

University of Nevada, Reno

Host use, mutualism and parasitism in the *Lycaeides* butterfly complex

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by

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ABSTRACT

Changes in host use in herbivorous insects can lead to diversification, speciation, and changes in other ecological interactions. In the first chapter, larval performance was investigated in three species of *Lycaeides* butterflies and placed into the context of other already studied ecological traits that potentially contribute to reproductive isolation in this system. For the larval performance experiment, caterpillars from seven populations were reared on five host plants, asking if host-specific, adaptive larval traits exist. We found large differences in performance across plants and fewer differences among populations. These patterns of performance are complex and suggest both conserved traits (i.e. plant effects across populations) and more recent dynamics of local adaptation, in particular for *L. melissa* that has colonized an exotic host. Finally, we put larval performance within the context of several other traits that might contribute to ecologically-based reproductive isolation in the *Lycaeides* complex. This larger context, involving multiple ecological and behavioral traits, highlights the complexity of ecological diversification and emphasizes the need for detailed studies on the strength of putative barriers to gene flow in order to fully understand the process of ecological speciation. One ecological factor that could affect diversification in this group is interactions with other trophic levels. In the second chapter, interactions with mutualistic ants and parasitoids were investigated in the context of current adaptation to a new host, alfalfa, within one of the species previously studied, *L. melissa*. Over the course of two summers, caterpillars and mutualistic ants were collected from sites with both native hosts and sites with an exotic host. Our understanding of the natural history of this system was broadened by identifying tending ants to species and parasitoids to subfamily. It was

found that parasitoid abundance and diversity varied considerably across space and time; however, the presence of mutualistic ants did not reduce parasitism rates. These studies highlight the importance of investigating ecological interactions, including interactions with other trophic levels, when studying host dynamics and diversification in herbivorous insects.

DEDICATION

For my mom.

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CHAPTER 1: Larval performance in the context of ecological diversification and speciation in *Lycaeides* butterflies

INTRODUCTION

Understanding the processes underlying diversification is a central question in evolutionary biology. Lineages diversify along multiple axes of variation, including morphological, physiological and ecological traits. With respect to diversification in ecological traits, many recent studies have found that ecological niches can be highly conserved from a macroevolutionary perspective (Peterson et al. 1999, Prinzing et al. 2001, Kozak and Wiens 2006). In other words, closely related species tend to utilize similar resources or occupy similar environments. In contrast, the field of ecological speciation suggests that ecological traits can evolve due to disruptive selection and drive the process of diversification (Dieckmann and Doebeli 1999, Schluter 2001, Ramsey et al. 2003, Funk et al. 2006, Matsubayashi et al. 2010). In herbivorous insects, evolution in response to habitat or host shifts is often thought to be a first step in the evolution of reproductive isolation (Dres and Mallet 2002, Nosil 2007). In most well studied systems, although exceptions exist (Ramsey et al. 2003, Martin and Willis 2007), niche conservatism and niche evolution are often characterized by a small number of ecological traits, such as habitat preference or physiological performance (Wiklund 1975, Wasserman and Futuyma 1981, Craig et al. 1997). To understand the causes and consequences of evolution in ecological traits, more studies are needed of groups in which diversification is recent or ongoing and multiple ecological traits are studied. The study of multiple traits is particularly important for our understanding of ecological speciation. For example, it has been suggested that weak selection acting on a

multifarious suite of traits could be as important for speciation as strong selection acting on a single ecological trait (Nosil et al. 2009).

The butterfly genus *Lycaeides* (Lycaenidae) includes a complex of taxa in North America that has been the focus of studies investigating the evolution and ecology of host use, mate choice, and genitalic morphology, among other subjects (Fordyce et al. 2002, Nice et al. 2002, Lucas et al. 2008, Forister et al. 2009). In the context of diversification, this group is interesting because hybridization has been documented among multiple entities, with a variety of consequences (Gompert et al. 2006, Gompert et al. 2010), including the formation of at least one hybrid species in the alpine of the Sierra Nevada mountains (Gompert et al. 2006). The *Lycaeides* taxa in western North America (specifically *L. idas*, *L. melissa*, and the hybrid species) differ in many traits, some of which have been implicated in the evolution of ecological reproductive isolation in this system. For example, there is variation in the strength of host preference, which is often linked to reproductive isolation in herbivorous insects that mate on or near their host plants, as *Lycaeides* do (Nice et al. 2002). There are also potentially important differences in mate preference, phenology, and egg adhesion (Fordyce et al. 2002, Fordyce and Nice 2003). The latter trait is interesting with respect to the evolution of the hybrid species, which lacks egg adhesion (Gompert et al. 2006, Fordyce and Nice 2003). The eggs of the hybrid species fall from the host plants. This is presumed to be an adaptation to the characteristics of the alpine plants, for which the above-ground portions senesce and are blown by the wind away from the site of next year's fresh growth (thus eggs that fall off are well positioned for feeding in the spring). Since the eggs of lower-elevation *Lycaeides* taxa do adhere to hosts, this trait could serve as a barrier to gene flow

with respect to individuals emigrating from lower elevations.

Our state of knowledge for *Lycaeides* is unusual for well-studied groups of herbivorous insects in that we know a great deal about a diversity of traits, as discussed above, but we have not heretofore investigated larval performance across taxa in the context of ecological speciation, which is often one of the first traits studied in other insect groups (Jaenike 1990). This study has two goals, first to investigate larval performance and then to put this information in the context of other already-studied traits to investigate which traits might be important for reducing gene flow between populations and species in this system. We have focused on performance of caterpillars from both *L. idas* and *L. melissa* populations as well as from populations of the hybrid species. Beyond the inclusion of the hybrid taxon, of added interest is the fact that *L. melissa* has undergone a recent expansion of diet, encompassing exotic alfalfa (*Medicago sativa*) as a larval host plant across much of its range. Thus we are able to investigate variation in the key ecological trait of larval performance across multiple levels of diversification, including the differentiation of *L. idas* and *L. melissa*, the formation of a hybrid species, and a host expansion that has occurred within the last two hundred years (Gompert et al. 2006, Gompert et al. 2008, Forister et al. 2009).

Using individuals from two *L. idas*, three *L. melissa*, and two hybrid species populations, we conducted reciprocal rearing experiments using all five of the host species found at these focal populations. We assessed larval performance by examining survival, time to emergence (eclosion), and adult weight, and by comparing survival curves from different populations on the different plants. For each population, we contrasted larval performance on a natal host to performance on the plants of other

populations. Higher larval performance on natal host plants would support the hypothesis of local adaptation to host plant species. In the second part of the paper, these results are discussed both within the light of local adaptation in a diversifying group, and also within the context of possible reproductive isolation related to variation in ecological traits.

METHODS

Two of our focal taxa, *L. idas* and *L. melissa*, are widely distributed across western North America. Our study focused on populations of these two species and the hybrid species in northern California and Nevada (Figure 1). In this area, *L. idas* is found on the west slope of the Sierra Nevada, *L. melissa* is found on the eastern side, and the hybrid species is only found in the alpine zone. *Lycaeides* species use a variety of plants in the pea family, Fabaceae, as hosts, although (with few exceptions) specific *Lycaeides* populations generally utilize a single host plant species. The two *L. idas* populations studied were Yuba Gap (YG), which uses *Lotus nevadensis* as a host, and Leek Springs (LS) which uses the host *Lupinus polyphyllus* (Table 1). Both populations of the hybrid species, Mt. Rose (MR) and Carson Pass (CP), use *Astragalus whitneyi*. At Washoe Lake (WL), *L. melissa* uses the native host *Astragalus canadensis*; at Beckwourth Pass (BP), butterflies use both *A. canadensis* and alfalfa, *Medicago sativa*; and at Goose Lake (GLA), the only available host is alfalfa (Table 1).

Lycaeides idas and the hybrid species are univoltine, while *L. melissa* populations have at least three generations per year. Eggs from the univoltine populations have to be maintained under winter conditions (i.e. cold temperatures and darkness) in the lab for

experiments in the following spring. Females and eggs from univoltine populations were collected in the summer of 2008 to be reared in the summer of 2009, while *L. melissa* females and eggs were collected during the 2009 summer. Females were collected from the *L. idas* and hybrid species populations (32 from Yuba Gap, 50 from Leek Springs, 45 from Carson Pass and 40 from Mt. Rose) and caged individually or in small groups with host plants for a period of three days after which eggs were collected. Eggs were washed with a dilute (2%) bleach solution and held over the winter at 4-6°C. Eggs were removed from cold storage on May 27th, 2009, and the majority hatched within several hours. The number of caterpillars hatching synchronously required that the larvae be moved in groups of twenty to standard-sized petri dishes (100 mm diameter) with fresh plant material on the 27th and 28th. On the 29th and 30th of May, the groups of twenty were split into three dishes each containing three to seven individuals. An average of 6 caterpillars was added to 9 dishes per treatment (plant/population combination); in some cases fewer (but not less than three) caterpillars were added per dish to try to maximize the number of dishes, which is the unit of replication (see below). Once all the larvae in a petri dish reached the 3rd or 4th instar they were moved to larger petri dishes (170 mm diameter). These groups of individuals were considered a “rearing dish”, and dish was used as a random factor in statistical analyses (see below).

Females and eggs from the three *L. melissa* populations were collected following similar protocols (though without the necessity of overwintering). Seventeen females were collected from Beckwourth Pass during the last week of June, 27 were collected from Goose Lake the third week of July and 14 were collected Washoe Lake the last week of July. For these populations, larvae were added to standard-sized petri dishes in

groups of four to six individuals as soon as the eggs hatched. Again, caterpillars were transferred to a large petri dish once all the individuals in a dish reached the 3rd or 4th instar.

