much remains unknown about the impact of *S. robustus*. 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) suggests a deleterious effect of infection for northern flying squirrels, while Krichbaum et al. (2010) found no relationship between infection and body condition. 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 address the following questions: 1) Is *S. robustus* present at a high northern latitude?; 2) Do other sympatric squirrel species act as host reservoirs?; and 3) Does *S. robustus* negatively affect northern flying squirrel body condition? We hypothesize 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 squirrels. 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.

Methods

Trapping and Sample Collection

We trapped squirrels at 30 woodlots of varying patch size (1.1 - >1000 ha) in Peterborough, Ontario and the surrounding area (Fig. 3.1). Most sites were selected based on re-sampling sites from a study 14 years prior (Walpole and Bowman 2011). Given *S. robustus* has also been identified in eastern grey squirrels (*Sciurus carolinensis*; Espenshade and Stewart 2013), red squirrels (*Tamiasciurus hudsonicus*; Espenshade and Stewart 2013), 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 ~2m in trees. At each site, we set 20-30 traps with trap spacing of ~20 - 40m apart. Each site was subject to 3 days and nights of consistent trapping (Walpole and Bowman 2011). To capture both nocturnal (flying squirrels) and diurnal (grey squirrels, red squirrels, and chipmunks) squirrels, 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 long-term sites, 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. Further, 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 (Waksmonski et al. 2017). When possible, fresh fecal samples were collected directly while handling squirrels to reduce the possibility of parasite eggs hatching. 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).

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 four cardinal directions) and a distance between 1 and 10m 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 simple 360° prism sweep was performed to identify trees characterizing the woodlot (Husch, Miller, and Beers 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.

Fecal Flotation and Parasite Identification

All fecal samples were processed using a modified McMaster technique (Coombs 2010; see Appendix 3.1 for procedure details). 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 100X 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:

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

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.

Body Condition Index

To determine if infection with *Strongyloides 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 with the highest correlation 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* and all other parasites detected. We tested for differences in body condition using one-tailed Wilcoxon Rank Sum tests.

Generalized Linear Models

To test for evidence of competitive exclusion by parasite-mediated competition within woodlots, we ran a suite of competing generalized linear models. For all models we used species presence (northern or southern flying squirrel) as the dependent variable. We fit 5 models for each species response including: (1) a null model; (2) squirrel co-occurrence model (capture numbers of northern or southern flying squirrel, grey squirrel, red squirrel, and chipmunk); (3) parasite model (presence/absence of *Strongyloides robustus*, *Eimeria* sp., *Capillaria* sp., and *Hymenolepis* sp.); (4) habitat model (proportion of hardwoods, DBH, and decay class); and (5) a global model with all variables for a total of 10 models. We fit models using the *brms* package (Bürkner 2017) in R (R Core Team 2020). To help with model convergence we used weakly informative, flat priors bounded at -10 and 10 for all models, along with a 5000 iteration warm-up and 5000 sampling iterations. For each model we calculated conditional R^2 with the *performance* package (Lüdecke et al. 2020). We also calculated leave-one-out information criterion (LOOIC) for each model to determine parsimony of candidate models.

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.

Results

Trapping

Overall we had 229 captures of 204 squirrels over 900 trap nights (TN). By species we captured 18 northern flying squirrels, 51 southern flying squirrels, 14 red squirrels, 64 grey squirrels, and 82 chipmunks. Of the 229 squirrels captured, we were able to collect fecal samples from 186 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 15 sites exhibited a transition in species occurrence over the 14 years (Table 3.1).

Fecal Flotations

We detected 4 genera of endoparasites across all samples analyzed: *Strongyloides robustus*, *Eimeria* sp., *Capillaria* sp., and *Hymenolepis* sp. *Eimeria* oocyts were present in nearly all samples analyzed, while tapeworm eggs were only detected in chipmunks. Eggs of *Strongyloides robustus* were present in all species except chipmunks. *Strongyloides robustus* was detected at sites with both species or flying squirrels, only northern flying squirrels, only southern flying squirrels, and neither species (Figure 3.1). Prevalence of *S. robustus* for northern flying squirrels, southern flying squirrels, red squirrels, and grey squirrels was 16.7%, 4.9%, 30.8%, and 4.7%, respectively (Figure 3.2; see Appendix 3.2 for details on other parasites). There was no pattern evident in the occurrence of *S. robustus* at a site and the transition of flying squirrel species at the site over 14 years (Table 3.1).

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: $W = 42$, $P = 0.33$; grey squirrels: $W = 23$, $P = 0.59$; Figure. 3.3A 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: $W = 22$, $P = 0.03$; Figure. 3.3C and D). No differences were detected in body condition of any squirrel species infected with *Eimeria* or *Capillaria* (Appendix 3.3), but chipmunks infected *with Hymenolepis* had lower body condition than those not (Appendix 3.3).

Generalized Linear Models

For all models, the relationships between northern and southern flying squirrel occurrence were not in the predicted direction and 95% credible intervals were overlapping 0 (Table 3.2; Table 3.3). While some relationships were in the predicted direction and with non-overlapping 95% credible intervals, we found the null model to be the most parsimonious according to LOOIC for occurrence of both northern and southern flying squirrels (Table 3.2; Table 3.3).

Ordinations

For the first ordination, species occurrence was not significantly related to habitat variables ($F = 1.66$, $P = 0.32$). The sum of all eigenvalues for this RDA was 0.093. The biplot of species and habitat showed an association between northern flying squirrels and mean tree decay (Figure 3.4A). The RDA for species constrained by parasites showed a significant relationship between squirrel species occurrence and parasite presence ($F = 1.88$, $P = 0.03$). The sum of all eigenvalues for this RDA was 0.14. The biplot for the relationship between squirrel species and parasites showed red squirrels grouping with *S. robustus* and chipmunks grouping with *Capillaria* sp. and *Hymenolepis* sp. (Figure 3.4B). Southern flying squirrels and grey squirrels were grouped on their own with no relationships to parasites, while northern flying squirrels were placed in the centre of the plot. The final ordination of species by spatial location was not significant (*F* = 1.18, *P* = 0.32). The sum of all eigenvalues for this RDA was 0.048. The biplot for species and spatial variables showed southern flying squirrels and chipmunks grouping together with no association to any spatial variables (Figure. 3.4C). Red squirrels had a weak association with latitude. Variance partitioning showed unique variation explained by habitat, parasite, and spatial variables as 4%, 14%, and 3%, respectively. Variation shared between all three suites of variables was shown to be 5% and unexplained variation was 84%.

Discussion

The presence of *Strongyloides robustus* at our study sites and within 4 of 5 squirrel species indicates the parasite can persist at northern latitudes and can be maintained by a variety of host species. Further, lower body condition in northern flying squirrels infected with *S. robustus*, but not southern flying squirrels supports the parasite-mediated competition hypothesis between flying squirrels, however presence of the parasite at sites where both species occur and at sites where only northern flying squirrels occur lends uncertainty to the magnitude of 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 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 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). Interestingly, we found the highest prevalence of *S. robustus* in red squirrels, followed by northern flying squirrels (~30% and 16%, respectively). As expected, prevalence in northern flying squirrels was lower compared to studies farther south (50% Pedder, Mahan, and VanKuren 2009; 75% Krichbaum et al. 2010), but higher than Pauli et al. (2004) who report a prevalence of 11% farther north in Wisconsin. The presence of *S. robustus* in several hosts suggests that if parasite-mediated competition takes place, interactions within the entire squirrel community should be considered.

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. We suggest the low prevalence of *S. robustus* found in southern flying squirrels may indicate that as the species has expanded north, *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 relatively accurate given the higher sample size compared to other species and still low detection. In addition, this finding is corroborated by previously low reporting by Coombs (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, we suggest that the parasite has long been present in northern populations of these squirrels for longer than expected and thus they may not be naïve hosts. However, our findings on body condition lend some uncertainty to this idea.

If parasite-mediated competition via *Strongyloides robustus* leads 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, Bowman, and Burness 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., Chapter 2, Garroway et al. 2010). It is possible that pathological effects might require higher parasite prevalence or more intense infections than occur at the northern latitudes of our study area.

