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Diet Breadth Evolution and Diversification of a Generalist Insect Herbivore

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Abstract

Insect herbivores are one of the most diverse groups of multicellular organisms, and the vast majority are specialists, which feed on only a few plant species. The factors that cause some herbivores to be specialists and others to be generalists are still unclear. It is known that the selective forces from natural enemies (top-down) and the host plants (bottom-up) influence an herbivore's diet breadth. In my meta-analysis evaluating the relative important of top-down and bottom-up forces on insect herbivore fitness, I found that herbivores usually have greater performance on better quality plants and in the absence or reduction of enemy pressure. Usually top-down forces were stronger than bottom-up forces, except when considering the diet breadth of herbivores. I found that specialists are more affected by bottom-up forces than top-down forces, whereas generalists had similar performance on different host plants. As specialists and generalists are differently affected by bottom-up and top-down forces, to understand the diet breadth evolution of herbivores, we should consider the impact of these two forces together on herbivore fitness. There is currently no method to measure this combined effect (tri-trophic), so I developed a new approach to calculate the fitness associated with tri-trophic interactions using bi-trophic slopes. I use the relationship between fitness associated with top-down and bottom-up forces as well as the frequency of host-plant use to calculate the top-down and bottom-up fitness slopes, which I then combine to obtain the tri-trophic slopes. I tested my approach using one of the most generalist herbivores as my model organism (fall webworm, *Hyphantria cunea*, Lepidoptera: Erebidae); I found that generalism is a good strategy for this herbivore, as populations with broader diet feed more frequently on the best hosts available.

One of the mechanisms that can lead to diversification of insect herbivores is local adaptation to their host plants. However, high gene flow between individuals feeding on different plants, as is the case for generalists, can lead to low levels of local adaptation. Using common garden and transplant experiments, I found mixed evidence for local adaptation of fall webworm. The red-headed type of fall webworm that is relatively less generalist had a higher level of local adaptation than the more generalist black-headed type. In addition to local adaptation, other factors such as host plant use, diet breadth, and geographic isolation can influence the diversification of herbivores. I performed genetic analyses of fall webworm using double-digest RADseq to test the relative importance of diet breadth, host plant use, and geographic distance on the genetic divergence of fall webworm populations. Using both red and black types from a broad geographic range, I found the two types to be genetically different, and both host plant use and geographic distance influenced the divergence of the red type, while only geographic distance was influential for the black type. Considering a fine geographic scale of the red type in Colorado, geographic distance was more influential than host plant use and diet breadth on the genetic divergence of individuals.

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Mayra Cadorin Vidal
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Advisor: Shannon M. Murphy

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CHAPTER 1: Bottom-up versus top-down effects on terrestrial insect herbivores: a meta-analysis

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Introduction

Insect herbivores are one of the most diverse groups of organisms known and are important model organisms for studies of resource specialization and niche breadth (e.g., Futuyma and Moreno 1988), ecological speciation (e.g., Rundle and Nosil 2005; Funk et al. 2002), coevolution (e.g., Ehrlich and Raven 1964), and food web dynamics (e.g., Schmitz 1994). Herbivorous insects are under strong selection from bottom-up forces via their host plants and top-down forces via natural enemies (Ehrlich and Raven 1964; Singer and Stireman 2005). Today it is accepted that both bottom-up and top-down selective forces influence an herbivore’s evolution, distribution and population dynamics; however, we still do not know how the relative importance of these selective forces varies across habitats, diet breadth and feeding guilds. The comparative importance of resource (“bottom-up”) and consumer (“top-down”) controls has been investigated for primary producers in terrestrial (Gruner et al. 2008) and marine (Poore et al. 2012) ecosystems, but not yet for higher trophic levels. Thus, we performed a meta-analysis to test the relative importance of bottom-up and top-down forces on the fitness of primary consumers, which have significant effects on abundance of primary producers (Gruner et al. 2008; Poore et al. 2012); we focused our analysis on herbivorous insects due to their enormous diversity compared to other primary consumers and long history of study.

Plant characteristics such as nutritional quality, chemical and mechanical defenses, distribution and abundance can have detrimental impacts on insect herbivore fitness and these bottom-up effects have received much attention throughout the history of studies of insect-plant interactions (Schoonhoven et al. 2005; Price et al. 2011 and references therein). Because plants are not equally suitable as hosts due to variation in chemical or mechanical defenses that hamper consumption by herbivores (Fraenkel 1959; Feeny 1970), bottom-up effects were long thought to be more important than top-down effects in regulating herbivore populations. Yet the idea that herbivores may not be limited by resources, but instead by higher trophic levels was proposed early in the literature ("the world is green hypothesis" Hairston et al. 1960) and received more attention after Price et al. (1980) and Bernays and Graham (1988) re-emphasized the importance of top-down forces on herbivore fitness. However, most hypotheses proposed and tested to investigate diet breadth evolution and herbivore macroevolution are based primarily on bottom-up forces (i.e., host plant use; e.g. Janz and Nylin 2008; Hardy and Otto 2014; Hardy et al. 2016; Mason 2016), whereas relatively fewer hypotheses have been proposed and tested that consider top-down forces (but see Jeffries and Lawton 1984; Singer and Stireman 2005; Mooney et al. 2012). Two examples of hypotheses that do consider tri-trophic interactions are the enemy-free space hypothesis, which predicts that insect herbivores should feed on host plants associated with lower susceptibility to natural enemies even if those host plants are otherwise poor quality (Jeffries and Lawton 1984), and the tri-trophic interactions hypothesis, which makes predictions about the interactive effects of host-plant quality, natural enemies and diet breadth on herbivore performance (Mooney et al. 2012). Studies have also shown that some herbivores face trade-offs between host quality and enemy escape when choosing a host plant (e.g. Thompson 1988; Thompson and Pellmyr 1991; Mira and Bernays 2002; Murphy 2004; Murphy and Loewy 2015).

The purpose of our meta-analysis was to quantitatively analyze the relative impacts of bottom-up and top-down forces on primary consumers, focusing on fitness-related measures for insect herbivores, and to test related paradigms. We evaluated research publications that measured both bottom-up and top-down effects on the same insect herbivore (or community of herbivores) at the same time. We analyzed the effect of bottom-up and top-down forces on measures of herbivore fitness, considering multiple moderators that can modulate these effects: 1) herbivore diet breadth (specialist vs. generalist), 2) feeding guild (sucking, chewing, gall-makers, miners), 3) habitat/environment (natural vs. controlled and subdivisions within each), 4) type of bottom-up effects (plant quality vs. habitat quality), 5) type of top-down effects (predator, parasitoid, pathogen) and 6) how the fitness effects are measured on the herbivore (abundance, development time, growth, mass, reproduction, survival). Each of these moderators has been well studied and alternative predictions exist as to how they may affect herbivore fitness (Table 1). Our meta-analysis will increase our understanding of tri-trophic interactions and how they are structured independently or interactively by bottom-up and top-down selective forces. Previous research supports the positive effects of host plant quality (e.g. Feeny 1970; Coley et al. 1985; Agrawal 1998; Eubanks and Denno 2000; Gruner et al. 2005) and negative effects of natural enemies (e.g. Holt and Lawton 1994; Marquis and Whelan 1994; Lill et al. 2002; Singer and Stireman 2003; Murphy 2004; Long and Finke 2014) on herbivore fitness, yet the relative importance of top-down and bottom-up selective forces on herbivores remains unknown. By conducting a meta-analysis that only included studies that measured both bottom-up and top-down effects on a focal herbivore, we were able to quantitatively assess the relative magnitudes of these selective forces for herbivorous insects. Comparing these results with model predictions will improve the development of ecological hypotheses to better understand diet breadth evolution and speciation of herbivores, and their macroevolutionary relationship with host plants and natural enemies.

Table 1: Predictions of the direction of response for each moderator that we analyzed.

Moderator	**Hypothesis**	**References**	**Results follow prediction?**	**Data used**	**Figure**
Force type	Bottom-up and top-down forces are both important to herbivore fitness	1-4	Y	All	2B
	Top-down forces have stronger effect on herbivores than bottom-up forces.	8,9 (but see 5-7)	Y		
Diet breadth	Top-down forces are stronger for specialists than generalists.	10 (but see 4,11)	N	Chewers	3A
	Bottom-up forces are stronger for generalists than specialists.	7,12	N		
Feeding guild	External feeders are more affected by top-down forces than internal feeders.	13	N	All	3B

	Sucking insects are less affected by bottom-up forces than other feeding guilds.	14,15	N		
Habitat	Bottom-up and top-down forces are stronger in controlled than natural environments.	16,17	N	All	3C-D
	Top-down forces are similar in natural and controlled environments.	13,18	Y		
Bottom-up type	Host plant and habitat quality have similar effects on herbivores.	19	Y	Bottom-up	4A
Top-down type	Parasitoids have stronger effect on herbivores than predators.	13	N	Top-down	5A-C
	A community of natural enemies has a stronger effect on herbivores than a single species.	16,20 (but see 21,22)	N		
	Invertebrate predators have stronger effect on herbivores than vertebrate predators.	23,24 (but see 25)	N	Predator (chewers only)	
Fitness measure	Impact of selective forces more apparent for direct fitness measures than indirect fitness measures.	26	N	Bottom-up (chewers and suckers only) or top-down	4B, 5D

1) Price et al. 1980, 2) Denno et al. 2005, 3) Singer and Stireman 2005, 4) Mooney et al. 2012, 5) Dethier 1954, 6) Fraenkel 1959, 7) Ehrlich and Raven 1964, 8) Hairston et al. 1960, 9) Bernays and Graham 1988, 10) Dyer and Gentry 1999, 11) Dyer 1995, 12) Cornell and Hawkins 2003, 13) Hawkins et al. 1997, 14) Raven 1983, 15) Peterson et al. 2016, 16) Letourneau et al. 2009, 17) Rowen and Kaplan 2016, 18) Halaj and Wise 2001, 19) Price 1991, 20) Griffiths et al. 2008, 21) Finke and Denno 2005, 22) Vance-Chalcraft et al. 2007, 23) Barber and Marquis 2011, 24) Mooney 2007, 25) Mooney et al. 2010, 26) Roitberg et al. 2001.

Methods

Data survey

In our data survey, we included only studies that measured both bottom-up and top-down effects on the same focal herbivore. We compiled publications from two sources: searches of Web of Science using different combinations of key-words of studies done from 2000 to 2015 and publications cited in the review by Walker and Jones (2001). We searched for publications using ISI Web of Science on January 6 and February 2-4, 2016. We used different combinations of the search terms: top-down, bottom-up, insect herbivore, tri-trophic, predator, parasitoid, pathogen, performance, and plant. Our initial survey yielded 1,617 publications. We then excluded any publications that did not focus on terrestrial systems (e.g. aquatic systems), did not measure both bottom-up and top-down forces, or were not in English. We also excluded studies of ant-plant mutualisms or competition between herbivores that had no measure of the effect of bottom-up and/or top-down forces on the focal herbivore outside the protective mutualist or competitive interaction; meta-analyses on these topics have already been done (e.g. Kaplan and Denno 2007; Chamberlain and Holland 2009). We also excluded publications that used indirect measures of bottom-up and top-down forces, such as herbivory, richness of herbivores, and/or abundance and richness of natural enemies without measures of attack or direct effects on herbivores. This first review winnowed the pool of 1,617 publications down to 178 publications. We then performed a secondary review to determine if each of these 178 studies included both bottom-up and top-down measures for the same herbivore, many of which did not. Any publication that was rejected during this secondary review for not including bottom-up and top-down measures for the same herbivore was independently read by both co-authors before being excluded. Our secondary review yielded 112 publications. However, of these 112 publications, many did not include measures of error on the top-down effect, which is required to weight individual case studies in meta-analyses; we were able to extract standard deviation from 75 of these publications, which were kept in our analysis. Citations for the 112 publications are listed in the Supplementary Information (Appendix Table 1).

Effect size measures

To calculate effect sizes for the meta-analysis, we used log response ratio (RR):

$$RR=\ln(\text{mean treatment}/\text{mean control})$$

Some researchers prioritize using RR because it is not biased by differences in sample size among experiments and usually follows a normal distribution (Hedges et al. 1999; Gruner et al. 2008).

For bottom-up effects, we considered the treatment as the effect that is expected to have a positive impact on herbivore fitness. For example, Murphy (2004) reared Alaskan swallowtail (*Papilio machaon aliaska*) larvae on different host plants: the ancestral host (*Cnidium cnidiifolium*), which was high quality, and a novel host that was low-quality (*Petasites frigidus*). In this example, larvae reared on plants of high quality (the ancestral host) would be the treatment and larvae reared on plants of low quality (the novel host) would be the control. Thus, a significant positive effect size for bottom-up forces means that herbivores had greater fitness on high quality host plants than on poor quality host plants.

For top-down effects, we considered the treatment as the condition (e.g., host plant or habitat) for which herbivores were less negatively-impacted by natural enemies (or where natural enemies were excluded) than in the control. For example, insects reared on plants where enemies were excluded would be the treatment and insects reared in the presence of enemies would be the control. As another example of a comparison in which natural enemies were not excluded, Murphy and Loewy (2015) found that fall webworm (*Hyphantria cunea*) larvae suffered fewer attacks from parasitoids on crabapple (Malus spp.), which we classified as our treatment, than on chokecherry (*Prunus virginiana*), which was our control. Thus, a significant positive effect size for top-down forces means that herbivores had greater fitness when natural enemies were absent or exerted lower pressure and therefore that natural enemies had a negative impact on herbivore fitness. When a study included development time of the herbivore or consumption by predators, we switched the treatment and control, since shorter development time or lower consumption by predators is associated with greater herbivore fitness (Price et al. 1980). When the top-down effect was measured as percentage parasitism or mortality, we used the percent survival (e.g., if 60% were parasitized, then 40% survived). In our figures, we show the positive effect of plants and negative effect of natural enemies both as positive effect sizes for ease of comparison.

Sometimes the treatment used to calculate the bottom-up effects was the same as the treatment used to calculate the top-down effects, however that was not the case for every study and it depended on how the experiment was designed. For example, in an experiment that used open and closed cages on different quality plants, we would use the results from only the closed cages (no enemies) to assess the different plant qualities for our bottom-up treatment and control, but we would use the results from the closed and open cages paired by host plant as our top-down treatment and control. Similarly, in an experiment that tested the natural occurrence of natural enemies, the plants with low and high enemy pressure were not necessarily the same plants used for the bottom-up effect size. Experimental design also affected the fitness measures taken on herbivores subjected to different top-down and bottom-up forces. For example, Murphy (2004) used pupal mass, growth rate and survival to pupation as bottom-up fitness measures, but larval survival in the field as the top-down fitness measure; thus, for this example, the fitness measures all differed between bottom-up and top-down treatments and controls. Additionally, there were also more data points for bottom-up than for top-down for this experiment as well as for a few others, which explains why we have greater sample size for bottom-up than top-down forces.

When publications included mean and standard error/deviation in the text, we used this information, whereas when publications included only mean and standard error/deviation on graphs, we assessed those values using PlotDigitizer®. Some studies included multiple bottom-up treatments (e.g. survival on multiple host plants), and in this case we compared only the two extremes (e.g. survival on the highest and lowest quality host plants) or the highest value compared with the second lowest value if the lowest value was zero. When measures were taken over time, we only included comparisons taken at the last time point (or the one before that if the last measure was zero). Some publications studied more than one herbivore species, and as long as each had independent bottom-up and top-down measures, we analyzed them as different entries. Many studies of tri-trophic interactions use percent survival or percent attack by natural enemies as a measure of fitness, however these percentages often do not include standard deviations; of the 112 publications that we found, 37 did not compute standard error/deviation and we could not include them in our analysis.

Impact of bottom-up and top-down measures on herbivores

We tested the overall strength of bottom-up vs. top-down forces and whether the strength of bottom-up or top-down forces varied by diet breadth (specialist, generalist), herbivore feeding guild (sucking, chewing, miner, gall-maker), herbivore taxonomy (order), and habitat/environment type (natural, controlled). Diet breadth is a continuum, but for simplicity we considered specialists as herbivores that feed on fewer than 3 plant families, while generalists feed on more than 3 families; we used this threshold because it has historically been used in other studies of insect-plant interactions (Bernays and Graham 1988; Ali and Agrawal 2012). We based classifications of diet breadth from the classification used in the study, or when not reported from our own knowledge or an internet search. The feeding guild (or feeding behavior) of gall-makers and miners can be considered as chewing herbivores by some authors since they use mandibles to grind the food (Schoonhoven et al. 2005). However, we considered these internal feeders as separate guilds from chewers because their relationship with the plant and natural enemies is expected to differ from chewing herbivores that feed externally (Table 1). Additionally, we considered the possible effect of bottom-up and top-down forces on the herbivore's order, which included Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera; however, because most orders are confounded with feeding guilds, we only show the results for taxonomic order in the supplementary material.

Lastly, we tested whether strength of bottom-up or top-down forces depended on whether the study was conducted in natural or controlled environments as well as subdivisions within each environment type.

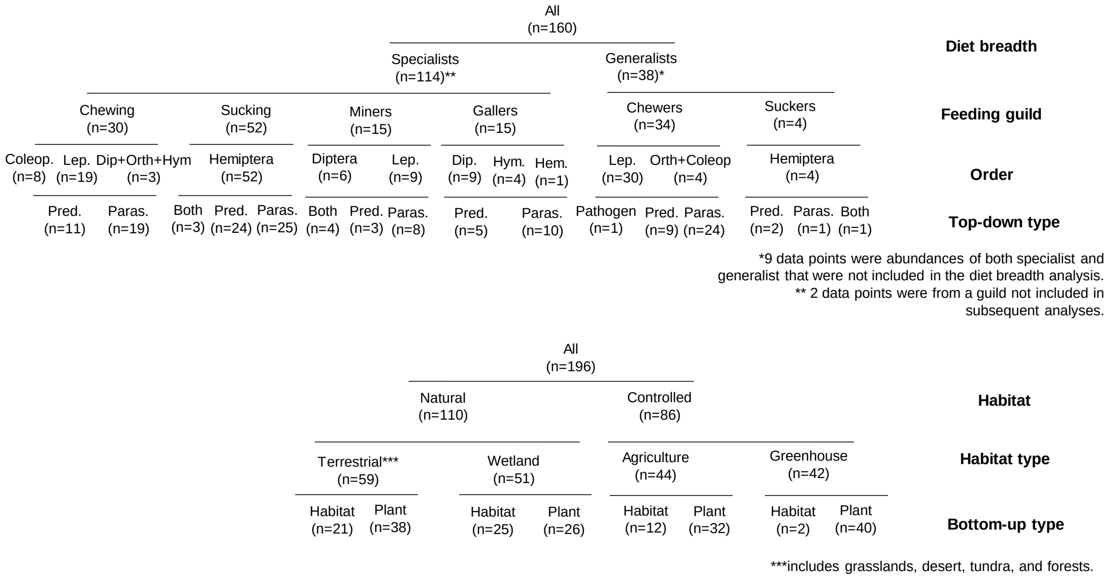
Influence of different types of selective forces and types of fitness-related measures

We tested whether the strength of bottom-up forces varied among the different types of bottom-up effects that ecologists study (host plant quality and habitat quality). We used host quality as any measure taken of characteristics intrinsic to the plant, whereas habitat quality measured environmental characteristics that may or may not affect plant quality. Host plant quality included any measure of plant age, architecture, genotype, height, nutritional quality, size, as well as the presence/absence and type of trichomes and/or chemical compounds. Habitat quality included agricultural type, CO2 variation, elevation, flood regime, fertilization, salinity, habitat complexity, pollution, spatial variation, temperature variation, and temporal variation; the threshold to define the treatment and control depended on the study system (see Appendix Table 2 for more information). We tested whether the strength of top-down forces varied among different types of natural enemy (parasitoid or predator; there were too few studies (n=3) on pathogens to include in the analysis), whether inflicted by a community of enemies or a single species, and whether inflicted by vertebrate or invertebrate predators. We also tested if the response variable used to assess bottom-up and top-down effects on herbivores (e.g. abundance, body mass, developmental time, growth rate, reproduction or survival rate) would elicit different results.

Statistical analysis

For our statistical analysis, we used multi-level error meta-analysis, with publication identity and case identity nested in publication identity as random factors, the moderators and interactions as fixed effects, and RR as the response variable. We used the inverse of variation from each effect size as our weight in the model. We did not consider moderator levels with 3 or fewer effect sizes because such small sample sizes can cause imprecision in the analysis using random models (Borenstein et al. 2009, Murphy et al. in press). We analyzed the type of selective force (top-down or bottom-up) as an individual moderator, and analyzed habitat/environment type and subtypes, diet breadth, feeding guild and taxonomic group as the interaction with the selective force type (main effects were also included). We analyzed top-down type (natural enemies type, level of organization, predator type, and fitness measure) only using effect sizes obtained from the top-down data, and similarly analyzed bottom-up type only with bottom-up data (Table 1). To test if strength of effect sizes were different, we compared effect sizes between top-down and bottom-up effects, and within bottom-up and top-down effects for each moderator using Tukey's HSD post-hoc test. For our post-hoc test, we used the package multcomp and the function ghlt to test linear hypotheses, and we used Bonferroni correction when there were more than six comparisons (Hothorn et al. 2008). The estimates and confidence intervals used in the post-hoc comparisons and in our figures were obtained from models including only the interactions compared to a zero intercept, without the main effects. We tested for publication bias using Rosenberg's fail-safe number (Rosenberg 2005) and tested asymmetry (funnel plot Fig. S1) of effect sizes by using a rma.mv model with the function "mod=vi" for each data-set. We performed the models using the metafor package with rma.mv function (Viechtbauer 2010). We performed all statistical tests in R environment 3.4.1 (R Development Core Team 2011). We deposited our data in the Dryad Repository: https://doi.org/10.5061/dryad.2ng06 (Vidal and Murphy 2017).

To control for confounded effects in our data (Fig. 1), we tested the effect of diet breadth only with chewing herbivores because they were the only guild with equal representation of both specialists and generalists. Similarly, we tested the effect of vertebrate and invertebrate predators only with chewing herbivores. We tested the influence of plant and habitat quality on herbivores using the bottom-up natural habitat data, because habitat quality had a good representation in natural habitats (Fig.1). All of the other moderators were well distributed among subgroups.



*9 data points were abundances of both specialist and generalist that were not included in the diet breadth analysis.
** 2 data points were from a guild not included in subsequent analyses.

***includes grasslands, desert, tundra, and forests.

Figure 1: The number of cases for each moderator that we analyzed (Table 1 shows each data-set that was used for specific analyses).

We carried out a sensitivity analysis to assess how our initial analysis was sensitive to key features of our dataset. We performed 3 separate tests to compare with our results:

1. We removed data from Moon and Stiling (2004), Parry et al. (2003) and Santolamazza-Carbone et al. (2014), which together had 77 effect sizes of top-down and bottom-up forces (22% of all data) and re-analyzed our data to determine if these studies may have significantly affected our results.
2. We compared the top-down effects from studies using artificial exclusion of natural enemies and studies using natural occurrence of natural enemies.
3. To test if considering only the extremes in cases with more than one treatment of bottom-up effects may have significantly affected our results, we removed those studies (n=60 effect sizes) and reanalyzed our data.

Results

We obtained 356 effect sizes from the 75 publications used in our analysis (Table S1). Fail-safe numbers indicate that the number of unpublished, non-significant studies that would need to be published to negate our significant results is more than 1,000 times greater than the number of studies included, and thus our findings are robust against publication bias (fail-safe number = 364,396, $P<0.0001$). We found asymmetry (i.e., some studies with relatively large sample size also had large residuals, Appendix Figure 1) for the whole data-set ($z=6.92$, $n=356$, $P<0.0001$), for the bottom-up data-set ($z=2.88$, $n=196$, $P=0.004$), and for the top-down data-set ($z=2.23$, $n=160$, $P=0.02$) (see Fig. 2A for effect size distribution and Appendix Figure 1 for funnel plot). All tests had QE with

P<0.0001, which shows that there is considerable unexplained heterogeneity among the studies. Most of the studies were conducted since 2000 (Appendix Figure 2), perhaps due to our survey method, and were performed in the United States (Appendix Figure 3A), with the majority from the east coast (Appendix Figure 3B).

Overall impact of top-down and bottom-up forces on herbivores

Top-down forces had a significantly greater effect than bottom-up forces on herbivore fitness (QM=66.4, df=1, P<0.0001, Fig. 2B). The effect size for top-down forces was positive, which means that herbivores had greater fitness in the absence or reduction of natural enemies. For bottom-up forces, the effect size was also positive, which means that herbivores had greater fitness on the highest quality plants or in the best habitats.

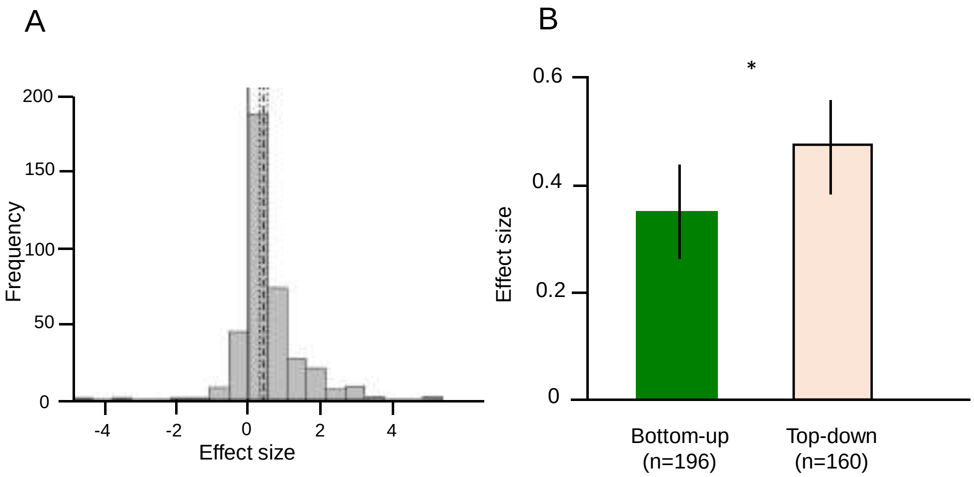


Figure 2: A) Histogram of effect sizes. Solid line represents zero, dashed lines represents the estimate size with 95% confidence intervals from a null model. B) Overall bottom-up and top-down effects on insect herbivores. Bars represent the estimate of the model, numbers represent the sample size for each test, and lines represent the 95% confidence interval of the model. If the line crosses zero, it means that the effect size was not significantly different from zero (P from z-test >0.05). Asterisk represents significant pairwise differences according to post-hoc Tukey’s test.

Interactions between selective force type and different moderators

Generalist and specialist herbivores were differently affected by top-down and bottom-up forces (QM=101.48, df=3, P<0.0001, Fig. 3A). For specialist chewing herbivores, bottom-up forces were stronger than top-down forces, and bottom-up forces were stronger for specialists than for generalists. Generalists had greater fitness in the absence/reduction of natural enemies, but were not affected by variation in bottom-up forces. Most studies included in our meta-analysis that tested bottom-up effects on specialists used a single host plant species that varied in quality (n=28 of the 33 bottom-up effect sizes), not multiple host plant species; however, we found no difference in effect size of bottom-up forces between studies done with either the same versus different host plants (QM=0.38, df=1, P=0.54).

The feeding guild of the herbivore affected the response of herbivores to selective forces (QM=122.7, df=7, P<0.0001). All feeding guilds were significantly affected by both bottom-up and top-down forces (Fig. 3B). Notably, top-down effects were stronger than bottom-up effects for chewing, sucking, and gall-making feeding guilds. The strength of top-down forces did not differ significantly among the feeding guilds, whereas bottom-up forces were stronger for miners than for chewing herbivores. Top-down effects were also greater than or equal to bottom-up effects for all taxonomic orders (Appendix Figure 4).

Top-down forces were significantly greater than bottom-up forces in both natural and controlled environments (QM=68.21, df=3, P<0.0001, Fig. 3C). The relative effect of top-down and bottom-up forces on herbivores also varied among the different types of controlled and natural environments (QM=83.66, df=7, P<0.0001, Fig. 3D). Top-down forces were significantly stronger than bottom-up for herbivores in wetland and greenhouse environments. We found no significant difference in the strength of top-down or bottom-up forces among the habitat types.

