Title: Phylogenetic community structure and stable isotope analysis of the parasitoid community associated with Eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae)

Authors

Christopher J. Greyson-Gaito[1], Sarah J. Dolson[1,2], Glen Forbes[3], Rosanna Lamb[3], Wayne E. MacKinnon[3], Kevin S. McCann[1], M. Alex Smith[1], Eldon S. Eveleigh[3,4]

*Corresponding Author - christopher@greyson-gaito.com

Affiliations

1. Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

2. Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada

3. Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, New Brunswick, E3B 5P7, Canada

4. Population Ecology Group, Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, E3B 5A3, Canada

ORCID

CJGG – 0000-0001-8716-0290

SJD – 0000-0001-9312-2282

KSM - 0000-0001-6031-7913

MAS – 0000-0002-8650-2575

ESE - 0000-0001-5060-8565

Abstract

1. Eastern spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), is a major pest of eastern North American forests. Outbreaks of spruce budworm occur every 30-40 years, causing high tree mortality.

2. Researchers have established that higher proportions of hardwood trees within stands (higher hardwood content) may reduce the defoliation and mortality of balsam fir and spruces during spruce budworm outbreaks. One mechanism posited to explain these patterns is that hardwood trees positively impacts the parasitoids of spruce budworm. Indeed, parasitism of spruce budworm by parasitoids has been found to be impacted by hardwood content. However, more research is needed to understand how hardwood content impacts the parasitoid community as a whole.

3. In this study, we trialled the use of two analyses, phylogenetic community structure and stable isotope analysis, to examine how hardwood content influenced the parasitoid community associated with spruce budworm.

4. We found that phylogenetic community structure differed between forest stands with different hardwood content. Furthermore, the trophic relationships between several parasitoids and caterpillars on balsam fir or hardwood trees changed within and between years.

5. Our study highlights the potential of these two analyses for understanding how hardwood content influences the parasitoid community associated with spruce budworm.

Keywords

Choristoneura fumiferana, *Abies balsamea*, hardwood, parasitoids, phylogenetic community structure, stable isotopes, trophic relationships

Introduction

Every 30–40 years, Eastern spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), have massive outbreaks in eastern North American forests (Royama *et al.*, 2017). These outbreaks last about 5-15 years, severely defoliating balsam fir and spruce trees and causing high growth loss and tree mortality (Hennigar *et al.*, 2008). Spruce budworm outbreaks have been known to damage millions of hectares of North American forests per outbreak and have large impacts on the forestry sector (Chang *et al.*, 2012). Consequently, finding methods to reduce the severity of spruce budworm outbreaks is important to maximize forestry economic activity while minimizing losses of balsam fir and species of spruce.

Hardwood trees have long been thought to reduce the severity of spruce budworm outbreaks. Since the 1920s, the importance of tree diversity to spruce budworm control has been periodically brought up (Miller & Rusnock, 1993). More recently, researchers have evaluated how the proportion of hardwood trees within stands (hardwood content) impacts the growth, defoliation, and mortality of balsam fir and spruces. Research on balsam fir growth found that spruce budworm-caused growth reductions of balsam fir during the 1972–1992 outbreak was significantly mitigated by hardwood content (Campbell, MacLean & Bergeron, 2008). Research on balsam fir defoliation found defoliation was lower in mixed forest stands containing hardwood trees compared to balsam fir dominated stands during spruce budworm outbreaks (Su, Needham & MacLean, 1996; Zhang *et al.*, 2018, 2020). In contrast, MacKinnon & MacLean (2003) found no effect of surrounding forest type on spruce budworm defoliation of balsam fir. Instead, MacKinnon & MacLean (2003) found that spruce budworm defoliation of white spruce was reduced in stands surrounded by mixed wood forest. Finally, research on balsam fir mortality found mortality due to spruce budworm defoliation was greater in extensive conifer stands than fir stands surrounded by deciduous forest or on islands in the middle of a lake (Cappuccino *et al.*, 1998). Researchers have also tested the effect of hardwood content on spruce budworm abundances and densities. Quayle *et al.* (2003) found that relative basal area of non-host tree species had a significant negative effect on the abundance of spruce budworm and Eveleigh *et al.* (2007) found lower outbreak peak spruce budworm densities in heterogeneous plots compared to homogeneous plots. Overall, the evidence points to a complicated yet important impact of hardwood content on spruce budworm outbreaks.

One proposed mechanism behind hardwood content impacting spruce budworm outbreaks is that hardwood content affects the community of insects that parasitize and then kill spruce budworm caterpillars (parasitoids). Among the natural enemies of spruce budworm, parasitoids have arguably the strongest impact on spruce budworm mortality causing between 30-90% mortality depending on the surrounding forest composition and the point in the spruce budworm cycle (Cappuccino *et al.*, 1998; Royama *et al.*, 2017). Several researchers, examining how hardwood content impacts the parasitism of spruce budworm, have found that, depending on the parasitoid species, there was either no effect of tree composition or an increase in parasitism with higher diversity of trees (Simmons, Leonard & Chen, 1975; Kemp & Simmons, 1978; Quayle *et al.*, 2003; Legault & James, 2018). However, these studies have examined parasitoid species individually. An important further research direction is how hardwood content influences the parasitoid community as a whole. Currently, we know the parasitoid community responds strongly to spruce budworm density with increases in diversity cascading up parasitoid trophic levels (the bird feeder effect) (Eveleigh *et al.*, 2007) and the parasitoid community responds largely indiscriminately to changing spruce budworm and other caterpillar abundances on balsam fir (Greyson-Gaito *et al.*, 2021). Indeed in an initial survey, Eveleigh *et al.* (2007) did find increased diversity and abundance of primary parasitoids in plots with greater proportions of hardwood trees. Marrec *et al.* (2018)also found that variation in spruce budworm parasitoid community composition was mostly explained by surrounding forest structure. Eveleigh *et al.'s* (2007) and Marrec *et al.'s* (2018) research show that examining how hardwood content influences the parasitoid community as a whole is a useful endeavour.

Analysing phylogenetic community structure could be useful in examining how hardwood content impacts the parasitoid community associated with spruce budworm. Phylogenetic community structure is defined as the nonrandom patterns of evolutionary relatedness between species in a community (Kraft *et al.*, 2007). These nonrandom patterns can be produced from the interaction of ecological processes, including habitat filtering and competitive exclusion, with the evolutionary history of species (i.e. how closely related are different species). With the assumption that closely related species have higher competition than distantly related species, the ecological processes can be inferred from the phylogenetic community structures found when sampling communities. Researchers test for three phylogenetic community structures: phylogenetic clustering, where communities are made up of closely related species; overdispersion, where communities are made up of distantly related species; and neither clustering nor overdispersion (Webb *et al.*, 2002) (Figure 1a). Clustering indicates that the habitat is filtering conserved traits within the species pool. In contrast, overdispersion can indicate either closely related species competitively excluding each other or distantly related species converging on similar niches. Finding neither clustering nor overdispersion generally indicates distantly related species with convergent traits are competitively excluding each other (Webb *et al.*, 2002). Overall, including the evolutionary history of species can illuminate fundamental processes behind the assembly of communities leading to key insights into how the community functions (Kembel & Hubbell, 2006; Ricklefs, 2006). Similarly, for the spruce budworm – parasitoid system including the evolutionary history of the parasitoids can help us to identify how hardwood content might be influencing the parasitoid community associated with spruce budworm leading to insights into how to use the parasitoid community to reduce the severity of spruce budworm outbreaks.