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, White, and Boots 2003), pheasants (Tompkins, Draycott, and Husdon 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 *Strongyloides 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 *Strongyloides robustus* on northern flying squirrels is too weak to lead to exclusion.

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 used by a study ~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 colonize. 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. Further research is required to understand this pattern, however, our findings would suggest *Strongyloides robustus* is not likely playing a significant role in exclusion of northern flying squirrels from woodlots.

Parasite-mediated competition via the intestinal nematode, *Strongyloides 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 a barrier to reproduction between the two. Our study highlights that *S. robustus* is able to 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.

Table 3.1 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 *S. robustus***
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

Table 3.2 Model summaries for northern flying squirrel (*Glaucomys sabrinus*) woodlot presence. Five models were fit for the binary response variable each with different sets of predictor variables, including 1) null model, 2) squirrel community model, 3) parasite community model, 4) vegetation model, and 5) global model. Significant variables are emboldened. Leave-one-out information criterion (LOOIC) indicates model parsimony, with the most parsimonious model indicated by an asterisk.

model	**fixed effect**	**coefficient**	**95% CI**		**conditional R^2**	**LOOIC**
			lower	**upper**		
Null	**none**	**-0.83**	**-1.76**	**-0.03**	0	34.3*
Squirrel	SFS	-0.40	-0.97	0.08	0.30	54.5
	Grey	0.35	0.00	0.83		
	Red	**1.33**	**0.04**	**2.86**		
	Chipmunk	0.26	-0.06	0.62		
	Area	0.00	0.00	0.01		
Parasite	*Strongyloides*	2.26	-0.04	4.92	0.24	47.2
	Eimeria	-0.10	-9.30	9.43		
	Capillaria	0.74	-1.40	2.88		
	Hymenolepis	1.42	-2.61	5.58		
	Area	0.00	0.00	0.00		
Vegetation	DBH	-0.05	-0.12	0.00	0.23	42.6
	Decay	3.03	-0.58	7.44		
	Proportion Hardwood	-1.77	-6.26	2.61		
	Area	0.00	0.00	0.00		
Global	**SFS**	**-0.80**	**-1.77**	**-0.02**	0.44	88.4
	Grey	0.61	-0.08	1.47		
	Red	1.53	-1.14	4.03		
	Chipmunk	0.15	-0.49	0.78		
	Strongyloides	2.32	-1.76	6.71		
	Eimeria	0.54	-9.21	9.63		
	Capillaria	2.84	-1.59	7.62		
	Hymenolepis	-1.12	-7.71	5.62		
	DBH	-0.07	-0.19	0.02		
	Decay	2.71	-4.26	9.01		
	Proportion Hardwood	1.78	-7.69	9.37		
	Area	0.00	0.00	0.01		

Table 3.3 Model summaries for southern flying squirrel (*Glaucomys volans*) woodlot presence. Five models were fit for the binary response variable each with different sets of predictor variables, including 1) null model, 2) squirrel community model, 3) parasite community model, 4) vegetation model, and 5) global model. Significant variables are emboldened. Leave-one-out information criterion (LOOIC) indicates model parsimony, with the most parsimonious model indicated by an asterisk.

model	fixed effect	coefficient	95% CI		conditional R^2	LOOIC
			lower	upper		
Null	none	0.48	-0.28	1.27	0	36.7*
Squirrel	**NFS**	**-2.29**	**-4.84**	**-0.51**	0.34	45.5
	Grey	-0.11	-0.52	0.25		
	Red	1.26	-0.31	3.45		
	Chipmunk	-0.08	-0.36	0.21		
	Area	0.00	0.00	0.00		
Parasite	*Strongyloides*	0.27	-1.90	2.82	0.19	41.5
	Eimeria	0.36	-9.32	9.68		
	Capillaria	0.24	-1.89	2.27		
	Hymenolepis	-6.01	-9.84	-0.85		
	Area	0.00	0.00	0.00		
Vegetation	DBH	-0.01	-0.04	0.03	0.18	46.3
	Decay	-2.35	-5.94	0.72		
	Proportion Hardwood	-1.98	-6.43	1.85		
	Area	0.00	0.00	0.00		
Global	NFS	**-4.76**	**-9.13**	**-1.27**	0.50	64.2
	Grey	0.07	-0.51	0.59		
	Red	2.45	-0.84	6.22		
	Chipmunk	**-0.76**	**-1.42**	**-0.08**		
	Strongyloides	-2.60	-8.28	4.20		
	Eimeria	-0.20	-9.38	9.40		
	Capillaria	**6.51**	**1.02**	**9.77**		
	Hymenolepis	**-6.98**	**-9.88**	**-1.54**		
	DBH	**-0.08**	**-0.16**	**-0.01**		
	Decay	-2.00	-7.38	3.00		
	Proportion Hardwood	-1.89	-9.56	8.33		
	Area	0.00	0.00	0.01		

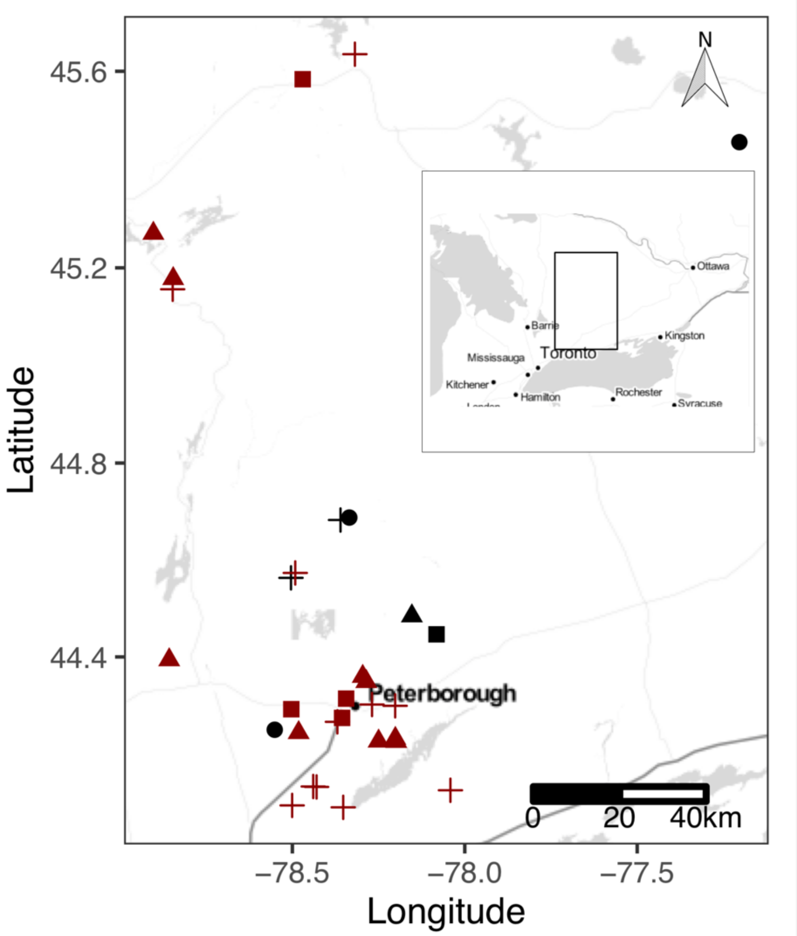


Figure 3.1 Map of sites used to survey the presence of the intestinal parasite *Strongyloides robustus* in northern and southern flying squirrels in Ontario from June - September 2019. Symbol shape represents squirrel species detected at a site: square = northern flying squirrel, cross = southern flying squirrel, circle = both, and triangle = neither. The colour of symbol indicates whether *S. robustus* was detected at a site: red = absent and black = present.