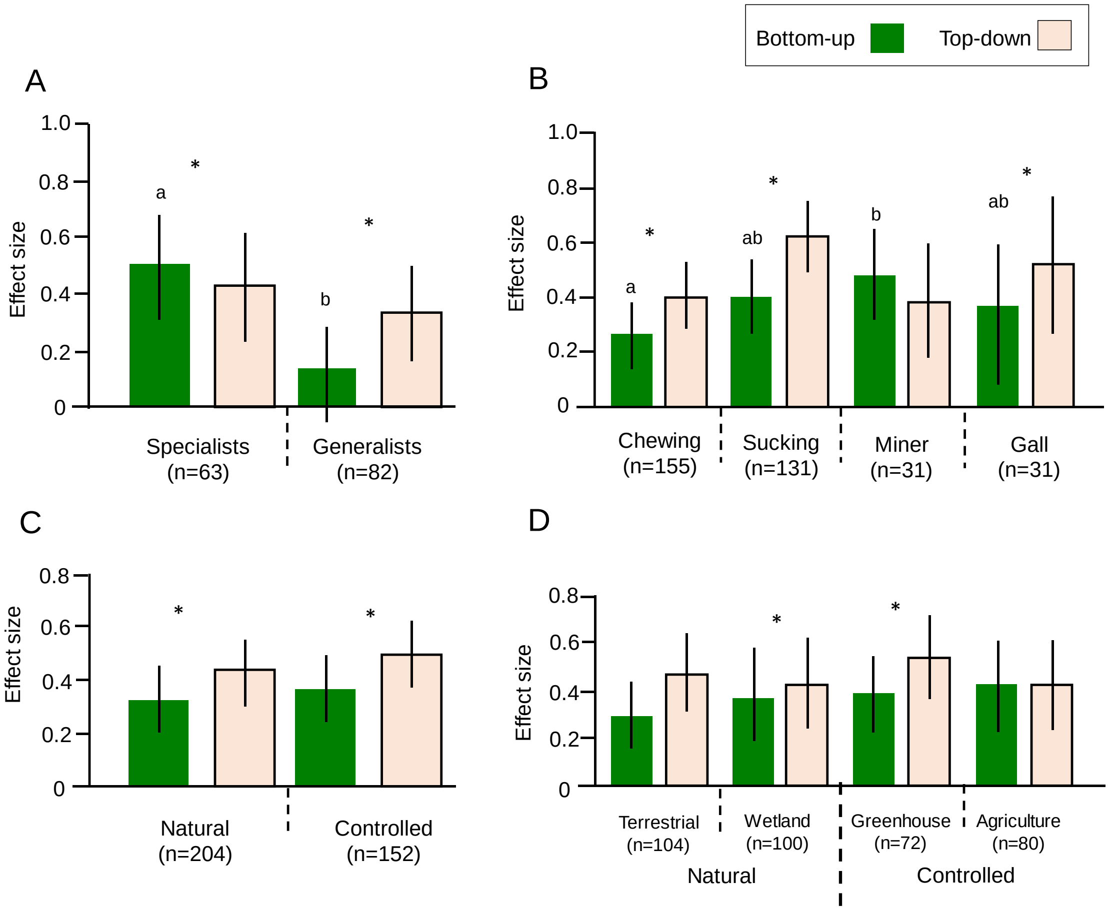


Figure 3: Bottom-up and top-down effects on insect herbivores: A) that vary in diet breadth (analyses conducted with chewing herbivores only), B) for each feeding guild, C) in natural and controlled environments, and D) in subdivisions of natural and controlled environments. Positive effect sizes for bottom-up and top-down forces show that the herbivore had greater fitness in the treatment (e.g., plant of better quality or in the absence/reduction of natural enemies) than in the control. Bars represent the estimate of the model, numbers represent the sample size for each test, and lines represent the 95% confidence interval of the model. If the line crosses zero, it means that the effect size was not significantly different from zero (P from z-test >0.05). Asterisks represent significant pairwise differences between bottom-up and top-down forces within each feeding guild or environment type, while letters represent significant pairwise differences within each force type according to post-hoc Tukey's test.

Type of bottom-up forces and fitness measures

The effects of bottom-up forces on herbivore fitness did not differ significantly between studies of habitat and host-plant quality (QM=1.3, df=1, P=0.25, Fig. 4A). We also analyzed the type of bottom-up forces with only natural habitat data, in which both habitat and host quality were equally represented, and we found the same pattern (QM=0.94, df=1, P=0.33). We found that the strength of bottom-up forces varied significantly among the types of fitness measure used (QM=112.86, df=5, P<0.0001); the strongest effects were on abundance and survival while the weakest was on development time (Fig. 4B).

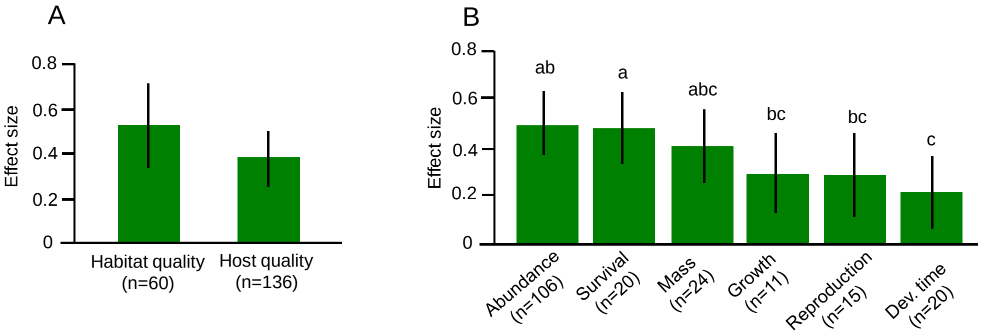


Figure 4: Bottom-up effects on insect herbivores: A) that vary in host plant quality or habitat quality, and B) for different measures of herbivore fitness. Bars represent the estimate of the model, numbers represent the sample size for each test, and lines represent the 95% confidence interval of the model. If the line crosses zero, it means that the effect size was not significantly different from zero (P from z-test >0.05). Letters represent significant differences among fitness measures according to post-hoc Tukey’s test.

Type of top-down forces and fitness measures

Variability in herbivore fitness was not significantly affected by the type of top-down forces (QM=1.82, df=3, P=0.61); predators, parasitoids and both together had equally negative effects on herbivores (Fig. 5A). Similarly, both single species and communities of natural enemies negatively affected herbivores but there were no differences in their effects on fitness (QM=0.997, df=1, P=0.32, Fig. 5B). Herbivore fitness was not differently affected by the type of predator (QM=0.69, df=1, P=0.41) with both vertebrate and invertebrate predators comparably affecting herbivores (Fig. 5C). Finally, the strength of top-down forces did not vary significantly among the type of fitness measure used (QM=2.55, df=2, P=0.28); however, abundance and survival of herbivores were positively affected by the absence or reduction of natural enemies, but there was no effect on development time (Fig. 5D).

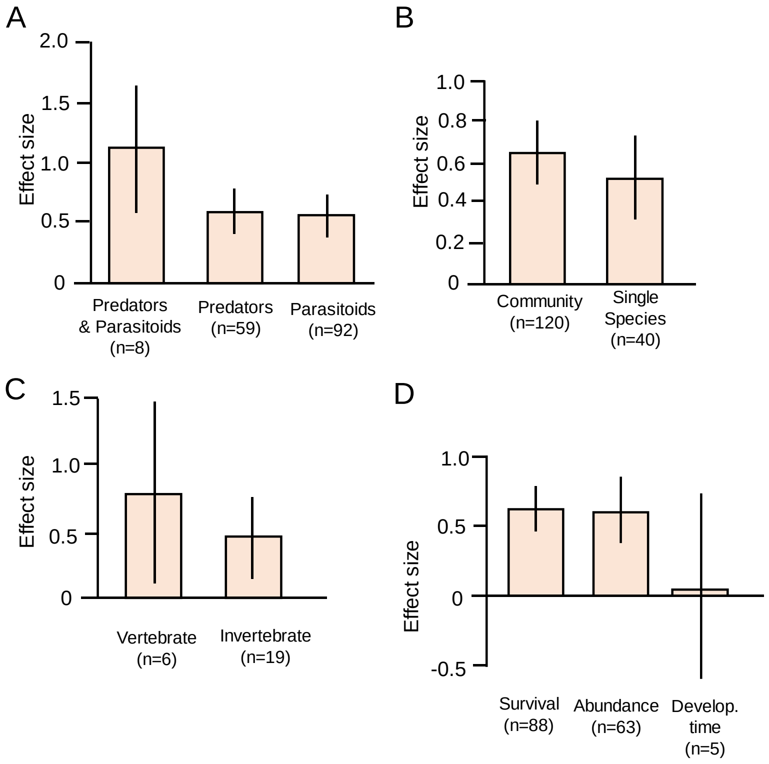


Figure 5: Top-down effects on insect herbivores: A) for the different kinds of natural enemy, B) whether a community or single species of enemy was studied, C) whether the predator was invertebrate or vertebrate, and D) for different measures of herbivore fitness. Bars represent the estimate of the model, numbers represent the sample size for each test, and lines represent the 95% confidence interval of the model. If the line crosses zero, it means that the effect size was not significantly different from zero (P from z-test >0.05).

Sensitivity analysis

When we removed the three publications that accounted for 22% of our effect sizes, we found that top-down forces were not significant for miners and growth as a measure of fitness for bottom-up effects was not different from zero. Bottom-up forces were significant for generalist chewing herbivores, but top-down forces were significantly stronger than bottom-up forces. For our second sensitivity analysis, we tested if the type of experimental design of top-down forces could have different effects, and we found that studies using natural occurrence or exclusion of natural enemies were not significantly different (QM=0.16, df=1, P=0.69). Thus, this result shows that whether natural enemies were excluded artificially or varied naturally in the environment (type of top-down experiment) does not affect our findings. For the third sensitivity analysis, we found the same significant effect of bottom-up forces on herbivore fitness even when we removed the studies that only included extreme comparisons of the best and worst quality host plants or habitats (z=9.54, n=307, P<0.0001, estimate=0.35, lower c.i.=0.25, upper c.i.=0.44).

Discussion

Our meta-analysis showed that bottom-up and top-down forces both have consistent impacts on herbivore fitness, but that the effect of top-down forces is significantly stronger than bottom-up forces. Although some historical studies argue that resources (i.e., plants) are the most important factor regulating populations of primary consumers (e.g., Lindeman 1942; White 1978), our meta-analysis showed that top-down forces are more important for most groups of insect herbivores. Generalist herbivores were only affected by variation in top-down effects, and top-down forces were significantly stronger than bottom-up forces for members of the chewing, sucking, and gall-making feeding guilds. Notably, top-down selective forces were stronger than bottom-up forces in both natural and controlled environments.

The influence of diet breadth, feeding guild and habitat on the response of herbivores to top-down and bottom-up forces

Contrary to the overwhelming effect of top-down forces on herbivore fitness for most moderators that we tested, we found a remarkably weak effect of bottom-up forces for generalists. Our results suggest that generalists may be the "jack of all trades, masters of all" because they did not experience significant bottom-up trade-offs among high and low quality host plants, were less affected by bottom-up forces than specialists, and their fitness did not differ significantly from the fitness of specialists for top-down forces. However, this result is sensitive to sample size as we found a significant effect of bottom-up forces on generalists when we removed the three studies that accounted for 22% of our data; yet, even excluding these studies, the effects of top-down and bottom-up forces on generalists remained similar to their effects on specialists. There is growing evidence that generalist herbivores do not suffer fitness trade-offs for feeding on different hosts. For instance, our finding that generalists are unaffected by bottom-up forces corresponds with the results of another recent meta-analysis that found that generalists are as likely as specialists to benefit from secondary metabolites of plants (Smilanich et al. 2016). Several studies also suggest that generalists are equally fit to feed on the many host plants that they may include in their diet (e.g. Futuyma and Philippi 1987; Agosta and Klemens 2009).

Specialist chewing herbivores were significantly affected by both bottom-up and top-down forces, and it was the only case in which bottom-up forces were stronger than top-down forces. Smilanich et al. (2009) found in their meta-analysis that specialists are more negatively affected by plant qualitative defenses than generalists, which corresponds with our finding of bottom-up forces being stronger for specialists than generalists. Although the delimitation of diet breadth can be challenging, our results would not have differed had we used a narrower delimitation of diet breadth for specialists given that we found significant bottom-up effects on specialist chewers for studies done with either the same versus different host plants. However, many specialists will choose to starve rather than feed on an unknown plant, which would have led to an even greater bottom-up effect if more studies had included non-hosts. Our results demonstrate that even when feeding on their adapted host plants, specialists are still affected by variations in plant or habitat type. However, we conducted the diet breadth analysis using the chewing feeding guild as it was the only guild that had equal representation of both specialists and generalists. Notably, the sucking feeding guild was composed almost entirely of specialists (n=123 of 131 effect sizes) and yet top-down forces were significantly greater than bottom-up forces for this guild. Interestingly, recent work comparing diet breadth evolution of Lepidoptera (which composed most of our chewing guild) and Hemiptera found that caterpillars suffer from more negative trade-offs on fitness when feeding on alternative hosts than true bugs (Peterson et al. 2016; Hardy et al. 2016). Therefore, to fully analyze the question of how bottom-up and top-down forces differentially affect generalist versus specialist herbivores, we need more studies on generalist herbivores in guilds other than chewers.

Our results lend additional support to the idea that specialist and generalist herbivores are similarly affected by top-down forces. Although specialist herbivores may have defenses against generalist natural enemies (e.g. ants, Dyer 1995), those defenses often have weak or no effect on specialist natural enemies (e.g. Thorpe and Barbosa 1986; Dyer and Gentry 1999). In contrast, generalist herbivores are often heavily affected by generalist predators (e.g. birds, Singer et al. 2014). Thus, if specialists are more affected by certain natural enemies and generalists by others, the end result may be a similar impact of top-down forces on both types of herbivores, which is what we found. Notably, top-down forces were stronger than bottom-up forces for chewing herbivores, the feeding guild that we used to test the interaction between diet breadth and force type. Chewers feed on the leaf surface, and are usually exposed and vulnerable to natural enemies (Bernays 1997; Sendoya and Oliveira 2017). Research on the ecology of fear demonstrates that even the mere perception of predation risk can detrimentally affect chewing herbivores (e.g. Schmitz et al. 1997; Kaplan et al. 2014). Miners were the only guild equally affected by bottom-up and top-down forces. Previous research has found that miners are less attacked by predators than external feeders, but can be heavily attacked by parasitoids (Hawkins et al. 1997). In our dataset, half of the top-down data for miners included predators, whereas for gall-makers, the other internal feeder guild, predators composed only one third of the top-down forces and that may explain why top-down forces were stronger than bottom-up forces for gall-makers but not for miners.

Top-down forces were stronger than bottom-up forces for both controlled and natural habitats, demonstrating the consistency of our findings across environments. We also found that each force type was surprisingly equal among habitats (i.e. bottom-up forces did not differ significantly among any of the habitat subdivisions, nor did top-down). A recent meta-analysis found that natural enemy diversity has a stronger negative effect on herbivores in cultivated than natural habitats (Letourneau et al. 2009), but the studies included in our analysis did not allow us to account for plant and natural enemy diversity so we could not test this directly. However, our results for top-down effects do agree with those of Halaj and Wise (2001) and Hawkins et al. (1997), in which they found similar effects of top-down forces on herbivores in crops and natural habitats. We note that, as is true for any meta-analysis, our results assume that the variation in strength of top-down and bottom-up forces tested in the literature reflects the variation found in nature. It is possible that experimental designs have been biased towards testing a greater proportion of variation for top-down forces than for bottom-up forces, but the design of our meta-analysis in which we used studies that tested the two forces on the same herbivore at the same time mitigates this potential effect.

Influence of different types of selective forces and fitness-related measures on herbivores

Insect herbivores are significantly impacted by bottom-up effects of both host quality and habitat quality, which is notable because our results indicate that both direct measures of plant quality as well as more indirect measures of habitat quality have similar overall effects on herbivores. Interestingly, although both host plant quality and habitat quality are bi-trophic measures, they are both known to affect tri-trophic interactions. Indeed, plant traits can have variable effects on insect herbivores, and even interact with higher trophic levels (e.g. volatiles, Rowen and Kaplan 2016; De Moraes et al. 1998). For instance, many studies included in our meta-analysis that investigated how variation in habitat quality affects the strength of selective forces were performed in salt marshes and tested the effect of fertilization and/or salinity on herbivores. Fertilization can increase the nutrients available in the plant, and therefore benefit herbivores, but fertilization can also positively affect higher trophic levels and thus regulation of herbivore populations (Wimp et al. 2010). For example, Murphy et al. (2012) found a positive effect of fertilization on herbivores and natural enemy populations, with stronger responses by predators than by herbivores to this bottom-up effect. Bottom-up and top-down cascades are both well studied, and it is clear that both forces can influence each other (e.g. Halaj and Wise 2001).

Predators and parasitoids both negatively affected herbivore fitness. The negative effects of communities or single species of natural enemies, as well as of vertebrate and invertebrate predators, were surprisingly equal. Although predators can have negative effects on parasitoids (intraguild predation, e.g. Snyder and Ives 2001), even for studies that tested parasitoids and predators together we found a similar and negative effect on herbivores compared to studies of either type of natural enemy separately. A community of natural enemies has sometimes been expected to have a more detrimental top-down effect on herbivores than a single enemy species, since a community would likely be composed by different types of natural enemies that can affect the herbivore differently, some being able to avoid herbivore protective mechanisms (Sih et al. 1998). However, the effect of a community of natural enemies can be either reduction or enhancement of risk, and so far studies have found support for both cases (Sih et al. 1998; Schmitz 2007). For example, Finke and Denno (2005) found that increasing the number of predators decreased prey suppression via intraguild predation. Similarly, vertebrate predators are often regarded as intra-guild predators that can suppress other natural enemies of the herbivores (Rosenheim 1998). By feeding on other natural enemies, vertebrate predators may end up lessening the pressure exerted by invertebrate natural enemies on herbivores, and thus may even have a positive or null effect on herbivores. However, a recent meta-analysis found an effect of vertebrate insectivores on both herbivores and their arthropod predators, but with a negative resulting effect on herbivores (Mooney et al. 2010).

Our meta-analysis suggests that abundance and survival are good measures of fitness to quantify the effects of bottom-up and top-down selective forces on herbivorous insects. Development time is commonly used as an indirect fitness measure in many studies (e.g. Murphy and Loewy 2015) and it is also the basis of the slow-growth high-mortality hypothesis (Price et al. 1980), which is a fundamental hypothesis in plant-insect interactions. However, we found that for top-down effects, development time in treatments and controls were indistinguishable and bottom-up effects also had the weakest effect on development time. Herbivores feeding on plants of lower quality are usually expected to have longer development time, which would indirectly decrease the chance of survival because of more time exposed to adverse weather and natural enemies (Feeny 1976; Price et al. 1980). Furthermore, natural enemies may indirectly influence the growth rate of herbivores, by decreasing the amount of time that herbivores spend feeding to avoid predation (Heinrich 1979). However, parasitoids and predators may have different effects on herbivores with varying development time; slow growing herbivores may suffer greater mortality from predators, but not from parasitoids (Williams 1999). Our sample size for development time was small and more studies are needed to determine its usefulness as a fitness measure (Murphy et al. in press). Although fitness measures are usually taken under more benign conditions than herbivores usually face in natural conditions (Agrawal et al. 2010), we found that there are significant differences in how much top-down and bottom-up forces can affect commonly used fitness proxies.

Further considerations

Our meta-analysis has important implications for future research on primary consumers and indicates areas of research in need of additional attention. For instance, it would be interesting to test if the same pattern that we found for diet breadth of chewing herbivores would be found for generalist and specialists from other feeding guilds such as sucking herbivores, but there are not yet enough studies with generalist herbivores to test this. The greater proportion of studies on specialists compared to generalists likely reflects the disproportionate diversity, but not importance, of each type of diet breadth found in nature. Additional studies are also needed on more diverse insect orders as studies with Lepidoptera and Hemiptera represented the majority of our data. We showed that the relative strengths of each type of selective force varies for most feeding guilds with top-down forces being stronger than bottom-up for all guilds but miners. These varied dynamics in different orders and guilds can provide an opportunity for experimental research on insect physiology as related to processing of plant-derived food, as well as behavioral ecology of defense by insect herbivores under variable ecological contexts. Comparative studies among orders and guilds will help us understand what regulates insect-plant interactions and how they may evolve. Similarly, future investigations on variation in the strength of top-down and bottom-up selective pressures and the evolution of diet breadth will advance our understanding of key evolutionary questions such as why there are so many more specialist than generalist herbivorous insects.

Future studies should also investigate the differential impact of parasitoids, predators and pathogens as enemies of insect herbivores, and the evolutionary dynamics between different types of natural enemies and their herbivorous prey. However, future studies should be careful in choosing the fitness measure to use and how to report it. A large problem that we encountered when performing this meta-analysis is that one third of the studies we found (33%) failed to include any measure of variance for their top-down effect even when they included variance for their bottom-up effect (e.g. Murphy 2004; Murphy and Loewy 2015). Standard error can easily be included in a study by measuring the survival/parasitism rate per maternal line, plant replicate or sampling period, for example, and it is unclear why so many previous studies have failed to do this for top-down measures when we as researchers are clearly thinking about it for bottom-up measures. It is imperative that researchers studying tri-trophic interactions include standard error in their representation of data for both their bottom-up and top-down effects so that future syntheses can include a wider range of studies.

More studies are also needed in different environments and especially in the tropics, as current studies are heavily biased towards temperate regions in North America. For most taxa, the tropics have significantly greater biodiversity than temperate regions and it would be interesting to test whether the strength of bottom-up and top-down selective forces differs among biomes, leading to variation in speciation rates. Our results are limited primarily to temperate regions, and the relative magnitudes of bottom-up and top-down effects may differ in tropical regions that are often more biologically complex. The strength of top-down and bottom-up forces can change with climate and latitude; for instance, both top-down and bottom-up effects on herbivores was shown to increase with temperature towards the tropics (Rodríguez-Castañeda 2013). A recent worldwide experiment demonstrated that caterpillars are more heavily predated in the tropics (Roslin et al. 2017). The strength of selective forces varies not only on latitudinal scales, but also regionally and locally, and other authors have already highlighted the importance of a landscape view of interactions (e.g. Gripenberg and Roslin 2007). More experiments are needed to account for landscape variability in tri-trophic interactions to help us understand geographic variation in diet breadth and local adaptation to host plants.

Although our meta-analysis improves our understanding of insect-plant interactions and the selective forces that affect herbivore fitness, we must be cautious with pitfalls associated with an undeveloped view of bi-trophic interactions. Top-down and bottom-up effects are usually interconnected and the separation into bi-trophic forces only makes sense for simplicity; whenever possible, a multitrophic perspective should be used for studies that measure herbivore response to host/habitat quality and natural enemies. Other interactions not accounted for in the bi-trophic (or even tri-trophic) approach may also influence the response of herbivores to top-down and bottom-up selective forces that regulate herbivores; examples would include competition with other herbivores (Kaplan and Denno 2007) and mutualistic partners of the plant (e.g. Koricheva et al. 2009; Vidal et al. 2016) or of the herbivore (e.g. Ferrari et al. 2004). Therefore, it is always advisable to use a food-web or a community module approach when possible, even though it is more time consuming and labor-intensive. Encouragingly, a few studies included in our meta-analysis did include more trophic levels than plant-herbivore-enemy, but not enough for a separate analysis. Another factor that we could not test in our meta-analysis is the importance of indirect interactions; top-down and bottom-up forces interact in many different ways to impact herbivores, and the effect of one force often directly or indirectly influences the strength of the other force or how the herbivore responds to that force. For instance, plant quality can influence how herbivores respond to predation risk, in which herbivores feeding upon a less nutritious plant might not be able to afford to stop feeding to hide from predators (Kaplan et al. 2014).

With these caveats in mind, we strongly recommend that a tri-trophic approach be used to study herbivore-plant interactions to understand what regulates consumer performance, as the bi-trophic approach that focuses only on consumer and resource is clearly too simplistic and ignores a critical part of most interactions, namely natural enemies (higher trophic levels). Our results demonstrate that both top-down and bottom-up effects must be considered when studying the evolution and population dynamics of insect herbivores. Historically, ecologists have argued about whether bottom-up or top-down effects were more important (e.g. Bernays and Graham 1988; Feeny 1970), but our meta-analysis clearly demonstrates that both are significant selective forces and, for most groups, top-down is more important. Many studies considering the macroevolution of insect-plant interactions involve only bottom-up effects, even though the importance of natural enemies on an herbivore's evolution was advocated more than 10 years ago (Singer and Stireman 2005). Other researchers have similarly highlighted the importance of a tri-trophic perspective (e.g. Price et al. 1980; Singer and Stireman 2005; Mooney et al. 2012), and here we demonstrate that the importance of top-down effects on insect primary consumers has been undervalued.

CHAPTER 2: Quantitative measure of fitness in tri-trophic interactions and its influence on diet breadth of insect herbivores

Introduction

Resource specialization is ubiquitous in many systems and is directly associated with many ecological and evolutionary mechanisms, such as ecological speciation (e.g., Funk et al. 2002; Rundle and Nosil 2005), coevolution (e.g., Ehrlich and Raven 1964), and food web dynamics (e.g., Schmitz 1994). Evolutionarily, resource use is a crucial factor influencing diversification. For instance, expansion onto new resources or environments is one of the key features of adaptive radiation (Schluter 2000; e.g., Winkler et al. 2009) and selective pressure due to competition for resources is a clear condition for natural selection to act (Darwin 1859). Insect herbivores are ideal model organisms to study resource specialization (e.g., Futuyma and Moreno 1988) because they represent one of the most diverse groups of organisms and the vast majority of herbivorous insects are specialists. Although the study of herbivore diet breadth has spanned many years and yielded exciting insights, we still do not fully understand why there are so many specialist species and so few generalists, as more than 90% of insect herbivores feed on fewer than four plant families (Forister et al. 2015). The proportion of specialists may be even greater if we consider that some species considered to be generalists might be composed by locally specialized populations (Fox and Morrow 1981) or be a complex of cryptic species (Hebert et al. 2004; Blair et al. 2005).

Most hypotheses proposed to explain diet specialization focus on the possible advantages of being specialists rather than generalists from the perspective of host plant use (Bernays 1998, 2001; Futuyma and Moreno 1988; Jaenike 1990; Joshi and Thompson 1995; but see Hardy et al. 2016). For instance, it has long been assumed that herbivores specialize on a specific host species because it facilitates best coping with the defenses of that host plant (Ehrlich and Raven 1964). As a result, specialists are expected to have fitness trade-offs when feeding on a non-host (as compared to the host plant to which they are adapted). Generalists, however, would not experience these trade-offs but instead would be expected to have generally lower fitness than specialists (but see Fry 1996). However, evidence from a recent meta-analysis and other experiments (Vidal and Murphy 2018; Agosta and Klemens 2009) suggests that generalists may be the “jack of all trades, master of all” because specialists are more affected by variation in bottom-up forces than generalists, even when feeding on their adapted host plant, and generalists seem able to feed relatively well on their many potential hosts. Another possible explanation for the observed greater proportion of specialists than generalists is that specialist lineages have had a greater speciation rate than generalists, while generalist lineages had fewer extinction events (Hardy and Otto 2014, but see Hardy et al. 2016). However, diversification rate does not completely explain the disparity in relative proportion of herbivorous insects with narrow versus broad diet breadth, especially given that the advantages of being specialist rather than generalist are not clear (Hardy et al. 2016).

One problem with studies meant to understand macroevolutionary patterns of herbivore diet breadth is that they usually only include the effect of plants, and neglect the effect of natural enemies (e.g., Janz and Nylin 2008; Hardy and Otto 2014; Hardy et al. 2016; Mason 2016. But see Singer and Stireman 2005; Mooney et al. 2012). The effects of plants (i.e., resource, bottom-up effects) and natural enemies (i.e. top-down effects) have important implications to the evolution and diet breadth of insect herbivores (Singer and Stireman 2005; Mooney et al. 2012; Vidal and Murphy 2018), and a tri-trophic approach is advisable to understand diet breadth patterns. Here, we introduce a new conceptual framework to measure the effect of tri-trophic interactions on herbivore fitness (called tri-trophic fitness slope) and we apply our framework to interpret 1) host shifts in different systems and 2) how diet breadth can change over time and space in natural populations of a generalist herbivore. Our quantitative measure of fitness associated with tri-trophic interactions has the potential to be used in other systems to advance our understanding of resource specialization and diet breadth evolution as well as other questions related to selective pressures.