Another analysis, stable isotope analysis, could similarly be useful for examining how hardwood content impacts the parasitoid community associated with spruce budworm. Stable isotope analysis aims to identify trophic relationships (Boecklen *et al.*, 2011) and involves measuring the ratio of heavy to light isotopes of different chemical elements (often carbon and nitrogen). In fact, the ratio of heavy to light carbon isotopes in a consumer will be similar to that of the consumer's diet and the ratio of heavy to light nitrogen isotopes increases at each level of a trophic food chain (Figure 1b). From this information, a food web of the different organisms measured can be elucidated (Boecklen *et al.*, 2011). Furthermore using carbon isotopes, researchers can examine whether the consumers feed on multiple resource compartments (food chains) within the food web, otherwise called coupling (McMeans *et al.*, 2016). Importantly for this study, the ratio of heavy to light carbon isotopes differs between softwood and hardwood trees (Brooks *et al.*, 1997; Risk, Kellman & Moroni, 2009). This difference is consistent even with environmental fluctuations and between different locations (Brooks *et al.*, 1997). Thus, if we measure the stable isotopes of parasitoids, we can essentially measure the relative attack rates of parasitoids on caterpillars, either spruce budworm or other species, on the softwood and hardwood resource compartments. Hardwood content likely influences these relative attack rates. Thus, stable isotope analysis can be used to examine the extent of coupling by parasitoids of the softwood and hardwood resource compartments.

In this study, we illustrate how phylogenetic community structure and stable isotope analyses could be used to examine the impact of hardwood content on the parasitoid community associated with spruce budworm. We provide some preliminary findings from these analyses. Specifically, using Malaise caught parasitoids from years where spruce budworm were at low density and reared parasitoids from years where spruce budworm were at high density, we tested whether the phylogenetic community structure differed along a hardwood gradient. Second, using stable isotope analysis of Malaise caught parasitoids sampled immediately prior to and after a spruce budworm outbreak peak, we identified how trophic relationships between parasitoids and caterpillars on balsam firs and on hardwood trees changed within and between years. Our preliminary findings indicate that hardwood content does impact the parasitoid phylogenetic community structure, and the utilization of caterpillars on balsam fir or hardwood trees changes, depending on the parasitoid, within and between years.

Methods

Phylogenetic community structure along a hardwood gradient

Low density spruce budworm

Sampling

Sampling was done in the Acadia Research Forest (ARF) near Fredericton (66^{0}25'W, 46^{0}00'N). The ARF is a 9,000 ha (22,230 ac) experimental forest with a mixture of softwood, hardwood, and mixed wood stands (Figure 2). Spruce (*Picea* spp.) and balsam fir (*Abies balsamea* (L.) Mill.) are the most abundant trees (Swift *et al.*, 2006).

All plots sampled in this study were outside areas of aerial application of insecticides for spruce budworm control. In 2014, nine 150 metre by 120 metre plots were selected, where three were balsam fir dominated (70% balsam fir), three were hardwood tree dominated (75% hardwood), and three had an even mixture of balsam fir and hardwood trees (40-60% balsam fir) (Figure 2). The nine plots were chosen using a forest cover map provided by the ARF, lidar maps, and ground truthing. In 2016, five balsam fir trees, at least 20 metres apart and with healthy crowns, were chosen within each plot in the ARF (45 trees total). In April of 2016, 2,000 2nd instar spruce budworm individuals were placed onto each of the 45 trees. Spruce budworm were implanted to effectively recreate the birdfeeder effect found in Eveleigh *et al.* (2007) and assess the parasitoid community associated with spruce budworm but now with low densities of spruce budworm. Spruce budworm individuals were reared by Insect Production Services (IPS) at the Great Lakes Forestry Centre in Sault St Marie, Ontario on a bed of gauze, which were cut up into squares of about 250 caterpillars (Roe, Demidovich & Dedes, 2018). We placed a total of eight squares on each of the 45 trees, with each square being pinned to the underside of single branch in the mid-crown layer that had new growth. Then to examine the spruce budworm-associated parasitoid community between these three types of stands, on May 19th 2016 we placed a Malaise trap in every plot chosen above close to one of the trees where spruce budworm individuals were implanted. The Malaise traps were taken down on August 11th 2016. The flying insects from the Malaise traps were sampled once a week during May and June, and once a month during July and August. We separated out individuals belonging to insect families that we knew contained species that attack spruce budworm. These families included, but were not limited to, Tachinidae, Sarcophagidae, Braconidae, and Ichneumonidae. We stored the collected parasitoids in 70% ethanol and in a refrigerator at 4°C, until they were barcoded. Note, we use the term spruce budworm-associated parasitoid community to acknowledge that although Malaise sampling will capture parasitoids attracted to the implanted spruce budworm, the Malaise sampling will also capture hyperparasitoids and other parasitoids that do not attack spruce budworm.

To examine phylogenetic community structure, we used DNA barcoding where a region of an organism's DNA is sequenced and compared to the same region in other organisms (Ratnasingham & Hebert, 2007). Tissue samples were taken using 1-6 legs and placed in 30 µL of 95% ethanol and stored at -20°C. Mitochondrial DNA from the cytochrome *c* oxidase I (COI) region (the standard animal DNA barcode locus) was amplified and sequenced at the Biodiversity Institute of Ontario (BIO; University of Guelph, Ontario). High resolution photographs were taken of wet specimens under a dissecting microscope using Leica Application Software V4.9. Sequences and photographs were uploaded to the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007). We used Barcode Index Numbers (BINs), a DNA-based delineation of species based on patterns of intra and interspecies variations outlined by Ratnasingham & Hebert (2013), to identify species using the BOLD database. We constructed a single-representative maximum likelihood tree in MEGA6 based on estimation of the best substitution models in MEGA6 (Nei & Kumar, 2000; Tamura *et al.*, 2013).

Statistical Analyses

To examine how hardwood content affected the phylogenetic community structure of spruce budworm-associated parasitoids, we calculated the mean nearest taxon distance (MNTD) using maximum likelihood trees between the three forest types for the Malaise caught parasitoids. Maximum likelihood trees used a general time reversible model with discrete gamma distribution under the assumption that sites were evolutionarily invariable (Nei & Kumar, 2000; Tamura *et al.*, 2013). The standard effect size of the MNTD was then calculated and phylogenetic clustering and dispersion assessed by performing 999 random permutations of hardwood content associations to simulate a distribution of MNTD for each community. The significance of the observed MNTD values for each community was examined with a two-tailed test of significance (p = 0.05) (function ses.mntd, R package Picante, version 1.7, (Kembel *et al.*, 2010)).

High density spruce budworm

Sampling

In the 1980s and 1990s when spruce budworm had high densities, three plots of approximately one hectare were established in balsam fir forests in New Brunswick, Canada. Plots 1 and 2 were in the ARF (Figure 2). Plot 3 was located approximately 170km north of plots 1 and 2, near Saint-Quentin (47^{0}29'N, 67^{0}15'W, see Figure 2). The tree basal area of these three plots were as follows: Plot 1, balsam fir 98%, Spruce 1%, Hardwood 1%; Plot 2, balsam fir 77%, spruce 8%, hardwood 14%; Plot 3, balsam fir 50%, spruce 36%, hardwood 14%. For further details of the three plots and all sampling and rearing procedures, see Lucarotti *et al.* (2004), Eveleigh *et al.* (2007) (SI Materials and Methods) and Royama *et al.* (2017). Twenty whole, nodal, mid-crown balsam fir branches from each plot were collected once prior to spruce budworm larval emergence from winter diapause and approximately daily thereafter until adult eclosion. Parasitoids were reared from both spruce budworm and other caterpillar species found on the sampled balsam fir branches. From this collection of parasitoids, Eveleigh *et al.* (2007) compared the richness of reared parasitoids between the three plots. A subset of these parasitoid species were preserved at -20ºC then DNA barcoded to explore how genetic estimates of isolation and species identification changed the estimates of food web connectance (connectance was reduced as the number of nodes increased) (Smith *et al.*, 2011). However, Smith *et al.* (2011) did not report estimates of phylogenetic community structure for the parasitoids of these three plots, and so in this study we add an examination of the phylogenetic community structure of parasitoids sampled in the 1980s when spruce budworm were at high density and compare with the phylogenetic clustering of parasitoids sampled along a hardwood gradient in 2016 when spruce budworm were at low density.