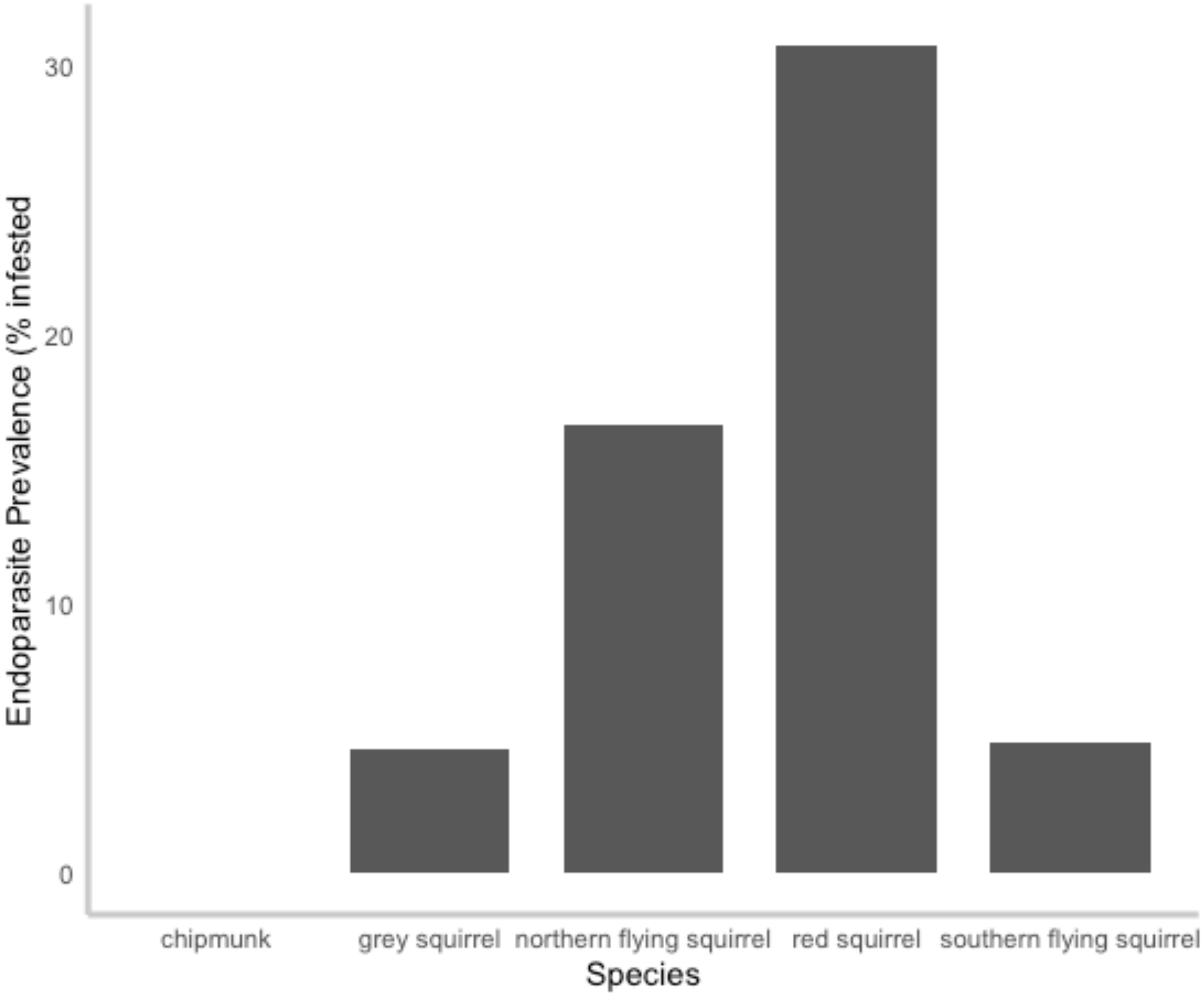


Figure 3.2 Prevalence of the intestinal parasite *Strongyloides robustus* in chipmunks (*n* = 70), grey squirrels (*n* = 43), northern flying squirrels (*n* = 18), southern flying squirrels (*n* = 41), and red squirrels (*n* = 13) captured near Peterborough, Ontario between June - September 2019. Prevalence for each species are as follows: chipmunks = 0%, grey squirrels = 4.7%, northern flying squirrels = 16.7%, red squirrels = 30.8%, and southern flying squirrels = 4.9%.

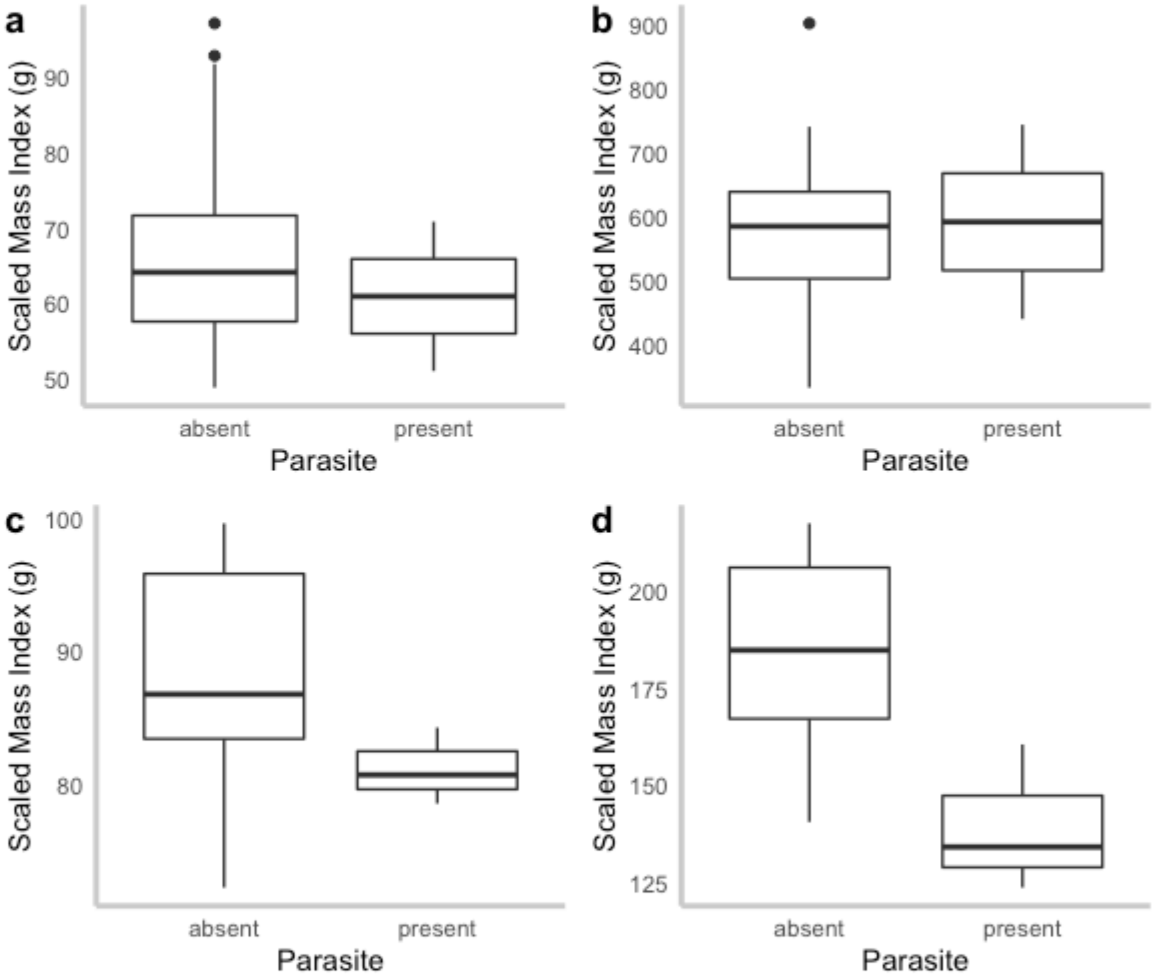


Figure 3.3 Body condition of A) southern flying squirrels ($n = 41$), B) grey squirrels ($n = 43$), C) northern flying squirrels ($n = 18$), and D) red squirrels ($n = 13$) infested with *Strongyloides robustus* compared to those not infested. Body condition was calculated for squirrels captured near Peterborough, Ontario between June - September 2019.