Larvae from each population were reared on all five plants, *Astragalus canadensis*, *Astragalus whitneyi*, *Lotus nevadensis*, *Lupinus polyphyllus* and *Medicago sativa*, with individual rearing dishes being assigned exclusively to a single plant throughout development. Caterpillars in the wild consume both vegetative and reproductive tissues, but only leaves were used in this study, as flowers would be difficult to standardize across plants (not being available synchronously for most species). For a study of this kind, ideally all plant material to be used in rearing would be collected from focal locations (where butterflies are flying) or grown in a common environment. However, many of these species are not easily propagated, and moreover our focal locations are widely dispersed geographically; these factors necessitated some compromise in collecting some of the plants. *A. canadensis* cuttings were obtained at the site of the butterfly populations at Beckwourth Pass and Washoe Lake and from the greenhouse (plants were grown from seeds collected at Washoe Lake). *Astragalus whitneyi* was collected from the site of the Mt. Rose hybrid species population and on a hillside adjacent to Carson Pass (38°42'23"/120°00'23"). All *Lotus nevadensis* were collected from Yuba Gap (YG). The only case in which plant material was collected from a site where the butterfly is not found is *Lupinus polyphyllus*. These plants were collected off I-80 at the Soda Springs exit (39°19'29"/ 120°23'25") and seven miles north of Truckee CA, off State Route 89 (39°25'59"/ 120°12'13"). *Medicago sativa* was obtained from Beckwourth Pass (BP), and from plants grown in the greenhouse with

seeds from BP. *M. sativa* was also collected from south of Minden, NV on State Route 88 (38°48'60"/ 119°46'46") and off of California State Route 49 in Sierra Valley, CA (39°38'35"/ 120°23'10"). Plant material was kept in a refrigerator and larvae were fed fresh cuttings whenever the plant material in petri dishes was significantly reduced or wilted, which was approximately every two to seven days. Each time caterpillars were given fresh plant material, the number of surviving caterpillars was recorded along with the date. All dishes were kept at room temperature, 20° to 23° Celsius, on lab benches. Newly emerged adults were individually weighed to the nearest 0.01 mg on a Mettler Toledo XP26 microbalance and sex was recorded.

Analyses

The strengths of our experiment were that we reared a large number of individuals from multiple taxa across five plants, but a weakness of our design was that not all rearing could be done simultaneously. As discussed further below, flowers were not included in the rearing, and plant material was collected from most but not all focal populations. Experiments were conducted in two phases, first involving the populations of the hybrid species and *L. idas*, being reared together and earlier in the spring, and second involving the three low-elevation *L. melissa* populations being reared later in the summer. This division into two rearing groups was largely a consequence of being constrained by the total number of caterpillars that could be handled and reared in the lab at any one time. Considering the possibility that phenological variation in plants could have implications for larval performance, we conducted analyses separately for the three butterfly species. Post-emergence adult weight, time to emergence as adult, and survival

to adult were recorded. Mortality (reflected in the survival data) included death associated with caterpillars that died while developing, individuals that pupated but failed to emerge, and disease; we did not distinguish between these sources of mortality. Data were standardized (Z transformed) within populations to facilitate comparisons among populations and taxa that may have inherent differences, such as in size or in development time. Z scores were used in analyses described below unless otherwise noted.

Dish was considered the unit of replication, thus percent survival was calculated per dish. For analyses of adult weight and time to emergence, dish was used as a random factor. Percent survival was analyzed using analysis of variance (ANOVA) with plant, population and the interaction between the two as predictor variables. Time to emergence and adult weight were both analyzed with ANOVA, using population, plant, the interaction between the two and sex as predictor variables, along with dish as a random factor nested within plant and population. For all of these analyses, ANOVA was performed a second time without the plant/population interaction if it was not significant at $\alpha < 0.05$. These analyses were performed using JMP software version 8.0.2 (SAS Institute).

Differences in survival were also investigated by generating and comparing survival curves. To create survival curves, individual caterpillars were assumed to be alive until the date they were found dead. Rather than analyzing survival curves on an individual-dish basis (where sample sizes were small), the number of individuals surviving on a given day was calculated for each plant/population combination, giving one curve per combination, as is often done in survival analysis (Klein and Moeschberger

2003). Survival curves were generated in R (2.12.2) using the packages *splines* and *survival*. The shapes of the curves were investigated within population using the packages *MASS* and *fitdistrplus*. Weibull distributions are commonly used to model survival using two parameters, shape and scale. The shape parameter measures where the inflection point occurs, or practically whether individuals are lost more at the beginning or end of a given time period, and the scale parameter characterizes the depth of the curve. We estimated the two Weibull parameters, shape and scale, that characterized the fitted curves using maximum likelihood. One-thousand bootstrap replicates were then used to generate 95% confidence intervals for the shape and scale parameters, so that they could be compared across plants within a given population.

RESULTS

We began the larval performance experiments with 2040 caterpillars in 357 dishes. Average survival to eclosion across all experiments was 23.4%. In general, differences in larval performance among plants were greater than differences between populations, which can be seen both in Fig. 2 and also by comparing variation partitioned by plants and population in Table 2. For example, survival was highest on *Lotus nevadensis* across all populations for all three taxa, with an average survival of 56.5% (survival on *Lupinus polyphyllus* was comparable for two of the three *L. melissa* populations). Survival on alfalfa was consistently the lowest of any plant across populations: only two caterpillars survived to eclosion (Figs. 2 and 3). Because survival was so low on alfalfa, it was excluded from most analyses and figures. The inferior nature of alfalfa as a host plant is consistent with previous studies, particularly when

caterpillars do not have access to flowers and flower buds. When flowers have been included in performance experiments, survival of *L. melissa* on alfalfa and *A. canadensis* was equal, although those individuals reared on alfalfa were significantly smaller adults (Forister et al. 2009).

Plant and the interaction between plant and population were significant predictors of survival for *L. idas*, *L. melissa* and the hybrid species (Table 2). Within taxa, there were differences among populations on certain plants. For example, survival for *L. idas* on *Lotus nevadensis* was greater for LS compared to YG, but the pattern was reversed for the host *Lupinus polyphyllus* (Fig. 2); in other words, each population had higher survival on the natal host of the other population. A different pattern can be seen across populations of *L. melissa* on *Astragalus canadensis*, where survival was highest for individuals from WL, a population whose natal host is *A. canadensis*. *L. melissa* survival on *A. canadensis* was lowest for GLA, which is a population associated with the exotic host alfalfa, and survival on *A. canadensis* is intermediate for BP, where both *A. canadensis* and alfalfa are utilized. Thus host use by *L. melissa* populations predicts variation in larval performance. Effects of plant and population were generally not as pronounced for either adult weight or time to emergence (for *L. idas*, the only significant predictors of adult weight were dish and sex); exceptions to this include the significant population by plant interaction for adult weight of *L. melissa*. As with survival, *L. melissa* performance was greater on *A. canadensis* for the population that is associated with that plant, WL (Fig 2d).

Consistent with results for survival to emergence as an adult, survival curves through time also showed pronounced differences among plants (Fig. 3; Table A1). For

example, the Weibull scale parameter for alfalfa was generally different compared to the other plants, reflecting early and pervasive mortality for individuals reared on that plant. Most but not all mortality was manifested quite early in development, particularly for the alpine and *L. idas* populations across plants. Mortality was more evenly distributed through time for *L. melissa* on all plants. In some cases, patterns of survival vary among plants within populations, even when overall survival was low. For example, survival curves for CP drop much less rapidly for three plants, one of which is the natal host *A. whitneyi* and another is the congeneric *A. canadensis* (the third is *L. nevadensis*). For *A. canadensis*, it is interesting to note that across all populations there was a drop off in survival near the end of development: many individuals made it to the pupal stage, but failed to emerge, perhaps suggesting a subtle nutritional challenge for successful completion of development presented by that plant.

DISCUSSION

The reciprocal rearing experiment detected strong host plant effects, and limited evidence of local adaptation to natal host plant species in the *Lycaeides* species complex. For example, development on *L. nevadensis* (the host of the *L. idas* population at YG) resulted in relatively high survival throughout the experiment, while development on *Medicago sativa* (the exotic host of *L. melissa* at GLA and BP) led to extremely low survival in all cases. These plant effects that transcend populations could be indicative of larval traits (such as high survival on *L. nevadensis*) that are conserved in the group and are not particularly labile. Our results could be influenced by the use of leaves but not flowers in larval rearing. Previous work has shown that survival is improved on

Medicago sativa for larvae that have access to flowers, but this is not true on *Astragalus canadensis* (Forister et al. 2009). We do not know if flowers are or are not important for larvae developing on the other plants.

In general, the survival that we report (23.4% throughout the experiment) could reflect the absence of flowers or other unfavorable lab conditions, and we do not at this time have life history data from the field for *Lycaeides* with which to compare our results. However, in interpreting results here and elsewhere (e.g., Forister et al. 2009), we make the assumption that lab experiments are informative with respect to relative performance across hosts. In other words, the consumption of *M. sativa* by *Lycaeides* caterpillars is associated with development into adults that are small relative to adults that develop on other plants. Without artifacts of lab rearing, it is possible that performance would generally be higher in the wild, but we would predict that performance on *M. sativa* would still be lower relative to performance on native hosts. An alternate possibility, which we can not test at this time, is that lab-rearing has plant-specific effects (i.e. *M. sativa* is a poor host only when used under artificial conditions).

For all the performance results, it is also important to note that phenological effects of changes in plant quality or suitability could be pronounced, but are not addressed by our experimental design. In particular, as noted above, our rearings were conducted in two phases due to logistical constraints; first including *L. idas* and populations of the hybrid species, and second including all three *L. melissa* populations. This is not a completely unnatural situation, as *L. idas* and the hybrid species are univoltine, while *L. melissa* populations are multivoltine. Thus *L. idas* and hybrid-species caterpillars are more likely to be exposed to only the early spring vegetation, as in

our experiment. The consideration of phenological effects in plants is most relevant when comparing performance among butterfly taxa (for example the performance of *L. melissa* versus *L. idas* on a particular plant) but is less important when making comparisons within a taxon (for example the performance of *L. melissa* on different plant species).