Statistical Analyses

We calculated the mean nearest taxon distance (MNTD) and assessed phylogenetic clustering and dispersion (function ses.mntd, R package Picante, version 1.7, (Kembel *et al.*, 2010)) of reared parasitoids collected from the three plots in Eveleigh et al. (2007).

Stable isotope analysis of parasitoid community trophic relationships

Sampling

All parasitoid sampling was performed in a single balsam fir dominated plot in ARF for the years of 1982, 1983, 1986, and 1987 (in this plot, spruce budworm peaked in 1985).

Because this analysis used historical parasitoid sampling, the sampling was limited to the single plot and time points from the original study. This plot was 98% *Abies balsamea*, 1% *Picea rubens* Sarg., and 1% *Acer rubrum* L. by basal area (Lethiecq & Regniere, 1988). Parasitoids were collected using modified 1 m^3 Malaise traps (Nyrop & Simmons, 1982). A Malaise trap was placed with the open sides perpendicular to the tree trunk at the top, middle, and lower crown levels of three balsam fir trees separated by approximately 100 metres (i.e. 3 traps at each crown level, 9 traps in total). The Malaise traps were placed in the same trees every year beginning in May and ending in September. Flying insects were collected daily, immediately stored in 70% ethanol, and frozen at -7°C until preparation for stable isotope analysis in 2017 (except insects collected in 1982 which were stored without ethanol but still in the freezer).

In 2017, as an initial attempt to understand how parasitoids with different life cycles utilize caterpillars, either spruce budworm or other species, on balsam fir and hardwood trees, we separated the 1980s Malaise caught parasitoids into three groups (see Table S1): Group 1, univoltine parasitoid species that attack one caterpillar species within a year and do not require an alternate caterpillar in which to overwinter (Elliott, Simmons & Sapio, 1987; O’Hara, 2005); Group 2, multivoltine parasitoid species that overwinter away from a host or where overwintering status was unknown; and Group 3, multivoltine parasitoid species that require an alternate caterpillar in which to overwinter (Thireau & Régnière, 1995; O’Hara, 2005). All parasitoid species are common parasitoids of spruce budworm. These parasitoid species are capable of attacking multiple caterpillar species but differ in the frequency and life cycles of attacking spruce budworm and other caterpillar species. The parasitoids had previously been identified using representative specimens provided by taxonomists from the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC). These three groups were then further split into three periods to capture the phenology of the parasitoid emergences from spruce budworm and other caterpillar species: May/June, July, and August/September. When there were fewer than 50 total individuals in a group and sampling period, all individuals were used for stable isotope analysis. When there were more than 50 total individuals in a group and sampling period, we randomly selected 50 individuals and ensured the proportions of selected individuals of each species matched the proportions of total number of individuals for each species (within the group and sampling period). We removed legs and wings from all individuals, keeping the mass of legs and wings approximately constant between individuals and species. Legs and wings were combined for each group and sampling period and were dried at 60°C for at least 48 hours. We used legs and wings because many parasitoids as adults consume non-host nutrient sources, and legs and wings have a slower turnover rate compared to other body parts (Gratton & Forbes, 2006; Benelli *et al.*, 2017)

In stable isotope analysis, carbon and nitrogen stable isotopes are measured in samples from resources at the bottom of the food chain (basal resources) and from intermediate consumers of each resource compartment (food chain) (see Figure 1b). From these measurements, called baselines, researchers can deduce the trophic relationships of the focal organisms. In this study, balsam fir plus its inhabitant caterpillars and hardwood trees plus their inhabitant caterpillars were the two resource compartments. Thus, our baselines consisted of balsam fir and hardwood foliage, and caterpillars from these sampled foliage. In 2017 beginning on May 30th and ending on June 27th, once a week we sampled one metre long, mid-canopy branch from 5 balsam fir trees in each of the nine plots used to study the phylogenetic community structure (one branch per tree, five trees per plot, 45 branches per week). Each week, we also sampled one metre long branch from multiple hardwood tree species in each plot. These multiple hardwood species were the most abundant in each plot as found by the original plot ground truthing. On the 17th July and on the 4th August, we randomly sampled a single balsam fir branch from each plot, and we sampled branches from the same hardwood species as we sampled in June (a branch per species in each plot). We sampled foliage without any noticeable herbivory damage from all branches. This foliage was rinsed with distilled water and dried at 60°C for at least 48 hours. We ground the foliage and ensured that the combination of different hardwood species in each plot's ground sample matched the proportions of hardwood trees found in each plot. This was repeated for June, July and August. From the balsam fir branches and the hardwood branches, we collected all caterpillar individuals and separated them into caterpillars from balsam fir or hardwoods and by plot and by sampling period. The caterpillar samples were dried at 60°C for at least 48 hours. All parasitoid, caterpillar and foliage samples were analyzed for carbon and nitrogen isotope ratios at the University of Windsor GLIER (Windsor, ON, Canada) laboratories.

Statistical Analyses

Normal practice when using stable isotopes is to use mixing models, where both δ13C and δ15N are included to establish the trophic levels and percentage of diet from multiple resource pathways (Phillips *et al.*, 2014). However, the δ13C of the parasitoid samples were enriched by 16% compared to the foliage and caterpillar baselines probably because the parasitoid samples were stored in ethanol and frozen for about 30 years whereas the foliage and caterpillars were sampled in 2017 (Jesus *et al.*, 2015). Because mixing models are unable to account for this enrichment, we were not able to use mixing model analyses with both δ13C and δ15N. Instead, we used δ13C only by comparing δ13C between years, sampling periods, and groups because we knew that there were consistent differences in δ13C between hardwood and softwoods which were transferred to the caterpillars (Balsam fir and hardwood foliage Welch t-test: t = 2.813, df = 40.219, P = 0.00756. Balsam fir caterpillars and hardwood caterpillars Welch t-test: t = 3.161, df = 39.161, P = 0.00303). Note, from the three sampling periods above (May/June, July, August/September), we simplified the periods into two sampling periods, May/June and July/August/September, by averaging the δ13C values of the July and August/September periods. These two sampling periods were chosen to coincide with when spruce budworm were larvae (approximately May/June) and when they were moths/eggs/L1 (approximately July/August/September). We ran a generalized least squares regression to test the effects of year, sampling period (May/June or July/August/September), parasitoid group, and all interactions on the δ13C of sampled parasitoid legs and wings (function gls, R package nlme, version 3.1-137, (Pinheiro *et al.*, 2018)). We added a varIdent variance structure to account for the different variation in the residuals between the sampling periods. We fitted the full model using maximum likelihood estimation and then used backwards selection with log likelihood ratio tests to select the final fixed effects. We refitted the final model using restricted maximum likelihood estimation to give unbiased maximum likelihood predictors (Zuur *et al.*, 2009) .

Results

Phylogenetic community structure along a hardwood gradient

Low density spruce budworm

Phylogenetic clustering was found in the balsam fir dominated plots with Malaise caught parasitoids from 2016 (Balsam Fir: MNTD z =-2.502, P = 0.009. Figure 3a). Neither phylogenetic clustering nor dispersion were found in the mixed forest plots and the hardwood dominated plots with Malaise caught parasitoids from 2016 (Mixed: MNTD z = 1.135, P = 0.877. Hardwood: MNTD z = -1.368, P = 0.087. Figure 3a).

High density spruce budworm

Phylogenetic clustering was found (marginally significant) in Plot 1 from the 1980s (MNTD z = -1.601, p = 0.055, Figure 3b). Neither phylogenetic clustering nor dispersion were found in the two other plots from the 1980s (Plot 2: MNTD z = -1.497, p = 0.075. Plot 3: MNTD z = -0.518, p = 0.303. Figure 3b).