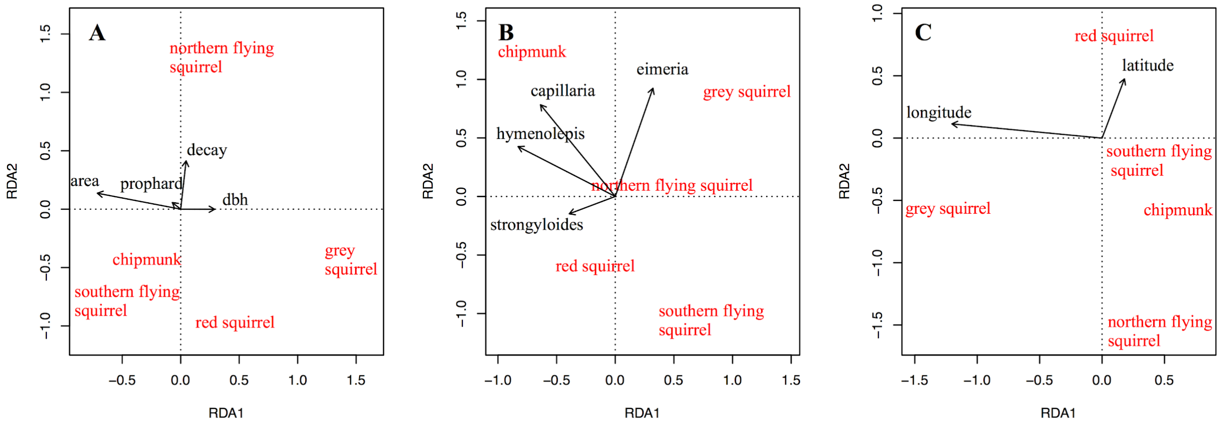


Figure 3.4. Ordination biplots for presence of squirrel species at woodlots based on live-trapping surveys conducted near Peterborough, Ontario during the summer of 2019. Biplots show A) the species and habitat matrices, B) the species and parasite matrices, and C) the species and spatial matrices.

Chapter 4: General Discussion

Species may respond to climate change by expanding or contracting their range to track shifting environmental changes. Such range shifts can bring recently diverged species into secondary contact. In Ontario, secondary contact of northern and southern flying squirrels has led to hybridization in areas of sympatry. Reinforcement is thought to 'finish' the speciation process through selection against maladaptive hybridization. In chapter 2 and 3 of my thesis I explore potential species barriers between sympatric flying squirrels. Specifically, I examine this by looking at summer habitat use and parasite-mediated competition.

In Chapter 2, I examined differences in summer habitat use by northern and southern flying squirrels through time to test for divergence. Specifically, I looked at fine-scale habitat use within and across sympatric and allopatric sites to determine if divergent selection could act on differences in habitat use. I hypothesized that if hybrids are less fit than parental species in parental environments, then I would expect to see increased differentiation in the microhabitats used by northern and southern flying squirrels in areas of co-occurrence. I also hypothesized that across sites, occurrence of northern and southern flying squirrels would be predicted by broad-scale habitat features. Within sites, I did not find evidence of divergence in microhabitat use by northern and southern flying squirrels. Across sites, I found that latitude and not broad-scale habitat features best explained variation in the distribution of flying squirrel species among woodlots.

Bowman et al. (2005) found that the shifting of the northern range boundary of southern flying squirrels in Ontario was influenced by annual temperatures and precipitation and time-lagged mast crop success. That I found latitude was the strongest predictor of woodlot species occurrence makes sense given that latitude is often a proxy for climate. Further, Holloway and Malcolm (2007) found that microhabitat variables poorly predicted local processes, such as nest site selection. Therefore, by examining microhabitat use of northern and southern flying squirrels within sites, I may have been unable to detect evidence of reinforcement. I suggest that future research examining divergence in habitat use should use a broader scale of habitat within sites (e.g., home range). If divergent selection is acting on habitat use of northern and southern flying squirrels, it may be more likely to be detected at the stand-level rather than microhabitat level. Moreover, research could focus more specifically on squirrels behaviours and daily activities within woodlots (e.g., nesting or foraging).

In Chapter 3, I tested the parasite-mediated competition hypothesis. I hypothesized that if the intestinal parasite, *Strongyloides robustus*, is able to persist in northern populations of flying squirrels and negatively affects northern, but not southern flying squirrels, then parasite-mediated competition could serve as a reproductive barrier. Presence of *S. robustus* at multiple sites and within 4 of 5 squirrel species studied, indicates it is able to persist at northern latitudes and can be maintained by a variety of hosts. Further, my results suggest the existence of an asymmetrical tolerance to *S. robustus*, however, modelling results do not support hypotheses of competitive exclusion mediated by parasites.

Northern and southern flying squirrels have been used as a key example of parasite-mediated competition since research by Weigl (1968). Numerous studies since have provided support for the hypothesis and suggested *S. robustus* infection is a factor contributing to the decline of northern flying squirrels in parts of their distribution (Pauli et al. 2004, Krichbaum et al. 2010). Perhaps most convincingly, Krichbaum et al. (2010) found *S. robustus* in sympatric populations of northern and southern flying squirrels in Pennsylvania, but no parasite in an allopatric population of northern flying squirrels in adjacent New York state. In Ontario, I found low prevalence of *S. robustus* in southern flying squirrels, a finding corroborated by previous work in the region (Coombs 2010). Interestingly, I still found relatively higher prevalences of *S. robustus* in northern flying squirrels and red squirrels. The low prevalence in southern flying squirrels suggests a low likelihood of the parasite leading to competitive exclusion in areas where northern and southern flying squirrels co-occur. My finding that northern flying squirrels and red squirrels exhibit higher prevalence of *S. robustus* is interesting given the long-standing sympatry between the two. I suggest the possibility that northern flying squirrels in Ontario may not be a naïve host to *S. robustus*, thus reducing the magnitude of the proposed competition between northern and southern flying squirrels mediated by *S. robustus*.

Speciation by reinforcement occurs when selection against hybridization increases reproductive isolation between hybridizing populations (Butlin 1987). Past literature has focused heavily on intrinsic genetic incompatibilities, such as hybrid inviability or sterility. More recently, extrinsic incompatibilities, such as lower hybrid fitness in association with certain environmental conditions, are important in contributing to reinforcement (Servedio and Noor 2003). Hatfield and Schluter (1999) eloquently showed how selection against hybrids could arise from environmental differences between parental species, such that intermediate hybrid offspring suffered fitness costs in parental habitats. Differences in diet and nesting preferences of northern and southern flying squirrels provided possible traits on which divergent selection could reinforce reproductive isolation. Despite these species-specific ecological differences, I did not find any support for my hypothesis that northern and southern flying squirrels in areas of sympatry have diverged in fine-scale microhabitat use through time. However, recent research in the area suggests some weak evidence of divergence in winter nest selection over 10 years (O'Brien et al., *in review*).

Servedio and Noor (2003) suggest that detecting reinforcement in areas of secondary contact may be difficult, particularly when hybrid zones occur at the range edge of species. Close proximity to the range edge increases the opportunity for migrants from areas of allopatry to disperse into sympatric areas, which may impede divergence. Thus, detecting reinforcement in this flying squirrel hybrid zone may be difficult given that it lies at the northern edge of the southern flying squirrel range boundary. Moreover, Wang et al. (1997) found that sagebrush hybrids were more fit than parent species in hybrid zones, while parent species were most fit in their respective habitat types. In such cases, hybrid zones could remain stable and low selection pressure may hinder reinforcement. Despite the lack of support for my hypotheses, I think divergence in ecological traits remains a promising area of study for flying squirrels given the low frequency of hybridization detected in Ontario. Future research should examine the fitness of hybrids in parental habitats to determine if extrinsic genetic incompatibilities exist.