In contrast to the general result of strong plant effects across taxa and populations, one result suggestive of local adaptation is the performance (survival and adult weight) of *L. melissa* on the native host *A. canadensis* (Kawecki and Ebert 2004). Performance was highest on the native for the population that utilizes that host in the wild, and lowest for a population associated with the exotic host alfalfa. Performance on *A. canadensis* is intermediate for the population where both hosts are used. These results raise a number of possibilities, including a scenario in which genetic variants associated with higher performance on an ancestral host were lost in the transition to the exotic host, which could be a consequence of relaxed selection or a population bottleneck in the new environment. Another explanation could involve a change in gene regulation associated with performance, rather than a loss of alleles. In any event, the transition to the novel host has apparently not been accompanied by an increase in performance on alfalfa. One caveat to this conclusion is that the *M. sativa* used in experiments was collected at one of the focal locations (BP), but could not, for logistical reasons, be collected from GLA. The latter population (GLA) is the population associated only with *M. sativa*, thus the conclusion that performance has not increased following the colonization of the novel host could have been different if local plant material from that location had been used in experiments; however, we have found consistently low performance on *M. sativa* in other

experiments (Forister et al. 2009), suggesting generality to the result of low performance on that plant.

Variation in host preference has previously been documented among populations of *Lycaeides* butterflies, with populations of the hybrid species in particular exhibiting strong preferences for their natal host, *A. whitneyi*, (Nice et al. 2002, Gompert et al. 2006), relative to the hosts of other *Lycaeides* populations. However, we found low survival and low adult weights for individuals of the hybrid species reared on *A. whitneyi* (Fig. 2). It is possible that laboratory conditions were a poor reflection of appropriate abiotic conditions for the hybrid species individuals adapted to an alpine environment. It is also possible that other factors, such as the absence of flowers in experiments or induced defenses in leaves, could be important in *A. whitneyi*, which supported poor growth for larvae from all populations. In any event, the patterns of performance that we report are not consistent with an expected preference-performance paradigm for host shifts leading to the evolution of reproductive isolation (Gripenberg et al. 2010). Variation in both adult preference and larval performance are discussed further in the following section considering ecological traits and hypotheses relating traits to reproductive isolation.

Ecology and diversification

Although many studies of herbivorous insects have focused on larval performance with respect to local adaptation and ecological speciation, populations of herbivorous insects (or of any organism) can of course differ in numerous ways, some related to resource use but also to other aspects of the environment. Nosil et al. (2009) have suggested a number of scenarios in which multiple traits could be important in the

evolution of reproductive isolation. In particular, natural selection acting on a single ecological trait or a single niche dimension could be important for initiating speciation, while the evolution of differences along multiple ecological axes might often be needed for complete reproductive isolation (Nosil et al. 2009). Multiple ecological axes could be different aspects of, for example, resource use (Forister et al. 2007, Nosil and Sandoval 2008), or they could be more disparate traits, such as mate finding or predator avoidance. In either case, the idea is that selection along one axis might be insufficient for reproductive isolation, but selection acting along multiple axes might confer a high, overall level of reproductive isolation.

Considering the potential importance of multiple traits in ecological speciation, Fig. 4 and Table 3 summarize information from this and other studies in *Lycaeides* and present hypotheses regarding multiple ecological and behavioral traits and how these might interact with ecological, reproductive isolating processes in this system. Specifically, Figure 4 explores hypotheses about reduced gene flow, represented by faded arrows, between the taxa due to the ecological differences of a given trait. For example, the model shown for egg adhesion posits that variation in adhesion could be a barrier to gene flow going from *L. idas* and *L. melissa* populations into populations of the hybrid species. *L. idas* and *L. melissa* females lay eggs that adhere to plants. As discussed above, the alpine host plants senesce and are blown from the area, thus removing any attached eggs from the site of next-spring's fresh plant growth (Fordyce and Nice 2003). Differences in host preference might also affect patterns of gene flow between the species. The host of the hybrid species populations is readily accepted by ovipositing females from all *Lycaeides* examined thus far (Nice et al. 2002), thus it would likely be

accepted by females from *L. idas* and *L. melissa* populations arriving at a population of the hybrid species. In contrast, the hosts of the *L. idas* populations are not preferred by females of either the hybrid species or *L. melissa* (Nice et al. 2002, Forister et al. 2009). The arrows pointing towards *L. melissa* assume the presence of only the native host *A. canadensis*, not the exotic *M. sativa* (excluding the exotic is a simplifying assumption for Fig. 4, but also appropriate given that ecological diversification occurred before the recent introduction of alfalfa). *Astragalus canadensis* and *A. whitneyi* are equally acceptable for oviposition by hybrid species individuals (Forister, unpubl. data), and we assume the same equivalence for *L. idas* (i.e. we assume *L. idas* females would readily accept *A. canadensis*, just as they do *A. whitneyi*, thus an arrow without a barrier pointing from *L. idas* to *L. melissa* in the host preference diagram).

Similar to host preference, variation in male mate discrimination potentially presents barriers only between hybrid species and *L. idas* populations, and between *L. idas* and *L. melissa* populations but not between hybrid species and *L. melissa* populations (Nice et al. 2002). *L. melissa* males will readily approach either *L. idas* or females of the hybrid species, while *L. idas* males discriminate against females from the other two taxa, and hybrid species males discriminate against *L. idas* females. This behavioral variation among taxa, reported in Fordyce et al. (2002), comes from choice tests involving dead and paper-model females presented in experimental arrays in the field. It is important to note that being a less-preferred mate is of course not the same as not being mated. In other words, a virgin *L. melissa* female that immigrated into an *L. idas* population might be a low-ranked mate for male *L. idas* relative to local females, but it is possible that she would eventually find a mate. However, the patterns of gene flow

shown in Fig. 4 are meant to be hypotheses for potential barriers to gene flow within a given trait. A *L. melissa* female immigrating into a population of the hybrid species would be mated more readily (and thus be more likely to contribute to the gene pool) relative to the dynamic just described (an *L. melissa* female arriving at an *L. idas* population). It is possible to imagine all of the traits depicted in Fig. 4 being involved in either pre- or postzygotic isolation. For example, mate preference could act as just described on immigrant, virgin females, as in the immigrant inviability concept of Nosil et al. (2005). An alternative but similar scenario could involve the offspring of an immigrant; in this case, wing-pattern alleles (related to mate choice) would interact with mate choice in the next generation.

Variation in voltinism could affect gene flow from *L. melissa* into the other univoltine taxa. Because *L. melissa* populations are multivoltine, it is possible that an *L. melissa* female moving into populations of the other taxa would lay eggs that failed to diapause in habitats where the univoltine strategy is superior (such as in the alpine habitat where there is a short window for larval development, Somme 1989). Alternatively, diapause could be plastic, in which case the patterns of connectivity (hypothesized patterns of gene flow) pictured would be different.

We can now add larval performance to the suite of hypotheses linking ecology and gene flow in *Lycaeides*. In generating hypotheses relating larval performance to gene flow, we have used this criterion (focusing on survival, rather than adult weight, as the most straightforward metric of performance): if foreign larvae (i.e. the offspring of a recently arrived female) have lower survival on the local host relative to local individuals, we hypothesize a relative reduction in gene flow associated with performance. For gene

flow between *L. idas* and *L. melissa*, the survival of *L. idas* larvae on the host of *L. melissa* is lower than the survival of *L. melissa* caterpillars on the same plant (see mean survival values in Fig. 3). Interestingly in the context of hybrid speciation, our results suggest that gene flow from both *L. idas* and *L. melissa* would be unimpeded into populations of the hybrid species relative to the reverse, meaning that the two parental species had higher survival (relative to hybrid individuals) on the alpine host, and that hybrid individuals had relatively inferior performance on the two parental species' hosts. Of course, this could be different if another trait, for example egg adhesion, had a stronger effect or acted before larval performance in restricting gene flow (see Ramsey et al. (2003) and Martin and Willis (2007) for examples of the complexities of estimating components of reproductive isolation associated with a suite of traits). We stress that these are hypotheses that bear further investigation, as we know that larval performance is complex, being affected not only by variation in host quality (i.e. the availability of flowers, Forister et al. 2009) but also the presence of mutualistic ants and natural enemies (Forister et al. 2011).

CONCLUSION

We conducted a performance experiment for seven populations from three species within the *Lycaeides* species complex, *L. idas*, *L. melissa* and the hybrid species, on five different plants. Our primary results include large plant effects, with *L. idas* hosts being generally superior for larval development, and the exotic host of *L. melissa* being extremely poor, both for *L. melissa* and the other taxa. In general, there is little evidence of local adaptation in these performance data. This conclusion is perhaps consistent with

the fact that these butterfly taxa are associated with multiple hosts throughout their geographic ranges. Thus gene flow could limit local adaptation to any particular plant species. As a consequence, variation in larval performance across multiple hosts is unlikely to be the dominant mechanism of reproductive isolation between populations and taxa.

Our results (including some evidence for local adaptation among *L. melissa* populations for their native host, *A. canadensis*) together with previously published data (Fordyce et al. 2002, Nice et al. 2002, Forister et al. 2009, Gompert et al. 2006, Fordyce and Nice 2003) were integrated to build a hypothetical model relating ecology to reproductive isolation and diversification in *Lycaeides*. The model presented in Fig. 4 describes a system that is well poised for a test of the “multifarious selection” hypothesis (Nosil et al. 2009). One hypothesis that can be generated from Fig. 4 is that there might not be one single trait that could act as a barrier to gene flow between all three taxa, and most traits might only act to reduce gene flow asymmetrically. For example, egg adhesion could affect gene flow from both *L. idas* and *L. melissa* into the hybrid species, but would not necessarily be effective in the opposite directions (from the hybrid species into *L. idas* and *L. melissa*). More generally in the context of ecological speciation, a greater number of traits might increase the possibility that a hybrid “falls between” the niches represented by the two adaptive peaks occupied by the species or incipient species (Orr 2000, Gavrillets 2004). In a relatively simple example involving two traits, hybrids between populations of *Mitoura* butterflies associated with different host plant species inherit a maladaptive mis-match of traits: hybrid individuals have higher performance on

one of the parental hosts, but express an oviposition preference for the other host (Forister 2005).