Stable isotope analysis of parasitoid community trophic relationships

The final model explaining δ13C included year, group, sampling period (May/June or July/August/September), and the interactions of year with group (year: group interaction, L = 13.230, P = 0.0013, df = 1, log likelihood ratio test, Figure 4) and group with sampling period (group: sampling period interaction, L = 28.900, P < 0.0001, df = 1, log likelihood ratio test, Figure 4, see Table 1 for ANOVA output of model). Group one parasitoids became slightly more negative by approximately 0.5% each year, and group one parasitoids caught when spruce budworm were absent had more negative δ13C values by 2.4% compared to group one parasitoids caught when in May/June. δ13C values for group two parasitoids became less negative overtime by approximately 1.6% each year. Group three parasitoids showed a difference of 12.2% in δ13C between May/June and July/August/September. In May/June, group three parasitoids had more negative δ13C values. In July/August/September, group three parasitoids had less negative δ13C values. In comparison to the difference in δ13C between May/June and July/August/September, δ13C for group three parasitoids changed little with no noticeable trend between years.

Discussion

We trialled the use of phylogenetic community structure and stable isotope analyses to illustrate their potential in spruce budworm research. Using Malaise caught and reared parasitoids, the phylogenetic community structure of the parasitoid community was consistently clustered in balsam fir dominated plots when spruce budworm were at low and high density. From comparing the stable isotopes of parasitoids during a spruce budworm outbreak, we found that several parasitoids changed their attack rates between caterpillars, including spruce budworm, on balsam fir and caterpillars on hardwoods within and between years. Taken together, our study highlights the potential for these analyses to illuminate how hardwood content could impact the severity of spruce budworm outbreaks through affecting the parasitoid community.

The hardwood content of the stands did appear to impact the spruce budworm-associated parasitoid phylogenetic community structure. We found that the balsam fir dominated plots exhibited phylogenetic clustering in 2016 when spruce budworm were at low densities and in the 1980s when spruce budworm were high densities. Clustered phylogenies indicate that habitat filtering is a major factor determining the community composition (Webb *et al.*, 2002). Because closely related parasitoid species are more likely to share host species or search within the same plant species than distantly related parasitoid species (Ives & Godfray, 2006), we speculate that the habitat filtering is likely due to the differences in caterpillar composition maintained by balsam fir dominated stands compared to stands with greater hardwood content (Summerville & Crist, 2008). Potentially, balsam fir dominated plots host a subset of caterpillar species thus filtering closely related parasitoid species. Similarly, Marrec *et al.* (2018) found environmental (habitat) filtering to be important in shaping spruce budworm parasitoid communities. One caveat to our habitat filtering pattern is that our sampling does not differentiate between primary parasitoids and hyperparasitoids. Because hyperparasitoids may be key in driving spruce budworm outbreaks (Nenzén, Martel &

Gravel, 2018), examining the differential impacts of hardwood content on primary parasitoids and hyperparasitoids is critical. Overall, our phylogenetic community structure analysis indicates that hardwood content likely impacts the spruce budworm-associated parasitoid community through influencing the caterpillar communities. Further research should extensively sample caterpillar communities on all tree types along a hardwood gradient as well as sample and differentiate between primary parasitoids and hyperparasitoids.

Our preliminary stable isotope analysis found that our three groups of parasitoids differed in how they utilized caterpillars on balsam fir and hardwood trees within and between years. The parasitoids that within a single year must attack caterpillars at the beginning of the summer, usually spruce budworm on softwoods, and then overwinter in other caterpillar species usually on hardwoods (group three) provide us with the clearest comparison of trophic relationships between balsam fir and hardwood. The δ13C of group three parasitoids sampled in May/June was more negative than the δ13C of group three parasitoids sampled in July/August/September. Our sampled hardwood foliage was similarly more negative in δ13C compared to our sampled balsam fir foliage (hardwood foliage = -30.222 δ13C, balsam fir foliage = -29.521 δ13C). This correspondence of the differences between group three in the two sampling periods and the differences in balsam fir and hardwood δ13C matches what we know of the life history of group three parasitoids because, in May/June, group three parasitoids emerge from other caterpillar species often on hardwood trees to attack caterpillars, usually spruce budworm on balsam firs and other softwoods. Then in July (within the July/August/September sampling period), group three parasitoids emerge from these caterpillars to attack other caterpillars often on hardwoods. Therefore, we suggest any comparable changes in δ13C for the other groups should be due to the parasitoids changing their attack rates on caterpillars, including spruce budworm, on balsam fir and other caterpillar species on hardwoods.

The parasitoids that attack one caterpillar species within a year (group one) seemingly did not change their relative utilization of caterpillars, either spruce budworm or other species, on balsam fir and caterpillars on hardwoods within a year nor between years. Group one parasitoids not changing relative utilization within a year is unsurprising because these parasitoids are univoltine. Group one parasitoids not changing utilization between years as spruce budworm densities change is consistent with other studies that concluded that these parasitoids attack spruce budworm more than other caterpillar species (O'Hara, 2005; Cossentine *et al.*, 2007). Furthermore, the populations of group one parasitoids are supported by other caterpillar species that feed on balsam fir as suggested by *Apanteles fumiferanae* Vier. (Hymenoptera: Braconidae) and *Glypta fumiferanae* Vier. (Hymenoptera: Ichneumonidae) attacking other caterpillar species on balsam fir (Greyson-Gaito *et al.*, 2021). In contrast to group one parasitoids, the multivoltine parasitoid species that overwinter away from a host or where overwintering status was unknown (group two) exhibited greater change in δ13C between years, from more to less negative, suggesting that these parasitoids likely attacked caterpillars on hardwoods when spruce budworm had lower densities and then attacked spruce budworm (or other caterpillar species) on balsam fir when spruce budworm had higher densities. Overall, there are indications that certain parasitoids may be coupling the softwood and hardwood resource compartments within and between years. However, increased resolution of this stable isotope analysis is required and we encourage future researchers to measure the stable isotopes of individual parasitoid, caterpillar, and tree species within a year and between years. We also recommend that researchers include understory plants as stable isotope baselines because parasitoids gain nutrients from non-host sources including nectar from understory plants (Benelli *et al.*, 2017) and caterpillars consume understory plants (Seifert *et al.*, 2020).

Two techniques that would complement stable isotope analysis for examining how parasitoid utilize caterpillars on softwoods and hardwoods are fatty acid analysis and the quantitative polymerase chain reaction (qPCR) TaqMan assay. Fatty acid analysis has the same overall goal of stable isotope analysis because fatty acid compositions often differ between different resources and these differences get passed onto any consumers. Indeed, fatty acid compositions differ between softwoods and hardwoods more than δ13C (Mueller *et al.*, 2012) and thus fatty acid analysis could be powerful to unpack the trophic relationships of the spruce budworm-associated parasitoids. The qPCR TaqMan assay can be used to identify individual species from bulk samples with high accuracy. In the spruce budworm system, a qPCR TaqMan assay has been created to determine whether and by what a spruce budworm larva has been parasitized (Nisole *et al.*, 2020). So far this method is limited to 20 common natural enemies of spruce budworm as a compromise between time/costs and broad applicability. Yet, this assay has great potential to quickly identify a parasitoid species attacking spruce budworm sampled from the field. This assay similarly has great potential to be used to quantify the relative attack rates of parasitoids on spruce budworm and other caterpillar species. Thus, we suggest that DNA libraries of spruce budworm parasitoids be expanded to include representation from hardwood forest parasitoid communities. Overall, comprehensive sampling of parasitoids and caterpillars on softwoods and hardwoods throughout the spruce budworm cycle is required to evaluate the trophic relationships between parasitoids and the caterpillars on softwood and hardwood trees. Stable isotope analysis, fatty acid analysis and qPCR would all be highly complementary techniques.