Range expansions can lead to secondary contact of recently diverged species, but also provides the possibility for invaders to introduce novel parasites or pathogens to native species (Romeo et al. 2014; Tompkins, White, and Boots 2003). Parasites can shape interactions between invasive and native species that can ultimately affect higher-up community processes (Prenter et al. 2004). If species are in secondary contact, parasites may be able to mediate reproductive isolation, thereby reinforcing species barriers. A review by Karvonen and Seehausen (2012) outlines mechanisms by which parasites can mediate ecological speciation. I tested the parasite-mediated competition hypothesis between northern and southern flying squirrels, but found weak evidence that the shared parasite, *S. robustus*, can maintain species barriers through apparent competition. Research has found that *S. robustus* is intolerant to below freezing temperatures (Wetzel and Weigl 1994), which led to the belief that it may not be able to persist at northern latitudes. Previous research in our study area found a low prevalence of *S.*

robustus in northern flying squirrels (~1%) and no parasites in the southern flying squirrels sampled. In comparison, I found a prevalence of ~5% and 16% in southern flying squirrels and northern flying squirrels, respectively. This increase in prevalence may suggest that as we experience warmer winters in Ontario, *S. robustus* can persist and maintain higher populations, a pattern that has been observed in other northern parasite/host communities (Jenkins et al. 2006). If this is the case, we may continue to see *S. robustus* prevalence, or perhaps more importantly, intensity, increase to levels that may have stronger effects on flying squirrel populations. Future research should continue to monitor parasites in squirrel populations given their ability to influence interspecific interactions and ecological communities. Further, understanding the tolerance of hybrids to *S. robustus* may be an important research question. If hybrids are tolerant of *S. robustus*, then hybridization could lead to resistance to the parasite. Alternatively, hybrids could serve as an additional host reservoir that could contribute to any negative effects on northern flying squirrels.

My findings do not support the competitive exclusion of northern flying squirrels from woodlots by southern flying squirrels mediated by *S. robustus*. Despite this lack of evidence, there does appear to have been partial or complete species turnover (i.e., northern flying squirrel to southern flying squirrel or sympatry) for a subset of woodlots I surveyed as compared to a survey 14 years prior. The cause of these turnovers is unknown, but based on my findings, are not likely the result of parasite-mediated competition. Moreover, how these species turnovers will affect the surrounding community is unclear and will likely be an important area of research going forward. For instance, northern flying squirrels are considered to be important dispersers of fungal spores and in particular, hypogeous mycorrhizal fungi (Maser, Maser, and Trappe 1985; Li et al. 1986). As such, they play an important role in nitrogen cycling within forests (Maser, Trappe, and Nussbaum 1978; Li et al. 1986). Turnover of woodlots from northern flying squirrels to southern flying squirrels may consequently have an impact on the availability of nitrogen within these forests.

The outcome of secondary contact between northern and southern flying squirrels in Ontario is still unclear, however frequency of hybridization remains low suggesting some species barriers, albeit imperfect. My research suggests that divergence in microhabitat use and parasite-mediated competition are not contributing to reproductive isolation between northern and southern flying squirrels. How species respond to secondary contact is a complex question and one that will be critically important in the context of species conservation as our climate continues to warm and more species respond by shifting their ranges. While hybridization can lead to a loss of biodiversity, hybrids may also be better able to adapt to our rapidly changing climate.

Literature Cited

Arbogast, Brian S. 2007. “A Brief History of the New World Flying Squirrels: Phylogeny, Biogeography, and Conservation Genetics.” *Journal of Mammalogy* 88 (4): 840–49.

Banfield, A.W.F. 1987. *The Mammals of Canada*. Toronto, ON: University of Toronto Press.

Bendel, Peter R, and J Edward Gates. 1987. “Home Range and Microhabitat Partitioning of the Southern Flying Squirrel (Glaucomys Volans).” *Journal of Mammalogy* 68 (2): 243–55.

Beysard, Mathias, Rebecca Krebs-Wheaton, and Gerald Heckel. 2015. “Tracing Reinforcement through Asymmetrical Partner Preference in the European Common Vole Microtus Arvalis.” *BMC Evolutionary Biology* 15 (1): 1–8. https://doi.org/10.1186/s12862-015-0455-5.

Bowman, Jeff, Graham J Forbes, and Tim G Dilworth. 2001. “The Spatial Component of Variation in Small- Mammal Abundance Measured at Three Scales.” *Canadian Journal of Zoology* 79: 137–44.

Bowman, Jeff, Gillian L Holloway, Jay R Malcolm, Kevin R Middel, and Paul J Wilson. 2005. “Northern Range Boundary Dynamics of Southern Flying Squirrels: Evidence of an Energetic Bottleneck.” *Canadian Journal of Zoology* 83 (11): 1486–94. https://doi.org/10.1139/z05-144.

Bürkner, Paul-Christian. 2017. “Brms: A R Package for Bayesian Multilevel Models Using Stan.” *Journal of Statistical Software* 20 (1): 1–20. https://doi.org/doi:10.18637/jss.v080.i01.

Bush, Albert O., Kevin D. Lafferty, Jeffrey M. Lotz, and Allen W. Shostak. 1997. “Parasitology Meets Ecology on Its Own Terms: Margolis et Al. Revisited.” *The Journal of Parasitology* 83 (4): 575. https://doi.org/10.2307/3284227.

Butlin, Roger. 1987. “Speciation by Reinforcement.” *Trends in Ecology and Evolution* 2 (1): 8–13. https://doi.org/10.1016/0169-5347(87)90193-5.

Carey, Andrew B., Todd M. Wilson, Christine C. Maguire, and Brian L. Biswell. 1997. “Dens of Northern Flying Squirrels in the Pacific Northwest.” *Journal of Wildlife Management* 61 (3): 684–99.

Coombs, Andrea B. 2010. “Competition between Parapatric Flying Squirrel Species: Nest Use, Parasitism, and the Implications of Climate Change.” M.Sc. Thesis, Trent University, Peterborough, Ontario, Canada.

Coyne, Jerry A, and H. Allen Orr. 2004. *Speciation*. Sinauer Associates.

Diggins, Corinne A, and Mark W Ford. 2007. "Microhabitat Selection of the Virginia Northern Flying Squirrel (Glaucomys Sabrinus Fuscus Miller) in the Central Appalachians." *Northeastern Naturalist* 24 (2): 173–90.

Dolan, Patricia G, and Dilford C Carter. 1977. "Glaucomys Volans." *Mammalian Species*. https://doi.org/10.2307/3504026.

Espenshade, Jessica L, and Richard L Stewart. 2013. "Prevalence of Strongyloides Robustus in Tree Squirrels (Sciuridae) in South-Central Pennsylvania and Potential Impacts for the Endangered Northern Flying Squirrel, *Glaucomys Sabrinus*." *Journal of Student Research* 2 (1): 43–47.

Garroway, Colin J., Jeff Bowman, Tara J. Cascaden, Gillian L. Holloway, Carolyn G. Mahan, Jay R. Malcolm, Michael A. Steele, Gregory Turner, and Paul J. Wilson. 2010. "Climate Change Induced Hybridization in Flying Squirrels." *Global Change Biology* 16 (1): 113–21. https://doi.org/10.1111/j.1365-2486.2009.01948.x.

Gooderham, Krista, and Albrecht Schulte-Hostedde. 2011. "Macroparasitism Influences Reproductive Success in Red Squirrels (Tamiasciurus Hudsonicus)." *Behavioral Ecology* 22: 1195–1200. https://doi.org/10.1093/beheco/arr112.

Hatfield, Todd, and Dolph Schluter. 1999. "Ecological Speciation in Sticklebacks: Environment-Dependent Hybrid Fitness." *Evolution* 53 (3): 866–73.

Holloway, Gillian L., and Jay R. Malcolm. 2007. "Nest-Tree Use by Northern and Southern Flying Squirrels in Central Ontario." *Journal of Mammalogy* 88 (1): 226–33.

Hudson, Peter, and Jon Greenman. 1998. "Competition Mediated by Parasites: Biological and Theoretical Progress." *Trends in Ecology and Evolution* 13 (10): 387–90. https://doi.org/10.1016/S0169-5347(98)01475-X.

Husch, Bertram, Charles I. Miller, and Thomas W. Beers. 1972. *Forest Mensuration*. Second. John Wiley & Sons.