However, the importance of multiple traits for ecological speciation in *Lycaeides* must wait on estimates of historical and contemporary gene flow between pairs of populations, and analyses of those estimates in light of variation in ecological and behavioral traits (Lu and Bernatchez 1999). The inclusion of such comparative data, particularly for a larger suite of populations, would perhaps reveal the influence of a single trait for explaining a majority of the variation in reproductive isolation. It is also possible that a key trait for reproductive isolation remains unstudied in this system. For future studies in this group, it will also be important to sample populations widely throughout the geographic ranges of the focal butterfly species (Fig. 1), as dynamics of local adaptation and diversification can be affected by geographic context, particularly proximity to the edge of a range and potentially marginal habitats. Beyond the details of ecological diversification in *Lycaeides*, our results should generally stress the importance of delving deeper than the traditional “preference-performance relationship” when investigating ecological speciation in herbivorous insects.

LITERATURE CITED

- Craig, T. P., J. D. Horner, and J. K. Itami. 1997. Hybridization studies on the host races of *Eurosta solidaginis*: Implications for sympatric speciation. *Evolution*. 51:1552-1560.
- Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. *Nature*. 400:354-357.
- Dres, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*. 357:471-492.
- Forister, M. L. 2005. Independent inheritance of preference and performance in hybrids between host races of *Mitoura* butterflies (Lepidoptera : Lycaenidae). *Evolution*. 59:1149-1155.
- Forister, M. L., C. C. Nice, J. A. Fordyce, and Z. Gompert. 2009. Host range evolution is not driven by the optimization of larval performance: the case of *Lycaeides melissa* (Lepidoptera: Lycaenidae) and the colonization of alfalfa. *Oecologia*. 160:551-561.
- Forister, M. L., Z. Gompert, C. C. Nice, G. W. Forister, and J. A. Fordyce. 2011. Ant association facilitates the evolution of diet breadth in a lycaenid butterfly. *Proceedings of the Royal Society B-Biological Sciences*. 278:1539-1547.
- Forister, M. L., A. G. Ehmer, and D. J. Futuyma. 2007. The genetic architecture of a niche: variation and covariation in host use traits in the Colorado potato beetle. *Journal of Evolutionary Biology*. 20:985-996.

- Fordyce, J. A., and C. C. Nice. 2003. Variation in butterfly egg adhesion: adaptation to local host plant senescence characteristics? *Ecology Letters*. 6:23-27.
- Fordyce, J. A., C. C. Nice, M. L. Forister, and A. M. Shapiro. 2002. The significance of wing pattern diversity in the Lycaenidae: mate discrimination by two recently diverged species. *Journal of Evolutionary Biology*. 15:871-879.
- Funk, D. J., P. Nosil, and W. J. Etges. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America*. 103:3209-3213.
- Gavrilets, S. 2004. *Fitness landscapes and the origin of species*. Princeton University Press, Princeton, NJ, USA.
- Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters*. 13:383-393.
- Gompert, Z., C. C. Nice, J. A. Fordyce, M. L. Forister, and A. M. Shapiro. 2006. Identifying units for conservation using molecular systematics: the cautionary tale of the Karner blue butterfly. *Molecular Ecology*. 15:1759-1768.
- Gompert, Z., L. K. Lucas, J. A. Fordyce, M. L. Forister, and C. C. Nice. 2010. Secondary contact between *Lycaeides idas* and *L. melissa* in the Rocky Mountains: extensive admixture and a patchy hybrid zone. *Molecular Ecology*. 19:3171-3192.
- Gompert, Z., J. A. Fordyce, M. L. Forister, A. M. Shapiro, and C. C. Nice. 2006. Homoploid hybrid speciation in an extreme habitat. *Science*. 314:1923-1925.
- Gompert, Z., J. A. Fordyce, M. L. Forister, and C. C. Nice. 2008. Recent colonization and

- radiation of North American *Lycaeides* (*Plebejus*) inferred from mtDNA. *Molecular Phylogenetics and Evolution*. 48:481-490.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*. 21:243-273.
- Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters*. 7:1225-1241.
- Kozak, K. H., and J. J. Wiens. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. *Evolution*. 60:2604-2621.
- Klein, J. P., and M. L. Moeschberger. 2003. *Survival analysis: techniques for censored and truncated data, second ed.*, Springer, New York, NY, USA.
- Lu, G., and L. Bernatchez. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution*. 53:1491-1505.
- Lucas, L. K., J. A. Fordyce, and C. C. Nice. 2008. Patterns of genitalic morphology around suture zones in North American *Lycaeides* (Lepidoptera : Lycaenidae): Implications for taxonomy and historical biogeography. *Annals of the Entomological Society of America*. 101:172-180.
- Martin, N. H., and J. H. Willis. 2007. Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. *Evolution*. 61:68-82.
- Matsubayashi, K. W., I. Ohshima, and P. Nosil. 2010. Ecological speciation in phytophagous insects. *Entomologia Experimentalis Et Applicata*. 134:1-27.
- Nice, C. C., J. A. Fordyce, A. M. Shapiro, and R. Ffrench-Constant. 2002. Lack of

- evidence for reproductive isolation among ecologically specialised lycaenid butterflies. *Ecological Entomology*. 27:702-712.
- Nosil, P. 2007. Divergent host plant adaptation and reproductive isolation between ecotypes of *Timema cristinae* walking sticks. *American Naturalist*. 169:151-162.
- Nosil, P., L. J. Harmon, and O. Seehausen. 2009. Ecological explanations for (incomplete) speciation. *Trends in Ecology & Evolution*. 24:145-156.
- Nosil, P., and C. P. Sandoval. 2008. Ecological niche dimensionality and the evolutionary diversification of stick insects. *Plos One*. 3.
- Nosil, P., T. H. Vines, and D. J. Funk. 2005. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*. 59:705-719.
- Orr, H. A. 2000. Adaptation and the cost of complexity. *Evolution*. 54:13-20.
- Peterson, A. T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. *Science*. 285:1265-1267.
- Prinzing, A., W. Durka, S. Klotz, and R. Brandl. 2001. The niche of higher plants: evidence for phylogenetic conservatism. *Proceedings of the Royal Society of London Series B-Biological Sciences*. 268:2383-2389.
- Ramsey, J., H. D. Bradshaw, and D. W. Schemske. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution*. 57:1520-1534.
- Schluter, D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution*. 16:372-380.
- Somme, L. 1989. Adaptations of terrestrial arthropods to the alpine environment.

Biological Reviews of the Cambridge Philosophical Society. 64:367-407.

Wasserman, S. S., and D. J. Futuyma. 1981. Evolution of host plant utilization in laboratory populations of the Southern cowpea weevil, *Callosobruchus maculatus fabricius* (Coleoptera: Bruchidae). *Evolution*. 35:605-617.

Wiklund, C. 1975. Evolutionary relationship between adult oviposition preferences and larval host plant range in *Papilio machaon* L. *Oecologia*. 18:185-197.

Table 1: Locations of populations (see also Fig. 1) and hosts associated with the seven populations studied.

Taxon	Location	Latitude/longitude	Host
<i>L. idas</i>	Leek Springs (LS)	38°38'8" N/120°14'25" W	<i>Lupinus polyphyllus</i>
	Yuba Gap (YG)	39°19'24" N/120°35'60" W	<i>Lotus nevadensis</i>
Hybrid species	Carson Pass (CP)	38°42'28" N/120° 0' 28" W	<i>Astragalus whitneyi</i>
	Mt. Rose (MR)	39°19'21" N/119°55'47" W	<i>Astragalus whitneyi</i>
	Washoe Lake (WL)	39°13'59" N/119°46'46" W	<i>Astragalus canadensis</i>
<i>L. melissa</i>	Beckwourth Pass (BP)	39°46'55" N/120°4'23" W	<i>Astragalus canadensis</i> and <i>Medicago sativa</i>
	Goose Lake (GLA)	41°59'9" N/120°17'32" W	<i>Medicago sativa</i>

Table 2. Results from analyses of variance for the three measures of performance: percent survival, adult weight and time to emergence. In all cases dish was used as the unit of replication. Most population/plant combinations had 9 dishes, except for the following: YG/Ac 12 dishes, YG/Ln 12 dishes, YG/Lp 12 dishes, YG/Ms 12 dishes and all plant combinations for CP and MR had 12 dishes. The total number of dishes was 357.

	SS	F Ratio _{df}	P
Survival <i>L. idas</i>			
Plant	48.62	42.02 _{3,73}	< 0.0001
Population	0.02	0.06 _{1,73}	0.81
Plant x Population	4.00	3.46 _{3,73}	0.02
Survival hybrid species			
Plant	30.93	16.33 _{3,88}	<0.0001
Population	0.00	0.00 _{1,88}	1.00
Plant x Population	7.50	3.96 _{3,88}	0.01
Survival <i>L. melissa</i>			
Plant	44.95	29.32 _{3,96}	<0.0001
Population	0.03	0.03 _{2,96}	0.97
Plant x Population	9.99	3.26 _{6,96}	0.006
Adult Weight <i>L. idas</i>			
Plant	2.11	0.52 _{3, 33.47}	0.67
Population	0.49	0.14 _{1, 19.38}	0.71
Dish[Plant,Population]	47.78	4.04 _{26, 74.00}	<0.0001
Random			
Sex	10.81	11.87 _{2, 74.00}	<0.0001
Adult Weight hybrid species			
Plant	7.25	2.81 _{3, 48.09}	0.049
Population	0.53	0.61 _{31, 48.45}	0.44
Dish[Plant,Population]	14.90	0.74 _{20, 30.00}	0.76
Random			
Sex	1.29	1.28 _{1, 30.00}	0.27
Adult Weight <i>L. melissa</i>			
Plant	21.22	7.25 _{3, 132.13}	0.0002
Population	8.62	4.54 _{2, 154.10}	0.01
Dish[Plant,Population]	89.56	1.44 _{84, 217.00}	0.02
Random			
Plant x Population	11.24	15.15 _{1, 217.00}	0.0001
Sex	25.93	4.39 _{6, 126.13}	0.0005
Time to Emergence <i>L. idas</i>			
Plant	20.32	8.91 _{3, 59.17}	<0.0001
Population	0.69	0.99 _{1, 4.11}	0.37
Dish[Plant,Population]	19.34	0.94 _{26, 75.00}	0.55
Random			
Sex	3.87	2.45 _{2,75.00}	0.09
Time to Emergence hybrid species			
Plant	5.30	3.28 _{2, 25.82}	0.05
Population	0	0 _{0, 28.00}	1.00
Dish[Plant,Population]	16.06	0.95 _{20, 28.00}	0.54