Conceptual framework

We propose a simple and straightforward way to measure tri-trophic fitness by adding the resulting slope of the two bi-trophic fitness slopes (adapted from Singer and Stireman 2005). The addition of the slopes results in a measure similar to the mean of the fitness associated with each bi-trophic force. Here, we explain our conceptual framework and provide examples using insect herbivores, but our conceptual framework could be used with different systems to test a variety of questions, which we address later. To calculate our proposed tri-trophic fitness slope, herbivores need to use at least two host plants or have a variable measure of bottom-up effect or top-down effect (e.g., same host species but of different quality or same host species but with spatial variability in enemy exposure). We present a case (Figure 6) that measures fitness associated with bottom-up and top-down forces of herbivores feeding on two different host plant species, while our conceptual framework (Figure 7) considers frequency of host plant use, therefore different measures of host use can be used. For each host plant, we first calculate the bi-trophic bottom-up effect (bi-trophic because here we consider only the herbivore and its host plant) on herbivore fitness for both host plants (e.g. solid black line in Figure 6). Then we calculate the bi-trophic top-down effect (bi-trophic because here we consider only the herbivore and its natural enemies) on herbivore fitness (e.g. dotted line in Figure 6). Finally, to calculate the tri-trophic fitness slope (dashed lines in the right side of Figure 6), we add the two bi-trophic slopes, which is the same as taking the mean of the two bi-trophic fitness components. Fitness from tri-trophic interactions could alternatively be calculated by multiplying the bottom-up fitness component by the top-down fitness component, which results in a similar direction of tri-trophic slope (i.e. positive, null, or negative). We test this latter measurement with our case study using populations of a generalist herbivore (test II).

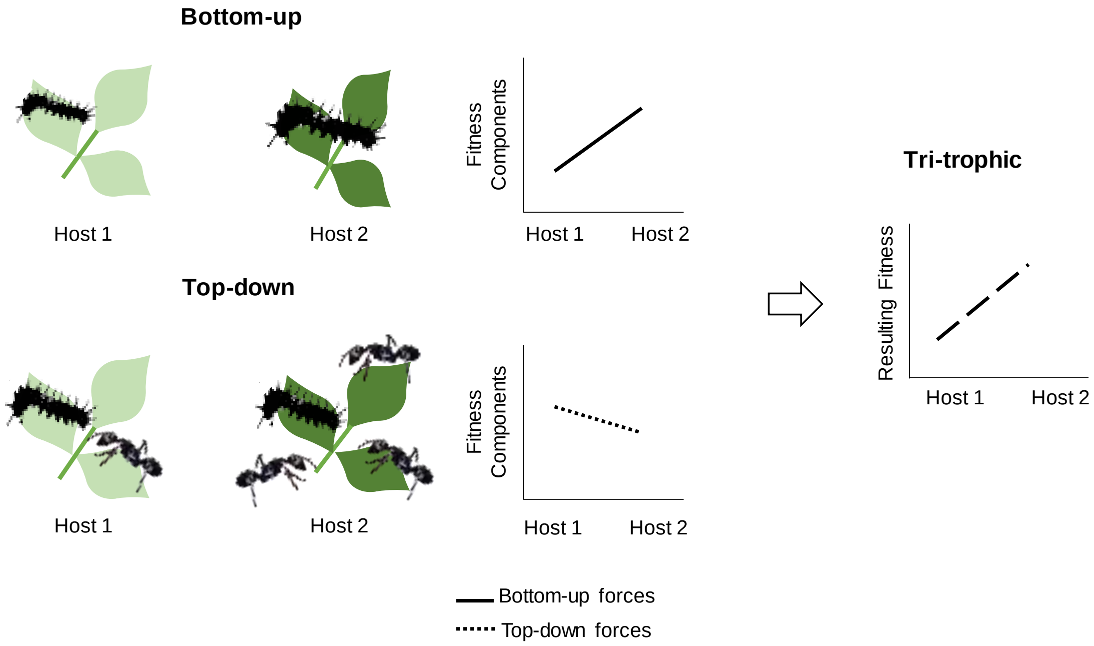


Figure 6: Example of a positive tri-trophic fitness slope. An herbivore has two potential host plants. Host 1 (light green) is of lower quality than host 2 (dark green), and as a result the herbivore grows more when feeding on host 2. When plotting the bi-trophic fitness (bottom-up effects) of the herbivore in relation to the two hosts, we find a positive relationship (solid line). However, the herbivore suffers more attacks by natural enemies (e.g. ants) on host 2 than on host 1, so when we plot the bi-trophic fitness (survival from escaping enemies, top-down effects) in relation to the two hosts, we find a negative relationship (dotted line). In this example, the positive bottom-up slope is steeper than the negative top-down slope, so the resulting tri-trophic slope is also positive (dashed line).

We consider all possible combinations of bi-trophic slopes, which results in three possible tri-trophic fitness scenarios:

I) Positive tri-trophic slope: the population feeds most frequently on the host on which they have higher tri-trophic fitness compared to the overall fitness associated with less frequently used hosts. When a positive bi-trophic slope is steeper than the negative bi-trophic slope (i.e., greater difference in top-down or bottom-up fitness across frequency of host use) or when both bi-trophic slopes are positive, it results in a positive tri-trophic slope (Figure 7, top row). In the case of multiple hosts being compared, the fitness associated with the selective forces might be high for some plants that are used in low frequency, however when considering all plants, the trend would be to have higher fitness associated with the tri-trophic interaction on the plants used more frequently.

II) Null tri-trophic slope: the population feeds on different hosts that have equal tri-trophic fitness. When the negative and the positive bi-trophic slopes have equal value or cancel each other, the resulting tri-trophic slope would be null (Figure 7, middle row). Again, the variation in fitness found when studying herbivore performance on multiple host plants may show the herbivore to have higher fitness on some hosts compared to others, but our slopes consider the overall trend across all hosts and not individual comparisons between hosts.

III) Negative tri-trophic slope: the population tends to feed most frequently on the hosts on which they have lower tri-trophic fitness. If the negative bi-trophic slope is steeper than the positive bi-trophic slope (i.e., lower top-down or bottom-up fitness on the most frequently used host) or both bi-trophic slopes are negative, the resulting tri-trophic slope would be negative (Figure 7, bottom row).

Different measures of top-down and bottom-up forces can be used with our conceptual framework, and it would be advisable to use measures known to have significant fitness impacts in the specific study system investigated. For example, in systems in which top-down forces are exerted mainly by predators and exclusion experiments are used to measure predator impact, researchers could use relative survival rate in predator-exposed versus predator-excluded treatments as a top-down fitness measure. Similarly, bottom-up fitness could also be measured as growth rate, body mass or reproduction rate, if those measures are known to be associated with plant quality and herbivore fitness. It is important to choose appropriate fitness measures as bottom-up and top-down forces have different impacts on different fitness measures (Vidal and Murphy 2018). In our examples, we mostly use survival and a measure of mass, which are fitness proxies well supported in studies with insect herbivores (Honěk 1993; Crone 2001). Notably, the measures of fitness associated with the top-down and bottom-up forces must be of similar scale. For example, if survival used to measure top-down forces is measured as a percentage, bottom-up effects should vary between 0-100 as well. Another option is to use eigenvalues from principal component analysis when there are multiple fitness measures (we discuss this further in the "Limitations" section). If the measures are not of similar scale, the slopes would be of different scale, which might result in one slope having stronger influence than the other without biological precedence. Further, we sometimes refer to bottom-up, top-down, or tri-trophic fitness, and although fitness is a measure at the individual level relative to other members of the population, we use this terminology to refer to fitness resulting from those selective forces or interactions.

We describe below two tests of our conceptual framework, first using data from the literature that tested herbivore host shifts, and second using natural populations of a generalist herbivore that we spent several years studying. For herbivores, our model can be easily used with specialist herbivores to investigate potential host-shifts or host-expansion, or simply to measure the resulting tri-trophic fitness from studies testing top-down and bottom-up effects. Our proposed conceptual framework could be used to infer tri-trophic fitness in different systems, including non-herbivores; we present limitations and possible applications of our conceptual framework beyond herbivore diet breadth.

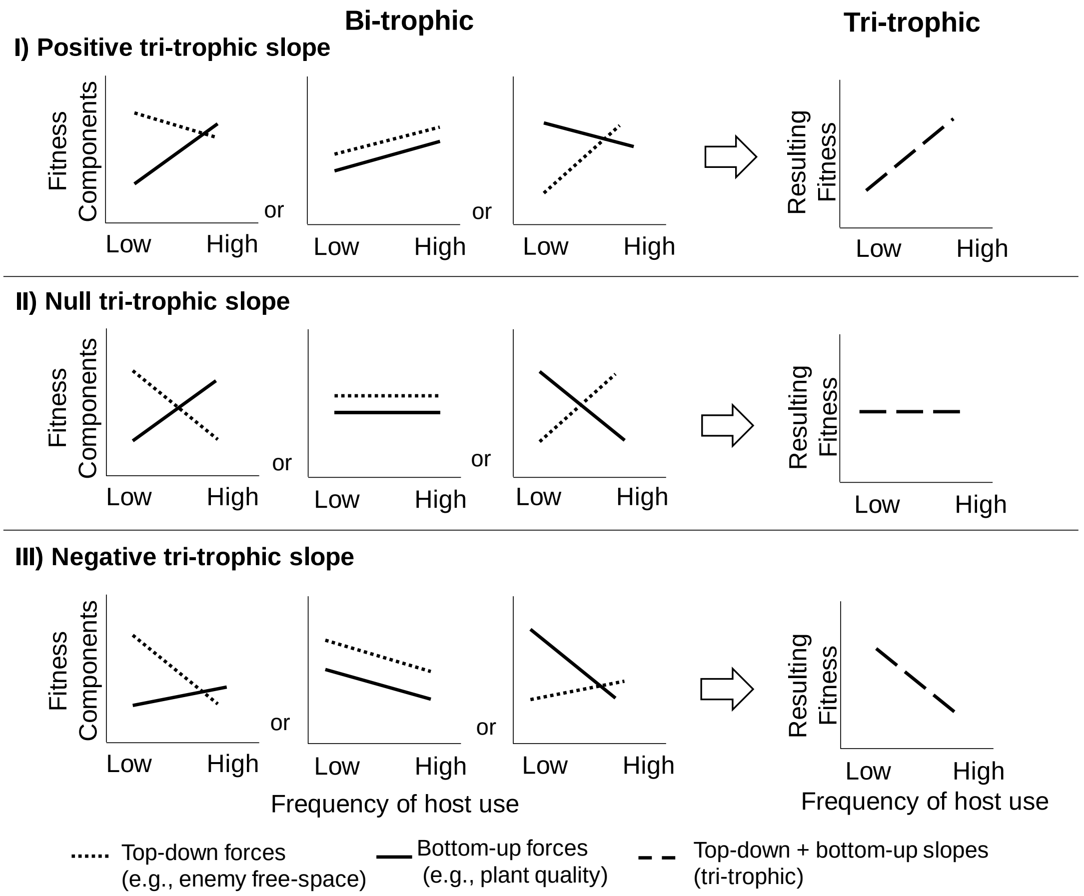


Figure 7: Conceptual framework of how to measure tri-trophic fitness based on all possible cases of bi-trophic interactions that consider only top-down (TD; e.g. EFS) or bottom-up (BU; e.g. host plant quality on larval fitness) forces. We consider both TD and BU as positive effects on the herbivore. For example, for the BU force lines, high quality host plants result in greater fitness components (e.g. growth rate) for the herbivore. Similarly, for the TD force lines, host plants where the herbivore escapes enemies (e.g. EFS; the herbivore suffers reduced predation/parasitism) result in greater fitness components (e.g. survival) for the herbivore. In the bi-trophic figures, TD or BU selective forces are shown separately with respect to frequency of host plant use in a given time. The tri-trophic figures show the separate bi-trophic slopes added together (similar as the mean of bi-trophic fitness components); the direction of the tri-trophic slope is similar (positive, null, or negative) if fitness components are multiplicative (top-down fitness as a portion of bottom-up fitness, not shown here). Considering tri-trophic fitness, we have three possible scenarios: I) TD, BU or both are significantly greater for the most frequently used host resulting in positive tri-trophic fitness; II) TD and BU cancel each other, or there is no difference among hosts; III) the host plant most frequently used is associated with lower TD and/or BU effects, resulting in negative slope of the tri-trophic interaction. See Figure 1 for a descriptive example.

Test I - Proof of concept using host shifts by multiple insect herbivore taxa

A consistent conceptual framework should be applicable to different taxa and independent of sampling methods. To test the consistency of our conceptual framework we applied it to tests of herbivore host shifts from the published literature; we used studies that measured both top-down and bottom-up forces on the herbivore. Host shift is one of the mechanisms that can lead to speciation in herbivorous insects by the formation of host races (Drès and Mallet 2002). Herbivores can experience increased fitness on a novel host compared to an ancestral host through reduced attack from natural enemies (e.g., Murphy 2004). Considering the enemy-free space hypothesis, we would expect that the advantage of escaping enemies should trump the disadvantage of feeding on a poor quality plant (Berdegue et al. 1996). Thus, the top-down slope should be positive and have a greater absolute value than the bottom-up slope, which should be negative according to the enemy-free space hypothesis (the herbivore experiences a trade-off between poor host plant quality and survival from natural enemies on the novel host). Therefore, the herbivore’s tri-trophic fitness should be greater on the novel than in the ancestral host, and hence the resulting tri-trophic slope would be positive (Figure 7 top row).

We consider a host shift to be the inclusion of a novel host in the herbivore’s diet, regardless if the herbivore continues to use the ancestral host or not (sensu Agosta 2006; sometimes termed a diet breadth expansion). We searched for papers that tested herbivore host shifts using both top-down and bottom-up forces with Web of Science® on March 29 and 30, 2017, and used combinations of search terms including: enemy-free space, host shift, host expansion, host race, host switch, insect herbivor*, natural enem*, and tri-trophic. We only used papers that had clear novel and ancestral hosts and had direct measures of bottom-up and top-down effects on both hosts. With these criteria, we found 14 papers (13 cases) for our analysis (Table 2). To obtain the slope of each bi-trophic measure, we plotted the novel and ancestral hosts on the x-axis and fitness on the y-axis. We added the slope from the equation of the line from both top-down and bottom-up graphs to derive the tri-trophic slope.

Table 2: Summary of the data collected from published papers testing host shifts by herbivorous insects for both naturally occurring and artificial host shifts.

Paper	**Naturally occurring host shift?**	**Herbivore**	**Ancestral host**	**Novel host**	**BU slope**	**TD slope**	**Tri-trophic slope**
Gratton and Welter 1998, 1999	N	*Liriomyza helianthi*	*Helianthus annuus*	*Centaurea solsitialis*	-0.25	0.14	-0.11
Gross et al. 2004a,b	N (this population)	*Chrysomela lapponica*	*Salix borealis*	*Betula pubescens*	-0.84	0.1	-0.74
Mira and Bernays 2002	Y	*Manduca sexta*	*Datura wrightii*	*Proboscidea parviflora*	-0.45	0.43	-0.02
Vosteen et al. 2016	Y	*Acyrthosiphon pisum*	*Vicia faba*	*Trifolium pretense*	-0.1	0.2	0.10
				Pisum sativum	0	0.07	0.07
				Medicago sativa	-0.38	0.74	0.36
Murphy 2004	Y	*Papilio machaon*	*Cnidium cnidiifolium*	*Artemisia arctica, Petasides frigidus*	-0.22	0.29	0.07
Feder 1995, Prokopy et al. 1988	Y	*Rhagoletis pomonella*	*Crataegus* spp. (hawthorn)	*Malus pumila* (apple)	-0.25	0.32	0.08
Diamond and Kingsolver 2010	Y	*Manduca sexta*	*Nicotiana tabacum*	*Cotesia congregate*	-0.29	0.4	0.11
Mulatu et al. 2004	Y	*Phthorimaea operculella*	*Solanum tuberosum* (potato)	*S. lycopersicum* (tomato)	-0.13	0.27	0.14
Brown et al. 1995	N	*Eurosta solidaginis*	*Solidago altissima*	*S. gigantea*	-0.14	0.32	0.18
Meijer et al. 2016	Y	*Rhagoletis alternata*	*Rosa canina*	*R. rugosa*	0.23	0.06	0.29
Torres-Vila and Rodriguez-Molina 2013	Y	*Lobesia botrana*	*Daphne gnidium*	*Vitis vinifera*	0.69	0.04	0.73

As predicted, we found that all cases of host shift in which the herbivore used the novel host in nature had a positive or null tri-trophic fitness slope (Table 2). Of the 13 cases, 10 had a positive tri-trophic fitness slope, 1 had a null slope (-0.02 in Table 2) and 2 had negative slopes (Table 2, -0.1 and -0.74). Considering the 2 cases with negative slope, one was from an experimental host shift in which the insect was switched to a plant it would not use naturally (Gratton and Welter 1999), while the other was from a population that did not experience a host shift (Gross et al. 2014a,b). Both top-down and bottom-up slopes had a significant relationship with tri-trophic slopes (r^2=0.57, P=0.003 and r^2=0.7, P=0.0004, respectively). Interestingly, all cases had a positive top-down slope, which shows that for all of these host shifts, the herbivore suffered lower attack from natural enemies on the novel host and suggests that mortality from natural enemies is a driving factor in diet breadth expansions. Most of the cases had a negative bottom-up slope, except for 2 that were positive and 1 that was null. Thus for 10 of the 13 cases we found trade-offs between top-down and bottom-up forces (positive top-down and negative bottom-up). The trade-offs and positive top-down slopes follow the requirements to be considered a test of enemy-free space, wherein the novel host on which an herbivore escapes enemies must be of lower quality compared to other hosts (Berdegue et al. 1996).

Test II - Case study using populations of a generalist herbivore with variable diet breadth

For the second test of our conceptual framework, we present a case study in which we used the tri-trophic fitness slope to interpret and predict diet breadth evolution of a generalist herbivore. Fall webworm (*Hyphantria cunea*, Erebidae, Lepidoptera; hereafter FW) is an insect herbivore native to North America and is a dietary generalist that feeds on more than 600 host plant species over its geographic range (Warren and Tadić 1970). As a species, FW are dietary generalists, but individual larvae feed only on the plant species that their mother chose as an oviposition site. FW is an ideal model organism to study diet specialization because the diet breadth of populations varies considerably across their geographic range (see for example Murphy and Loewy 2015, Mason et al. 2011). With our conceptual framework we investigate why some populations are more specialized or generalized than others; for a species like FW that is a true generalist and seems to remain so over time, we would predict that the maintenance of generalism would require populations that are more generalized in their diet breadth to have greater tri-trophic fitness than populations that are more specialized in their diet breadth. Further, we make predictions about how population diet breadth may change over time. In our model we use mean fitness for individuals within a population and diet breadth at the population level because most insect herbivores feed during their entire larval stage on only one host plant individual, and thus we cannot calculate individual diet breadth measures. However, for the case of generalist individuals that can move from one host species to another (e.g., adult beetles, Orthoptera, some caterpillars), the measure of diet breadth could be assessed at the individual level.

Field Survey

To analyze how spatial difference in strength of bottom-up and top-down forces influences diet breadth, we sampled 10 different populations of FW in Colorado in the summers of 2011, 2012 (data from Murphy and Loewy 2015), 2014, 2015 and 2016. We considered the 10 sampling locations as different populations because female FW are known to not fly very far from their emergence location and males can fly only up to 23 km (Yamanaka et al. 2001), a smaller distance than our two nearest locations (48 km). Female moths lay eggs in a clutch on a single host plant and sibling larvae feed in aggregation, forming a web that encompasses the host plant branches, usually including hundreds of sibling larvae. In each population, we looked for webs near roads and rivers where FW usually occur (Murphy and Loewy 2015). When we found a web, we identified the host plant species and established two 10 m transects on each side of the host; along the transects we recorded the identity of all plant species, excluding plants that rarely serve as FW hosts (e.g., gymnosperms and grasses). We sampled at least 21 webs in each population, and we collected 10 larvae from each web to rear in our laboratory to measure percent parasitism, pupal mass and survival. In the laboratory, we fed all larvae with leaves from the same host plant species collected from the location from which the larvae were found. We used the same protocol for data collection and larval rearing as described in Murphy and Loewy (2015), and thus were able to include additional data from Boulder and part of Larimer and Jefferson populations from that study.

Bi-trophic measures

We used plant abundance and plant quality (measured as herbivore pupal mass and survival) as measures of bottom-up forces and percentage of larvae that escaped parasitoids as a measure of top-down force. Plant abundance has been shown to influence host plant use by FW (Mason et al. 2011; Murphy and Loewy 2015). Pupal mass and survival are fitness measures related to plant quality (Awmack and Leather 2002); larvae reared on plants of high quality have greater survival and greater pupal mass than larvae reared on poor quality hosts, and since pupal mass is directly related to fecundity, it is a reliable fitness proxy (Loewy et al. 2013). FW larvae are greatly affected by parasitoids and are less affected by invertebrate and bird predation (Morris 1972, 1976), therefore parasitism rate is a reliable measure of the strength of top-down forces.

We calculated relative abundance and relative host use following Mason et al (2011) and Murphy and Loewy (2015) and we analyzed the relationship between host plant use and abundance using linear regression. We only tested this relationship for populations that used 3 or more host plant species. To test if pupal mass, survival and parasitism rates vary among populations and host plant species, we used a (generalized) linear mixed model with pupal mass, survival or parasitism as the response variable, host plant and population as fixed factors, and the web of origin was a random factor (to control for genetic similarity within webs). For the models including survival and parasitism rates, we used a binomial distribution. We performed our analyses in R environment 3.4.1 (R Development Core Team 2011), and we used the package afex v. 0.18-0 with function mixed (Singmann et al. 2017). To calculate each bi-trophic fitness slope, we determined the relationship between the fitness score for each host plant species with respect to frequency of host plant use for each population. Then, we used the additive result of the two bi-trophic slopes as the tri-trophic fitness relationship among host plants for each population.

We found significant variation across all populations and across hosts for parasitism rate (Population: X^2=60.47, df=9, P<0.0001, Host: X^2=55.56, df=29, P=0.002), for pupal mass (Population: $F_{9,376.23}$=3.76, P=0.0001, Host: $F_{29,435.56}$=1.63, P=0.02), and for survival rate (Population: X^2=52.36, df=9, P<0.0001, Host: X^2=43.2, df=29, P=0.04). The variation in plant quality and parasitoid pressure fits well with the conditions of our model, as we need to have variation in selective pressure and host plant use among populations to be able to compare the effect of variation in tri-trophic fitness on different diet breadths. Host plant abundance is a predictor of host plant use for all locations (r^2 values range from 0.38 to 0.89, all P<0.05). Therefore, there is a strong correlation between abundance and frequency of host use, as was also found by Murphy and Loewy (2015) and Mason et al. (2011). However, most populations in our study had one host plant species with a higher frequency of host use than the other species, and when we removed this "outlier", we found no relationship between host use and abundance, so the relationship was mainly driven by the most frequently used host (as was also found by Murphy and Loewy 2015). Only 3 populations had negative bottom-up slope (triangles in Figure 8), which means that most populations fed most frequently on the hosts associated with greater pupal mass and survival. However, all populations had negative top-down slope, which means that all populations fed most frequently on host plants associated with higher parasitism rate (see Appendix Figures 5 and 6 for all bi-trophic slopes, host abundance and host use, and distribution of all 10 populations).

Tri-trophic fitness and diet breadth

Although diet breadth has different types of measures, the most acceptable view of diet breadth is as a continuum between generalism and specialism, especially considering that diet might vary in a species depending on location or time (Forister et al. 2015). We used 3 continuous measures of diet breadth: (1) relative generalism, which considers the number of hosts used relative to the number of hosts available, (2) the number of plant species included in the diet of each population, and (3) Ordinated Diet Breadth (ODB), which calculates diet breadth relative to the identity of plants used in each population compared to the hosts used by other populations. With ODB, when a population uses more plants that are dissimilar to those used by another population, it gets a higher diet score (Fordyce et al. 2016). The number of plants used and ODB are expected to yield similar trends, because they are similar measures of diet breadth (Fordyce et al. 2016). We used the package ordiBreadth (Fordyce 2015) to calculate ODB in R. We analyzed how tri-trophic fitness slope influences diet breadth by using a linear regression.

As an additional test of our conceptual framework, we performed another analysis with an indirect measure of fitness associated with tri-trophic interactions, which we will refer as “tri-trophic score”. In each population, we obtained the mean pupal mass per host plant species and multiplied it by the proportion of survival from escaping natural enemies on the same host species (without considering mortality by other factors). Since this measure does not take into consideration the frequency of host plant use as our tri-trophic slope does, we used another measure of diet breadth that included this frequency, the Shannon-Weiner Diversity Index (H’). To calculate the diversity of diet breadth in each population, we summed the multiplication between proportion (i.e., frequency) of use and the natural log of the proportion for all hosts used in each location, and therefore we considered both the number of plants used and the frequency by which they were used.

Considering the combined effects of different bottom-up and top-down forces on diet breadth of FW, we found a positive relationship between tri-trophic slopes and both the number of plants used and ODB (plant number: r^2=0.6, P=0.008; ODB: r^2=0.53, P=0.018; Fig. 8A, only ODB shown because figures nearly identical for the two measures), in which populations with positive slopes had a broader diet, while populations with negative slopes had narrower diet. We found the same pattern when we considered the relationship between diversity of diet breadth and tri-trophic score (Fig. 8B, r^2=0.13, P<0.001), in which populations that had higher mean "tri-trophic fitness" also had more generalist diets. However, when considering relative generalism as diet breadth measure, we found no relationship between diet breadth and tri-trophic slope (r^2=0.003, P=0.87). We found 5 populations with a negative tri-trophic fitness slope (i.e., individuals feed more frequently on the host associated with lower tri-trophic fitness), and 5 with positive tri-trophic slope (i.e., individuals feed more frequently on the host associated with greater tri-trophic fitness). There was a positive relationship between the number of webs sampled and number of plants used (r^2=0.46, P=0.03), but there was no relationship between sample size and tri-trophic slope (r^2=0.19, P=0.2). Bottom-up slope was correlated with tri-trophic slope (r^2=0.93, P<0.0001), but top-down was not (r^2=0.001, P=0.92). Therefore, in this case tri-trophic fitness might be driven by bottom-up forces.

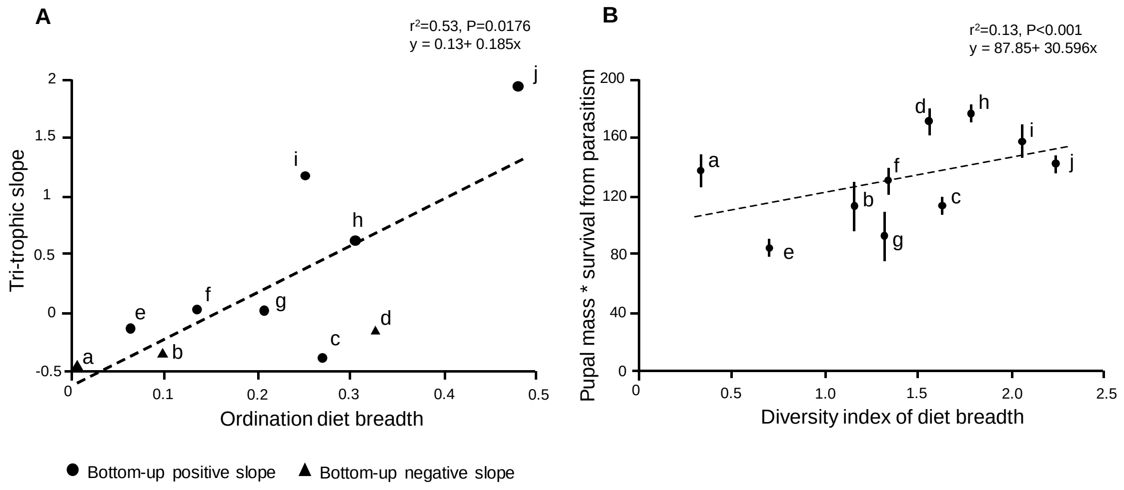


Figure 3: Relationship between diet breadth and measures of fitness associated with tri-trophic interactions for fall webworm populations. A) Diet breadth measured using Ordinated Diet Breadth (Fordyce et al. 2016) and fitness measured as the resulting tri-trophic slope as explained in figures 1 and 2; circles represent populations with positive bottom-up slope and triangles represent populations with negative bottom-up slope. B) Diet breadth measured as Shannon-Wiener Diversity index (which considers the frequency of host use) and fitness measured as the multiplication of pupal mass and proportion survival from natural enemies (i.e., tri-trophic score), lines across the circles represent standard error; we show here the mean and standard error, however the statistical analysis was done with all points. Each data point is a different population and letters represent the county where the population was located in Colorado: a) Arapahoe, b) Las Animas, c) Jefferson, d) Mesa, e) Garfield, f) El Paso, g) Chaffee, h) Baca, i) Larimer, j) Boulder.