Jenkins, E. J., A. M. Veitch, S. J. Kutz, E. P. Hoberg, and L. Polley. 2006. "Climate Change and the Epidemiology of Protostrongylid Nematodes in Northern Ecosystems: Parelaphostrongylus Odocoilei and Protostrongylus Stilesi in Dall's Sheep (Ovis d. Dalli)." *Parasitology* 132 (3): 387–401. https://doi.org/10.1017/S0031182005009145.

Jorgensen, Eric E. 2004. "Small Mammal Use of Microhabitat Reviewd." *Journal of*

Mammalogy 85 (3): 531–39.

Karvonen, Anssi, and Ole Seehausen. 2012. “The Role of Parasitism in Adaptive Radiations- When Might Parasites Promote and When Might They Constrain Ecological Speciation?” *International Journal of Ecology* 1–20. https://doi.org/10.1155/2012/280169.

Krichbaum, Kristle, Carolyn G Mahan, Michael a Steele, Gregory Turner, and Peter J Hudson. 2010. “The Potential Role of *Strongyloides Robustus* on Parasite-Mediated Competition between Two Species of Flying Squirrels (*Glaucomys*).” *Journal of Wildlife Diseases* 46 (1): 229–35. https://doi.org/10.7589/0090-3558-46.1.229.

Li, C Y, C Maser, Z Maser, and B A Caldwell. 1986. “Role of Three Rodents in Forest Nitrogen Fixation in Western Oregon: Another Aspect of Mammal-Mycorrhizal Fungus-Tree Mutualism.” *Great Basin Naturalist* 46 (3): 411–14.

Lüdecke, Daniel, D. Makowski, P. Waggoner, and I. Patil. 2020. “Performance: Assessment of Regression Models Performance.” *CRAN*. https://doi.org/10.5281/zenodo.3952174.

MacArthur, R.H. 1972. *Geographical Ecology: Patterns in the Distribution of Species*. New York: Harper and Row.

Maser, C., James M. Trappe, and Ronald A. Nussbaum. 1978. “Fungal-Small Mammal Interrelationships with Emphasis on Oregon Coniferous Forests.” *Ecology* 59 (4): 799–809. https://doi.org/10.2307/1938784.

Maser, Z., C. Maser, and James M. Trappe. 1985. “Food Habitats of the Northern Flying Squirrel (Glaucomys Sabrinus) in Oregon.” *Canadian Journal of Zoology* 63: 1084–88. https://doi.org/10.1139/z85-162.

Matute, Daniel R. 2010. “Reinforcement of Gametic Isolation in Drosophila.” *PLoS Biology* 8 (3). https://doi.org/10.1371/journal.pbio.1000341.

Meyer, Marc D, Douglas A Kelt, and Malcolm P North. 2007. “Microhabitat Associations of Northern Flying Squirrels in Burned and Thinned Forest Stands of the Sierra Nevada.” *American Midland Naturalist* 157 (1): 202–11.

Morris, Douglas W. 1987. “Ecological Scale and Habitat Use.” *Ecology* 68 (2): 362–69.

Noor, Mohamed A.F. 1999. “Reinforcement and Other Consequences of Sympatry.” *Heredity* 83: 503–8.

Noor, Mohamed A. 1995. “Speciation Driven by Natural Selection in Drosophila.” *Nature* 375: 674–75.

O'Brien, Paul Philip, Jeff Bowman, Andrea B. Coombs, Sasha L Newar, and Colin J. Garroway. n.d. "Winter Nest Trees of Sympatric Northern and Southern Flying Squirrels: A Test of Reinforcment in a Hybrid Zone." *Journal of Mammalogy*.

Oksanen, J., F. Guillaume Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, Peter R. Minchin, et al. 2019. "Vegan: Community Ecology Package." R package version 2.4-6. https://cran.r-project.org/package=vegan.

Olson, Megan N., Jeff Bowman, and Gary Burness. 2018. "Social Thermoregulation Does Not Explain Heterospecific Nesting in North American Flying Squirrels." *Biological Journal of the Linnean Society* 123 (4): 805–13. https://doi.org/10.1093/biolinnean/bly014.

Parmesan, Camille. 2006. "Ecological and Evolutionary Responses to Recent Climate Change." *Annual Review of Ecology, Evolution, and Systematics* 37 (1): 637–69. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100.

Parmesan, Camille, Nils Ryrholm, Constantı Stefanescu, Jane K Hill, Chris D Thomas, Henri Descimon, Brian Huntley, et al. 1999. "Poleward Shifts in Geographical Ranges of Butterfly Species Associated with Regional Warming." *Nature* 399: 579–83. https://doi.org/10.1038/21181.

Parmesan, Camille, and Gary Yohe. 2003. "A Globally Coherent Fingerprint of Climate Change Impacts across Natural Systems." *Nature* 421: 37–42.

Patrick, Michael J. 1991. "Distribution of Enteric Helminths in Glaucomys Volans L . (Sciurdiae): A Test for Competition." *Ecology* 72 (2): 755–58.

Pauli, Jonathan N, Shelli A Dubay, Eric M Anderson, and Stephen J Taft. 2004. "*Strongyloides Robustus* and the Northern Sympatric Populations of Northern (*Glaucomys Sabrinus*) and Southern (*G. Volans*) Flying Squirrels." *Journal of Wildlife Diseases* 40 (3): 579–82. https://doi.org/10.7589/0090-3558-40.3.579.

Pedder, S., Carolyn G. Mahan, and A. VanKuren. 2009. "Prevalence of Parasites Including Strongyloides Robustus and Citellinemia Bifurcatum in the Endangered Northern Flying Squirrel and Other Members of the Squirrel Family." [Abstract] In: Proceedings of The Pennsylvania Chapter of the Wildlife Society. 2009.

Peig, Jordi, and Andy J Green. 2009. "New Perspectives for Estimating Body Condition from Mass / Length Data : The Scaled Mass Index as an Alternative Method." *Oikos* 118 (12): 1883–91.

Peterson, R.L. 1966. *The Mammals of Eastern Canada*. Oxford: Oxford University Press.

Phillips, Ben L., Crystal Kelehear, Ligia Pizzatto, Gregory P. Brown, Di Barton, and Richard Shine. 2010. "Parasites and Pathogens Lag behind Their Host during Periods of Host Range Advance." *Ecology* 91 (3): 872–81. https://doi.org/10.1890/09-0530.1.

Prenter, John, Calum MacNeil, Jaimie T.A. Dick, and Alison M. Dunn. 2004. "Roles of Parasites in Animal Invasions." *Trends in Ecology and Evolution* 19 (7): 385–90. https://doi.org/10.1016/j.tree.2004.05.002.

Price, Peter W., Mark Westoby, and Barbara Rice. 1988. "Parasite-Mediated Competition: Some Predictions and Tests." *The American Naturalist* 131 (4): 544–55. https://doi.org/10.1086/521238.

R Core Team. 2020. "R: A Language and Environment for Statistical Computing." R Foundation for Statistical Computing, Vienna, AustriaR. https://www.r-project.org/.

Rhymer, Judith M, and Daniel Simberloff. 1996. "Exinction by Hybridization and Introgression." *Annual Review of Ecology and Systematics* 27: 83–109.

Romeo, Claudia, Lucas A Wauters, Nicola Ferrari, Paolo Lanfranchi, Adriano Martinoli, Damiano G Preatoni, and Nicola Saino. 2014. "Macroparasite Fauna of Alien Grey Squirrels (*Sciurus Carolinensis*): Composition , Variability and Implications for Native Species." *PLoS Biology* 9 (2): 1–8. https://doi.org/10.1371/journal.pone.0088002.

Rundle, Howard D., and Patrik Nosil. 2005. "Ecological Speciation." *Ecology Letters* 8 (3): 336–52. https://doi.org/10.1111/j.1461-0248.2004.00715.x.

Rundle, Howard D., and Dolph Schluter. 1998. "Reinforcement of Stickleback Mate Preferences: Sympatry Breeds Contempt." *Evolution* 52 (1): 200–208. https://doi.org/10.2307/2410935.