Random			
Sex	1.20	1.41 _{1,28.00}	0.25
Plant x Population	6.04	3.61 _{2, 42.94}	0.04
Time to Emergence <i>L. melissa</i>			
Plant	9.36	2.41 _{3, 106.23}	0.07
Population	1.66	0.61 _{2, 99.01}	0.54
Dish[Plant,Population]	125.50	2.34 _{84, 223.00}	<0.0001
Random			
Sex	24.18	37.87 _{1, 223.00}	<0.0001

Table 3. Details of behavioral and ecological variation among taxa, specifically as variation relates to potential barriers to gene flow; see Fig. 4 for a graphical interpretation of these traits in relation to gene flow. The descriptions of male mate discrimination refer to preferences of males for females of the other taxa. Details for larval performance refer to performance on the hosts of the other taxa relative to performance of the other taxa on those same plants.

	<i>L. idas</i>	Hybrid species	<i>L. melissa</i>
Egg adhesion	Yes	No	Yes
Preference for natal host(s)	Moderate	High	Low to high
Male mate discrimination	Against <i>L. melissa</i> and hybrid species	Against <i>L. idas</i>	None
Voltinism	Univoltine	Univoltine	Multivoltine
Larval performance	Poor on hosts of <i>L. melissa</i> ; superior on host of hybrid species	Poor on hosts of both <i>L. melissa</i> and <i>L. idas</i>	Equivalent on hosts of <i>L. idas</i> and superior on host of hybrid species
Further information on specific behavioral and ecological variables (other than larval performance, reported here) can be found as follows: egg adhesion [23], preference for natal hosts [17, 19, 22], male mate discrimination [16], voltinism [23].			

Table 4. Survival curves for each host-population combination. A weibull distribution was fitted to each with 1000 bootstrap replicates. We report shape and scale parameters along with bootstrapped confidence intervals. Upper case and lower letters following shape and scale values correspond to 95% confidence intervals that do not overlap (upper case letters for shape and lower case letters for scale) within populations based on the thousand bootstrapped replicates. See Fig. 3 for graphical representation of survival curves.

Population	Host	Shape	Scale	
CP	Ac	1.47 (1.25-1.75)	B	31.16 (26.22-36.37) b
CP	Aw	1.61 (1.35-1.97)	B	23.53 (20.27-27.09) b
CP	Ln	0.82 (0.71-0.95)	A	63.75 (42.86-110.98) c
CP	Lp	1.38 (1.12-2.62)	B	11.73(9.61-14.66) a
CP	Ms	1.67 (1.34-2.42)	B	10.86 (9.41-12.76) a
BP	Ac	1.50 (1.17-2.01)	B	51.38 (41.18-64.28) b
BP	Aw	1.31 (1.07-1.61)	B	39.56 (31.44-50.49) b
BP	Ln	0.44 (0.38-0.52)	A	1128.76 (307.38-7477.83) c
BP	Lp	0.44 (0.39-0.51)	A	2152.31 (501.95-21856.68) c
BP	Ms	2.65 (2.00-4.07)	BC	26.36 (23.90-28.98) a
GLA	Ac	2.154(1.76-2.69)	B	40.31 (34.73-46.00) b
GLA	Aw	0.88 (0.74-1.07)	A	39.16 (25.84-61.45) b
GLA	Ln	0.66 (0.53-0.86)	A	236.85 (115.08-750.35) c
GLA	Lp	0.94 (0.68-1.50)	A	134.35 (82.65-321.98) c
GLA	Ms	1.70 (1.44-2.10)	AB	20.27 (16.98-23.59) a
LS	Ac	1.57 (1.21-2.86)	A	10.70 (8.96-13.28) a
LS	Aw	2.31 (1.97-3.09)	B	10.43 (9.22-11.71) a
LS	Ln	0.88 (0.67-1.24)	A	189.07 (104.65-511.29) c
LS	Lp	1.58 (1.33-2.17)	AB	19.51 (16.28-23.62) b
LS	Ms	2.70 (2.20-3.70)	B	9.52 (8.62-10.63) a
MR	Ac	1.11 (0.97-1.23)	A	24.07 (18.38-31.14) b
MR	Aw	1.84 (1.59-2.16)	B	21.37 (18.59-24.33) b
MR	Ln	1.29 (1.08-1.59)	A	22.56 (17.80-29.12) b
MR	Lp	1.788 (1.38-5.18)	A	10.23 (8.83-11.96) a
MR	Ms	2.73 (2.26-4.42)	AB	8.54 (7.71-9.45) a
WL	Ac	1.65 (1.03-3.29)	A	82.61 (62.56-145.41) c
WL	Aw	1.20 (0.92-1.57)	A	41.69 (31.24-56.54) b
WL	Ln	0.77 (0.57-1.38)	A	302.19 (125.45-1912.44) c
WL	Lp	0.77 (0.56-1.24)	A	144.13 (79.93-404.52) c
WL	Ms	1.43 (1.22-1.89)	A	22.96 (18.23-28.27) a
YG	Ac	1.29 (1.17-1.45)	A	22.20 (18.23-27.03) b
YG	Aw	1.34 (1.12-1.88)	A	17.23 (13.41-22.86) b
YG	Ln	1.05 (0.89-1.21)	A	71.74 (50.50-111.48) d
YG	Lp	1.07 (0.94-1.23)	A	36.16 (27.35-49.30) c
YG	Ms	2.45 (2.12-3.32)	B	9.25 (8.30-10.29) a

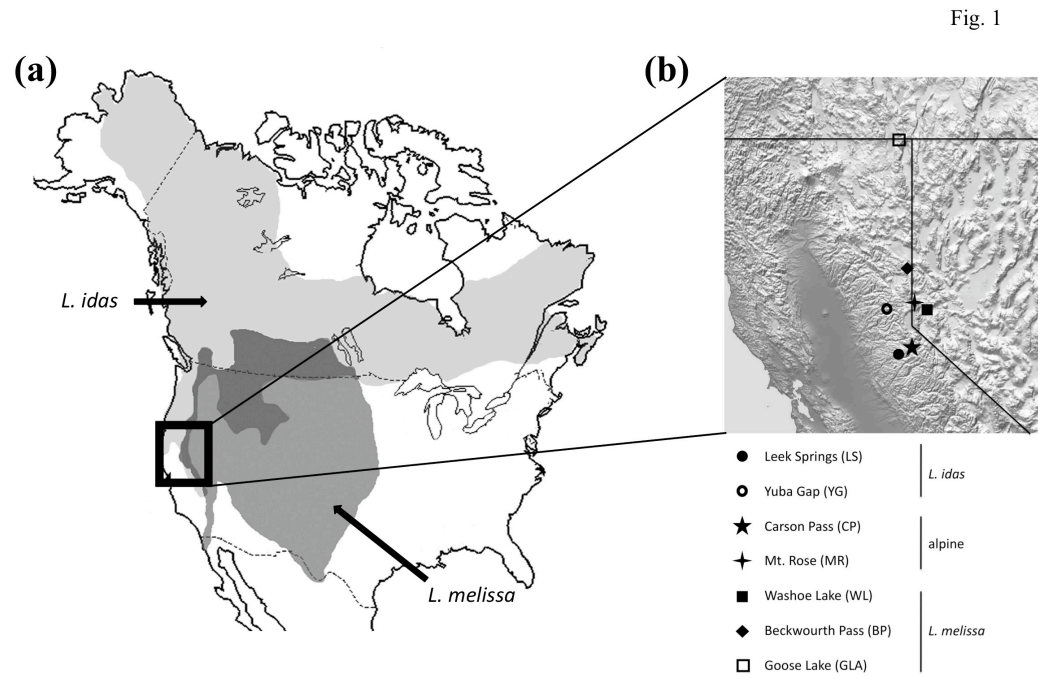
Figure legends

Figure 1: (a) Ranges of *L. idas* and *L. melissa* across North America, darker shaded regions correspond to ranges of overlap, which includes alpine populations of the hybrid species considered here (CP and MR). (b) Map of sampled *Lycaeides* populations in Northern California and Nevada, USA. Symbols correspond to populations and taxa.

Figure 2: Survival and emergence weights from rearing experiments: (a and b) *L. idas*, (c and d) hybrid species, (e and f) *L. melissa*. Values are standardized (Z-transformed). Shading of bars indicates plant species, see legends and Fig. 1 for population locations. See Table 2 for associated model details, including plant and population effects. Significant differences ($P < 0.05$) for population effects within plants are indicated here with small letters near bars. Black dots identify natal host associations for each population. Host plant abbreviations as follows: Ac, *Astragalus canadensis*; Aw, *Astragalus whitneyi*; Ln, *Lotus nevadensis*; Lp, *Lupinus polyphyllus*. Results from alfalfa, *M. sativa*, are not shown here because survival was very low, see main text for details.

Figure 3: Survival curves for the seven populations studied. Colors indicate survival associated with a given plant; letters next to each plant in legends correspond to differences in the shape (upper case) and scale (lower case) of each curve indicated by non-overlapping 95% confidence intervals from bootstrapped parameter values (see Table A1 for more details). Black dots indicate native host association for each population. Average, final survival is shown to the right of each graph for nonzero results. Plant abbreviations as follows: Ac, *Astragalus canadensis*; Aw, *Astragalus whitneyi*; Ln, *Lotus nevadensis*; Lp, *Lupinus polyphyllus*; Ms, *Medicago sativa*.