The positive relationship between diet breadth and tri-trophic slope demonstrates that populations that use the host associated with greater fitness most frequently have a broader diet, but it also shows that in a given population, the more variation in fitness among hosts (i.e., steeper slope), the more host plants the population uses. However, there is not necessarily a cause-consequence relationship between diet and tri-trophic fitness slope, as it is not clear if the fitness slope would be the cause or the consequence of variation in diet breadth. For our most generalist population (Boulder, letter j in Fig. 8), it is possible that their infrequent use of hosts associated with low tri-trophic fitness represents a transition towards not using them at all. Evidence for this argument is seen when we consider the relative generalism of this population, which used only 40% of the plants available, and thus it is possible that the population is transitioning to a narrow diet. It is also possible that the females still recognize the plants associated with lower tri-trophic fitness as potential hosts, and this might be the case especially if the change in plant quality/natural enemy attacks occurred recently or if the females are not choosy when selecting an oviposition site. In contrast, Arapahoe (letter a in Fig. 8) was our population with the narrowest diet when considering number of plants used and ODB, but had the highest relative generalism score (67%); this population had few options of host plants to use (larvae were using 2 out of 3 plants available), and the negative tri-trophic fitness slope might be because individuals use host plants that they have available, but that are not necessarily good hosts. Furthermore, all 10 populations had negative top-down slopes, which means that FW feeding on hosts used in lower frequency had lower parasitism than FW feeding on more frequently used hosts. This result suggests that FW feeding on low-use host plants may escape their enemies, and thus that these little-used host plants may offer enemy-free space. Another possibility is that parasitoids are preferentially attacking herbivores on hosts that are used more frequently (as host-plant dependent parasitism, e.g., Lill et al. 2002, or density-dependent parasitism, e.g., Singer et al. 2012).

It should be noted that we present here a snapshot of the FW populations at a specific time. Our model, however, can also be used to show variation over time, which could increase our understanding of how diet breadth evolves. From our model, we would expect that populations with a steep positive slope that are generalists (Boulder and Baca populations for example; in Fig. 3 letters j and i respectively) may eventually stop using the hosts that they use in low frequency and on which herbivores have low tri-trophic fitness (top-down fitness is high, but bottom-up fitness is very low). Those populations may therefore become more specialized (use fewer hosts) and thus their tri-trophic slope may become closer to zero because as the low-frequency hosts are eliminated from the diet, the diet would include only the formerly medium-frequency and the high-frequency hosts with tri-trophic fitness more similar to each other; in other words, the trade-off lessens and becomes more similar across host plants. Alternatively, if the selective advantage of enemy-free space is great enough, there may be selection for individuals that perform well on the low-quality host plants. Murphy (2004) suggested that enemy-free space should be ephemeral because selection for improved physiological performance on low-quality hosts should be high if the relief from natural enemies is great enough. Thus, the bi-trophic bottom-up slope would become less steep as low-frequency host plants became higher-quality and the tri-trophic slope would also decrease. The populations at the other end of the spectrum that frequently use hosts on which the herbivores have low fitness (for example, Las Animas and Jefferson; letters b and c in Fig. 8), should be selected upon to use more frequently the best hosts and therefore would eventually have a null or positive tri-trophic fitness slope. However, because those populations with negative tri-trophic fitness slopes are also using more frequently the most abundant hosts, it is possible that those populations would not completely stop using the abundant, yet poor-quality hosts, and therefore would still have a generalist diet. Overall, we would expect that over time populations would move along the dashed regression line in Fig. 8, “sliding” from the lower left to the upper right or vice versa.

Implications for diet breadth evolution of insect herbivores

Assessments of tri-trophic effects on herbivore fitness have been done qualitatively by inferring the combined effect of quantitative measures of bottom-up and top-down forces on herbivore fitness. However, the quantitative resulting effect of those two selective forces can differ from just qualitative comparisons. Here, we propose a way to quantitatively measure tri-trophic effects on herbivores fitness by adding the bi-trophic fitness slopes as a means of integrating the relationship between variation in selective forces and a measure of host plant use. We showed that for a generalist herbivore (FW), the tri-trophic fitness slope positively influenced diet breadth: populations using a greater number of different plants (i.e., generalists) have greater fitness associated with tri-trophic interactions on the plants that the individuals use more frequently. We also tested our model using published data on host-shifts that described bottom-up and top-down forces on both the ancestral and novel hosts, and we found that studies testing naturally occurring host shifts had greater tri-trophic fitness on the novel hosts than on the ancestral hosts, as we predicted. Our quantitative measure of fitness associated with tri-trophic interactions will allow us to better understand why and how diet breadth of herbivores change over space, and we could make predictions of how natural populations of a generalist herbivore might change their diet breadth over time. Assuming that natural selection is acting on the individuals of a population, selection should favor individuals that have greater tri-trophic fitness on a particular host, which would lead to a change in the pattern of host plant use according to the tri-trophic fitness in each population. In the case of a generalist herbivore such as FW, our finding suggests that generalism is maintained because there is clear fitness advantage of having a broader diet than a narrower diet for the populations of this species. This gives further support to the idea that being a generalist is not necessarily a disadvantageous strategy.

We found that trade-offs between top-down and bottom-up forces can be very common. Indeed, for our host shift analysis of published studies, we found trade-offs for 10 of the 13 cases; bottom-up slopes were mostly negative and top-down slopes were all positive. Interestingly, we found positive or null tri-trophic slope for all cases in which the shift was a naturally occurring host shift by the herbivore. This gives support to the idea that specialist herbivores include new hosts in their diet when the host is advantageous, and in these cases the benefit of escaping enemies on the new host trumps the disadvantage of the novel hosts being of worse bottom-up quality. For our FW data, most populations had positive bottom-up slopes and negative top-down slopes (Fig. 3, circles). Trade-offs between top-down and bottom-up forces when choosing a host plant have been shown to occur in other systems as well (e.g., Thompson 1988; Thompson and Pellmyr 1991; Mira and Bernays 2002; Murphy 2004). Therefore, it seems likely that trade-offs in top-down and bottom-up fitness will be found for most populations.

Our model to calculate tri-trophic fitness can be helpful to interpret pattern amidst many data points. In our model FW system, we had almost 4,000 data points (individual FW larvae feeding on different host plants across sites), and great variation in bi-trophic forces within and across populations and host plants. Using tri-trophic fitness slopes, the pattern for each population became clear and we could infer how selective forces influenced diet breadth. Although selective forces are expected to vary in strength over time and space, few papers on top-down and bottom-up forces have adopted this view (Gripenberg and Roslin 2007; but see Heard et al. 2006). Notably, our study is the first to use a geographic mosaic of selection (Thompson 2005) to investigate diet breadth evolution. With the geographic mosaic of selection and tri-trophic fitness slope, we can better understand diet breadth evolution and what leads some populations to have broader diet than others depending on their local selective forces. Furthermore, we showed that our quantitative measure of fitness works well with other systems given that we obtained the expected pattern with host-shift data. We believe that our quantitative measure of tri-trophic fitness will be useful in future research testing top-down and bottom-up effect in herbivore-plant interactions.

Conceptual framework limitations

Our conceptual framework is a first step towards a quantitative measure of fitness associated with tri-trophic interactions or multiple selective forces measures. Due to its simplicity, the framework has a few limitations that can be potentially explored in future research. One of the limitations is that we assume a linear relationship between fitness and a measure of host use, which is not necessarily true. In the case of only two variables in the x-axis, the linear relationship will be supported, as was the case with our first test using published data on host shifts. However, in our second test, we had many data points for most of the FW populations, and even though in most cases the relationship between fitness and host use was linear, there were some cases in which the intermediate-frequency host would be the best host. The calculations using non-linear relationships are more difficult, but they are still possible and can be used to make predictions (e.g. mutualism dynamics Holland and DeAngelis 2010, body size in trophic cascades DeLong et al. 2015).

Another pitfall of our conceptual framework is that we assume an additive effect of bi-trophic slopes, which is not always the case. This limitation is easy to deal with if the system of study and the responses to the different selective forces are well known. For example, in the risk allocation hypothesis, it is expected that prey response will depend on both the level of risk and the amount of time that predators are present (Lima and Bednekoff 1999). However, the effect of the level of risk and exposure time is not additive, as extremes in both risk and exposure can lead to weak prey response (Ferrari et al. 2008). In this case, we might use division instead of addition of the slopes to get to the resulting effect of both selective forces. Similarly, if there are selective forces that have negative effects on the focal organism, but the resulting effect is expected to be positive, one could multiply the slopes.

As we already mentioned above, a problem may result when the fitness measures differ between the two selective forces. This is a problem because the slope is directly related to the scale of the y-axis, if one uses numbers that range from 0 to 1, the slope value will also be in that range. Therefore, it is advisable to use similar ranges to measure bi-trophic fitness slopes. In our case study, we used survival for top-down forces that usually ranged from 50 to 100, and we considered the multiplication of proportion survival by pupal mass as our bottom-up force, which ranged from 30 to 160. It is still a similar range, however, we found that bottom-up forces were the main driver of tri-trophic slopes, which might be because of it having a broader range. One way to deal with this problem is to use the log of the slopes to get a similar value between them. If there is more than one fitness measure per selective force, one can also use eigenvalues from principal component analysis, given that the vectors used explain a good portion of the data. But we advise that thought should be put into what fitness measure to use before conducting an experiment, as the fitness proxy used should be well connected to the fitness of the study organism and to make calculations easier.

Finally, another limitation is that our conceptual framework does not directly consider variation in the data. This again might be a problem with multiple data points, as the line will consider the entire distribution of points but will not necessarily be a good fit. One way to deal with this problem is to calculate a variation measure for tri-trophic slopes from the variation found in the bi-trophic data. Another option is to use a measure of fit associated with the tri-trophic slope as a weight in the model, for example by multiplying each bit-trophic slope by its value of r2, that way slopes that have a better fit will have more weight in the calculation of the tri-trophic slope.

Potential applications and concluding remarks

Since our framework is simple and straightforward, there are many options of applications and modifications that can be made, and here we suggest only a few. Any research question that has measures of two selective forces in similar conditions can use our framework to obtain the resulting effect of these two forces together. For example, we can envision applications for questions of niche specialization, mutualistic interactions or tests of sexual vs. natural selection (Figure 9).

One of the challenges in ecology and evolution is to find pattern in the initial chaos that may appear when natural communities are first examined. In an attempt to study these patterns, we as ecologists and evolutionary biologists tend to simplify possible interactions and selective forces to interpret one or a few at a time, such as the many studies that focus only bi-trophic interactions when we now know that bi-trophic interactions can only be interpreted in a tri-trophic (or multi-trophic) context (Vidal and Murphy 2018). Our conceptual framework is a first step towards a more complex integration of multiple selective forces, in which we intend to analyze the fitness consequence of at least two selective forces acting together on the focal organism. We believe that our conceptual framework can open possibilities for the development of more complex frameworks and can be applied to studies that measure multiple selective forces to understand the evolution of interactions.

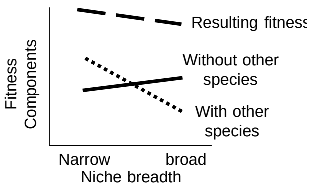
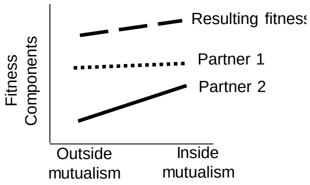
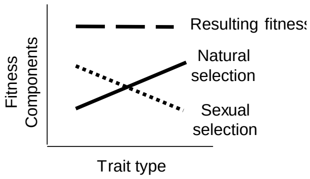
Theme	Example of problems	Example of application	Example of conceptual framework
Niche specialization	(1) Competition leads to niche specialization; (2) Top-down and bottom-up forces (or other selective forces) lead to expansion or retraction of niche.	(1) Competition for resources can lead to niche contraction[1]. If the resulting fitness slope from the fitness with or without competing species is negative (as in example), then niche specialization is expected. While if it is null or positive, niche expansion is expected.	
Mutualistic interactions	(1) Mutualism and antagonism interface; (2) Maintenance of mutualism over time.	(1) Facultative mutualisms are context dependent and might become antagonistic when one of the partners is harmed[2]. If the resulting slope is null or negative, partners have higher fitness outside the mutualism which would exemplify antagonism, whether if it is positive, might be a mutualistic interaction (as example). The steepness of the slope can also inform about the asymmetry of the relationship. In the example, Partner 2 might depend more on the interaction than Partner 1, as the latter has almost a null slope.	
Sexual vs. Natural selection	Which selective force drives the trait evolution.	Natural and sexual selection acting in opposite ways can lead to genetic variation in traits[3]. If the resulting slope of the two selective forces is null, then high genetic variation (or trait variation) is expected (as in example). While if it is positive or negative, one of the selective forces is stronger and thus driving selection, leading to directional selection of that particular trait.	

Figure 9: Examples of possible applications of our conceptual framework. [1] Futuyma and Moreno 1988, [2] Chamberlain et al. 2014, [3] Gray and Cade 1999.

CHAPTER 3: Local adaptation in a widespread generalist herbivore

Introduction

Local adaptation is a common outcome of natural selection (Reznick and Ghalambor 2001; Whitlock 2015). It has been found to occur in 65% of the cases studied, including in several species of herbivorous insects (Hereford 2009). Local adaptation is likely to occur in insect herbivores because they often spend the entirety of their development on the same plant individual (Funk et al. 2002). At the extreme, not only can insects be adapted to a local host plant species but to local or individual genotypes (Mopper 1996). Both the degree of gene flow and specificity can predict how fast and likely the herbivore is to be locally adapted to its host(s); when the host is not coevolving with the parasite (e.g., insect herbivore), high gene flow and low specificity of the parasite are expected to counteract local adaptation (Gandon and Van Zandt 1998; Gandon 2002; Kawecki and Ebert 2004). Generalist herbivores that feed on different host plant species in one location and freely mate with each other are expected to be under weak selective pressure from the host plant and have high gene flow among individuals feeding on different hosts, thus we would expect loose local adaptation of generalists to their hosts. Currently, there is no direct test of how much diet breadth influences local adaptation of herbivores, and although there are a few studies that considered gene flow (or geographic distance), none used an extreme generalist herbivore.

Fewer than 10% of herbivores are known to be generalists (arbitrarily defined as feeding on >4 plant families, Forister et al. 2015), and some ‘generalists’ may in fact be composed by multiple locally specialized populations (Fox and Morrow 1980) or even be host-specific cryptic species (e.g., Hebert et al. 2004). Theoretical models of local adaptation by parasites imply host specificity for local adaptation to occur in host-parasite systems, and thus a generalist diet is expected to limit local adaptation and specialist herbivores are expected to experience local adaptation more strongly than generalist species (Gandon 2002; Kawecki and Ebert 2004). There are exceptions to this prediction, as there are examples of local adaptation in generalist herbivores (e.g., Hanks and Denno 1994) and a lack of local adaptation in specialist herbivore species (e.g., Strauss 1997). Vidal and Murphy (2018) found in a meta-analysis that dietary generalists were less likely to experience trade-offs in fitness among different quality host plants than specialists. Because trade-offs are less likely for generalists, this could influence the evidence of local adaptation in generalists since trade-offs are expected for locally adapted organisms, manifested as greater fitness in their local environment than in another environment (Kawecki and Ebert 2004).

Another factor known to influence local adaptation is gene flow, the magnitude of which usually decreases with increasing geographic distance (Vekemans and Hardy 2004). The spatial scale at which local adaptation occurs has long been advocated as an important aspect of the mosaic of local adaptation in host-parasite systems (Gandon and Van Zandt 1998). Evidence for local adaptation is expected to be strongest when comparing populations at larger versus smaller geographic distances from the local population (Richardson 2014; Hereford 2009). Geographic distance has been included in a few studies testing the scale at which local adaption occurs in herbivores. These studies usually report that the strength of local adaptation increases with the distance from the local host plant population (Hanks and Denno 1994; Cogni and Futuyma 2009; Kalske et al. 2016; Tack and Roslin 2010).

In this study, we start with a literature review of local adaptation experiments focusing on insect herbivores in order to see the relative proportion of studies done with generalists and if the evidence of local adaptation varies according to the diet breadth of the focus herbivore. We further test our question of how diet breadth affects local adaptation by using both common garden and transplant experiments (*sensu* Kawecki and Ebert 2004) over more than a 2,500 km range to investigate the pattern of local adaptation on an extreme generalist herbivore. Insect herbivores can be under coevolutionary arms race with their host plants (Ehrlich and Raven 1964). The relative rate at which the interacting species are in coevolution with one another is an important aspect in local adaptation studies; in the case of long-lived plants and short-lived insects, insects might be locally adapted to the host plant population, while the long-lived partner would not be locally adapted to the herbivore, thus leading to a possible arms race advantage to the herbivore (Kawecki and Ebert 2004). To reliably say that a herbivores is locally adaptation, one should show evidence of the local herbivore having higher performance on the local population of hosts than on another population of hosts (home vs. away criterion), but most importantly, the local herbivore should have better performance on the local hosts compared to a herbivore from another location on the same hosts (local vs. foreign criterion) (Kawecki and Ebert 2004). Thus, with our common garden experiment we test the local vs. foreign criterion, and with our transplant experiment we test the home vs. away criterion.

In our experiments, we use fall webworm (hereafter FW, *Hyphantria cunea*, Erebidae, Lepidoptera), which is an extreme generalist over its entire geographic range (feeding on >650 plant species worldwide), but contains numerous local populations known to have narrower diets (e.g., Murphy and Loewy 2015). FW is an ideal model organism to study the influence of diet breadth and geographic distance on local adaptation because there are two sister species recently shown to be genetically distinct (Yang et al. 2017) and that vary in diet breadth, but are closely related enough to compare their performance on similar hosts. Furthermore, FW is a widespread herbivore, occurring across the North American continent, which makes it possible to test the home vs. away criterion in a broad geographic range. We ask the following questions in this study: 1) Can an extreme dietary generalist be locally adapted to different host plant species? 2) Do geographic distance and diet breadth influence local adaptation of generalist herbivores?

Methods

Literature review

To qualitatively evaluate the evidence for local adaptation in herbivorous insects, we performed a literature review by doing a Web of Science search using the terms local *adaptation and insect* herbivor*, performed on January 22, 2018. Papers were then read and categorized based on the diet breadth of the herbivore and whether they showed support, no support, or mixed results for local adaptation. We scored an experiment as showing *support* when the herbivore had greater fitness on the local host than on another host, or when the native population of the herbivore had better performance on their local host compared to a foreign population (Fig. 10, case I). We scored a study as *no support* when there was no difference in performance on the different hosts, or when a host plant was of better quality regardless of the herbivore origin (Fig. 10, case II). When multiple experiments or different conditions found different results supporting or not supporting local adaptation, we scored the study as *mixed evidence* (Fig. 10, case III). We classified if the study was directly testing geographic distance or not and we defined the diet breadth of the herbivore based on how the authors classified it in the paper. For the latter, the authors usually classified herbivores as generalists when they fed on multiple host plant species (>2 hosts, being from the same family or not), therefore it is a more conservative classification of diet breadth than the one we used previously (>4 plant families to be considered a generalist).

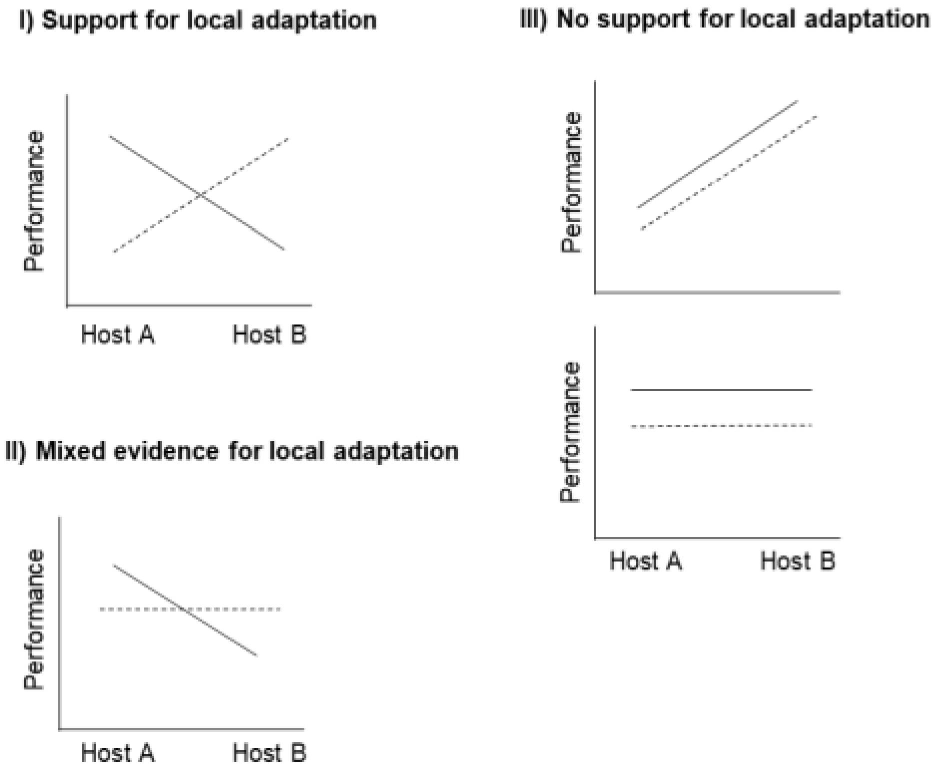


Figure 10: Classification of papers from the literature that test local adaptation in insect herbivores. For all cases, host A is the local host plant (familiar) for the focal herbivore and host B is the different host plant (unfamiliar). I) Support of local adaptation: the local herbivore (solid line) has better performance on the local host plant (host A) than on a different host (host B) in comparison to a foreign herbivore (dashed line) which has better performance on its local host (B) than on host A. II) Mixed evidence for local adaptation: a case shows that the local herbivore (solid line) has better performance on the local host plant (host A) than on a different host (host B), however another case finds that the herbivore has similar performance on the hosts being compared (dashed line). III) No support for local adaptation: either the herbivores being tested have better performance on the best quality host (host B) regardless of population origin, or the herbivores have similar performance on the hosts being compared.

Case study using fall webworm

Study system

Fall webworm is a highly generalist insect herbivore native to North America that feeds on more than 600 host plant species over its geographic range (Warren and Tadić

1970). As a species, FW are dietary generalists, but individual FW larvae feed only on the plant species that their mother selected as an oviposition site. Larvae of FW pupate in mid to late summer and overwinter as pupae. The adults emerge early May to mid-July, females lay eggs in a single large clutch on a host plant leaf and sibling larvae feed gregariously, forming a web that encompasses the host plant branches, usually including hundreds of sibling larvae.

There are two species of FW, easily distinguished by the color of their head capsule in the larval stage, black- and red-headed species. Black- and red-headed FW were considered to be two biotypes, however recent genetic analysis using mitochondrial CO1 and genomic data showed clear genetic differentiation of the two types, which indicates that they are different species (Yang et al. 2017; Chapter 4). The two sister species can be found in sympatry (e.g., both red- and black-headed are found together in the east and southeast US) or in isolation (e.g., in Colorado only the red-headed species is present, while in the northeast US only the black-headed species occurs), but the two species usually differ in the host plants that they use even when they co-occur, with few overlaps (e.g., Oliver 1964). Mason et al. (2011) and Murphy and Loewy (2015) showed that populations of black-heads from the eastern U.S. and red-heads from Colorado use most frequently the most abundant host plant species of those that are locally acceptable, even when those common species are not necessarily the best hosts (considering parasitism rate and plant quality). At least three clear geographic barriers isolate FW populations in North America: the Appalachian Mountains, the central plains and the Rocky Mountains (Fig. 11). These three barriers are likely to impede gene flow among populations separated by them, thus increasing the likelihood that populations are locally adapted to a set of local host plants.

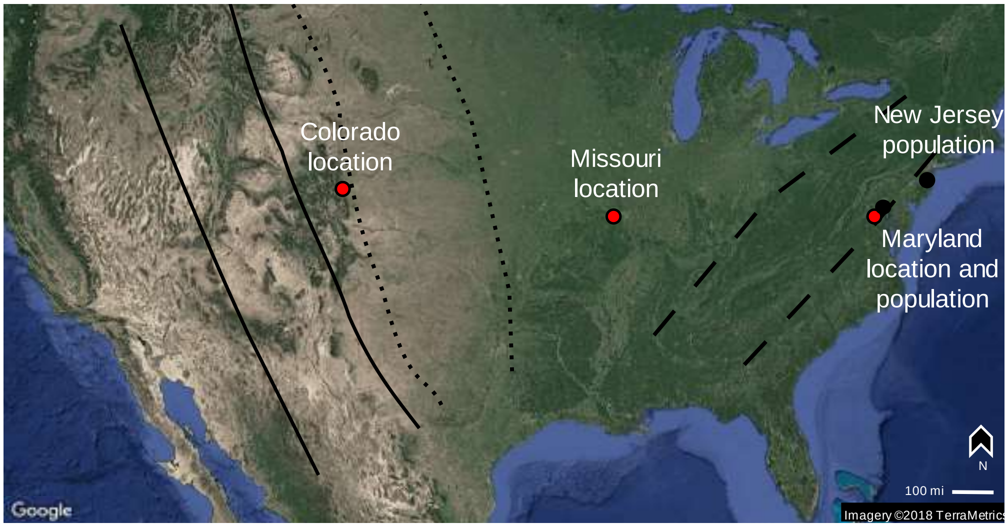


Figure 11: Map showing the geographic barriers among populations of fall webworms; solid line = Rocky Mountains, dotted line = central plains, dashed line = Appalachian Mountains. Black circles represent populations of black-headed fall webworms used in Experiment 1, while red circles represent the locations of the host plants used by red-headed fall webworms in Experiment 2. Map from Google Earth®.

Experiment 1: Influence of diet breadth on local adaptation of red and black-headed fall webworm

The diet breadth of FW varies considerably across their geographic range and between the two FW species. Colorado red-headed FW feed on fewer host plants than do black heads from east coast populations; Murphy and Loewy (2015) found red-headed FW feeding on 17 host plant species along the Colorado Front Range, but most commonly on only two host species [choke cherry (*Prunus virginiana)* and narrow leaf cottonwood (*Populus angustifolia*)], which is fewer hosts than Mason et al. (2011) found black-headed FW feeding on in Connecticut (23 hosts used total and 10 hosts with high frequency of use). Unfortunately, there is no clear description of the diet breadth of red-heads in the east coast, where they live in sympatry with black-heads. However, in Louisiana where the two species occur together, red-headed larvae were described as feeding on 10 plant species and black-headed larvae on 34 host plants, with 7 plant species used by both species (Oliver 1964). Therefore, we can consider the red-headed species as being less polyphagous than the black-headed species, but both species are clearly still generalists.