Rushton, S.P., P.W.W. Lurz, J. Gurnell, P. Nettleton, C. Bruemmer, M.D.F. Shirley, and A.W. Sainsbury. 2006. "Disease Threats Posed by Alien Species : The Role of a Poxvirus in the Decline of the Native Red Squirrel in Britain." *Epidemiology and Infection* 134: 521–33. https://doi.org/10.1017/S0950268805005303.

Schall, Jos J. 1992. "Parasite-Mediated Competition in Anolis Lizards." *Oecologia* 92 (1): 58–64. https://doi.org/10.1007/BF00317262.

Servedio, Maria R., and Mohamed A.F. Noor. 2003. "The Role of Reinforcement in Speciation: Theory and Data." *Annual Review of Ecology, Evolution, and Systematics* 34 (1): 339–64. https://doi.org/10.1146/annurev.ecolsys.34.011802.132412.

Sexton, Jason P., Patrick J. McIntyre, Amy L. Angert, and Kevin J. Rice. 2009. “Evolution and Ecology of Species Range Limits.” *Annual Review of Ecology, Evolution, and Systematics* 40: 415–36. https://doi.org/10.1146/annurev.ecolsys.110308.120317.

Smadja, C, and R K Butlin. 2009. “On the Scent of Speciation : The Chemosensory System and Its Role in Premating Isolation.” *Heredity* 102: 77–97. https://doi.org/10.1038/hdy.2008.55.

Stabb, M. 1988. “COSEWIC Status Report on the Southern Flying Squirrel *Glaucomys Volans* in Canada.”

Thomas, J.W., R.G. Anderson, C. Maser, and E.L. Bull. 1979. “Snags.” In *Wildlife Habitats in Managed Forests - the Blue Mountains of Oregon and Washington*, 60–77. Washington, D.C.

Tompkins, D.M., R.A.H. Draycott, and P.J. Husdon. 2000. “Field Evidence for Apparent Competition Mediated via the Shared Parasites of Two Gamebird Species.” *Ecology Letters* 3: 10–14.

Tompkins, D.M., A.R. White, and M. Boots. 2003. “Ecological Replacement of Native Red Squirrels by Invasive Greys Driven by Disease.” *Ecology Letters* 6: 189–96.

Trapp, Stephanie E, Winston P Smith, and Elizabeth A Flaherty. 2017. “Diet and Food Availability of the Virginia Northern Flying Squirrel (Glaucomys Sabrinus Fuscus): Implications for Dispersal in a Fragmented Forest.” *Journal of Mammalogy* 98 (6): 1688–96. https://doi.org/10.1093/jmammal/gyx115.

Trudeau, Caroline, Louis Imbeau, Pierre Drapeau, and M.J. Mazerolle. 2011. “Site Occupancy and Cavity Use by the Northern Flying Squirrel in the Boreal Forest.” *Journal of Wildlife Management* 75 (7): 1646–56. https://doi.org/10.1002/jwmg.224.

Utzinger, J., M. Booth, E. K. N’Goran, I. Müller, M. Tanner, and C. Lengeler. 2000. “Relative Contribution of Day-to-Day and Intra-Specimen Variation in Faecal Egg Counts of Schistosoma Mansoni before and after Treatment with Praziquantel.” *Parasitology* 122 (5): 537–44. https://doi.org/10.1017/S0031182001007752.

Vlas, S. J. de, and B. Gryseels. 1992. “Underestimation of Schistosoma Mansoni Prevalences.” *Parasitology Today* 8 (8): 274–77. https://doi.org/10.1016/0169-4758(92)90144-Q.

Waksmonski, Sarah N, Justin M Huffman, Carolyn G Mahan, and Michael A Steele. 2017. “An Examination of Endoparasites and Fecal Testosterone Levels in Flying Squirrels (*Glaucomys* Spp .) Using High Performance Liquid Chromatography-Ultra-Violet (HPLC-

UV).” *International Journal for Parasitology: Parasites and Wildlife* 6 (2): 135–37. https://doi.org/10.1016/j.ijppaw.2017.05.003.

Walpole, Aaron A., and Jeff Bowman. 2011. “Patch Occupancy by Squirrels in Fragmented Deciduous Forest: Effects of Behavior.” *Acta Theriologica* 56 (1): 63–72. https://doi.org/10.1007/s13364-010-0010-0.

Wang, Han, E Durant Mcarthur, Stewart C Sanderson, John H Graham, and D Carl. 1997. “Narrow Hybrid Zone Between Two Subspecies of Big Sagebrush (Artemisia Tridentata : Asteraceae). IV . Reciprocal Transplant Experiments Published by : Society for the Study of Evolution Stable URL : Http://Www.Jstor.Org/Stable/2410963.” *Evolution* 51 (1): 95–102.

Weigl, Peter D. 1968. “The Distribution of the Flying Squirrels, Glaucomys Volans and Glaucomys Sabrinus.”

———. 2007. “The Northern Flying Squirrel (*Glaucomys Sabrinus*): A Conservation Challenge.” *Journal of Mammalogy* 88 (4): 897–907. https://doi.org/10.1644/06-MAMM-S-333RR.1.

Weigl, Peter D. 1978. “Resource Overlap , Interspecific Interactions and the Distribution of the Flying Squirrels, <i>Glaucomys Volans<i/> and <i>G. Sabrinus<i/>.” *American Midland Naturalist* 100 (1): 83–96.

Wells-Gosling, N., and L.R. Heaney. 1984. “Glaucomys Sabrinus.” *Mammalian Species*.

Wetzel, Eric J, and Peter D Weigl. 1994. “Ecological Implications for Flying Squirrels (*Glaucomys Spp.*) of Effects of Temperature on the in Vitro Development and Behavior of *Strongyloides Robustus*.” *American Midland Naturalist* 131 (1): 43–54. https://doi.org/10.2307/2426607.

Zweep, James S, Christopher N Jacques, Sean E Jenkins, Robert W Klaver, and Shelli A Dubay. 2018. “Nest Tree Use by Southern Flying Squirrels in Fragmented Midwestern Landscapes.” *Wildlife Society Bulletin* 42 (3): 430–37. https://doi.org/10.1002/wsb.901.

Appendix 3.1. Fecal flotation procedure

All fecal samples were processed using a modified McMaster Technique. The procedure outlined by Gorrell (pers. comm.) calls for 0.5g of feces, but given the variability in fecal size among squirrel species and the amount able to be collected, this mass was not always achievable. We used either half of a sample if enough feces was available, otherwise the entire sample was used. The following procedure was used:

1) Blot fecal sample dry using filter paper and take mass of dried sample.
2) Measure 10 mL of Sugar Sol fecal flotation solution.
3) Place feces in a strainer over top of a mortar and begin rinsing with 10 mL of solution.
4) Gently crush the sample with a pestle while continuing to rinse with remaining solution.
5) Use the last of the solution to rinse the pestle.
6) Place strainer over clean beaker and pour solution in mortar back through strainer.
7) Measure another 5 mL of solution.
8) Place 4 layers of cheesecloth in a new strainer over a clean beaker.
9) Rinse mortar with 5 mL of solution.
10) Strain solution from beaker (10mL) and mortar (5mL) through cheesecloth.
11) Gently swirl the beaker in circular motion.
12) Fill a pipette while continuing to stir the beaker.
13) Fill both chambers of the McMaster slide with solution and place on the microscope stage.
14) Wait 5 minutes for eggs to float to surface and view under 100X total magnification.

Appendix 3.2. Prevalence of other sciurid parasites

Table 1. Prevalence of endoparasite eggs determined through flotation of fecal pellets collected from chipmunks ($n = 70$), grey squirrels ($n = 43$), northern flying squirrels ($n = 18$), southern flying squirrels ($n = 41$), and red squirrels ($n = 13$) captured near Peterborough, Ontario between June - September 2019.