Figure 4: Summary of hypotheses relating ecological traits to reproductive isolation between taxa based on the current and past studies [16, 17,19, 22, 23]. Arrows joining two taxa correspond to greater gene flow and those that are faded represent gene flow that could be prevented or reduced by a given trait. For details see text.



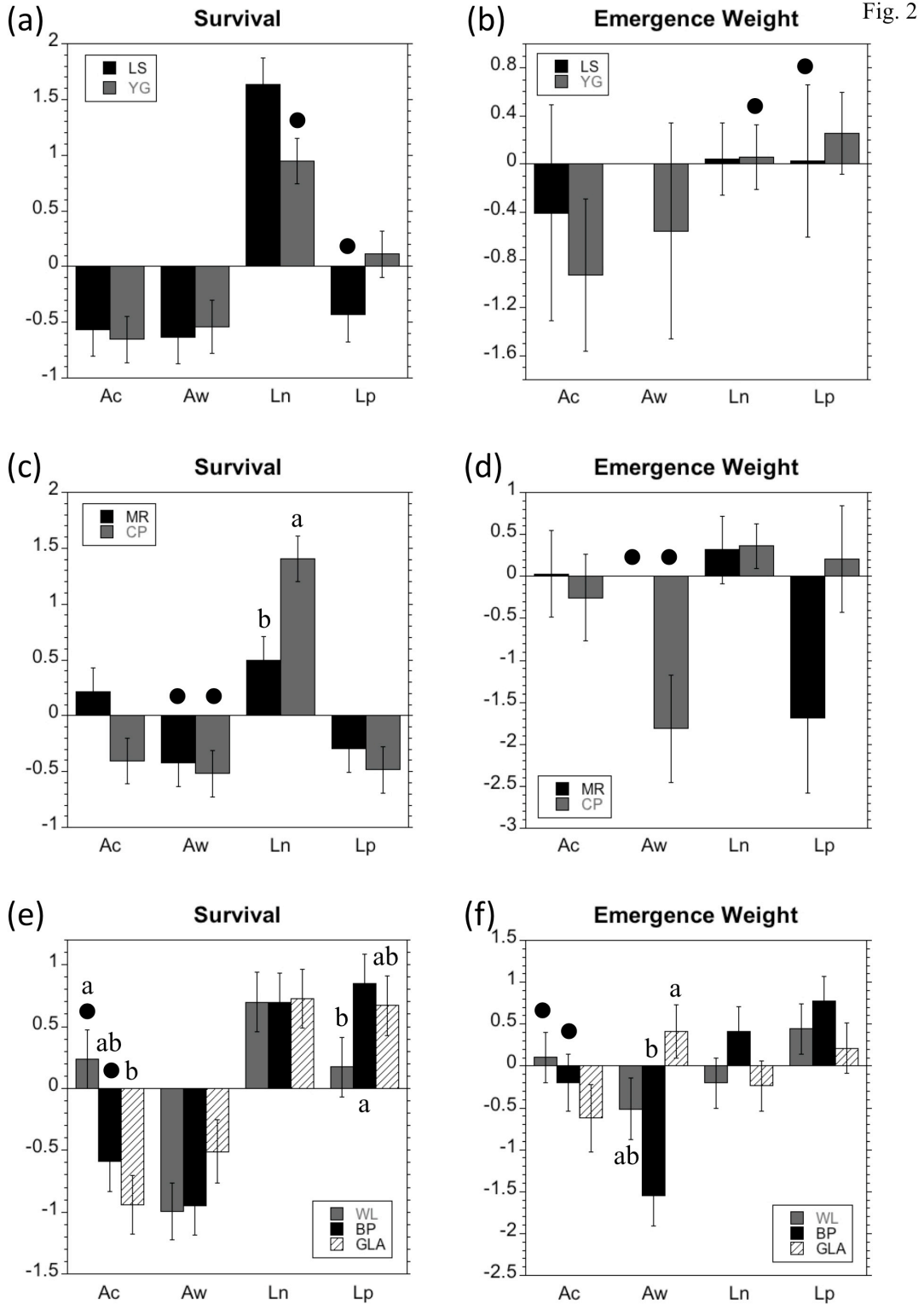


Fig. 2

Fig. 3

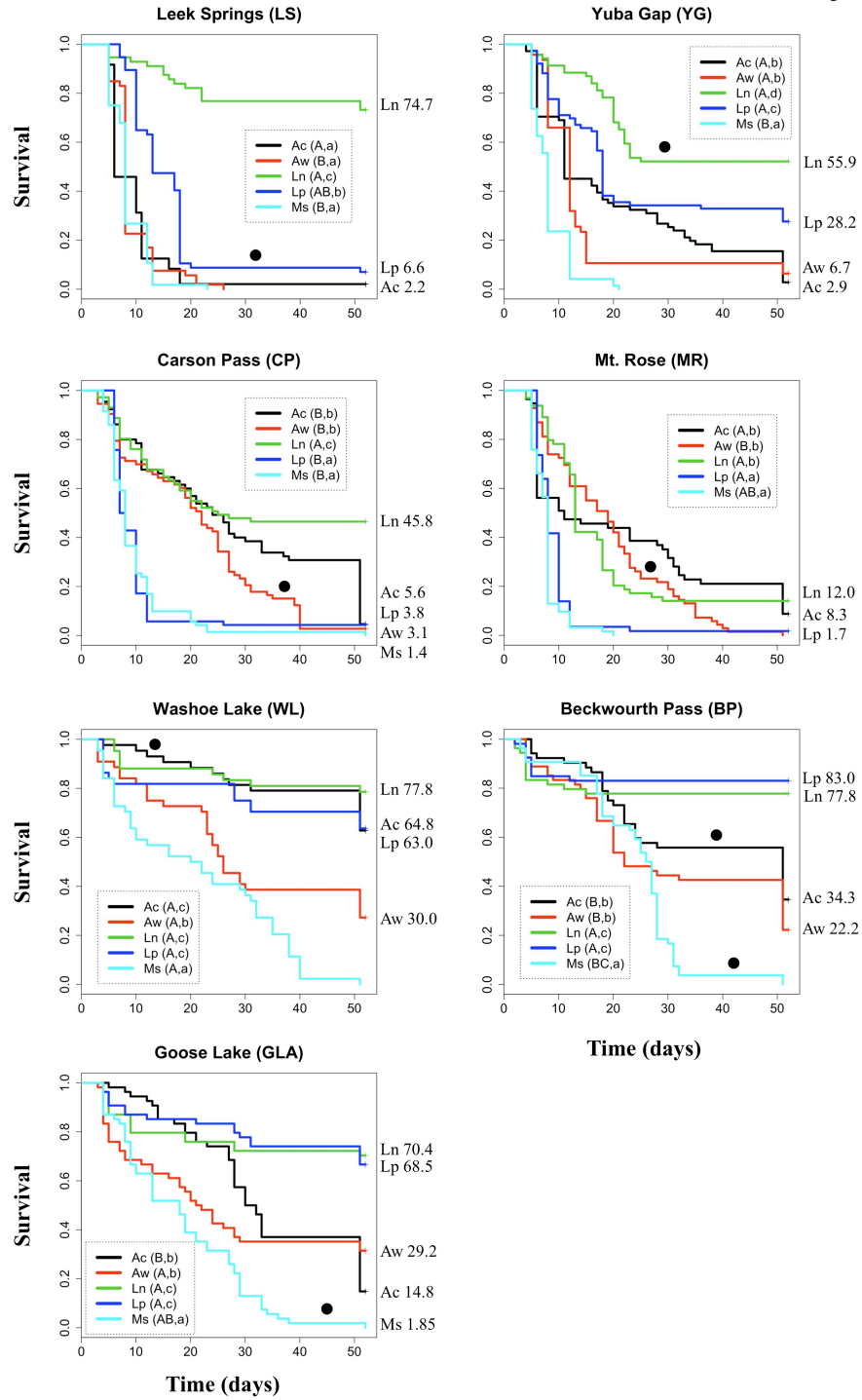
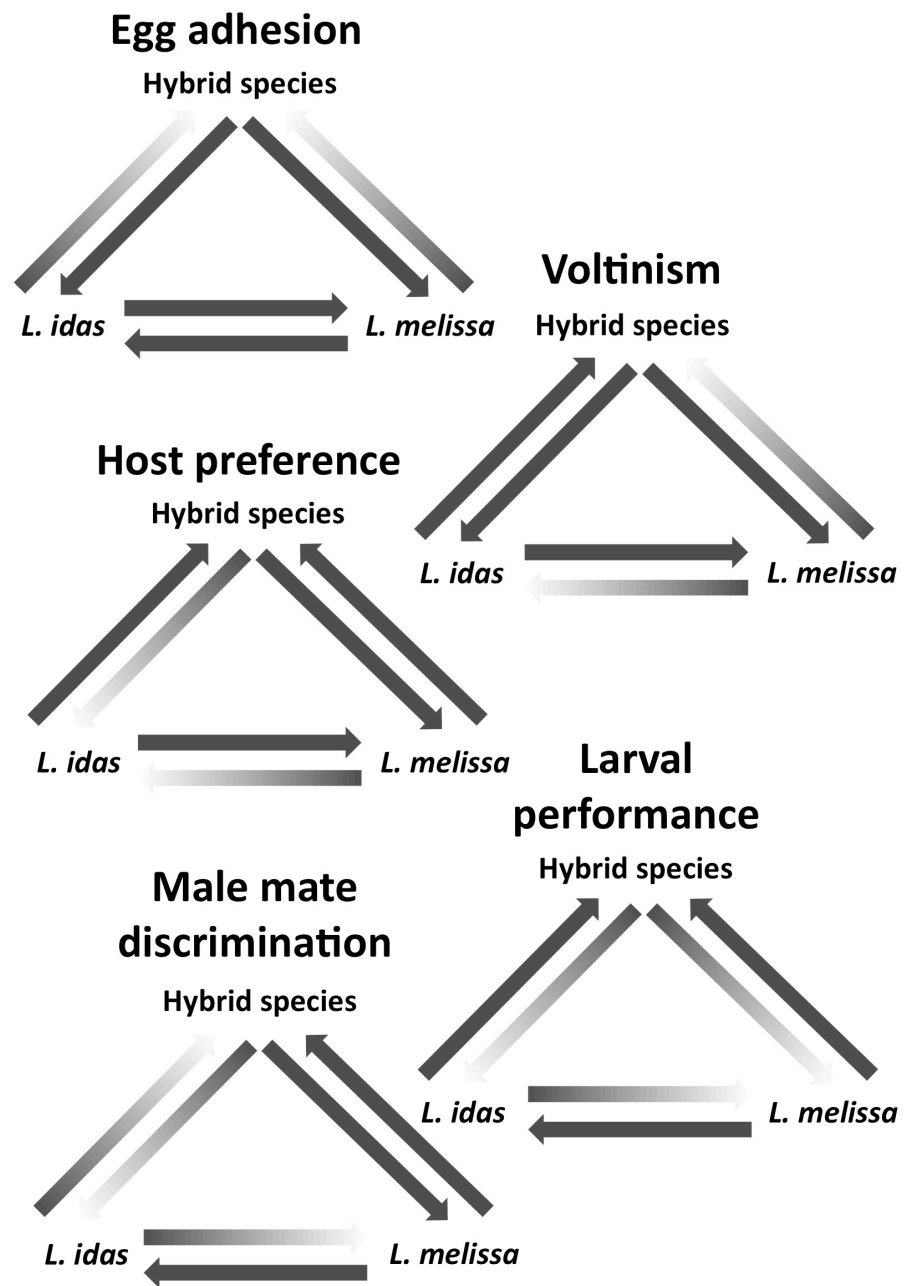