Hardwood trees in forest stands have long been thought to be important to reducing the severity of spruce budworm outbreaks. Key to reducing the severity of outbreaks could be hardwood trees impacting the abundances and composition of the parasitoids of spruce budworm. In this study, we have highlighted two useful analyses that we encourage spruce budworm researchers use to examine how hardwood content impacts the spruce budworm-associated parasitoid community: phylogenetic community structure analysis and stable isotope analysis. Our preliminary exploration using these analyses found that hardwood content influenced the phylogenetic structure of parasitoid communities and several parasitoids change their relative utilization of caterpillars on balsam fir and hardwoods within and between years. Taken together, we have shown some potential uses of the phylogenetic community structure and stable isotope analyses with some preliminary findings that point to the important influence of hardwood content on the spruce budworm-associated parasitoid community.

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Author contributions

ESE designed the initial studies. ESE, WM, GF, RL, CJGG, and SJD did the field and laboratory work. CJGG did the statistical analyses with assistance from ESE, MAS, SJD, and KSM. CJGG wrote the first draft and all authors contributed to editing the manuscript.

Data accessibility

All sequences and photographs are publically available on BOLD. All data and code (v3.0) to reproduce the reported results are publicly available on GitHub and have been archived on Zenodo.

References

Benelli, G., Giunti, G., Tena, A., Desneux, N., Caselli, A. & Canale, A. (2017) The impact of adult diet on parasitoid reproductive performance, *Journal of Pest Science*, **90**, 807–823.

Boecklen, W.J., Yarnes, C.T., Cook, B.A. & James, A.C. (2011) On the use of stable isotopes in trophic ecology, *Annual Review of Ecology, Evolution, and Systematics*, **42**, 411–440.

Brooks, J.R., Flanagan, L.B., Buchmann, N. & Ehleringer, J.R. (1997) Carbon isotope composition of boreal plants: functional grouping of life forms, *Oecologia*, **110**, 301–311.

Campbell, E.M., MacLean, D.A. & Bergeron, Y. (2008) The severity of budworm-caused growth reductions in balsam fir/spruce stands varies with the hardwood content of surrounding forest landscapes, *Forest Science*, **54**, 195–205.

Cappuccino, N., Lavertu, D., Bergeron, Y. & Régnière, J. (1998) Spruce budworm impact, abundance and parasitism rate in a patchy landscape, *Oecologia*, **114**, 236–242.

Chang, W.-Y., Lantz, V.A., Hennigar, C.R. & MacLean, D.A. (2012) Economic impacts of forest pests: a case study of spruce budworm outbreaks and control in New Brunswick, Canada, *Canadian Journal of Forest Research*, **42**, 490–505.

Cossentine, J., Bennett, A., Goulet, H. & O'Hara, J. (2007) Parasitism of the spring leafroller (Lepidoptera: Tortricidae) complex in organically managed apple orchards in the north Okanagan valley of British Columbia, *The Pan-Pacific Entomologist*, **83**, 276–284.

Elliott, N.C., Simmons, G.A. & Sapio, F.J. (1987) Honeydew and wildflowers as food for the parasites *Glypta fumiferanae* (Hymenoptera: Ichneumonidae) and *Apanteles fumiferanae* (Hymenoptera: Braconidae), *Journal of the Kansas Entomological Society*, 25–29.

Eveleigh, E.S., McCann, K.S., McCarthy, P.C., *et al.* (2007) Fluctuations in density of an outbreak species drive diversity cascades in food webs, *Proceedings of the National Academy of Sciences*, **104**, 16976–16981.

Gratton, C. & Forbes, A.E. (2006) Changes in δ13C stable isotopes in multiple tissues of insect predators fed isotopically distinct prey, *Oecologia*, **147**, 615–624.

Greyson-Gaito, C.J., McCann, K.S., Fruend, J., Lucarotti, C.J., Smith, M.A. & Eveleigh, E.S. (2021) Parasitoid community responds indiscriminately to fluctuating spruce budworm and other caterpillars on balsam fir., *The Canadian Entomologist*, 1–15. doi:10.4039/tce.2021.14.

Hennigar, C.R., MacLean, D.A., Quiring, D.T. & Kershaw, J.A. (2008) Differences in spruce budworm defoliation among balsam fir and white, red, and black spruce, *Forest Science*, **54**, 158–166.

Ives, A.R. & Godfray, H.C.J. (2006) Phylogenetic analysis of trophic associations, *The American Naturalist*, **168**, E1–E14.

Jesus, F.M., Pereira, M.R., Rosa, C.S., Moreira, M.Z. & Sperber, C.F. (2015) Preservation methods alter carbon and nitrogen stable isotope values in crickets (Orthoptera: Grylloidea), *PLOS ONE*, **10**, e0137650.

Kembel, S.W., Cowan, P.D., Helmus, M.R., *et al.* (2010) Picante: R tools for integrating phylogenies and ecology, *Bioinformatics*, **26**, 1463–1464.

Kembel, S.W. & Hubbell, S.P. (2006) The phylogenetic structure of a neotropical forest tree community, *Ecology*, **87**, S86–S99.

Kemp, W.P. & Simmons, G.A. (1978) The influence of stand factors on parasitism of spruce budworm eggs by *Trichogramma minutum*, *Environmental Entomology*, **7**, 685–688.

Kraft, N.J.B., Cornwell, W.K., Webb, C.O. & Ackerly, D.D. (2007) Trait Evolution, Community Assembly, and the Phylogenetic Structure of Ecological Communities, *The American Naturalist*, **170**, 271–283. doi:10.1086/519400.

Legault, S. & James, P.M.A. (2018) Parasitism rates of spruce budworm larvae: Testing the Enemy Hypothesis along a gradient of forest diversity measured at different spatial scales, *Environmental Entomology*, **47**, 1083–1095.

Lethiecq, J.L. & Regniere, J. (1988) Comparative description of the physical characteristics and vegetation of six sites used by the Canadian Forestry Service in the study of spruce budworm population dynamics. Rapport d’information LAU-X - Laurentian Forest Research Centre.

Lucarotti, C.J., Eveleigh, E.S., Royama, T., *et al.* (2004) Prevalence of baculoviruses in spruce budworm (Lepidoptera: Tortricidae) populations in New Brunswick, *Canadian Entomologist*, **136**, 255–264.

MacKinnon, W.E. & MacLean, D.A. (2003) The influence of forest and stand conditions on spruce budworm defoliation in New Brunswick, Canada, *Forest Science*, **49**, 657–667.

Marrec, R., Pontbriand-Paré, O., Legault, S. & James, P.M.A. (2018) Spatiotemporal variation in drivers of parasitoid metacommunity structure in continuous forest landscapes, *Ecosphere*, **9**, e02075.

McMeans, B.C., McCann, K.S., Tunney, T.D., *et al.* (2016) The adaptive capacity of lake food webs: from individuals to ecosystems, *Ecological Monographs*, **86**, 4–19.

Miller, A. & Rusnock, P. (1993) The rise and fall of the silvicultural hypothesis in spruce budworm (*Choristoneura fumiferana*) management in eastern Canada, *Forest Ecology and Management*, **61**, 171–189.

Mueller, K.E., Polissar, P.J., Oleksyn, J. & Freeman, K.H. (2012) Differentiating temperate tree species and their organs using lipid biomarkers in leaves, roots and soil, *Organic Geochemistry*, **52**, 130–141.

Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. New York, New York, USA.: Oxford University Press.

Nenzén, H.K., Martel, V. & Gravel, D. (2018) Can hyperparasitoids cause large-scale outbreaks of insect herbivores?, *Oikos*, **127**, 1344–1354.

Nisole, A., Stewart, D., Kyei-Poku, G., *et al.* (2020) Identification of spruce budworm natural enemies using a qPCR-based molecular sorting approach, *Forests*, **11**, 621.

Nyrop, J.P. & Simmons, G.A. (1982) *Measurement and analysis of the activity of adult spruce budworm parasitoids.* CANUSA Tecnical Report 82-12. East Lansing, Michigan: Michigan State Government.