Since the two species of FW appear to use different host plants, we experimentally tested the performance of the black and red-headed species of FW from different geographic regions on host plants that are known to be used in different frequencies by black-headed and red-headed larvae. We used red-heads from the Colorado Front Range (n=12 maternal lines), and black-heads from New Jersey (n=7 maternal lines) and Maryland (n=3 maternal lines). We reared all larvae in the same ambient conditions (L14:D10 and 27:19.5°C) at the same time period, using two host plants from Colorado that are known to be good quality for local FW (choke cherry, *Prunus virginiana*; narrow leaf cottonwood, *Populus angustifolia*) and two that are infrequently used by Colorado populations, but that are frequently used by black-headed FW populations along the east coast (box elder, *Acer negundo*, and green ash, *Fraxinus pennsylvanica*) (Mason et al. 2011; Murphy and Loewy 2015). We would expect to find stronger fitness tradeoffs for the red-headed species that is less polyphagous, in which larvae would have better performance on choke cherry and narrow leaf cottonwood (commonly used hosts), compared to green ash and box elder (occur locally, but not used as hosts by red-heads). If there is a negative correlation between diet breadth and local adaptation, we would predict the more polyphagous black-headed species to show weaker fitness tradeoffs between the host species that they use and do not use in comparison to the red-heads.

We divided all egg clutches from each maternal line onto the four host plant treatments and reared the larvae in containers with an average of two larvae per container, providing foliage *ad libitum*. Early-instar larvae do not survive well by themselves, therefore, we started the experiment with multiple larvae in the same container and split them into individual containers as the larvae grew. We checked larvae every other day and changed or included new leaves when necessary, and we cleaned frass weekly (rearing methods followed Loewy et al. 2013). We recorded the development time, pupal mass and survival of 10-15 larvae from each maternal line reared on each host plant, totaling 60 larvae per maternal line (1270 larvae total). Development time is an important characteristic for larval survival in natural conditions, as the longer the time spent in the larval stage, the longer the larvae are exposed to predation and parasitism (Price et al. 1980). We classified the sexes following Loewy et al. (2013) and measured pupal mass to the nearest 0.01mg, 10 (±1) days after pupation. Pupal mass and survival are fitness measures related to plant quality; larvae reared on plants of high quality have greater survival and greater pupal mass than larvae reared on poor quality hosts, and since pupal mass is directly related to female fecundity, it is often used as a reliable fitness proxy (Loewy et al. 2013). We also used a combined measure of fitness, which we called “fitness score” (e.g. Murphy and Loewy 2015), by multiplying the mean pupal mass per maternal line on each host by the proportion of surviving individuals and divided by the mean development time for that same maternal line.

To test for local adaptation, we first tested if FW had higher performance on familiar vs. unfamiliar plants. Here we refer to unfamiliar plants as plant species used by other populations of the same FW species, or by the other sister species, but that are not known to be used by the local population. Choke cherry and narrow leaf cottonwood were classified as familiar for Colorado red-headed FW and as unfamiliar for black-headed FW from the eastern US, while box elder and green ash were unfamiliar for red-heads and familiar for black-heads (following Mason et al. 2011; Murphy and Loewy 2015). We used generalized linear mixed models (GLMMs) to test the prediction that larvae would have better performance on familiar host plants, with 1 = familiar and 0 = unfamiliar; maternal line was considered as a random effect. For pupal mass, development time, and fitness score, we conducted a linear mixed model with a normal distribution. For survival we used a generalized linear mixed model with a binomial distribution (or "likelihood-ratio test" method when using the *mixed* function, see below). We compared the performance of red- vs. black-headed species, for which we used a (generalized) linear mixed model with pupal mass, development time or survival as response variables, head-color (species) as a fixed effect and maternal line as a random effect. We further tested the performance of FW on each host plant used. The fixed effects were *rearing host* and *population*, as well as *rearing host*population* interactions, with *maternal line* as a random effect. We included sex of moth as a random effect in the models testing pupal mass, as usually females have higher values of pupal mass than males (Loewy et al. 2013), and we also included it in the model testing development time, as there was a significant effect of sex. For the fitness score measurement, we performed an ANOVA with the interaction between *source population* and *rearing host* *treatment* as the fixed effect. We performed Tukey's HSD pairwise comparisons with all our models to determine on which hosts larvae had higher performance. All models were performed using the package *afex* v 0.18-0 with function *mixed* (Singmann et al. 2017) and package *lme4* v. 1.1-14 with function *lmer* or *glmer* depending on the response variable (Bates et al. 2015). Mean comparisons were made with Tukey's HSD post-hoc test using the package *multcomp* with function *ghlt* to test linear hypotheses for the mixed models, and we used Bonferroni correction when there were more than six comparisons (Hothorn et al. 2008). All analyses were performed in R environment 3.4.1 (R Development Core Team 2011).

Experiment 2: Influence of geographic distance on local adaptation of red-headed fall webworm

We conducted a transplant experiment to test how geographic distance influences local adaptation using only red-headed FW from Colorado. We did not perform the same experiment with black-headed FW because this species does not occur in Colorado. We reared 10 maternal lines of red-headed FW from Colorado, in which the egg clutch from each maternal line was divided into three parts (Appendix Figure 7), with one part reared in Colorado, one part reared in Missouri (University of Missouri-St. Louis, reared 9 of the 10 maternal lines) and another part reared in Washington DC (George Washington University; while the larvae were reared in DC, the host plants were collected from nearby sites in Maryland where red-headed FW are found in the field and we refer to the larvae reared at this site as being from Maryland). Maryland is approximately 2,600 km from Denver, while Missouri is approximately half that distance (1,300 km). Of the 10 maternal lines of FW, four were obtained from the lab colony at the University of Denver (previous generation of wild-caught larvae that were feeding on *Alnus* sp., *Populus angustifolia*, *Prunus virginiana*, or *Salix* sp.), and six were obtained in the field as neonate larvae; all maternal lines originated from Colorado (9 from Boulder, 40.090013, -105.359962, or Jefferson County, 39.746944, -105.210833, one from Garfield County, 39.7273511, -108.6020411). Of the six maternal lines collected in the field, four were collected from choke cherry and two were collected from narrow leaf cottonwood. Subdivisions of each maternal line were shipped as eggs or neonate larvae to Missouri or Maryland using overnight shipping. Each subdivision of each maternal line (field collected and lab colony) was then subdivided again at each site (CO, Missouri and Maryland) onto the 3 host plant treatments used in the specific location (Appendix Figure 7). In each location, we used host species that are commonly used by red-headed FW at each specific location and hosts that are shared among locations. In Colorado, host plants were choke cherry (*Prunus virginiana*), broad leaf cottonwood (*Populus deltoides*), and narrow leaf cottonwood (*Populus angustifolia*). In Missouri, host plants were broad leaf cottonwood, black cherry (*Prunus serotina*), and persimmon (*Diospyros virginiana*). In Maryland, the host plants were persimmon, black cherry, and black walnut (*Juglans nigra*). Therefore, Colorado and Missouri shared broad leaf cottonwood as a host plant, Missouri and Maryland shared persimmon, and the genus *Prunus* (either *P. serotina* or *P. virginiana*) was common to all locations. While black cherry and choke cherry are clearly different species, our results from previous rearing efforts of FW from Colorado suggest FW larvae exhibit similar performance on the two congeners. Our rearing methods and data collection were the same as described above in Experiment 1, with the exception that development time for maternal lines collected as larvae in the field could not be recorded in the absence of hatching dates. Therefore, our fitness score in this experiment considered only mean pupal mass multiplied by survival rate for each maternal line per host plant treatment. All larvae were reared in similar temperature and day length across sites (L12:D12; 25°C).

We performed similar statistical analyses as Experiment 1. We first tested if FW had higher performance on familiar vs. unfamiliar plants using GLMMs. All cherries (*Prunus* sp.) and cottonwoods (*Populus* sp.) were considered to be familiar for Colorado FW, while persimmon and black walnut were classified as unfamiliar (following Murphy and Loewy 2015). We further tested the performance of FW on each host plant used in which the fixed effects were *rearing host* and *rearing location*, as well as *rearing host*rearing location*, with *maternal line* and *sex* as random effects. For the fitness score measurement, we performed an ANOVA with the interaction between *rearing location* and *rearing host treatment* as the fixed effect. We performed Tukey's HSD pairwise comparisons with all our models to determine on which hosts larvae had higher performance.

Using the field-collected larvae that were then split into host treatments, we were able to test if switching the larvae to a new host had an effect on their pupal mass and survival rate. For this test, all maternal lines collected from the field were assigned as "field" and the others were assigned as "lab". We used (generalized) linear mixed models with *maternal line* and *rearing location* as random effects, and the interaction between *origin* (lab vs. field) and *rearing host plant* as a fixed effect. We then selected only larvae from the field to see if they had greater pupal mass when reared on the same host plant that they were originally feeding on in the field when we collected them compared to the new host species that we fed them in the lab. We used a linear mixed model with *pupal mass* as response variable, the interaction between *plant of origin* (narrow leaf cottonwood or cherry) and *rearing host treatment* as the fixed effects, and *rearing location* and *maternal line* as random effects.

Test of local adaptation strength according to diet breadth and geographic distance

As we found significant interactions between host plant species and population of origin (or rearing location) indicating possible local adaptation, we used effect sizes (similarly as Denno et al. 2003) to directly test the degree of local adaptation in our experiments according to host plant species, diet breadth, and geographic distance. We used response ratios of the fitness scores, calculated as the natural logarithm of the ratio between the two numbers being compared. For Experiment 1, we tested the *local-foreign* criterion of local adaptation (Kawecki and Ebert 2004), in which it is assumed that a local population of herbivores will have better performance on their home plants in comparison to a foreign population. For each host plant treatment, we ranked from lowest to highest the fitness scores within each maternal line in each location, and compared the fitness scores between Colorado maternal lines and east coast maternal lines. Therefore, we only compared within each host the fitness scores between local and foreign population according to the ranking of the fitness scores. In cases in which the fitness score was zero, we added 0.01 to make calculations possible. Fitness scores of Colorado red-headed FW (the local population) were always the numerator, and thus we expect to find higher values of response ratios on the plants that Colorado FW use frequently (narrow leaf cottonwood and choke cherry), and lower on rarely used hosts (box elder and green ash). To test if the response ratios were different from each other, we used ANOVA and Tukey’s HSD with the functions *aov* and *TukeyHSD* in R, and we used t-test to test if the response ratios were different from zero (function *t.test*). A negative value of effect size would mean that east coast FW (a foreign population) had better performance on the local plants than Colorado FW, and hence would suggest local maladaptation, while a positive value would mean that Colorado FW had greater fitness on their local host than a foreign population, supporting local adaptation. A value not different from zero means that the performances of reds and blacks were similar.

Using data from Experiment 1, we also tested the degree of local adaptation according to diet breadth. To do that, we divided the fitness score of the best commonly used host by the worst rarely used host for each maternal line. Although red and black FW both had better performance on green ash, we did not consider this host because it seems that the result we found was due to green ash being of extreme good quality, and not necessarily showing local adaptation. Thus, for red-headed Colorado FW, we divided the fitness score on narrow leaf cottonwood by box elder, and for east coast black-headed FW we divided the fitness score on box elder by narrow leaf cottonwood. Thus, the larger the effect size value, the stronger the trade-offs the herbivore experiences between these two hosts. In this case, we would expect a higher value of response ratio for red-headed than black-headed FW, since they are less polyphagous and expected to experience stronger fitness tradeoffs between hosts. We tested effect sizes using ANOVA and t-test as mentioned above.

For Experiment 2, we tested the *home-away* criterion (Kawecki and Ebert 2004), in which herbivores are expected to have better performance on local plants than on plants from farther away. We only included hosts that were shared between two locations (black cherry, broad leaf cottonwood, and persimmon). We did not include comparisons between black cherry and choke cherry because although FW larvae seem to perform well on both species, the effect of possible difference in quality might be more apparent in direct comparisons, which might make interpretations difficult. For each host plant, we calculated the response ratio of the fitness scores between locations within each maternal line. In cases in which all larvae from a maternal line died on the plant treatment, we added 0.01 to make calculations possible. We would expect to find higher values of response ratios (i.e., greater difference in performance) when comparing distant populations of hosts with the local population. Response ratio values might not be different from zero when the numbers being compared are similar, i.e., there is not enough variation in performance between larvae on near and far hosts, or when there is too much variation in the effect sizes among maternal lines.

Results

Literature review

Our literature search yielded 167 papers, of which only 33 tested local adaptation (Table 3). Most studies were excluded because they did not test local adaptation or were not using herbivores (e.g., local adaptation in plants). Of the 33 papers, 12 tested generalist herbivores, 19 tested specialists and 2 tested both. Only 8 papers showed unequivocal support for local adaptation (native herbivore had higher fitness on the local plant than on another host and/or had higher fitness than a foreign population on the local host), half of these papers tested generalists and half specialists. Most papers found mixed evidence of local adaptation (n=13), followed by no evidence (n=12). All of the papers that tested geographic distance, except for one (Strauss 1997), found evidence for local adaptation when comparing the most distant host population, and lack of local adaptation when comparing close by host plant populations.

Table 3. Papers that tested local adaptation to host plants by insect herbivores.

Diet Breadth	**Local adaptation**	**Insect herbivore**		**Tested geo distance?**	**Reference**
Specialists	Support	*Tetraneura yezoensis*	Hemiptera	N	Akimoto 1990
		Belonocnema treatae	Hymenoptera	N	Egan and Ott 2007
		Stilbosis quadricustatella	Lepidoptera	N	Mopper et al. 1995
		Acyrthosiphon pisum	Hemiptera	N	Via et al. 2000
	Mixed evidence	*Utetheisa ornatrix*	Lepidoptera	Y	Cogni and Futuyma 2009
		Pyrrhalta viburni	Coleoptera	N	Desurmont et al. 2012
		Leptinotarsa decemlineata	Coleoptera	N	Izzo et al. 2014
		Abrostola asclepiadis	Lepidoptera	N,Y	Kalske et al. 2016, Laukkanen et al. 2012
		Multiple species	Lepidoptera, Hymenoptera	Y	Tack and Roslin 2010
		Megacerus eulophus	Coleoptera	N	Stotz et al. 2013
		Cactoblastis cactorum	Lepidoptera	N	Varone et al. 2013
		Thaumetopoea pityocampa	Lepidoptera	Y	Zovi et al. 2008

	No support	*Aphis nerii*	Hemiptera	N	Bukovinszky et al. 2014
		Chionaspis pinifoliae	Hemiptera	N	Glynn and Herms 2004
		Adelges japonicus	Hemiptera	N	Ozaki 1997
		Battus polydamas	Lepidoptera	N	Rios et al. 2016
		Brevicoryne brassicae	Hemiptera	N	Ruiz-Montoya and Nunez-Farfan 2013
		Blepharida rhois	Coleoptera	Y	Strauss 1997
Generalist	Support	*Ostrinia nubilalis*	Lepidoptera	N	Calcagno et al. 2007
		Melitaea cinxia	Lepidoptera	N	Kuussaari et al. 2000
		Lochmaea capreae	Coleoptera	N	Soudi et al. 2015
		Hesperotettix viridis	Orthoptera	N	Traxler and Joern 1999
	Mixed evidence	*Cephalelus uncinatus*	Hemiptera	N	Augustyn et al. 2017
		Pseudaulacaspis pentagona	Hemiptera	Y	Hanks and Denno 1994
		Oreina elongata	Coleoptera	N	Ballabeni et al. 2003
	No support	*Epirrita autumnata*	Lepidoptera	N	Alonso et al. 2001
		Stator limbatus	Coleoptera	N	Amarillo-Suárez and Fox 2006, Fox et al. 1994
		Saissetia coffeae	Hemiptera	N	Spitzer 2006
		Operophtera brumata	Lepidoptera	N	Tikkanen et al. 2000
Both	Mixed evidence	Multiple species	Coleoptera, Lepidoptera, Orthoptera	N	Garrido et al. 2012
	No support	Multiple species	Coleoptera, Hemiptera, Orthoptera	N	Ho and Pennings 2013

Case study using fall webworm

Experiment 1: Influence of diet breadth on local adaptation of red and black-headed fall webworm

We found evidence consistent with local adaptation to different host plants for black and red-headed FW. Both species of larvae had significantly higher performance on familiar than on unfamiliar hosts (Table 4). Across all rearing host plants, black-headed larvae had lower pupal mass ($F_{1,173}$=153.01, P<0.0001), decreased survival (X^2=11.51, df=1, P=0.0007), and shorter development time ($F_{1,452.63}$=78.11, P<0.0001) than red-headed larvae. Notably, there was a significant interaction between rearing host and population (CO, NJ, MD) for pupal mass, survival, and development time (Table 4). Red-headed larvae from Colorado had lower pupal mass, took more time to develop, and had lower survival on box elder than on the other three rearing hosts (Fig. 12). Black-headed larvae from New Jersey took more time to develop on narrow leaf cottonwood than on green ash and box elder (Fig. 12b). Black-headed larvae from Maryland had lower survival on choke cherry and narrow leaf cottonwood than on green ash (Fig. 12c). Although pupal mass of FW from Maryland reared on narrow leaf cottonwood was about half that of pupae reared on the other plants, the pairwise comparison was not significant, probably because only two larvae survived to pupation (Fig. 12a); almost 90% of Maryland larvae died when feeding on narrow leaf cottonwood (Fig. 12c). When considering all fitness measure together in our fitness score measurement, box elder was still a worse host for Colorado FW than the other three hosts, and for Maryland and New Jersey, narrow leaf cottonwood was a worse host than the other three hosts (Fig. 12d).

Table 4: Statistical tests summary from (generalized) linear mixed models. Evidence of local adaptation is shown by a significant test of performance on familiar vs. unfamiliar host, and by a significant interaction between host plant and site.

	Experiment 1			**Experiment 2**		
Fixed effects	d.f.	F-stat	P-value	d.f.	F-stat	P-value
Pupal mass						
Familiar vs. unfamiliar	1, 654.7	12.84	0.0004	1, 480.7	29	<0.0001
Host-site[1] interaction	6, 654.3	16.04	<0.0001	2, 213	20.65	<0.0001
Host plant	3, 652.6	22.72	<0.0001	4, 213	4.65	0.001
Site[1]	2, 71.3	142.2	<0.0001	2, 213.5	0.71	0.49
Development time						
Familiar vs. unfamiliar	1, 653.9	83.8	<0.0001	1, 14.9	4.04	0.06
Host-site[1] interaction	6, 645.6	47.45	<0.0001	2, 215.6	22.42	<0.0001
Host plant	3, 645	14.54	<0.0001	4, 214.7	4.66	0.001
Site[1]	2, 48.7	47.45	<0.0001	2, 215.3	0.50	0.61
Fitness score						
Familiar vs. unfamiliar	1,83.3	11.74	0.001	1,84	23.94	<0.0001
Host-site[1] interaction	6,75	6.03	<0.0001	2,77	7.33	0.001
Host plant	3,75	20.09	<0.0001	4,77	8.56	<0.0001
Site[1]	2,75	0.57	0.56	2,77	8.16	0.0006

Fixed effect	d.f.	X^2	P-value	d.f.	X^2	P-value
Survival						
Familiar vs. unfamiliar	1	32.06	<0.0001	1	11.54	0.0007
Host-site[1] interaction	6	161.11	<0.0001	2	15.23	0.0005
Host plant	3	96.97	<0.0001	4	36.56	<0.0001
Site[1]	2	20.16	<0.0001	2	55.96	<0.0001

[1] Site for black vs. red experiment was the origin of the FW population (Colorado, New Jersey or Maryland), while for the red type experiment, it was the rearing site (Colorado, Missouri, or Maryland).

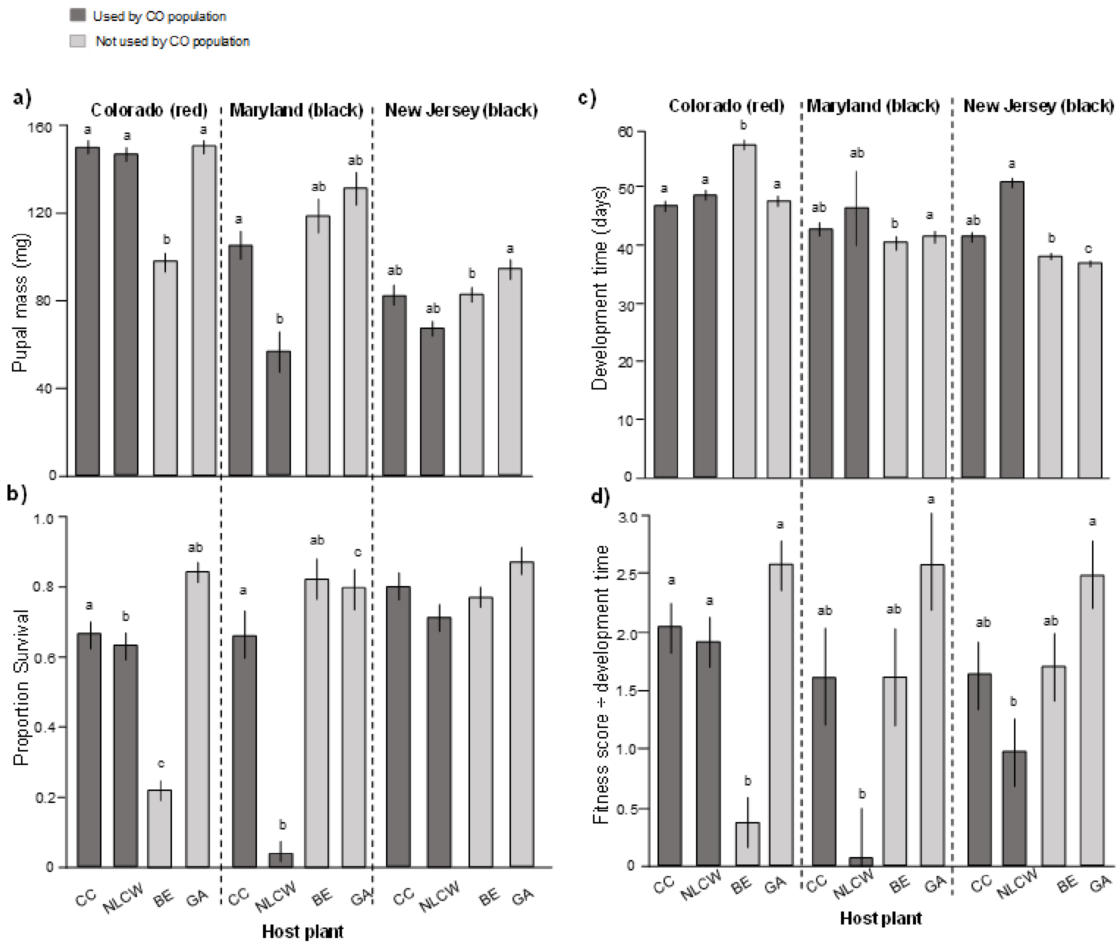


Figure 12: Performance of red-headed and black-headed fall webworm reared in Colorado measured as a) pupal mass, b) proportion survival, c) development time, and d) fitness score (survival*pupal mass/development time per maternal line). Dark grey bars represent hosts used by red-headed fall webworm in Colorado, while light grey bars represent hosts not used or rarely used by Colorado fall webworm, but frequently used by black-headed fall webworm in the eastern US. Letters represent pairwise comparisons within each source population. All Tukey's pairwise comparisons were Bonferroni corrected. Abbreviations of host plants used: CC = choke cherry, NLCW = narrow leaf cottonwood, GA = green ash, BE = box elder.

Experiment 2: Influence of geographic distance on local adaptation of red-headed fall webworm

We found evidence of local adaptation to host plants for red-headed FW from Colorado, but the strength of local adaptation was a function of the geographic distance.

FW larvae had better performance when reared on familiar than on unfamiliar plants (Table 4). However, rearing location was also an important factor as we found a significant interaction between rearing host and rearing site for pupal mass, development time and survival (Table 4). There was a negative effect of distance of rearing site (from Colorado, the original population site) on performance in some cases; FW had lower survival on black cherry in Maryland than on the choke cherry in Colorado, but there was no difference in survival between choke cherry from Colorado and black cherry from Missouri (Fig. 13c). Larvae had shorter development time and higher survival on broad leaf cottonwood from Colorado than from Missouri, and higher survival on persimmon from Missouri than from Maryland (Fig. 13b and c). However, there was a positive effect of distance when we consider the pupal mass and development time of larvae that fed on cherry; FW on black cherry from Maryland and Missouri had greater pupal mass and shorter development time than on choke cherry in Colorado (Fig. 13a and b), however, it could be a difference in plant quality between the different species. In Colorado, FW performed better on cottonwoods than on choke cherry, whereas black cherry was the best host in both Maryland (for pupal mass and development time) and Missouri (for survival) (Fig. 13). When considering pupal mass and survival together (fitness score), FW from Colorado had better performance on black cherry from Missouri than on choke cherry from Colorado, with no difference between black cherry from Maryland and choke cherry from Colorado (Fig. 13d). Furthermore, FW performed better on persimmon from Missouri than from Maryland (Fig. 13d).

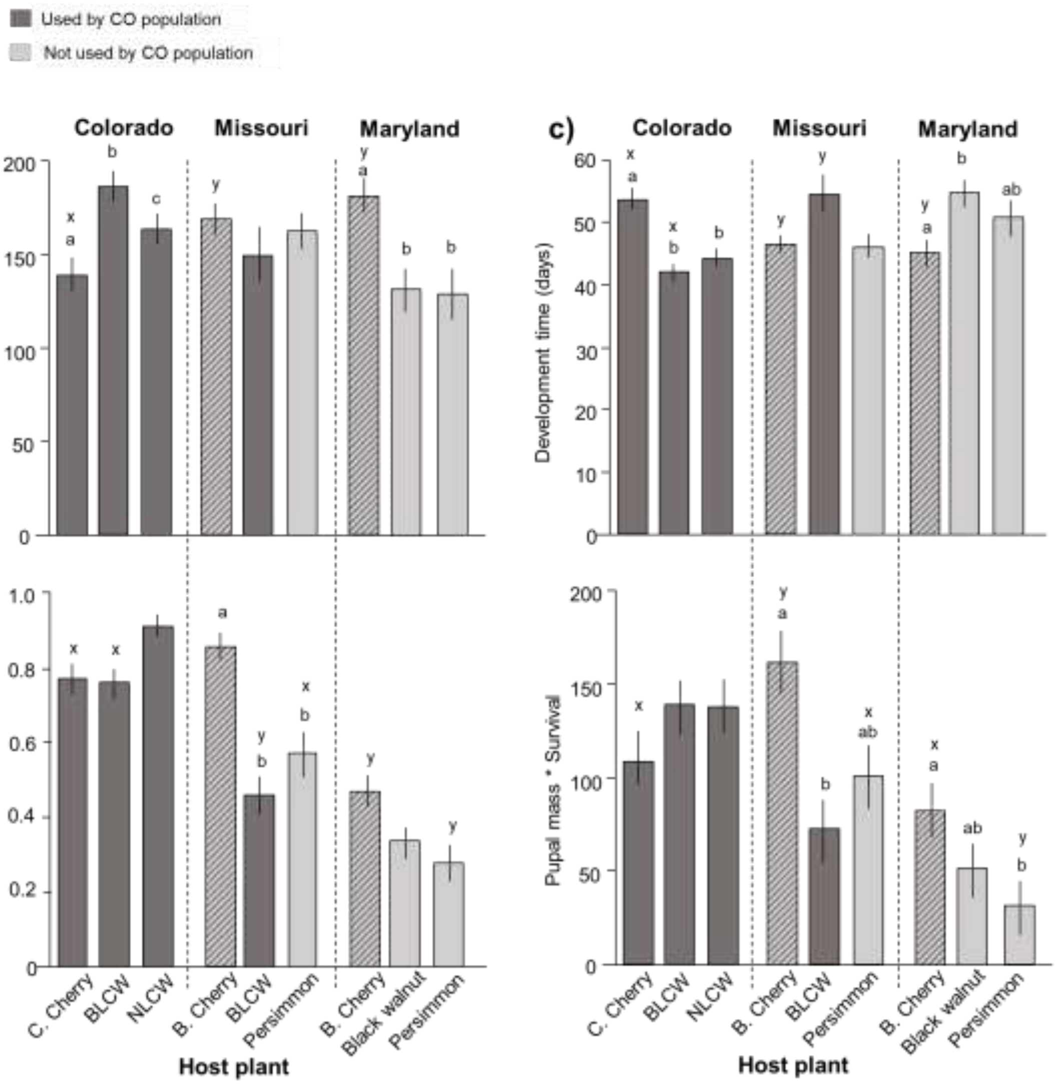


Figure 13: Performance of red-headed fall webworm from Colorado reared in Colorado, Missouri or Maryland measured as a) pupal mass, b) survival rate, c) development time, d) overall performance (survival*mass per maternal line). Dark grey bars represent hosts used by red-headed fall webworm in Colorado, light grey are hosts that are used by red-headed fall webworm in Missouri or Maryland, but not by fall webworm in Colorado, and stripped bars represent a genus commonly used, but we used different species in the locations. Letters x and y represent significant pairwise comparison between larvae from different rearing locations feeding on the same plant species (e.g., Persimmon in Missouri and Maryland), while letters a, b and c represent pairwise comparisons within each rearing location. All Tukey's pairwise comparisons were Bonferroni corrected. Abbreviations of host plants used: C. Cherry = choke cherry, B. Cherry = black cherry, NLCW = narrow leaf cottonwood, BLCW = broad leaf cottonwood.