Species	**Prevalence (% individuals infested/species)**		
	Eimeria spp.	*Capillaria* spp.	*Hymenolepis* spp.
Northern flying squirrels (*Glaucomys sabrinus*)	100	11.1	-
Southern flying squirrels (*G. volans*)	95.1	4.9	-
Grey squirrels (*Sciurus carolinensis*)	95.3	4.7	-
Red squirrels (*Tamiasciurus hudsonicus*)	100	-	-
Eastern Chipmunks (*Tamias striatus*)	75.7	24.3	2.9

Appendix 3.3. Effect of other sciurid parasites on body condition

We found no difference in scale mass index (SMI) of southern flying squirrels and chipmunks infested with *Eimeria* spp. compared to those not infested (Fig. 1). Comparisons for northern flying squirrels and red squirrels could not be made as 100% of individuals were infested. Similarly, we found no difference in SMI of any species infested with *Capillaria spp.* compared to those not infested (Fig. 2). Chipmunks infested with *Hymenolepis* spp. appear to have low body condition compared to those not infested, however, these results were marginally insignificant (Fig. 3).

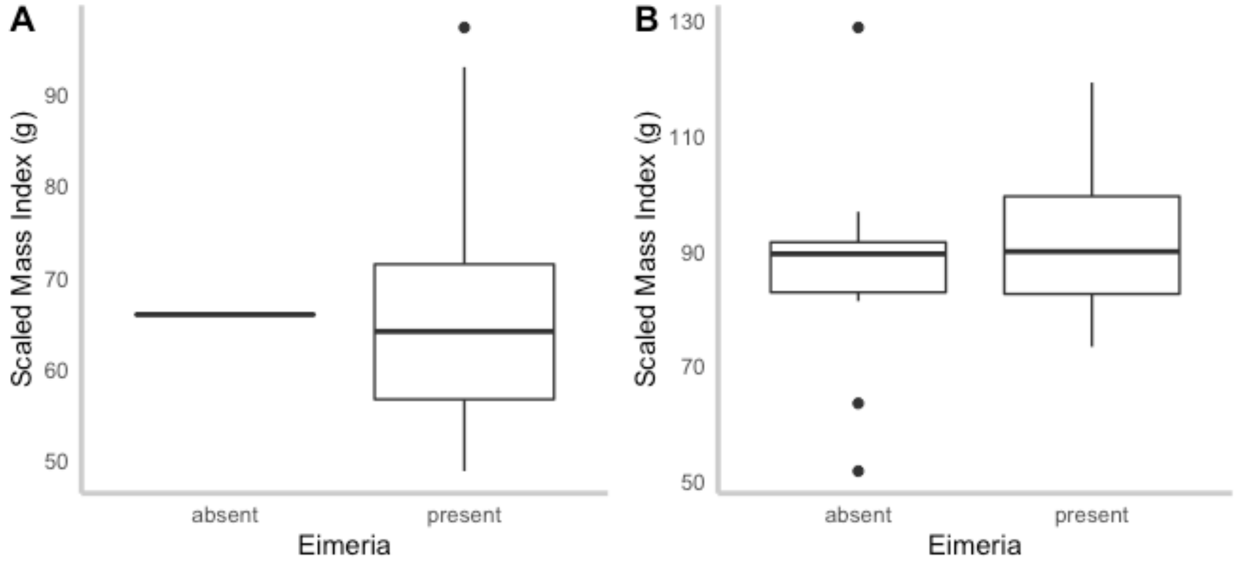


Figure 1. Body condition of **A** southern flying squirrels (*n* = 41, *W* = 22, *P* = 0.37) and **B** chipmunks (*n* = 70, *W* = 307, *P* = 0.78) infested with *Eimeria* spp. compared to those not infested. Body condition was calculated for squirrels captured near Peterborough, Ontario between June - September 2019.

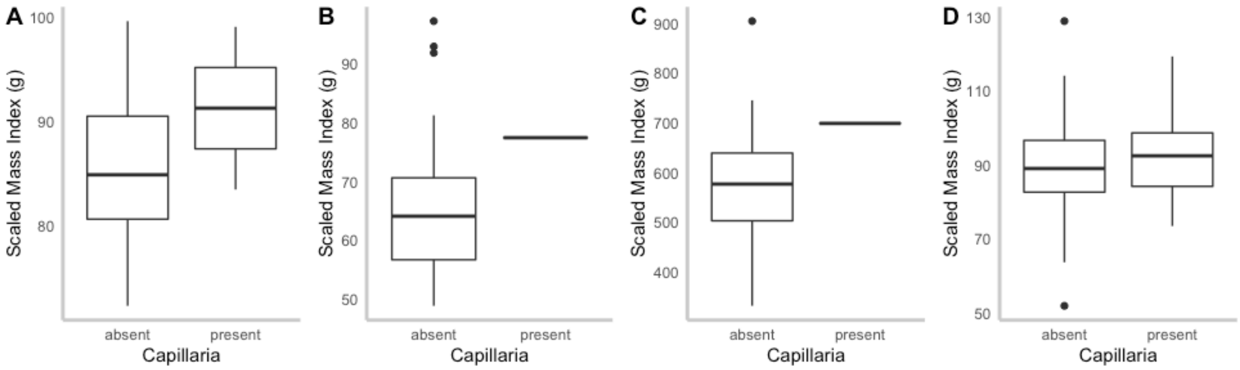


Figure 2. Body condition of **A** northern flying squirrels (*n* = 18, *W* = 10, *P* = 0.76), **B** southern flying squirrels (*n* = 41, *W* = 6, *P* = 0.88), **C** grey squirrels (*n* = 43, *W* = 4, *P* = 0.89), and **D** chipmunks (*n* = 70, *W* = 283, *P* = 0.85) infested with *Capillaria* spp. compared to those not infested. Body condition was calculated for squirrels captured near Peterborough, Ontario between June - September 2019.

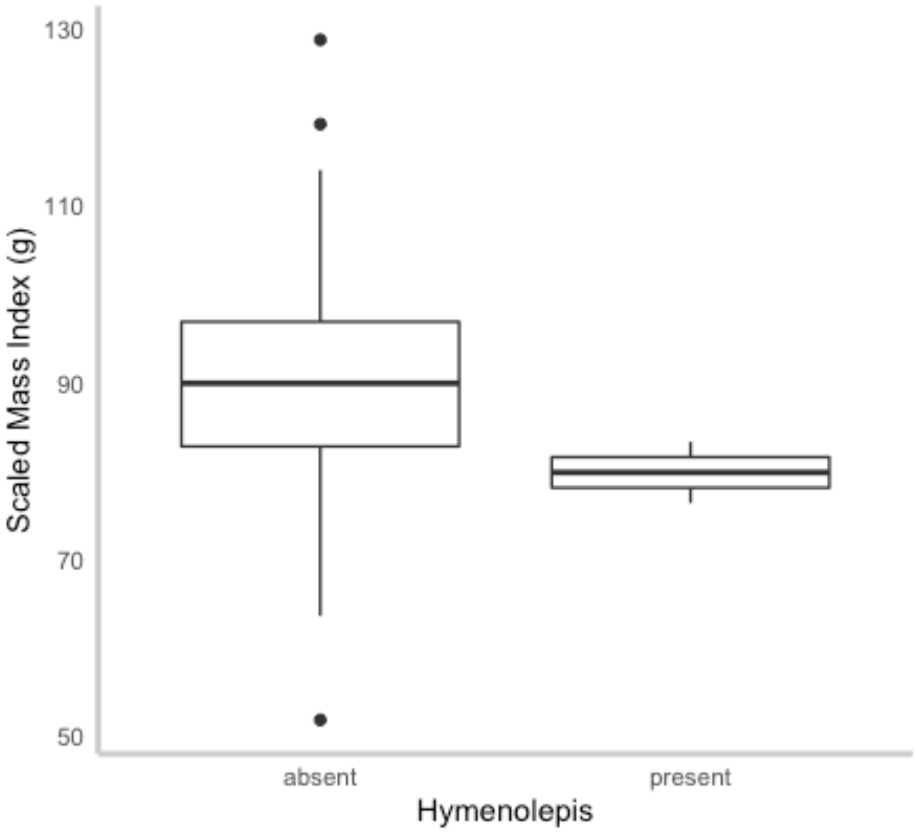


Figure 3. Body condition of chipmunks (*n* = 70) infested with *Hymenolepis* spp. (*W* = 98, *P* = 0.07) compared to those not infested. Body condition was calculated for squirrels captured near Peterborough, Ontario between June - September 2019.