O'Hara, J.E. (2005) A review of the tachinid parasitoids (Diptera: Tachinidae) of Nearctic *Choristoneura fumiferana* species (Lepidoptera: Tortricidae), with keys to adults and puparia, *Zootaxa*, **938**, 1–46.

Phillips, D.L., Inger, R., Bearhop, S., *et al.* (2014) Best practices for use of stable isotope mixing models in food-web studies, *Canadian Journal of Zoology*, **92**, 823–835.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team (2018) *nlme: Linear and*

Nonlinear Mixed Effects Models. Available at: https://CRAN.R-project.org/package=nlme.

Quayle, D., Régnière, J., Cappuccino, N. & Dupont, A. (2003) Forest composition, host-population density, and parasitism of spruce budworm *Choristoneura fumiferana* eggs by *Trichogramma minutum*: *Parasitism of* C. fumiferana *eggs by T. minutum*, *Entomologia Experimentalis et Applicata*, **107**, 215–227.

Ratnasingham, S. & Hebert, P.D.N. (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org), *Molecular Ecology Notes*, **7**, 355–364.

Ratnasingham, S. & Hebert, P.D.N. (2013) A DNA-based registry for all animal species: The barcode index number (BIN) system, *PLoS ONE*, **8**.

Ricklefs, R.E. (2006) Evolutionary diversification and the origin of the diversity-environment relationship, *Ecology*, **87**, S3–S13.

Risk, D., Kellman, L. & Moroni, M. (2009) Characterisation of spatial variability and patterns in tree and soil δ ^{13} C at forested sites in eastern Canada, *Isotopes in Environmental and Health Studies*, **45**, 220–230.

Roe, A.D., Demidovich, M. & Dedes, J. (2018) Origins and history of laboratory insect stocks in a multispecies insect production facility, with the proposal of standardized nomenclature and designation of formal standard names, *Journal of Insect Science*, **18.** doi:10.1093/jisesa/iey037.

Royama, T., Eveleigh, E.S., Morin, J.R.B., *et al.* (2017) Mechanisms underlying spruce budworm outbreak processes as elucidated by a 14-year study in New Brunswick, Canada, *Ecological Monographs*, **87**, 600–631.

Seifert, C.L., Lamarre, G.P.A., Volf, M., *et al.* (2020) Vertical stratification of a temperate forest caterpillar community in eastern North America, *Oecologia*, **192**, 501–514.

Simmons, G.A., Leonard, D.E. & Chen, C.W. (1975) Influence of tree species density and composition on parasitism of the spruce budworm, *Choristoneura fumiferana* (Clem.), *Environmental Entomology*, **4**, 5.

Smith, M.A., Eveleigh, E.S., McCann, K.S., Merilo, M.T., McCarthy, P.C. & Van Rooyen, K.I. (2011) Barcoding a quantified food web: crypsis, concepts, ecology and hypotheses, *PLoS ONE*, **6**, e14424.

Su, Q., Needham, T.D. & MacLean, D.A. (1996) The influence of hardwood content on balsam fir defoliation by spruce budworm, *Canadian Journal of Forest Research*, **26**, 1620–1628.

Summerville, K.S. & Crist, T.O. (2008) Structure and conservation of lepidopteran communities in managed forests of northeastern North America: a review, *The Canadian Entomologist*, **140**, 475–494. doi:10.4039/n07-LS06.

Swift, D.E., Kilpatrick, B., Murray, T., Toole, D., Henderson, J. & Pitt, C. (2006) Acadia Research Forest: a brief introduction to a living laboratory, in Irland, L.C., Camp, A.E., Brissette, J.C., and Donohew, Z.R. (eds) *Long-term Silvicultural and Ecological studies: Results for Science and Management.* Yale University, School of Forestry and Environmental Studies, Global Institute of Sustainable Forestry, New Haven, Connecticut, USA, 104–118.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular evolutionary genetics analysis Version 6.0, *Molecular Biology and Evolution*, **30**, 2725–2729.

Thireau, J.-C. & Régnière, J. (1995) Development, reproduction, voltinism and host synchrony of *Meteorus trachynotus* with its hosts *Choristoneura fumiferana* and *C. rosaceana*, *Entomologia Experimentalis et Applicata*, **76**, 67–82.

Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M.J. (2002) Phylogenies and community ecology, *Annual Review of Ecology and Systematics*, **33**, 475–505.

Zhang, B., MacLean, D., Johns, R. & Eveleigh, E. (2018) Effects of hardwood content on balsam fir defoliation during the building phase of a spruce budworm outbreak, *Forests*, **9**, 530.

Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S. & Edwards, S. (2020) Hardwood-softwood composition influences early-instar larval dispersal mortality during a spruce budworm outbreak, *Forest Ecology and Management*, **463**, 118035.

Zuur, A., Leno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) *Mixed effects* *models and extensions in ecology with R (1st ed.)*. New York, New York, United States of America: Springer-Verlag New York.

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Tables

Table 1 ANOVA output for model with δ13C from 1980s Malaise caught budworm parasitoids as the response variable and Year, Sampling Period, Parasitoid Group, Year:Parasitoid Group, Group:Sampling Period as explanatory variables.

Predictor variables	**df**	**F value**	**P value**
Intercept	1	115952.08	**<0.0001**
Year	1	3.15	0.0964
Sampling Period	1	28.14	**0.0001**
Parasitoid Group	2	2.50	0.1159
Year:Parasitoid Group	2	5.50	**0.0162**
Group:Sampling Period	2	36.67	**<0.001**

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Figures

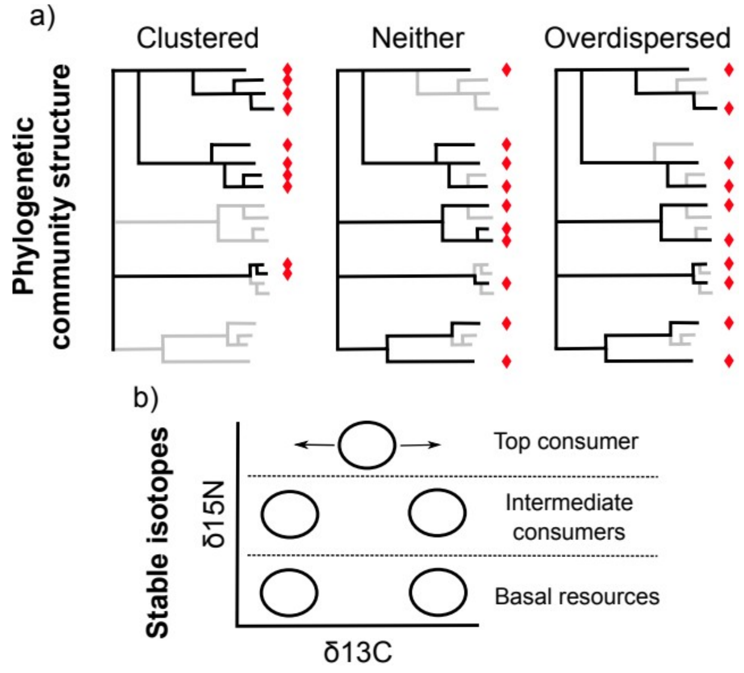

Figure 1 a) Hypothetical phylogenies showing either Clustered, Overdispersed or Neither phylogenetic community structures. In these hypothetical communities, the “sample” has 10 species from the 20 potential species in the species pool. Presence of a species is denoted by diamonds and black branches with absence denoted by grey branches. Note how closely related the “sampled” species are in the Clustered structure compared to the Overdispersed structure. b) Conceptual diagram illustrating the stable isotopes (δ15N & δ13C) of basal resources, intermediate consumers and a top consumer for two resource compartments (food chains). Note in this example, the top consumer is coupling the two resource compartments. The δ13C of this top consumer can even change depending on which resource compartment the top consumer is feeding on in time and space.