Larvae collected from the field and larvae from the laboratory colonies had similar survival (X^2=0.88, df=4, P=0.93). However, there was a significant interaction between the origin of the larvae and host plant treatment for pupal mass ($F_{4,481.3}$=4.35, P=0.002), in which pupal mass of larvae that fed on cherry was greater when the larvae came from the field than from the laboratory colony. We then investigated if the greater pupal mass on cherry from field-collected larvae was because the larvae were originally feeding on choke cherry and thus if there was an effect of host switching. There was a significant interaction between original host and treatment host ($F_{4,255.87}$=3.41, P=0.01), but not in the direction expected; when FW were reared on narrow leaf cottonwood as the treatment, they had greater pupal mass if they were originally on choke cherry than if they were originally feeding on narrow leaf cottonwood from egg hatch in the field, but for FW reared on cherry as the treatment, there was no difference if they were originally on cherry or narrow leaf cottonwood.

Test of local adaptation strength according to diet breadth and geographic distance

When using the response ratios for fitness scores, we found evidence only for the influence of diet breadth on local adaptation. When comparing the same rank of performance, the local red-headed FW had better performance than the foreign black-headed FW only on green ash, red-headed FW showed maladaptation on box elder, and had no significant difference between red and black-headed FW on the two commonly used hosts (Figure 14a, $F_{3,36}$=3.4, P=0.03). The difference between red and black FW was too variable to detect an effect on choke cherry and narrow leaf cottonwood, possibly due to some maternal lines having complete mortality on some hosts. We found that the less polyphagous red-headed FW had more variation in fitness between the best and worst host plant than the more polyphagous black-headed FW (Figure 14b, $F_{1,20}$=6.34, P=0.02). Overall, FW had too variable effect sizes among maternal lines when comparing fitness scores in near and far host populations (Figure 14c, t-test=1.6, df=22, P=0.125), regardless of host species (cherry: t-test=2.21, df=7, P=0.06; persimmon: t-test=1.98, df=6, P=0.09; broad leaf cottonwood: t-test=0.36, df=7, P=0.73). Thus, there was no clear trend of FW performance on local or distant populations of hosts.

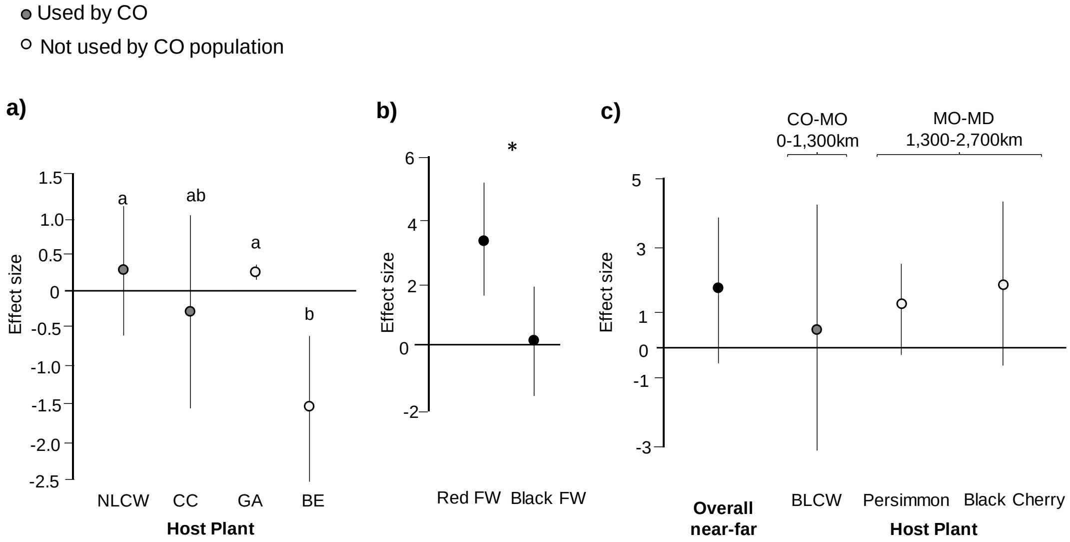


Figure 14: Effect size (response ratio) comparisons of fitness scores for: a) experiment 1, red and black-headed FW feeding on each host plant species, a negative value means that black FW had better performance on the Colorado local plants than red FW, and hence would suggest local maladaptation, while a positive value means that red FW had better performance than black FW, supporting local adaptation, and a value not different than zero means that overall there was no different in fitness between black and red FW; b) experiment 1 between best and worst host plant within each FW species, a positive value means that FW had greater fitness on the best host compared to the worst host, while a null value means that there was no detectable difference in fitness (possibly due to high variation among families); c) experiment 2, red-headed FW from Colorado feeding on the same host plant species from different locations, a positive value means that FW had greater fitness on local than distant host populations, while a null value means that there was no detectable difference in fitness (possibly due to high variation among families). Letters or asterisk represent pairwise comparisons within each source population performed using Tukey’s HSD.

Discussion

We found mixed evidence for local adaptation in one of the most generalist insect herbivore species known and that diet breadth influences the degree of local adaptation. We found a significant interaction between host plant species and population of origin, which suggests that the performance of FW on the host plants being compared varies depending on the population of FW being tested. Each of the two species of fall webworm (one more polyphagous than the other) performed significantly better on familiar than on unfamiliar hosts used by their close relatives, and host plant identity strongly influenced all measures of performance. However, the less polyphagous species (red-headed FW) showed more variation in the fitness scores between commonly used host and un-used host than the more polyphagous species, as expected. Because our study was restricted to a single interspecific comparison, inference into the role of diet breadth on facilitating or constraining local adaptation is limited. Geographic distance of the host also influenced the performance of FW, but the effect was less clear. We found a significant interaction between host plant species and location of rearing, which suggests that the performance of FW on the host plants being compared varies depending on the population of the host plant used. Although overall the mean performance was better on local hosts, the variance among maternal lines was too great to detect difference in performance when considering effect sizes. Overall, our findings support part of our initial prediction; we did find evidence that diet breadth influences the strength of local adaptation, and there was also evidence of local adaptation to different plant species within a location.

Contrary to expectations, FW from Colorado performed well on green ash, a host not commonly used in the Colorado Front Range (Murphy and Loewy 2015). However, we have observed *Fraxinus* sp. used by other populations of FW in Colorado (not in the Front Range), whereas box elder is only used rarely in the Front Range and not in other Colorado locations (Chapter 2). It is possible that the Front Range population does not use box elder because they perform poorly on them and might not use green ash because this plant occurs in low frequency in the Front Range. Additionally, in our Experiment 2, larvae reared in Colorado had lower performance on choke cherry than on black cherry from Missouri and similar performance on black cherry from Maryland; choke cherry is a host plant commonly used in the Colorado Front Range, but it is also the only locally abundant cherry species. Both black cherry and choke cherry have cyanogenic leaves, but FW can suppress the conversion of cyanogen to cyanide in its gut, and therefore are not harmed by it (Fritzgerald 2008). There is evidence that early season herbivory by western tent caterpillars (*Malacosoma californicum*, Lasiocampidae) on choke cherry makes the leaves tougher (Barnes and Murphy 2018), which may negatively affect FW later in the season. Although we considered choke cherry and black cherry as similar hosts to do our comparisons, they are different species. Thus, the difference in performance of FW on black cherry and choke cherry might be because of difference in the quality of the two species. The high performance of Colorado FW on black cherry suggests that the diet breadth of populations of FW might be broader than expected, as some populations can use plants that do not occur in their location. Thus, the diet breadth that we see is only the realized niche of the FW population.

Theoretical work proposes that parasites are more likely to be locally adapted to nearby hosts than to distant populations of hosts (Gandon et al. 1996). The meta-analysis by Hoeksema and Forde (2008) showed no influence of spatial scale on the degree of local adaptation detected in the studies included, however, the maximum distance recorded was 1,573 km. Cogni and Futuyma (2009) found evidence of local adaptation of *Utetheisa ornatrix* (Erebidae) to the sympatric host only at the continental scale (>6,000 km), while there was no evidence of local adaptation when testing more proximately located populations (160 to 200 km apart). Similarly, Kalske et al. (2016) found that the strength of local adaptation increases with the increase of distance between the hosts being compared. Although individual fitness measures showed better performance of FW on some of the near hosts compared to far host populations, using fitness score and response ratio, we found no evidence of fitness tradeoffs between near and far host populations due to the high degree of variation among maternal lines on each host. Generalist herbivores usually have a broader geographic range than specialists (Hardy et al. 2016) and can use a wide range of host populations. This variation in fitness that overall led to no difference between locations might facilitate the wide geographic distribution of generalist herbivores.

A meta-analysis conducted to test local adaptation of parasite-host systems found that gene flow predicts local adaptation, in which parasites with greater gene flow than their hosts are more likely to be locally adapted (Heoksema and Forde 2008). However, only one insect herbivore-plant interaction study was included in this meta-analysis. Although gene flow is an important predictor of local adaptation, the likelihood of local adaptation also depends on the strength of the selective force underlying the adaptation to local conditions (Kawecki and Ebert 2004). Considering host plants as environments for herbivores (Funk et al. 2002), there is considerable gene flow between FW individuals feeding on different host plant species in Colorado (Chapter 4). However, we found evidence that FW larvae are locally adapted to the multiple plant species that they use in high frequency in their home populations. This is evidence that herbivores might be under strong selection from their hosts, despite gene flow between populations using different sets of hosts. Although our most striking evidence of local adaptation was on the host not commonly used (greater performance of local FW on green ash compared to foreign FW), we should consider that in our experiments we only included bottom-up effects, and it is possible that FW suffer stronger top-down effects on green ash in nature, which would result in lower overall performance on this host (Chapter 2).

Of the 33 papers we found testing local adaptation in insect herbivores, only 8 found support for local adaptation, and 4 tested generalist herbivores (Table 1). Different reasonings were given for the lack of consistent support for local adaptation (25 of 33 papers), such as genetic variation of hosts and herbivores (Bukovinszky et al 2014), genetic independency of adaptation to different hosts (Fox et al. 1994), adaptation of the host not followed by adaptation of the herbivore (Spitzer 2006), non-adaptive evolutionary mechanisms (Strauss 1997), environmental heterogeneity (Tikkanen et al. 2000), enemy-free space (Verdon et al. 2007), maternal effects or plasticity leading to inconsistency of performance (Alonso 2001; Amarillo-Suárez and Fox 2006; Ruiz-Montoya and Nunez-Farfan 2013), and phenological characteristics of the plant (Stalhandske et al. 2016). These arguments show that different mechanisms might influence adaptation of insect herbivores to their host plants, with no consistent pattern.

In our experiments, phenotypic plasticity might have played an important role, as we found that there was great variation in performance among maternal lines.

Fall webworm is considered one of the most generalized insect herbivores, as the species can feed on more than 600 different plant species (and growing) from more than a dozen different plant families. While their geographic range is very broad, populations can feed on tens of plant species, being also local generalists. Our experiments give further evidence that generalist herbivores can be locally adapted to the hosts that each population uses, and that diet breadth can influence local adaptation. Our results follow the pattern described by Vidal and Murphy (2018) of generalists suffering less fitness tradeoffs than specialists, as we showed that this is the case even when considering different degrees of generalization (red vs. black FW). Even with gene flow between individuals feeding on different host species within populations, selection might be strong enough for local adaptation to occur in the populations we studied.

CHAPTER 4: Influence of geographic distance and host plant use on the genetic divergence of a generalist herbivore

Introduction

Insect herbivores are one of the most diverse groups of organisms known and are mostly composed of dietary specialists. Several factors have been proposed to drive such high levels of diversification including host plant use (Ehrlich and Raven 1964), diet breadth (Hardy and Otto 2014) and geographic isolation (Hardy et al. 2016). Recent phylogenetic research supports the idea of host plant use as an important driver of diversification of insect clades (Wiens et al. 2015), and host plant use has played a major role on the divergence of contemporary populations of insects (e.g., Funk 1998; Nosil et al. 2012; Powell et al. 2013). Diet breadth can also influence diversification of herbivores, as a narrow diet breadth has been shown to be associated with faster speciation rates than a generalist diet (Hardy and Otto 2014, but see Hardy et al. 2016). Furthermore, populations of generalist herbivores are expected to have greater genetic variation than specialist populations (Gloss et al. 2013), as the former are usually composed by individuals that feed on different hosts and freely mate with each other. Thus, generalists are expected to be under weaker divergent selecting from hosts and have greater realized population sizes than specialists. However, host plant use and diet breadth are not the sole factors influencing divergence of herbivores; for instance, geography along with local adaptation and demography were shown to influence the rate of differentiation associated with host plant in yucca moths (Darwell et al. 2014). Thus, diet breadth, host plant use, and geographic distance can influence divergence of insect herbivores, but it is unclear how much each of these factors contribute to the diversification of herbivores, especially for generalist herbivores.

A variety of macroevolutionary hypotheses have been proposed to explain how diet breadth might drive diversification of insect herbivores. Those hypotheses include the role of coevolutionary arms race between herbivores and their host plants (Ehrlich and Raven 1964, Escape and Radiate hypothesis) and local adaptation to host plants on geographic isolated populations (Stireman 2005, Serial Specialization hypothesis; and Janz and Nylin 2008, Oscillation hypothesis). These adaptive hypotheses assume that insect herbivores suffer fitness trade-offs on different host plant species and that speciation-via-specialization is the norm. However, a recent meta-analysis showed that although specialist herbivores suffer strong fitness trade-offs on different quality of host plants, generalists do not (Vidal and Murphy 2018). More recently, the Specialization by Drift hypothesis (Hardy et al. 2016) proposed that non-adaptive processes contribute to diet breadth evolution and diversification of herbivores. In this hypothesis, diversification would be positively related to diet breadth; generalist populations would tend to be more widespread and would continuously produce specialized small and geographically isolated populations. As generalist herbivores suffer weak trade-offs between different host plants (Vidal and Murphy 2018), we would expect that their diversification would be associated with geographic isolation and less with host plant use. Most studies to date that show diversification of herbivores were done using specialists and all adaptive macroevolutionary hypotheses of herbivore diversification assume a specialized phase for diversification to occur, therefore it is not clear if host plant use can lead to diversification of generalist herbivores without the retraction of their diet.

Geographic scale and isolation are thought to be critical factors in diversification of herbivorous insects for both adaptive and non-adaptive macroevolutionary hypotheses (Stireman 2005; Janz and Nylin 2008; Hardy et al. 2016). Isolation by distance has long been advocated as an important mechanism of evolutionary change in many species (Wright 1943), as gene flow is expected to be negatively correlated with geographic distance. We might find different patterns of divergence depending on the geographic scale being studied. For instance, grey white-eye birds were shown to be divergent in a very small geographic range (Milá et al. 2010), while the herbivore *Utetheisa ornatrix* (Lepidoptera) showed genetic divergence of populations only in a broad geographic range (>3,000km) and no consistent divergence in fine scale (Cogni et al. 2011). Furthermore, when considering both small and large geographic scale, we can test for both divergence across regions and within regions with a fine scale approach (Hutchison and Templeton 1999). However, geographic distance or isolation is only one component that can influence genetic differentiation between populations; other important factors are the environment and the ecological context. Populations might become isolated from one another (i.e., experience reduced gene flow) because of differences in the environment or in species interactions (e.g., host use), despite geographic distance (isolation by environment, IBE, Wang and Bradburd 2014). Recently, more evidence has been found for isolation by environment using new methods that test genetic divergence associated with variable environmental factors while controlling for geographic distance (review in Wang and Bradburd 2014). With these approaches we can test the relative importance of geographic isolation, host plant use, and diet breadth on the divergence of contemporary generalist herbivores on fine and broad geographic scales.

Fall webworm (*Hyphantria cunea*, Erebidae, Lepidoptera; hereafter FW) is an excellent model organism for testing how diet breadth, host plant use, and geographic distance influence the divergence of herbivores for several reasons. FW is one of the most generalist species known (Warren and Tadic 1967), yet its diet breadth varies considerably over its geographic range, with some populations being more specialized than others [e.g., Colorado (Murphy and Loewy 2015) vs. east coast (Mason et al. 2011)]. Furthermore, FW has a very broad geographic range, however, the use of some host species is conserved across sites (e.g., *Prunus* sp., *Ulmus* sp., and *Malus* sp. are used in the west and east of US, Murphy and Loewy 2015; Mason et al. 2011). FW seems to already be diversifying; there are two recognized types of FW that seem to be genetically different (Yang et al. 2018) and that differ in their life history traits, behavior, host plant use and geographic distribution. The two types are named based on the color of their head capsule as larvae, black-headed or red-headed (hereon referred as black and red, respectively). Blacks and reds can be found in sympatry in the east and southeast of US, but the types usually differ in the host plants that they use even when they co-occur (e.g., Louisiana, Oliver 1964). Using this extreme generalist species, we address the following questions: 1) What is the relative importance of geographic isolation, host plant use, and diet breadth on the genetic divergence of FW populations? 2) Does the pattern of divergence depend on geographic scale (e.g. within Colorado vs. >3,000 km, Fig. 15)?

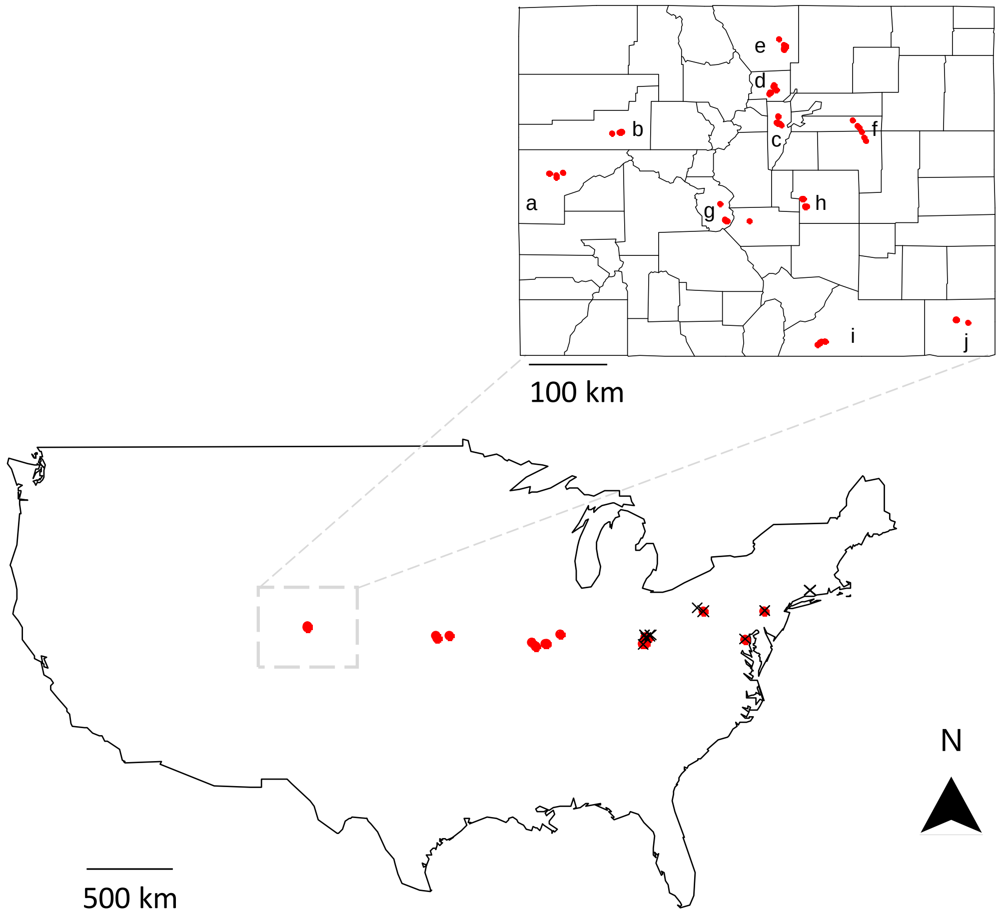


Figure 15: Map showing sampling locations. Red circles represent red fall webworm samples, while black xs represent black fall webworm samples. Map on right shows in detail sampling locations of Colorado counties: a - Mesa County, b - Garfield County, c+d+e - Front Range (Jefferson, Boulder and Larimer Counties, respectively), f - Arapahoe County, g - Chaffee County, h - El Paso County, i - Las Animas County, j - Baca County.

Methods

Morphometry

Although Yang et al. (2017) argued that the adults of the two types of FW are not morphologically different, they focused on wing length, which can vary with environmental and dietary factors (e.g. smaller individuals when feeding on low quality plant, Murphy and Loewy 2015). A more reliable way to measure morphological differences is to use landmarks to calculate the ratio of distances between these landmarks as a measure of overall body shape (Zelditch et al. 2004). We measured adult females and males of FW from Colorado (red, n=28), Missouri (red, n=5), New Jersey (black, n=12), and Maryland (red, n=13; black, n=12) following the criteria in Zelditch et al. (2004) to define our landmarks. These were demarcated as to junctures between different wing veins and between wing veins and wing borders on the right forewings (Fig. 16a). Wing shape in Lepidoptera is usually very variable between species and can be used to distinguish species and even families (Zhong et al. 2016). We used tpsUtil and tpsDIG (Rohlf, 2005) to extract the landmark coordinates from photos and MorphoJ (Klingenberg 2011) to analyze the data using Canonical Variate Analysis.

Sample collection and genotyping

Genetic analyses were performed on two separate sets of samples. With one set of samples (black and red FW over a broad geographic range), we addressed the question of the relative importance of host use and geographic isolation on population divergence using both types. We also analyzed the genetic divergence between the two types. With the other set of samples (red FW in Colorado), we analyzed the relative importance of diet breadth, host use, and geographic distance on genetic divergence in a fine geographic scale. We decided to analyze the two sets separately because we only had reliable diet breadth data for Colorado, and because including reds and blacks together might result in more SNPs being filtered out since the two types are expected to be divergent. One set included 61 samples with black and red types from different locations in a wide geographic scale, and the other included 126 samples only from Colorado.

For the broad geographic scale analysis, we had samples from Colorado (n=6 reds from Jefferson county), Connecticut (n=7 blacks), Illinois (n = 2 reds and 2 black), Kansas (n = 3 reds), Maryland (n = 5 reds), Missouri (n = 6 reds), New Jersey (n = 3 reds and 3 blacks), Ohio (n = 13 reds and 8 blacks), and Pennsylvania (n = 1 reds and 2 blacks) (Fig. 1). Our sampling included allopatric populations of red FW (Colorado) and of black FW (Connecticut), as well as sympatric populations of reds and blacks (all the others). For the fine geographic scale analysis, we used 10 locations of red FW from Colorado with 10-20 samples per location and used larvae from similar hosts across locations as much as possible, totaling 126 samples (Fig. 1). Larvae from Colorado were collected across the state in the following counties: Arapahoe, Baca, Boulder, Chaffee, El Paso, Garfield, Jefferson, Larimer, Las Animas, Mesa (letters a-j in Fig. 1). Sample and host plant data were collected as explained in Murphy and Loewy (2015). We used at least 2 larvae per web for each sample, and all larvae were placed on 95% alcohol immediately after collection.

We genotyped our 187 samples using double digest restriction site associated DNA sequencing (ddRAD-seq) described in Peterson et al. (2012), with slight modifications. We extracted DNA from head capsules of FW caterpillars (n=184) and whole pupae (N = 3) using a Qiagen DNA extraction kit. We followed manufacturer’s instructions but omitting the RNA step. We quantified DNA using the Quantifluor® dsDNA system (Promega Inc.) and digested 500 ng of DNA with Msp1 and MluC1 enzymes (New England Biolabs Inc., Beverly, MA) at 37 °C for 3h. We ligated adaptors and selected 300bp (±25bp) fragments using a Pippin Prep PR00953 (Sage Science, Inc.) following manufacturer's directions. Libraries were amplified for 9 cycles in 4-8 20 µL reactions using Phusion High Fidelity PCR® (ThermoFisher, Inc.) following the Peterson et al. (2012) protocol. Libraries were sequenced on an Illumina HiSeq 2500 by the Genomics and Microarray Core at University of Colorado Anschutz. Samples from the two analytical sets were intermixed in the two lanes that we used for sequencing.

We demultiplexed, filtered, and trimmed the raw reads using Stacks 1.46 (Catchen et al. 2013). We trimmed adaptors and removed low-quality bases (mean Phred score<10 along a sliding window of 20 bp) and reads with uncalled bases or ambivalent barcodes. Since FW does not have a reference genome, we performed *de novo* assembly using Stacks. We required a minimum of 3 identical reads to form a stack, a maximum distance of 2 between stacks, a maximum distance of 4 for secondary alignments, and a maximum of 3 stacks allowed per de novo locus. We used the program *populations* in Stacks to obtain a vcf file after filtering out loci with more than 2 alleles. We used vcftools 0.1.15 (Danecek et al. 2011) to remove loci that occur in fewer than 10% and more than 90% of populations, and remove 20% of missing loci. We compared different values of missing loci and found that 20-60% yielded similar trends, therefore we used the 20% value that retained more SNPs.

Population analyses

We conducted population genomic analyses for the two sets of data, with the two types of FW on a wide geographic scale, and with only the red-type in Colorado. All analyses, except Structure, were performed using R environment 3.4.1 (R Development

Core Team 2011). We evaluated genetic clustering with principle component analysis (PCA) and calculated pairwise Weir and Cockerham (1984) distances using the R package SNPRelate 1.10.2 (Zheng et al. 2012). Using the program *populations* from Stacks, we obtained the Structure file from the cleaned vcf file. We used Structure 2.3.4 (Pritchard et al. 2000) and StructureHarvester (Earl and VonHoldt 2012) to define the most likely number of populations that explain our data, and visualized the structure analysis result with the most likely K value ± 1 using Clumpak (Kopelman et al. 2015). For the structure analysis, we used 3 replicates per K value, and our K values ranged from 1 to 11, with 10,000 burn-ins followed by 50,000 Markov Chain Monte Carlo repetitions.

Genetic distance in a broad geographic scale

We analyzed the influence of host plant use and geographic distance on the divergence of FW using both red and black types across a broad geographic range (from Colorado to Connecticut, ~3,000 km apart, Fig. 1). We used multiple Moran's eigenvalues, distance-based redundancy analysis (db-RDA), and matrix regression to analyze the importance of geographic distance and host plant on genetic distance. For these analyses we used pairwise genetic distance between individual samples (identity by state, IBS), which we obtained using plink 1.07 (Purcell et al. 2007). To analyze how the geographic distance alone influences the genetic distance between individual samples, we used the R package *memgene* 1.0 (Galpern et al. 2014). This package takes into consideration the coordinates and genetic distances to calculate Moran's eigenvalues and show how similar (same size and color) samples are to each other in a map view. We tested the importance of host plant and color type on genetic distance using db-RDA with IBS as genetic distance. Assuming that geographic distance influences host plant distribution and potentially genetic distance, for the geographic distance, we used the significant eigenvalues from Principal Coordinates of Neighbourhood Matrix (*pcnm* function) as a condition in the model (Legendre and Fortin 2010), therefore geographic distance was parsed out before the constrained variables. We selected the variables that best explained the variance in genetic data using stepwise model selection with the function *ordiR2step*. Pcnm and db-RDA analyses were performed using the R package *vegan* 2.4-4 (Oksanen et al. 2007). We further tested the influence of geographic distance and host plant use on genetic distance (IBS) by performing matrix regression using the function *MRM* from the package *ecodist* 2.0.1 (Goslee and Urban 2007). Since black and red FW seem to be genetically distinct (Yang et al. 2017), we performed matrix regression using the two types together, and each type separately. To obtain the host plant distance used in matrix regression analyses, we coded the hosts shared between the two pairwise individual samples as 0 (i.e., no distance between hosts) when individuals were found on the same host plant, and when the individuals were using different hosts, we coded as 1 (i.e., distance between hosts) (as in Darwell et al. 2014).