Fig. 4



CHAPTER 2: Parasitism and mutualism in the butterfly *Lycaeides melissa*: temporal and geographic variation in parasitoid attack, but no evidence for ant protection

INTRODUCTION

A long-standing goal in ecology and evolutionary biology has been to understand how interactions between species promote or constrain diversification. This has been studied extensively in angiosperms and herbivorous insects, giving rise to the hypothesis that the complex interactions between flowering plants and insects have led to the great diversity of both groups (Erlich and Raven 1964, Mitter et al. 1988, Farrell and Mitter 1998, Janz 2011). For herbivores insects, a change in host plant use can lead to population divergence and eventually speciation (Bush and Smith 1998, Filchak et al. 2000, Dres and Mallet 2002). If shifts in host use are important for diversification in herbivorous insects, then a major goal for evolutionary ecology should be understanding the causes and consequences of changes in diet breadth. Many hypotheses have sought to explain changes in host use, or niche breadth, as a function of female choice (preference) and offspring fitness (performance) (Rausher 1988, Funk 1998). These traits have most often been studied in the laboratory, in conditions that exclude many if not most of the inter-specific interactions that herbivores experience in the wild (Forister et al. 2012). In particular, traditional assays of preference and performance do not account for trade-offs in host use that potentially involve natural enemies. Recently there has been a call to include more trophic levels when studying host shifts, thereby increasing our knowledge of how interaction complexity influences plant-herbivore dynamics (Singer and Stireman 2005, Forister et al. 2012).

The Melissa blue butterfly (*Lycaeides melissa*) is a well-studied and widespread Lycaenid butterfly native to western North America (Nice and Shapiro 1999, Forister et al. 2009, Forister et al. 2011, Scholl et al. 2012). *Lycaeides melissa* uses a variety of plants in the pea family (Fabaceae) as hosts, including the exotic host alfalfa (*Medicago sativa*) which was introduced to North America within the last 200 years (Michaud et al. 1988). Because alfalfa has been incorporated into the diet of *L. melissa* only recently, the system is well suited for studying current adaptation to a new host. In general, alfalfa is a poor host for caterpillars: individuals reared experimentally on alfalfa can be up to 70% smaller than individuals reared on a common native host, *Astragalus canadensis* (Forister et al. 2009). However, when alfalfa flowers are present, alfalfa is readily chosen as an oviposition site by females (Forister et al. 2009). *Lycaeides melissa* females from a population associated with both the native host, *Astragalus canadensis*, and the novel host prefer to lay eggs on the native host in choice tests when neither plant has flowers, but when flowers are present, the two hosts are equally used (Forister et al. 2009). This may in part be due to the fact that alfalfa flowers can be used as a nectar source while those of *A. canadensis* cannot. There is a severe cost to choosing alfalfa as an oviposition substrate: fewer butterflies emerge as adults and those that do emerge are much smaller (Forister et al. 2009, Scholl et al. 2012). As with many herbivorous insects, larger size has direct fitness benefits because larger females lay more eggs (Forister et al. 2009). Larval performance experiments have all been carried out in the lab, but larval fitness may be very different in the field where caterpillars are exposed to natural enemies. By switching hosts, some insects have been found to enter enemy-free space, or increase their fitness on an otherwise-inferior host by escaping from many of their native

predators (Mira and Bernays 2002, Murphy 2004).

About three fourths of lycaenid butterflies with well-studied life histories associate with ants; these relationships range from parasitic to obligate (Pierce et al. 2002). *L. melissa* caterpillars are facultatively myrmecophilous, providing secretions rich in sugars and amino acids in return for protection. Forister et al. (2011) found that on alfalfa, the presence of ants reduced predation rates for *L. melissa*, while the presence of ants did not affect predation rates on the native *A. canadensis*. Other studies have shown that ants can also protect lycaenids from parasitoids (Pierce and Mead 1981, Pierce and Easteal 1986 and Weeks 2003). The present study had two goals: first to broaden our knowledge of myrmecophily in this system by investigating the role of ants in protecting *L. melissa* caterpillars from parasitoids. Second, we lay the foundation for future studies of interaction ecology using *L. melissa* by reporting on the natural history of the system. Specifically, we identify species of parasitoids and species of ants tending caterpillars. We hypothesize that caterpillars found with tending ants will have lower parasitism rates than those found without ants, and that this result might be host specific, i.e. the presence of ants might be more beneficial on the exotic host, than on a native host. The latter prediction (a host-specific benefit of the ant mutualism) derives from the previously reported result that absolute numbers of insects and natural enemies are higher on the exotic host relative to the native host *A. canadensis*.

METHODS

In order to study attack by parasitoids, we collected caterpillars and ants from

sites with a native host or with the exotic host, alfalfa. Field sites were located either in typical Great Basin habitat where the dominant plant species are sagebrush (*Artemisia*) and rabbitbrush (*Ericameria*), or in invaded habitats (i.e. alfalfa along roadsides). Our goal was to collect caterpillars from twenty plants in three *L. melissa* populations that use native hosts and three that use alfalfa, in two sampling years, 2010 and 2011. Mutualistic ants were found by visually searching for and collecting ants that were actively tending caterpillars. We sampled five sites during the summers of 2010 and 2011 (Fig. 1 and Table 1). In 2011 we were able to re-sample four of the five sites from 2010. These four sites that were studied in both years included two sites where the native *A. canadensis* is used as a host, Washoe Lake (WLA) and Silver Lake (SLA), and two sites in Verdi, NV (VCP and VER) where alfalfa is used as a host. The fifth site, Beckwourth Pass (BWP), could not be re-sampled in 2011 due to intensive livestock grazing. This site has both the native host, *A. canadensis* and alfalfa, so data for this site were separated by host. In 2011, a site in Bishop, CA (BHP) was added, where the native host used is *Glycyrrhiza lepidota*. All sampling was performed in August or the first week of September.

The identity of host plants is inherently confounded with geography in this system (there is only one site where both hosts are present, and we were only able to sample it in the first year). Thus one would ideally sample a large number of locations and hosts. However, the collection of caterpillars is time intensive, and logistical constraints led us to focus on larger collections of caterpillars from fewer locations. It should be noted that the confounded nature of geography and host plant identity did not limit our ability to investigate the importance of ants for protection against parasitism, as ants were ubiquitous across sites and hosts.

Collection of caterpillars and ants

At each of our study sites (Fig. 1), the center of the area to be sampled was designated by selecting the center of a group of plants that appeared to be healthy and had evidence of caterpillar damage (*L. melissa* caterpillars leave signs of herbivory that are easily spotted). Individual plants to be searched were chosen in the following way: a line was extended out from the center of the area along a random compass direction, and walked until that line hit a host plant. Each plant was then inspected thoroughly for caterpillars. If no caterpillars were found, sampling was continued in the given direction until either a plant with caterpillars was found or the edge of the sampling area was reached at 25m (an arbitrary distance that we have found tends to encompass “patches” of host plants utilized by *L. melissa*). As plants were searched for caterpillars, any tending ants were collected before the caterpillar was removed.

After the foliage of each plant was thoroughly searched, the base of the plant was checked for larvae and pupae. The size of each plant (from which caterpillars were collected) was found by measuring the height, the width at the widest point, and the width perpendicular to the widest part. Plants were flagged to avoid re-sampling. Each site was sampled until either twenty plants with larvae were found or all the plants within the 25 m radius were searched. Data were collected differently for the two years – in 2010 data were collected by plant (i.e. caterpillars and ants were labeled only with reference to the plant that they were collected from) and in 2011 data were collected for each caterpillar (with ants and caterpillars labeled separately). The collections from the first year allowed us to ask if the percent of caterpillars being tended on a plant predicted parasitism rates for caterpillars on each plant. The data from the second year were

collected with individual-level resolution so that we could ask if the presence of ants for a particular caterpillar affected its probability of having been parasitized.

Caterpillar rearing

Caterpillars from the field were kept cool and brought back to a lab at the University of Nevada, Reno, and measured before being put individually into petri dishes (90x12mm). Larval age in lepidoptera can be determined with head capsule size (Dyar 1890), but we have not established the necessary standards for *L. melissa*. Instead, the total length of each caterpillar was taken to the nearest mm as a rough correlate to instar. Caterpillar instar or stage is important because some parasitoids attack specific stages of their hosts. Moreover, instar can be an important covariate for the probability of being parasitized, since later instars have simply had more time to be attacked.