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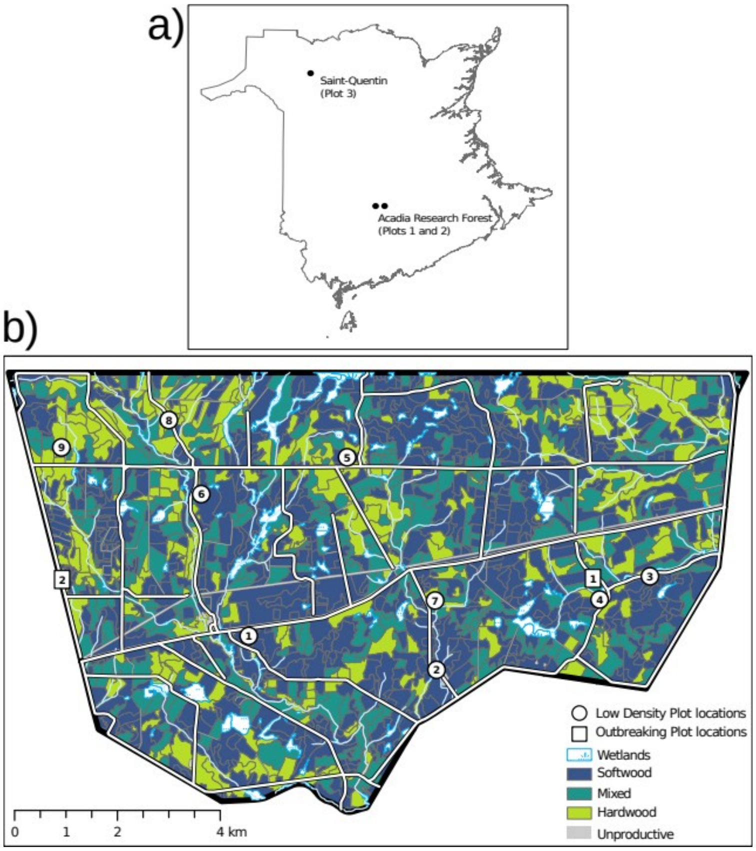

Figure 2 a) Map of New Brunswick, Canada with the three plots from the 1980s when spruce budworm were at high density indicated. b) Map of the Acadia Research Forest. The nine plots used in the 2016 Malaise trapping of parasitoids when spruce budworm were at low densities are indicated by circles. Plots 1, 2, 3 are balsam fir dominated, plots 4, 5, 6 are mixed wood plots, and plots 7, 8, 9 are hardwood dominated. Two of the three plots from the 1980s when spruce budworm were at high density are indicated by squares (the third plot is indicated in the map of New Brunswick).

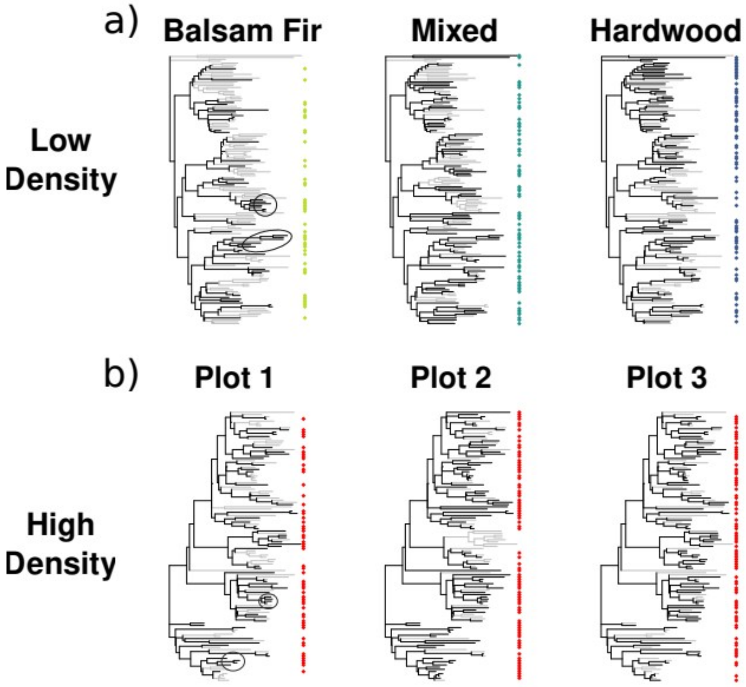

Figure 3 a) Phylogenies of Malaise caught parasitoid communities with presence denoted by diamonds and black branches in three balsam fir dominated plots, three mixed wood plots, and three hardwood dominated plots in Acadia Research Forest in 2016 when spruce budworm were at low density. b) Phylogenies of reared parasitoid communities with presence denoted by diamonds for Plots 1 & 2 (Acadia Research Forest), and 3 (Saint-Quentin) for all years sampled (1983-1995) when spruce budworm were at high density. Tree basal area, Plot 1: balsam fir 98%, Spruce 1%, Hardwood 1%. Plot 2: balsam fir 77%, spruce 8%, hardwood 14%. Plot 3: balsam fir 50%, spruce 36%, hardwood 14%. Examples of clusters of species are indicated with ellipses. Absence of parasitoid taxa are denoted by grey branches.

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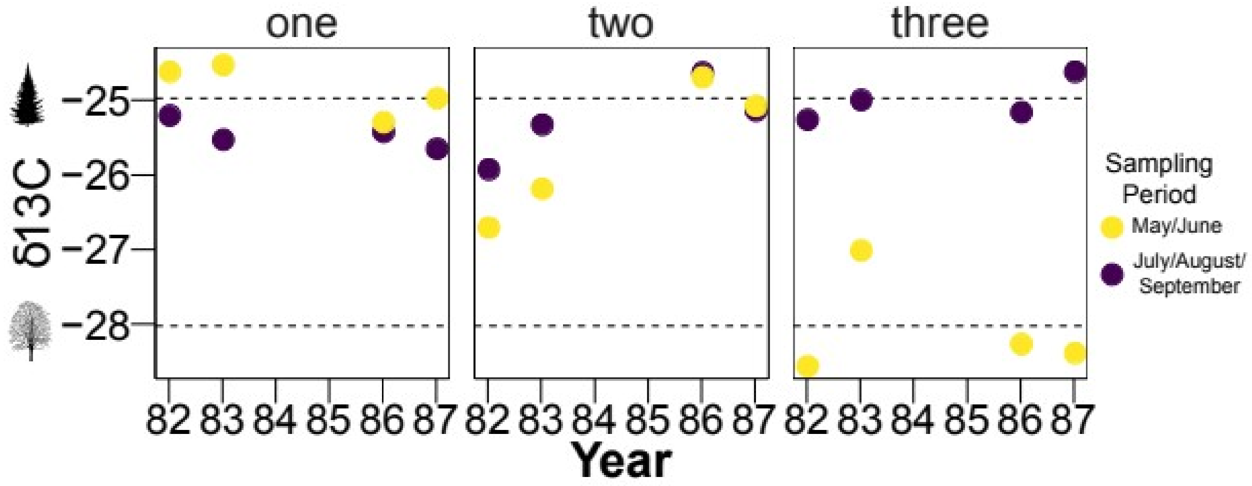

Figure 4 δ13C for three groups of parasitoid species: group one parasitoids are univoltine species that attack one type of caterpillar within a year (left plot); group two parasitoids are multivoltine species that overwinter away from a host or where overwintering status was unknown (centre plot); and group three parasitoids are multivoltine species that require an alternate caterpillar in which to overwinter (right plot). Spruce budworm populations peaked in 1985. δ13C was measured on parasitoids captured in the sampling periods of May/June and July/August/September. Dashed lines depict the average δ13C value for the group three parasitoids in May/June and July/August/September (used as estimates for the balsam fir and hardwood foliage δ13C values). See Figures S1, S2, S3 for time series of the proportions of the parasitoids in each group. Balsam fir and red maple images shown on the y-axis are publicly available from Natural Resources Canada, Canadian Forest Service.