Genetic distance in a narrow geographic scale

To test how host plant use, diet breadth and geographic distance influence genetic distance of FW in a narrow geographic range, we used red FW collected in different locations in Colorado (from 40 to 700 km apart, Fig. 1). We calculated fine scale geographic distance using the R package *geosphere* with function *distCosine*, which considers the shortest distance from one point to another. We used ordination diet breadth (Fordyce et al. 2016, hereon ODB) to calculate diet breadth; this measure takes into consideration the number of plant species used in a population and also the similarity of hosts in one population as compare to all other hosts used in the other populations tested. If a population uses few and similar plants compared to the other populations, it has a low value of ODB (close to 0, considered more specialist), whereas if a population uses many and different plants, it has a high value of ODB (close to 1, considered more generalist).

We repeated the above analyses (matrix regression, db-RDA and memgene) on this fine spatial scale. We tested for the relative importance of diet breadth and host plant use on the genetic distance of Colorado FW using db-RDA (with eigenvalues with $P<0.5$ from pcnm as geographic distance condition) and matrix regression. For both analyses we coded the distance of host plant use as 0 and 1 as explained above; 0 means that the individuals used the same plant species, while 1 means that the species were different. We also used a partial mantel test to analyze the influence of diet breadth on the divergence of populations while controlling for geographic distance, as diet breadth is a measure at the population level, and thus can be compared to a population's pairwise F_{ST}. To obtain pairwise comparison between populations using ODB, we used the absolute difference between the two values being compared. Therefore, a lower value of this difference would mean that the two populations being compared have similar diet breadths, whereas if the values are high, the populations have very different diet breadths. To perform the partial mantel test, we used the package *vegan*. We used the diet breadth at the population level, as FW individuals only feed on one host plant individual during their entire larval stage.

Results

Morphometry

The discriminant function analysis showed that the red and black FW are morphologically different (t-square=244.46, n=70, P<0.0001); the two types form distinct groups, and the two first axes represent 81% of the variance (Fig. 16a). In contrast, pairwise discriminant comparison between all geographic locations (without distinguishing color type for Maryland samples that had both types) did not show any shape difference (all P>0.05). A permutation test for Procrustes distance among groups had p-values less than 0.05 for all pairwise comparisons, except between black FW from Maryland and New Jersey. Therefore, when comparing the interposition of shapes formed by the landmarks, reds from different locations were different from each other, as well as from reds and blacks overall, while blacks from different locations were not different from each other.

Population Genomics

After demultiplexing, we obtained 340,184 to 9,024,092 reads per individual (median = 2,245,657, total = 480,351,136 reads) from our 187 individuals. From the *de novo* assembly that included the 61 samples of red and black FW, we obtained 56,515 SNPs, and the depth of coverage from processed samples of *de novo* assembly ranged from 8.05 to 31.88x per sample. For the Colorado locations, we used 126 samples with 60,737 SNPs, and the depth of coverage ranged from 5.52 to 37.48x.

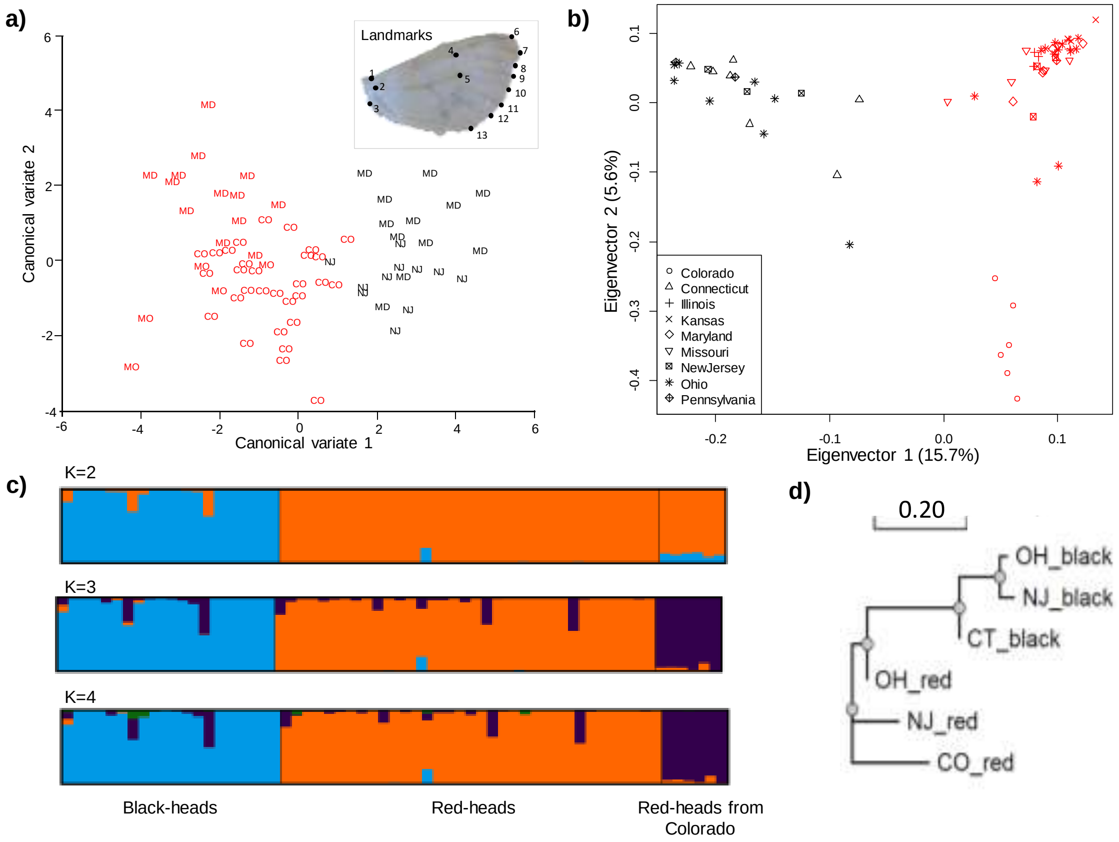


Figure 16: Morphometry and population genomics of the two color types of fall webworm. a) Morphometry of Colorado (CO), Maryland (MD), Missouri (MO) and New Jersey (NJ) fall webworm adults, image on top right shows the 13 landmarks in the forewings used. b) Principal Component Analysis of the 61 genetic samples of the two types (shown by different colors, red for red type and black for black type), symbols represent the different locations of the samples. c) Structure analysis for K=2-4 showing black-heads, red-heads from across our geographic sampling except Colorado, and red-heads collected from Colorado alone. d) Tree of Wier and Cockham’s FST pairwise comparisons for Colorado (CO), Connecticut (CT), New Jersey (NJ) and Ohio (OH), done using PRESTO.

With our 61 samples of red and black FW, we found that the two types are genetically different, and that the Colorado population is different from black FW and also from other red FW (Fig 16b-c). The two first axes of the principal component analysis explained more than 20% of the genetic variation, and there was a clear separation between red and black samples along the first axis (Fig. 16b). Samples were not grouped by host plant species (not shown) nor by location, except for Colorado samples that formed a distinct group along the second axis (circles in Fig. 16b). We found that the best number of K to group our samples was 3 (Delta K = 5613), and that each type clearly formed distinct populations. Further, Colorado was different from the two types, even though they are red FW (Fig. 16c, K=3). Using pairwise F_{ST}, the red samples from the sympatric populations (NJ and OH) were more similar to reds from other locations than to blacks from the same location (Fig. 16d).

For the 126 samples from Colorado, there was no clear distinction between samples in our PCA, however some samples from Front Range formed a group on the top right (letters *c* to *e* in fig. 17), samples from Mesa and Garfield formed another group on the bottom right (letters *a* and *b* in fig. 17) and the samples from the other 5 locations were all mixed on the left (Fig. 17a). However, the two axes explained only ~7% of the data. There was no clear grouping by host plant use. With the structure analysis, we found that the best K value to explain the genetic structure in Colorado was 2 (Delta K = 46.3); Mesa, Garfield and the northern Front Range are formed by a mix of the two genetic structures, while the other 5 sampling locations, which are found in eastern side of the Rocky Mountains and southern Colorado, are mostly composed by one cluster (Fig. 17c, light blue).

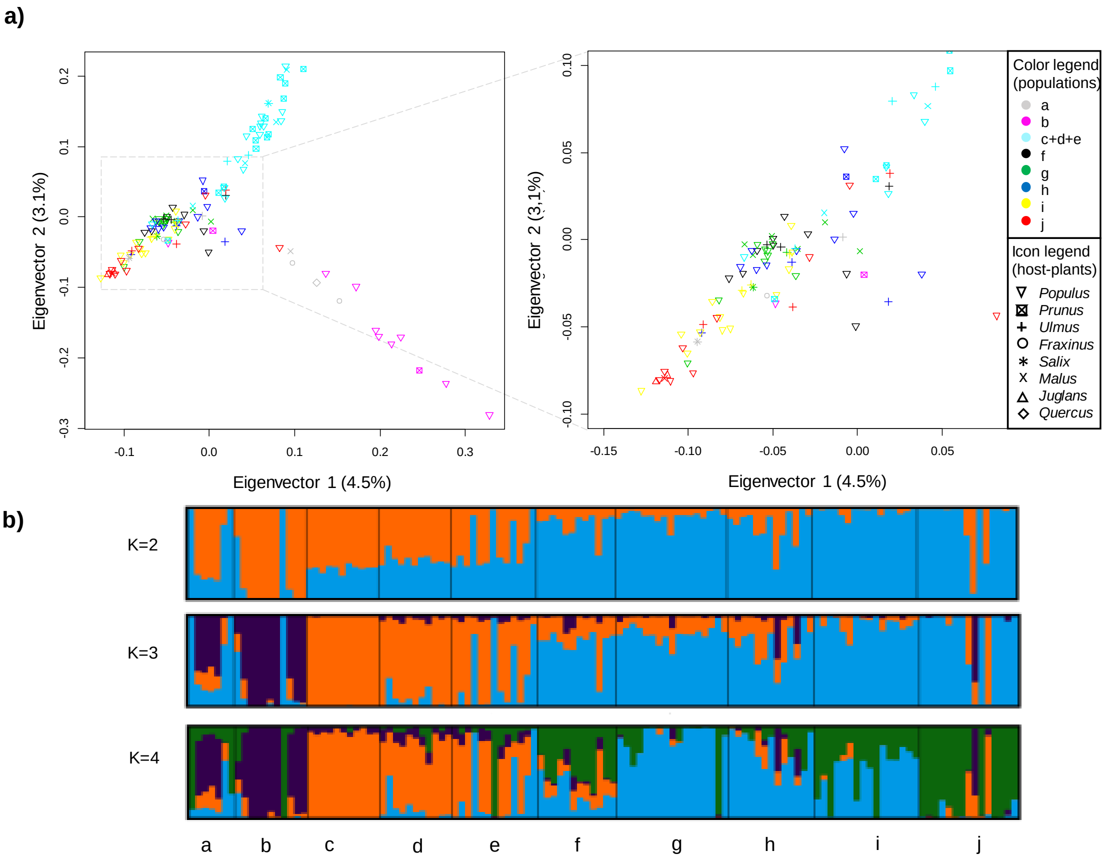


Figure 17: Population genomics of Colorado fall webworm populations. a) Principal Component Analysis of the 126 genetic samples. Different colors represent the different geographic locations of fall webworm (same as fig.15): a - Mesa County, b - Garfield County, c+d+e - Front Range (Jefferson, Boulder and Larimer Counties, respectively), f - Arapahoe County, g - Chaffee County, h - El Paso County, i - Las Animas County, j - Baca County. Different symbols represent the host plant genus that the larvae were using when collected. Graph on the right is a zoom in of the region outlined by the grey line in the graph on the left. b) Structure analysis for K=2-4, letters represent the same locations as in figure a.

Host plant use and geographic distance are predictors of divergence in a broad geographic range

We found that host plant use and geographic distance are predictors of genetic distance in our broad geographic range analysis. Using memgene, we found that 8% of the genetic variation can be explained by spatial patterns. As we can see in figure 18, most samples that are close together geographically are genetically similar (with similar size and color). In the db-RDA analysis, both color type and host plant use were kept in the model to explain individual pairwise genetic distance (Color: Adjusted $R^2 = 0.55$, F = 74.14, df = 1, P = 0.002; Host: Adjusted $R^2 = 0.03$, F= 1.28, df = 29, P = 0.004). Most of the genetic variation was explained by color type, as would be expected from what we found in the population genomic analysis.

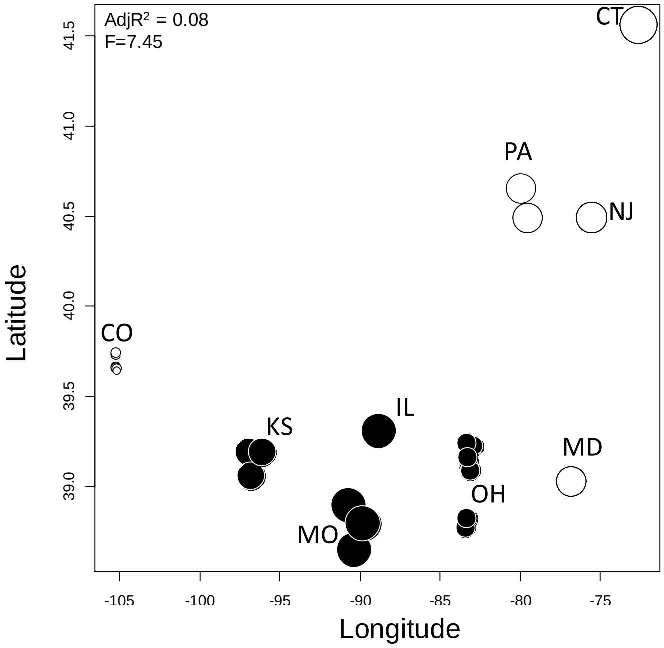


Figure 18: Memgene analysis showing genetic groupings of samples along the landscape, letters represent States, circles represent each sample, circles of same size and color show genetic similarity, while different sizes and colors show dissimilarity.

Since the two FW types seem to be highly differentiated and color type explains most of the variation, we performed our remaining analyses with the two types separately and with all samples together. Using individual pairwise comparisons (IBS) and matrix regression, only host plant distance was correlated with genetic distance when we analyzed the two types together (Table 5; r^2=0.015, P=0.03). When we analyzed the two types separately, only geographic distance influenced genetic distance for blacks (r^2=0.023, P=0.02) while both host plant use and geographic distance influenced genetic distance for reds (Table 5; r^2=0.55, P=0.01). The host plant and geographic distances explained 55% of the data for reds, while the variables explained less than 3% of the genetic distance for blacks and the two types together. Host plant distance was correlated with geographic distance for black FW (r^2=0.05, P=0.01), but not for red FW (r^2=0.002, P=0.18).

Table 5: Summary of the influence of geographic distance, host plant use, and diet breadth (for Colorado only) on genetic distance using matrix regression. Bold represents significant variables at P<0.05.

	Red and Black		Black type		Red type		Colorado red type	
	Coef.	P	Coef.	P	Coef.	P	Coef.	P
Intercept	0.85	1	0.33	0.93	**0.031**	**0.01**	0.5	1
Geo ~ Gen	0.0007	0.42	**0.0007**	**0.02**	**0.002**	**0.01**	**0.004**	**0.01**
Host ~ Gen	**0.08**	**0.02**	-0.0004	0.93	**-0.005**	**0.05**	0.0002	0.66
Diet ~ Gen	-	-	-	-	-	-	**0.02**	**0.01**
Model's r^2	0.015		0.023		0.55		0.11	

Host plant use and geographic distance are predictors of divergence in a narrow geographic range

For the Colorado samples, geographic distance was a more important factor than host plant use on the divergence of populations. We found that 11.3% of the genetic variation was explained by spatial pattern (Fig. 19a). This suggests that geographic profile is important, not altogether surprising as Mesa and Arapahoe are separated from the others by the Continental Divide formed by the Rocky Mountains and which demarcates the point at which drainages from North America move towards the Atlantic or Pacific sides of the continent. In the db-RDA model selection, diet breadth and host plant use were maintained in the model to explain genetic distance (Host: Adjusted R^2 = 0.033, F = 1.43, df = 10, P = 0.002, Diet breadth: Adjusted R^2 = 0.02, F = 3.45, df = 1, P= 0.002), however the two variables explained only 5.3% of the genetic distance. Thus, spatial pattern was >2x more important than host plant and diet breadth to explain genetic distance (11.3% vs. 5.3%). When we consider the individual genetic distance comparisons using matrix regression, geographic distance and diet breadth use were correlated with genetic distance, but host plant was not (Table 5; r^2=0.11, P=0.01). However, diet breadth is confounded with the sampling location, as it is a “population” measure and all individuals from the same location have the same value of diet breadth. In fact, both host plant (r^2=0.01, P=0.01) and diet breadth (r^2=0.03, P=0.02) are correlated with geographic distance. Using F_{ST} and partial mantel test for a population instead of individual comparisons, diet breadth pairwise comparison was not correlated with genetic distance (Fig. 19b).

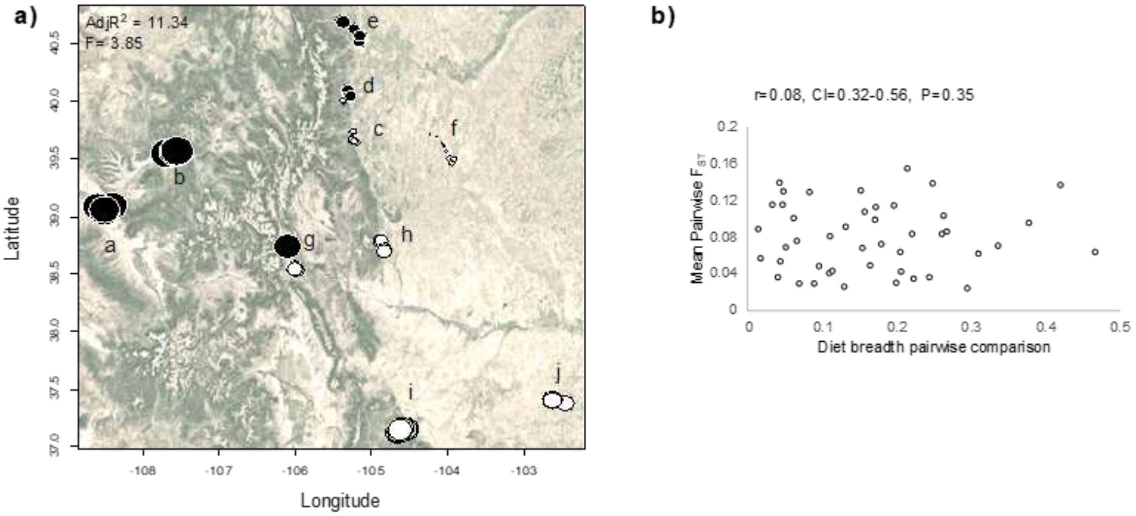


Figure 19: Influence of geography and diet breadth on genetic distance of Colorado populations. a) memgene showing genetic groupings of samples along the landscape (superposition of satellite topography of Colorado from Google Earth), letters represent the same populations as in Fig. 15 and 17, b) Partial mantel test for diet breadth pairwise comparisons.

Discussion

Geographic distance and host plant use were both correlated with genetic distance, but geographic distance accounted for more of the distribution of variation in genetic distance than did host plant use in both broad and narrow geographic scale. However, the effect of host plant use was stronger for red FW in a broad geographic range than for blacks and for Colorado reds. Diet breadth also explained genetic distance in Colorado reds, however, the diet breadth measure is confounded with sampling location, and thus is not a reliable measure. Interestingly, in the broad geographic scale analysis, reds are less polyphagous than blacks and were more influenced by host plant use than blacks. The evidence of geographic distance being more influential than host plant use on FW genetic divergence suggests that not all generalists are composed by cryptic species complex of specialists, as was previously suggested by Bickford et al.

(2006). FW is an example of a true extreme generalist species, especially if we consider Colorado samples that had no distinct population structure and individuals feed on different host plant species within and across locations.

We found that red and black FW are morphologically and genetically different from each other. Our results suggest that there is low gene flow between black and red types, even where they occur in sympatry, as local red FW were more similar to reds from other locations than to sympatric black FW. Recently, a study using mitochondrial DNA showed clear distinction between red and black morphs of FW, providing evidence that the two types may represent different species; however, the authors did not provide strong evidence of what might drive the divergence of this herbivore (Yang et al. 2017). The use of mitochondrial DNA to infer species delimitations can be misleading in cases where there is introgression, and for arthropods the pattern can be influenced by inherited symbionts (Hurst and Jiggins 2005; Toews and Brelsford 2012). In nature, red and black FW are usually reproductively isolated in time as the blacks usually emerge earlier in the season than reds and are multi-voltine, while reds are usually univoltine. However, we observed from our lab colonies that FW can be very sensitive to changes in light and temperature regime, which can influence the isolation in time between the two types. Although it is possible that the two types might overlap in time in rare cases, when the two types co-occur, they usually use different host plant species (Y. Ito and Warren 1973; Oliver 1964), and female moths are not very mobile (Yamanaka et al. 2001), which might further facilitate the isolation of red and black FW. Furthermore, bioassays showed that males of one type are usually more attracted by pheromones of females of the same type than of the other type, resulting in more intratype than intertype matings (McLellan et al.

1991). Hybrids between black females and red males are viable, but it is not clear if red females and black males can produce viable offspring (Ito and Warren 1973). With the body of evidence from natural history and genetic analysis accumulated during the past four decades, and our data on morphometry and nuclear genomic analysis using thousands of SNPs, there is ample evidence that the two types of FW are divergent lineages and likely different species.

Our data provide evidence of possible factors that can influence the divergence between the two types and among populations, but we should interpret these factors with caution. We showed that host plant and geographic distance are correlated with divergence of populations, however other factors such as competition between the two types or selective pressure from natural enemies could have also influenced their divergence. Yang et al. (2017) suggested that the separation of the two types occurred 1.2-1.6 million years ago. It is possible that the two types began the speciation process due to allopatric separation into different refugia and, after the Ice Age, established overlapping ranges. The different pattern of host plant use by the two FW types might have evolved due to the types occupying different regions during the Ice Age, and thus host plant use could be a consequence of geographic isolation and not the cause of speciation. Therefore, although we found evidence of host use being correlated with genetic distance, we cannot say that variation in host plant use was the cause of the divergence between black and red FW. However, we found evidence that host plant use influences divergence among populations of red FW, and in this case the pattern of host use was not correlated with geographic distance. Red FW are less polyphagous than black FW and we previously found that reds suffer stronger fitness trade-off when feeding on different quality plants than blacks (Chapter 3). Thus, it is possible that red FW are under stronger selective pressure from host plants than black FW.

It is intriguing that red FW from Colorado formed a distinct genetic group from the other collection sites. Colorado populations are isolated from eastern populations by the Central Plains, which is a dry region of grassland and where FW are rare (M. Vidal personal observation). FW usually feed on trees and shrubs and tend to fly along rivers and roads (e.g. Ito et al. 1970). Therefore, the migration of FW between eastern populations and Colorado might be limited to the few riverine corridors of riparian vegetation across the Central Plains, which might lead to genetic isolation of Colorado populations. The original colonization of Colorado populations by the red type might have been possible because of habitable corridors formed by the presence of cottonwoods along river banks. While red FW can use cottonwoods as a host plant, black FW are not known to use them in the wild and do not perform well on them in the lab (Chapter 3). In Colorado, the Rocky Mountains are clearly an important gene flow barrier between populations to the east or west of the Mountains. It would be interesting to compare the genetic structure of other western populations to see if the pattern holds in a larger geographic range.

Our results show that divergence of generalist herbivores is correlated with both host plant use and geographic distance, and the pattern might vary according to the geographic scale being considered. Usually it is assumed that generalist herbivores will be under selective pressure to specialize, and that specialization can eventually lead to speciation, via host race formation or other mechanism (Ehrlich and Raven 1964; Janz and Nylin 2008). But FW, one of the most generalist species known even at the population level, already has two very divergence lineages that are possibly different species, and host plant use influences the divergence of FW populations. Although it is not clear if host plant use was the driver of speciation of FW, we show here that host plant species is an important factor influencing divergence of current populations, even when we take geographic distance into consideration. Macroevolutionary hypotheses to explain the diversification of insect herbivores assume that herbivores go through a specialized phase to speciate (e.g., Ehrlich and Raven 1964; Stireman 2005; Janz and Nylin 2008), but we showed that this may not be a requirement for speciation of herbivores, as generalists can diverge without specializing. Furthermore, even when there are weak trade-offs in fitness of herbivores feeding on varied host plants, divergence is not only associated with geographic isolation as proposed by the Speciation-by-Drift hypothesis (Hardy et al. 2016), host plant use can also be influential.

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APPENDIX

Appendix Table 1: List of publications that test both bottom-up and top-down effects on a focal herbivore and whether each publication included a measure of standard deviation; we used the 75 papers that reported standard deviation (or for which we could calculate it) in the meta-analysis.

Publication	**Included st. dev.?**
Albarracin M.T. and P. Stiling (2006) Ecology 87: 2673-2679.	Yes
Annis, B. and L.E. O'Keeffe (1987) Environmental Entomology 16: 653-655.	Yes
Arthur, A.P. (1962) The Canadian Entomologist 94: 337-347.	No
Ballman, E.S. et al. (2012) Environmental Entomology 41: 1417-1425.	No
Barbosa, P. (2001) Ecology 82: 698-704.	No
Bierre, A. et al. (2002) Proceedings of the Royal Society of London B 269: 2197-2204.	Yes
Boege, K. and R.J. Marquis (2006) Oikos 115: 559-572.	Yes
Chaplin-Kramer, R. (2011) Journal of Applied Ecology 48: 880-887.	Yes
Chen, Y.H. and S.C. Welter (2002) Environmental Entomology 31: 626-636	Yes
Clancy, K.M. and P.W. Price (1986) Ecology 67: 1601-1607.	No
Connahs, H. et al. (2009) Journal of Insect Science 9:28.	No
Cornelissen, T. and P. Stiling (2006) Ecoscience 13: 66-74.	Yes
Costamagna, A.C. and D.A. Landis (2006) Ecological Applications 16: 1619-1628.	Yes
Costamagna, A.C. et al. (2012) PlosOne 8:e56394.	Yes
Danner, D.J. and A. Joern (2003) Oecologia 137: 352-359.	Yes
Daugherty, M.P. et al. (2007) Biological Control 43: 257-264.	Yes
Davalos, A. and B. Blossey (2010) Entomologia experimentalis et applicata 135: 85-95.	Yes
Degen, T. et al. (2012) PlosOne 7:e47589.	Yes
Denno, R. F. (1990) Ecology 71: 124-137.	No
Denno, R.F. et al. (2002) Ecology 83: 1443-1458.	Yes
Denno, R.F. et al. (2003) Ecology 84: 1032-1044.	Yes
Digilio, M.C. et al. (2012) Journal of Plant Interactions 7: 71-77.	Yes
Faeth, S.H. (1985) Ecology 66: 870-875.	No
Fatouros, N.E. et al. (2014) Proceedings of the Royal Society of London B 281: 20141254.	Yes
Ferrari, J. and H.C.J. Godfray (2003) Ecology Letters 6: 111-118.	No
Finke, D.L. and R.F. Denno (2006) Oecologia 149: 265-275.	Yes
Flaherty, L. et al. (2013) Environmental Entomology 42: 957-966.	No
Forkner, R.E. and M.D. Hunter (2000) Ecology 81: 1588-1600.	Yes
Fortuna, T.M. et al. (2013) Biological Invasions 15: 2387-2401.	Yes
Gassmann, A.J. (2010) Annals of the Entomological Society of America 103: 371-378.	No
Gols, R. et al. (2006) Basic and Applied Ecology 8: 421-433.	No
Gonthier, D.J. et al. (2011) Ecosphere 4: 107.	Yes

Griffin, C.A.M. and J.S. Thaler (2006) Ecology Letter 9: 338-346.	No
Gross, P. and P.W. Price (1988) Ecology 69: 1506-1516.	No
Hamm, J.J. and B.R. Wiseman (1986) The Florida Entomologist 69: 541-549.	No
Hare, J.D. and D.J.W. Morgan (2000) Ecology 81: 509-519.	Yes
Harmon, J.P. et al. (2003) Population Ecology 45: 75-81.	Yes
Havill, N.P. and K.F. Raffa (2000) Ecological Entomology 25: 171-179.	Yes
Helms, S.E. et al. (2004) Ecological Entomology 29: 44-51.	No
Hempel, S. et. al. (2009) Oecologia 160: 267-277.	Yes
Hopkins, G.W. and J. Memmott (2003) Ecological Entomology 28: 687-693.	No
Janmaat, A.F. and J.H. Myers (2007) Journal of Evolutionary Biology 20: 62-69.	Yes
Kahn, D.M. and H.V. Cornell (1989) Ecology 70: 1219-1226.	Yes
Kaplan, I. et al. (2007) Oecologia 152: 665-676.	Yes
Kareiva, P. and R. Sahakian (1990) Nature 345: 433-434.	No
Karimzadeh, J. and D.J. Wright (2008) Ecological Entomology 33: 45-52.	No
Kartohadjorno, A. and E.A. Heinricks (1984) Environmental Entomology 13: 359-365.	No
Klaiber, J. et al. (2013) Biological Control 66: 49-55.	Yes
Klemola, N. et al. (2008) Oikos 117: 926-934.	No
Klemola, N. et al. (2003) Ecological Entomology 28: 319-327.	No
Kos, M. et al. (2011) Functional Ecology 25: 1113-1124.	Yes
Krauss, J. et al. (2007) Functional Ecology 21: 107-116.	Yes
Le Guigo, P. et al. (2010) Basic and Applied Ecology 12: 72-79.	Yes
Li, S-J. et al. (2013) Basic and Applied Ecology 14: 670-679.	Yes
Lill, J.T. (2001) Evolution 55: 2236-2247.	No
Mason, P.A. et al. (2011) Oikos 1509-1518.	Yes
Mason, P.A. et al. (2014) Oecologia 176: 477-486.	Yes
Masters, G.J. et al. (2000) Oecologia 127: 246-250.	Yes
Miller, T.E.X. (2008) Ecological Entomology 33: 261-268.	Yes
Moon, D.C. and P. Stiling (2000) Ecology 81: 470-481.	Yes
Moon, D.C. and P. Stiling (2002) Oecologia 133: 243-253.	Yes
Moon, D.C. and P. Stiling (2002) Ecology 83: 2465-2476.	Yes
Moon, D.C. and P. Stiling (2002) Oikos 98: 480-490.	Yes
Moon, D.C. and P. Stiling (2004) Ecology 85: 2709-2716.	Yes
Moon, D.C. and P. Stiling (2005) Ecological Entomology 30: 642-649.	Yes
Mooney, K.A. et al. (2012) PlosOne 7: e34403.	Yes
Moreau,G. and C. Bjorkman (2012) Population Ecology 54: 125-133.	Yes
Moreira, X. et al. (2015) Ecological Entomology 40: 676-686.	Yes
Morse,D.H. (2009) Ecological Entomology 34: 246-253.	No
Muller, C. and K. Arand (2007) Entomologia Experimentalis et Applicata 124: 153-159.	No
Murphy, S.M and K.J. Loewy (2015) Oecologia 179: 741-751.	No*

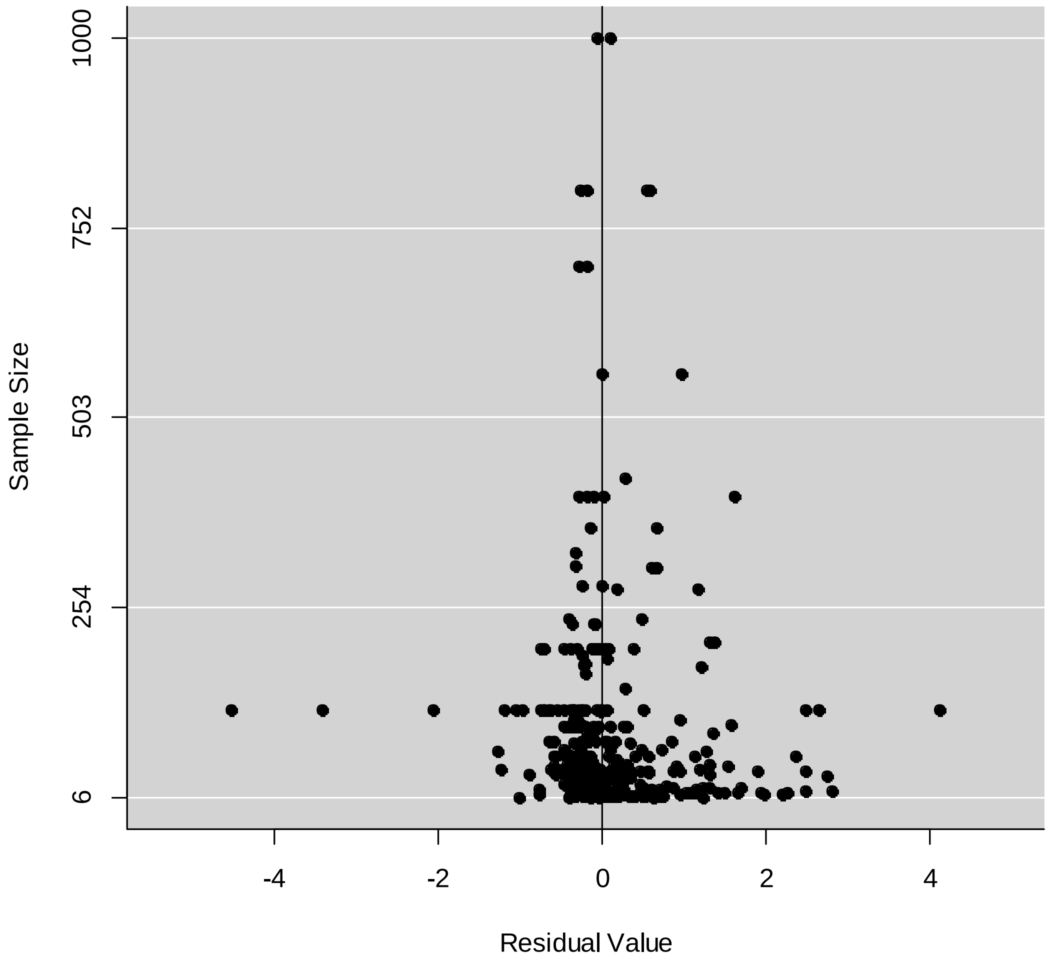
Murphy, S.M. (2004) Proceedings of the National Academy of Sciences 101: 18048-18052.	No*
Obrycki, J.J. et al. (1983) Journal of Economic Entomology 76: 456-462.	No
Oedekoven, M.A. and A. Joern (2000) Ecology 81: 66-77.	Yes
Oppenheim, S.J. and F. Gould (2002) Evolution 56: 679-689.	Yes
Orr, D.B. and D.J. Boethel (1985) Environmental Entomology 14: 612-616.	Yes
Ovadia, O. and O.J. Schmitz (2004) Oecologia 140: 398-406.	Yes
Parry, D. et al. (2003) Ecology 84: 1768-1783.	Yes
Pimentel, D. and A.G. Wheeler (1973) Environmental Entomology 2: 1-11.	No
Prado, S.G. and S.D. Frank (2013) Biological Control 66: 72-76.	Yes
Rahman, M. (1970) Journal of Economic Entomology 63: 820-821.	Yes
Richards, L.J. et al. (2010) Journal of Chemical Ecology 36: 1105-1113.	No
Richmond, D.S. et al. (2004) Ecological Entomology 29: 353-360.	Yes
Riihimaki, J. (2006) Ecological Entomology 31: 227-235.	Yes
Ritchie, M.E. (2000) Ecology 81: 1601-1612.	No
Rodriguez-Saona, C. et al. (2005) Oecologia 143: 566-577.	Yes
Rose, N.H. (2015) PlosOne 10: e0120769.	No
Rutledge, C.E. et al. (2003) Oecologia 135: 39-50.	Yes
Santolamazza-Carbone, S. et al. (2014) 174: 893-907.	Yes
Schadler, M. et al. (2010) Entomologia Experimentalis et Applicata 135: 162-169.	Yes
Shahjahan, M. and F.A. Streams (1973) Environmental Entomology 2: 921-925.	No
Shrewsbury, P.M and M.J. Raupp (2006) Ecological Applications 16: 262-272.	Yes
Singer, M.S. and J.O. Stireman III (2003) Oikos 100: 554-562.	No
Singer, M.S. et al. (2004) The American Naturalist 164: 423-429.	No
Singer, M.S. et al. (2012) The American Naturalist 179: 363-374.	Yes
Sipura, M. and J. Tahvanainen (2000) Oikos 91: 550-558.	Yes
Snyder, W.E. and A.R. Ives (2001) Ecology 82: 705-716.	Yes
Soufbaf, M. et al. (2012) Arthropod-Plant Interactions 6: 241-250.	Yes
Starks, K.J. et al. (1972) Annals of the Entomological Society of America 65: 650-655.	No
Stiling P.D. et al. (1982) Ecological Entomology 7: 447-452.	No
Stiling, P. et al. (2003) Ecological Entomology 28: 587-592.	Yes
Stiling, P. and D.C. Moon (2004) Oecologia 142: 413-420.	Yes
Stilling, P. and P.I. Bowdish (2000) Ecology 81: 281-285.	Yes
Styrsky, J.D. et al (2006) Biological Control 36: 375-384.	Yes
Tack, A.J.M. et al. (2012) Ecology Letters 15: 177-185.	Yes
Tilman, D. (1978) Ecology 59: 686-692.	No
von Merey, G.E. et al. (2012) Biological Control 60: 7-15.	Yes
Wies, A.E. and W.G. Abrahamson (1985) Ecology 1261-1269.	No
Wimp, G.M and T.G. Whitman (2001) Ecology 82: 440-452.	Yes
Woodman, R.L. and P.W. Price (1992) Ecology 73: 1028-1037.	Yes

Zovi, D. et al. (2008) Ecology 89: 1388-1398.	No
Zvereva, E.L. and M.V. Kozlov (2006) Oikos 115: 413-426.	Yes

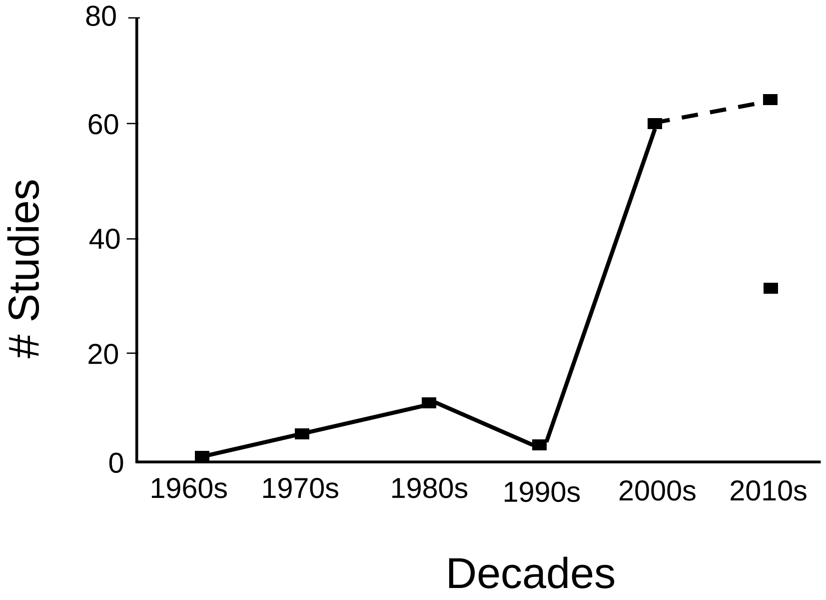
*Did not compute standard error for survival/parasitism, but we were able to obtain from original data, therefore were used in the analysis.

Appendix Table 2: Categories of host plant and habitat quality used in our analysis. For each type of habitat or plant quality used, we used the control and treatment as specified by expectations of the authors, or when expectations where not presented, we used the plant/habitat with greater herbivore fitness as our treatment and plant/habitat with lower fitness as control. For example, if plants grew better in lowland compared to upland areas, then that information determined which was treatment and which was control.

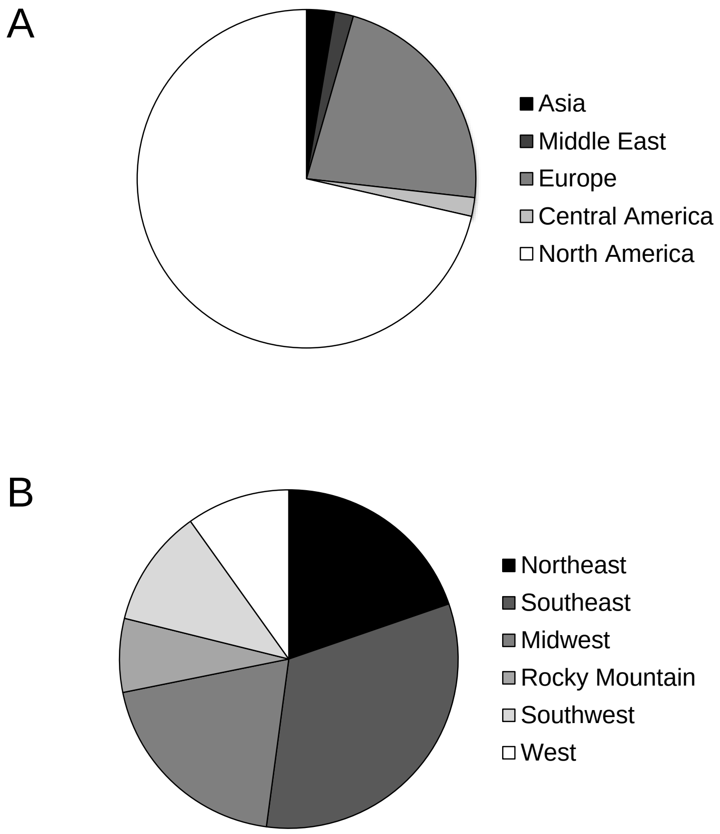
		Treatment	**Control**
Type of habitat quality variation	Agricultural type	Zero-chemical input Agriculture Monoculture	Conventional or no-till Wild Polyculture
	CO_2 variation	Normal CO_2	Elevated CO_2
	Flood regime	With flood	Without flood
	Fertilization	High Present	Low Absent
	Salinity	Low	High
	Habitat complexity	Thach absent	Thach present
	Pollution	Polluted	Clean
	Spatial variation	(different localities)	
	Temporal variation	(different years)	
	Temperature variation	(different temperatures)	
Type of host plant quality variation	Age	Young	Old
	Architecture	Normal	Reduced branching Reduced foliage
	Genotype	Different genotypes Male	Female
	Height	Different heights comparisons Tall	Short
	Nutritional quality	Healthy Girdled (stressed) Plant species of known different qualities for the focus herbivore	With disease Not girdled (healthy)
	Size	Greater area	Smaller area
	Trichomes	Variations in trichome density, herbivores can respond differently	
	Chemical compounds	Susceptible Control Without damage Without chemical addition Normal wax	Resistant Induced With damage With chemical addition Reduced wax



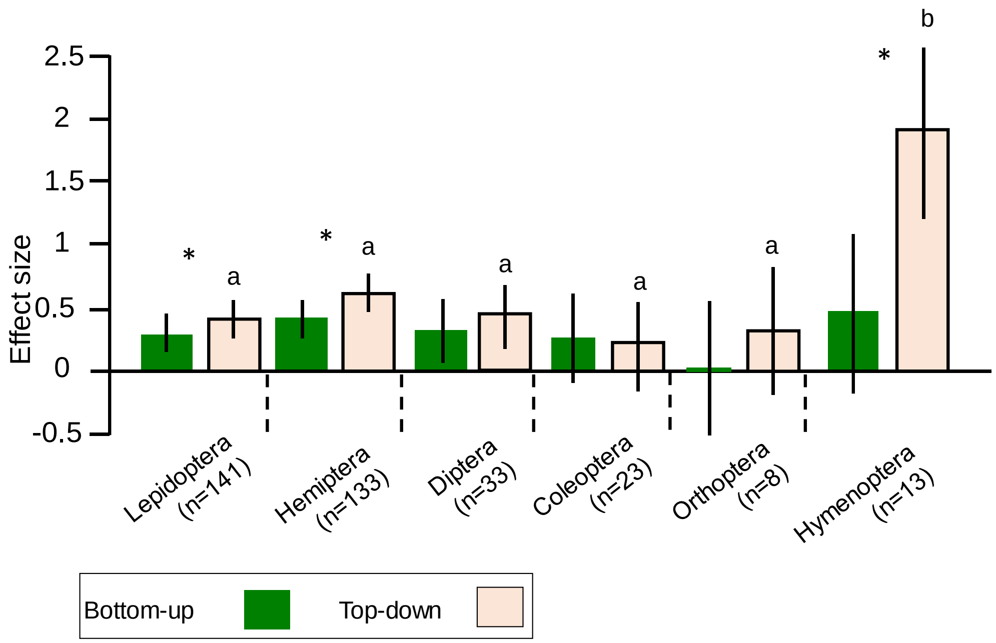
Appendix Figure 1: Funnel plot showing sample size in relation to effect size for each data point included in the meta-analysis.



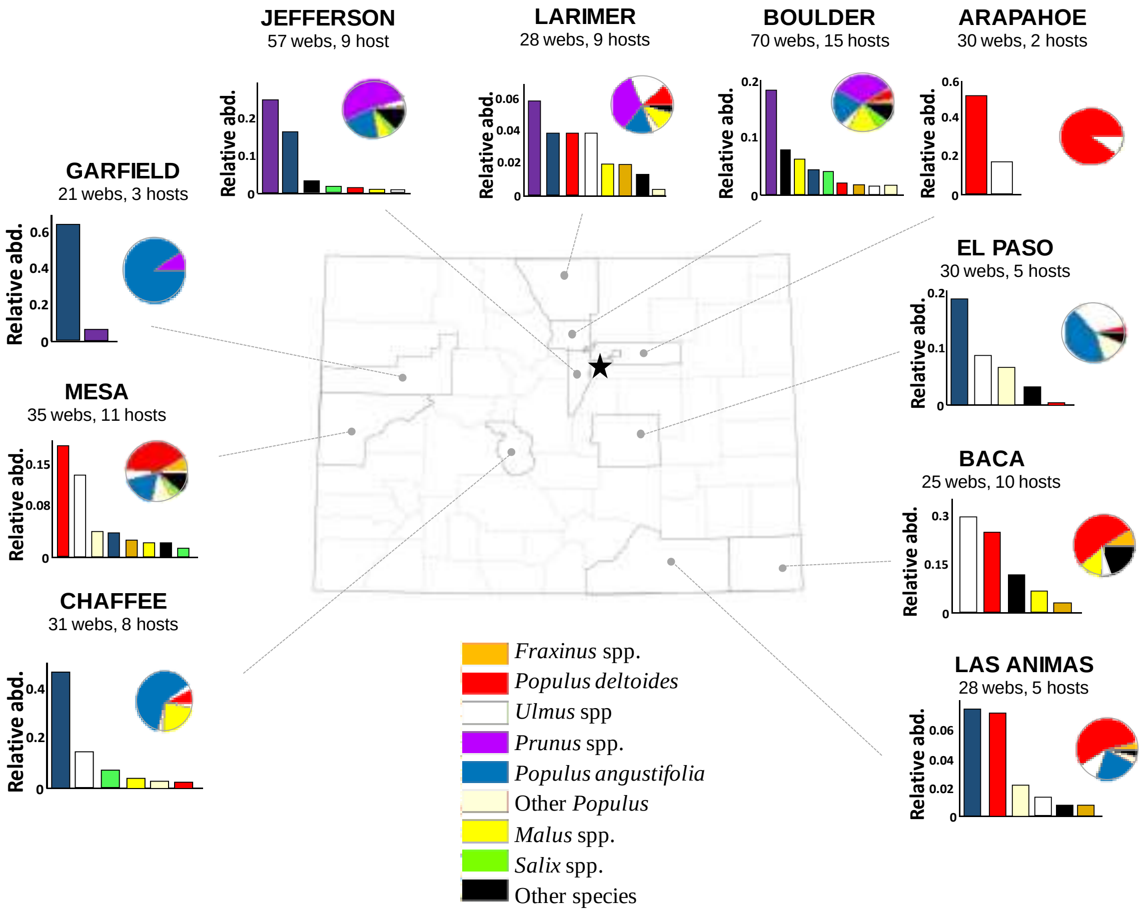
Appendix Figure 2: Number of studies conducted per decade. The point above the 2010s is the number of studies conducted from 2010-2015; the dashed line estimates the number of studies from 2010-2020 if current trends persist for the next 5 years**.**



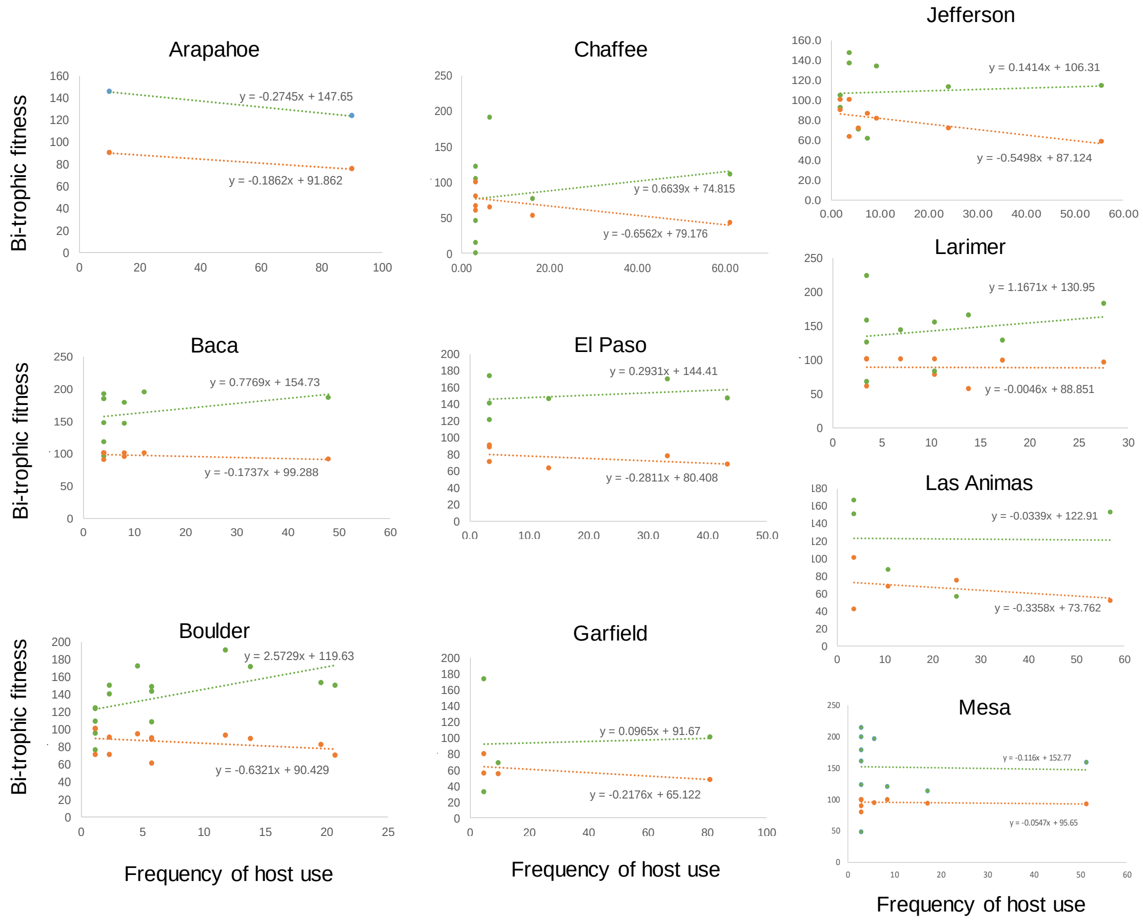
Appendix Figure 3: Proportion of studies that were conducted in A) different parts of the world and B) the United States. A) North America accounts for 71% of the studies, and 90% of the studies in North America occurred in the United States. Europe accounts for the next highest proportion of where studies were conducted at 22%. B) Within the United States, over half of the studies were conducted in the eastern US (52%) with Florida alone accounting for 17% of all studies in the United States, more than the number of studies conducted in the West, Southwest or Rocky Mountain state areas.



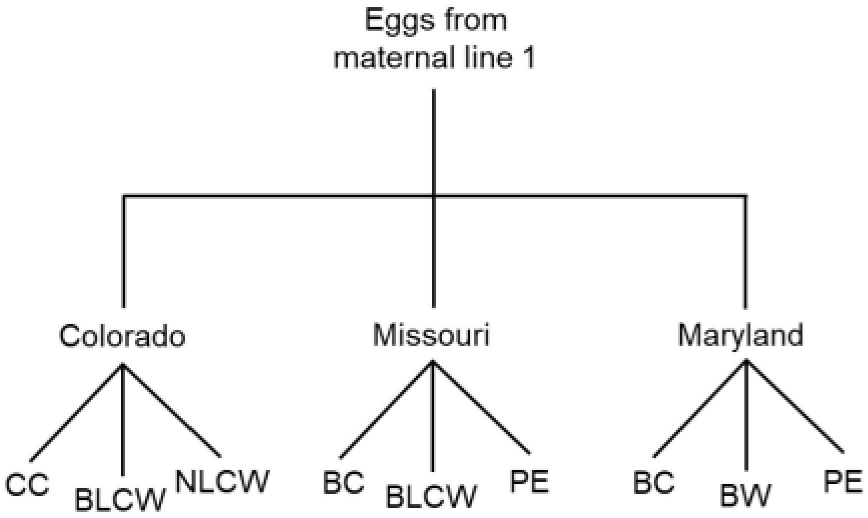
Appendix Figure 4: Bottom-up and top-down effects according to order of insect herbivores. Bars represent the estimate of the model, numbers represent the sample size for each test, and lines represent the 95% confidence interval of the model. Q_M=179.12, df=11, P<0.0001. If the confidence interval crosses zero, it means that the effect size is not different from zero (P from z-test >0.05). Asterisks represent significant Tukey's HSD pairwise comparisons between bottom-up and top-down forces for each order, while letters represent significant pairwise differences within each force type among orders.



Appendix Figure 5. Map of Colorado counties showing populations used in this study. Populations are named based on the counties in which they were collected. Each pie chart represents the proportion of host plants used in each population (host plant species used in fewer than 3 populations were included as “other hosts”) and bar graphs represent the relative abundance of the host plants in each population. Data from Boulder and partial data from Larimer and Jefferson populations were obtained from Murphy and Loewy 2015. The star represents Denver County.



Appendix Figure 6. Top-down and bottom-up slopes for each of the 10 populations of fall webworm sampled in Colorado. Orange represents the top-down forces and green the bottom-up forces.



Appendix Figure 7. Experimental design for Experiment 2 and how egg masses were split for each maternal line; the diagram shows how eggs were split for maternal line 1, but was repeated for all 10 maternal lines. The state names (Colorado, Missouri and Maryland) are where the larvae were reared and the host plant abbreviations below each state indicate on which plants the larvae were reared in each location. Abbreviation of host plants used: CC = choke cherry, BLCW = broad leaf cottonwood, NLCW = narrow leaf cottonwood, BC = black cherry, PE = persimmon, BW = black walnut.