Supporting Information For "Phylogenetic community structure and stable isotope analysis of the parasitoid community associated with Eastern spruce budworm (Lepidoptera: Tortricidae)"

1. Authors

Christopher J. Greyson-Gaito[1], Sarah J. Dolson[1,2], Glen Forbes[3], Rosanna Lamb[3], Wayne MacKinnon[3], Kevin S. McCann[1], M. Alex Smith[1], Eldon Eveleigh[3]

*Corresponding Author - christopher@greyson-gaito.com

2. Affiliations

1. Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

2. Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada

3. Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, New Brunswick, E3B 5P7, Canada

4. Population Ecology Group, Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, E3B 5A3, Canada

3. ORCID

CJGG – 0000-0001-8716-0290

SJD – 0000-0001-9312-2282

KSM - 0000-0001-6031-7913

MAS – 0000-0002-8650-2575

ESE - 0000-0001-5060-8565

Table 1: List of Malaise caught parasitoid species in each group. This list refers to the species caught in the 1980s. Previous names of species are provided in brackets if applicable. Group 1 are univoltine parasitoid species that attack one type of caterpillar within a year and do not require an alternate caterpillar in which to overwinter. Group 2 are multivoltine parasitoid species that overwinter away from a host or where overwintering status was unknown. Group 3 are multivoltine parasitoid species that require an alternate caterpillar in which to overwinter.

Group	**Species**	**Spruce Budworm Stage Attacked**
1	*Apantales fumiferanae* Vier. (Hymenoptera: Braconidae)	Early instar larvae
1	*Glypta fumiferanae* Vier.(Hymenoptera: Ichneumonidae)	Early instar larvae
1	*Lypha fumipennis* (*Lypha setifacies*) Brooks (Diptera: Tachinidae)	Late instar larvae
1	*Smidtia fumiferanae (Winthemia fumiferanae)* Tothill (Diptera: Tachinidae)	Late instar larvae
2	*Actia interrupta* Curran.(Diptera: Tachinidae)	Late instar larvae
2	*Agria affinis* (*Psuedosarcophaga affinis*) Fallén (Diptera: Sarcophagidae)	Late instar larvae
2	*Compsilura concinnata* Meigen (Diptera: Tachinidae)	Larvae
2	*Eumea caesar* Aldrich (Diptera: Tachinidae)	Late instar larvae
2	*Hemisturmia parva* (*Hemistermia tortricis*) Bigot (Diptera: Tachinidae)	Late instar larvae
2	*Nilea erecta* (*Pseudoperichaeta erecta*) Coquillett (Diptera: Tachinidae)	Late instar larvae

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2	*Sarcophaga aldrichi* Parker (Diptera: Sarcophagidae)	Pupae
2	*Tachinomyia nigricans* Webber (Diptera: Tachinidae)	Unknown
3	*Ceromasia auricaudata (Ceromasia aurifrons)* Townsend (Diptera: Tachinidae)	Late instar larvae
3	Madremyia saundersii Williston (Diptera: Tachinidae)	Late instar larvae
3	*Meteorus trachynotus Vier* (Hymenoptera: Braconidae)	Late instar larvae
3	*Nemorilla psyte* Walker (Diptera: Tachinidae)	Late instar larvae
3	*Phryxe pecosensis* Townsend (Diptera: Tachinidae)	Late instar larvae

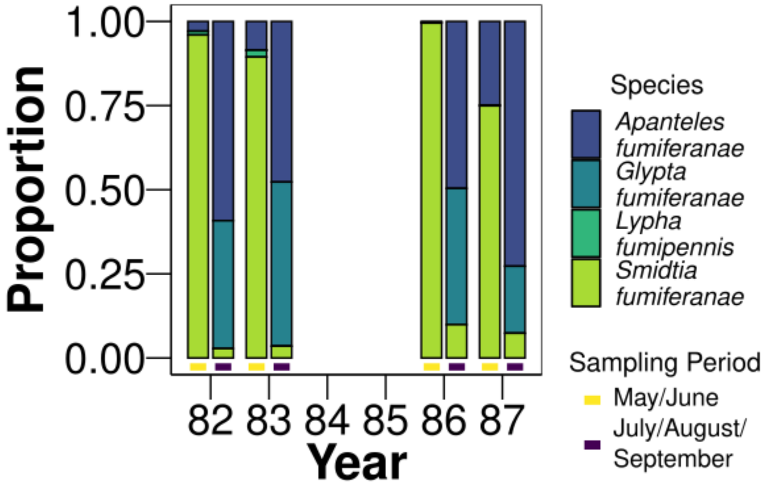

Figure S1 Proportion of each parasitoid species within group one that were Malaise caught in May/June or July/August/September for the years 1982, 1983, 1986, and 1987. To access the data behind this figure, please contact Eldon Eveleigh.

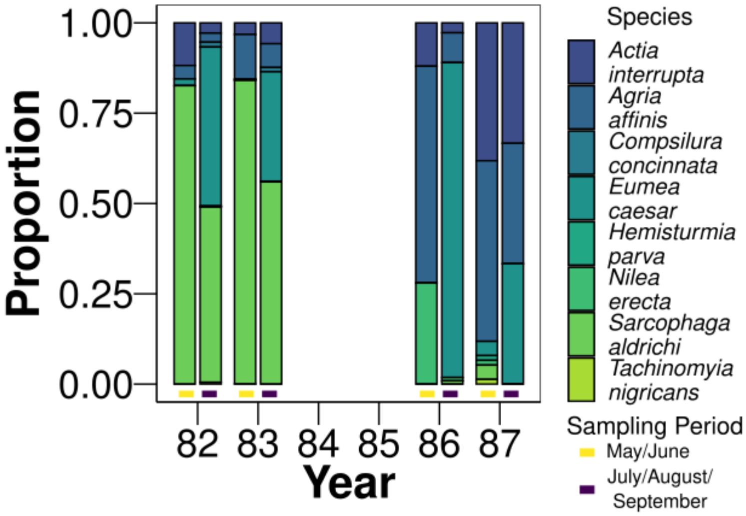

Figure S2 Proportion of each parasitoid species within group two that were Malaise caught in May/June or July/August/September for the years 1982, 1983, 1986, and 1987. To access the data behind this figure, please contact Eldon Eveleigh.

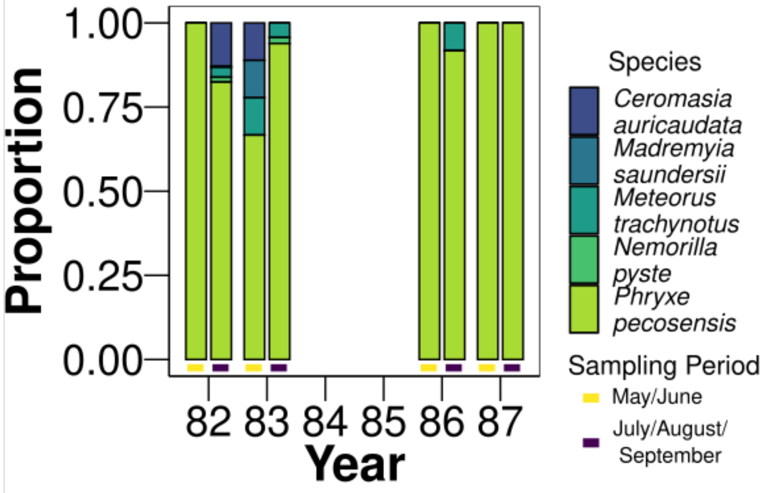

Figure S3 Proportion of each parasitoid species within group three that were Malaise caught in May/June or July/August/September for the years 1982, 1983, 1986, and 1987. To access the data behind this figure, please contact Eldon Eveleigh.