Our goal in rearing was to maximize caterpillar survival in order to maximize the chance of observing an emergent parasitoid (if a caterpillar died for reasons other than parasitism, a parasitoid might not be observed unless dissection was completed after death). The following host plants were used: *Lupinus polyphillus* in 2010 and *Lotus nevadensis* in 2011, both native hosts of the closely related butterfly species, *Lycaeides anna* (formerly *L. idas anna*). These hosts have been shown to be superior hosts relative to both the native host of *L. melissa*, *Astragalus canadensis* and the novel host, alfalfa (Scholl et al. 2012). While it is known that larval diet can impact parasitoid development and caterpillar immune response (Smilanich et al. 2009, Smilanich et al. 2011), switching all caterpillars to the same host should not affect comparisons across or within populations. *L. polyphillus* was collected approximately seven miles north of Truckee CA, off State Route 89 (39°25'59"/ 120°12'13") and *L. nevadensis* was collected at the

Hwy 20 exit off I-80 (39°19'24" N/120°35'60" W). Plant material was kept in a refrigerator until needed, with new plant material collected at least once a week. Caterpillars were checked daily and new plant material was provided as needed, but generally every second or third day. Caterpillars were reared until emergence as an adult or until a parasitoid emerged. All dishes were kept at room temperature, 20° to 23° Celsius, on lab benches with overhead lights set on a twelve-hour light/dark cycle. Parasitoid wasps were identified to subfamily or family using Wahl and Sharkey (1993). John O. Stireman III (Wright State University) identified tachinid flies. Ants in the *Fusca* group of *Formica* were identified using Francoeur (1973), while all other species were identified using Fisher and Cover (2007) and Wheeler and Wheeler (1986).

Statistical Analysis

Our primary objective was to address the potential influence of ant tending on parasitoid attack. Because data collection methods differed in the two years (see above) all analyses were run separately for the two years. We used a model comparison approach with generalized linear models with binomial error to compare parasitism across sites (Burnum and Anderson 2002) using the *MuMIn* package in R (2.14.0). A model was considered competitive if it was within 3 ΔAIC_c of the top model. Akaike weights or normalized relative likelihoods (w_i) were also calculated to corroborate model selection and to judge the relative importance of each factor (by summing the Akaike weights for all the models with a given covariate, Burnum and Anderson 2002). A null model with no factors was used for comparison; support for the null model that is comparable to other models suggests that there are no significant predictors of the response variable (i.e. experimental models should have much smaller ΔAIC_c values than the null). Subsequent

to the model comparison approach across sites, logistic regression, also performed in R, was used to compare parasitism rates within sites where parasitoids were found.

The covariates compared using the model comparison approach for 2010 were site, size of the plant and the presence of tending ants found on a plant, with the response being percent parasitism of caterpillars on a plant. Data were recorded for larvae individually in 2011, which changed the factors examined to site, size of the plant, size of the caterpillar, and the whether or not the caterpillar was found tended, with the response being whether or not an individual was parasitized. For within site analyses where parasitoids were found, the same factors (minus site) were used in logistic regressions with the same response variables. For all analyses, plant size was calculated by multiplying the height by the two width measurements. Caterpillar instar was estimated by creating four size classes, 1-4mm, 5-7 mm, 8-11mm and any larvae over 12mm and those that were pupating. Caterpillars were removed from analyses if they died in the lab or if they failed to emerge as adults.

RESULTS

We collected a total of 526 caterpillars, 424 of which survived and were included in analyses (213 caterpillars from 2010 and 211 from 2011). The average number of caterpillars found per plant (including only plants with caterpillars) was 2.3, however this ranged from 1 individual to 17 found on a single *A. canadensis* plant at BWP in 2010. Tending ants were found at all sites. Results from the model comparisons for 2010 show the top model as Site + Ant, with an Akaike weight (w_i) of 0.46, meaning it has a likelihood of 46% of being the correct model (Table 2). There are three other models

within 3 ΔAIC_c of the top model, all of which contain site as a factor. The relative importance of site was found to be 1 by summing all the Akaike weights of all models with site as a factor. The top model for 2011 contained only site, with an Akaike weight of 0.28 (Table 3). The four other models for 2011 within 3 ΔAIC_c of the top model all contained site as a factor. Again the relative importance of site was found to be 1.

In contrast to the considerable variation among sites (Table 2 and 3, Fig. 2 and 3), we found no evidence that ants provided any protection against parasitoids. The logistic regressions within sites showed plant size and the presence of tending ants were not significant predictors of percent parasitism in 2010 (Table 4). For the 2011 within-site comparisons, plant, plant size, presence of tending ants and caterpillar size were similarly not significant predictors of parasitism (Table 5).

The 48 parasitoids that emerged encompassed three subfamilies of parasitic wasps, Campopleginae, Rogadinae and Microgastrinae, and one subfamily of parasitic fly, Exoristinae (Figure 4, Table 6). Two sites had parasitoids in 2010 (WLA and VCP) while parasitoids were found at four sites in 2011 (SLA, WLA, BHP and VCP). The diversity of parasitoids also increased in 2011 with all four groups of parasitoids represented, instead of only Campopleginae and Exoristinae. Parasitism rates differed drastically between years, for example it increased at WLA from 15.4% in 2010 to 65.7% in 2011.

We collected and identified 288 ants, from ten species in five genera, actively tending caterpillars (149 from 2010 and 139 from 2011, Figure 4 and Table 7). Across sites in 2010, 60% of plants with caterpillars had tending ants and in 2011, 42.7% of the caterpillars found were tended. Ants in the genus *Formica* made up the vast majority of

ants found tending (96.4%). Of these, *Formica oreas* was most common, comprising 60% of the ants collected. This species was found at all but one (SLA) of the sites sampled. *Formica lepida* was the next most common species, making up 19.4% of the ants found tending, and was found at every site except for WLA in 2011. Ants from other genera, *Camponotus vicinus*, *Myrmecina americana*, *Monomorium minimum*, and *Tapinoma sessile* were each found only at one site during one year of sampling (Table 7). *Formica oreas* might have enslaved colonies of *Formica lepida* at several sites. The genus *Formica* is split into two groups, Rufa and Fusca, and members of the Rufa group are known to enslave members of the Fusca group. Both species, *Formica oreas* (Rufa group) and *Formica lepida* (Fusca group), were often encountered tending on the same plant, and in one case members of both species were found tending the same ant. Overall we found variation across years and sites in the species of ants tending, however major differences are not apparent in the community of ants tending on native hosts as compared to the exotic host. However, the present study was not designed explicitly to study diversity or community composition; rather identifications are useful for natural history context and to provide a foundation for future studies.

DISCUSSION

We did not find support for the hypothesis that the presence of mutualistic ants decreases parasitism rates of *L. melissa* caterpillars, at either the plant (2010) or individual level (2011). We found that parasitism was highly variable by site, ranging from none at several sites to 65.7% parasitized at WLA in 2011 (Fig. 2 and Fig. 3); the importance of site in relation to parasitism was reflected in the presence of site as a factor

in all top models across years. The top model for 2010 included ant tending as a covariate; however, within site results for the two sites with parasitoids showed no evidence that tending was an important factor in predicting percent parasitism. It is likely that the inclusion of ant tending in the top model was driven by a positive relationship between the presence of ants and parasitism at the two sites with parasitoids in 2010: 9 out of 10 plants with parasitized caterpillars had tending ants. Other studies have found mixed results for the role of mutualistic ants in protecting caterpillars from parasitoids. Protection from parasitoids by ants has often been found by experimental exclusion of tending ants (Pierce and Mead 1981, Pierce and Eastal 1986 and Weeks 2003). Pierce and Mead (1981) also collected final instars in the field from unmanipulated plants and found that those found untended or tended by only one ant were more likely to be parasitized than those found being tended by three or more ants. Devries (1991), on the other hand, found that ants protect caterpillars from predatory wasps but not from tachinid flies. Likewise Savignano (1994) found the presence of ants did not reduce parasitism but did reduce predation in *Lycaeides samuelis* (formerly *L. melissa samuelis*; Forister et al. 2011). Combined with the results from Savignano (1994) and a previous study from one of our focal sites (Forister et al. 2011), it seems likely that the presence of ants may reduce predation, but not parasitism in *L. melissa*.

We found variation in the species of ants tending across sites and years. It has been shown in other ant-caterpillar (Fraser et al. 2001) and ant-plant (Palmer and Brody 2007, Palmer et al. 2010) mutualisms that different ant species exhibit varying levels of protection. Caterpillars can also elicit changes in the intensity of tending behavior by varying the amount of reward they produce (Axén 2000, Fraser et al. 2001). In the *L.*

melissa system it would be interesting to better understand and quantify both the larval reward provided and the intensity of tending for different species of ants. It might be the case that some ant species are better at protecting *L. melissa* larvae from parasitoids than others, and that a failure to account for this source of variation could explain the lack of evidence we find for the protection against parasitoids. In a different system involving acacia trees and symbiotic ants, Palmer et al. (2010) found that the interaction complexity created by the presence of different ant species was key to understanding the evolution and persistence of the interaction.

We observed considerable variation in parasitoid abundance and diversity across space and time. VER and VCP are geographically very close, 200 meters apart, however parasitoids were only found at VCP. The highest rate of parasitism was at WLA in 2011 where 65.7% of caterpillars were parasitized by three different parasitoids. Interestingly there was only one caterpillar found being tended at this site for 2011 (WLA has a sandy substrate that is seasonally inundated with water, which might limit ant presence). Other studies of parasitism have also found large differences in parasitism rates across space and time (Pacala and Hassel 1991, Stireman and Singer 2002, Heard et al. 2006). Using theoretical population models, this variation has been shown to stabilize host-parasitoid population dynamics (Hassel and May 1973, Hassel et al. 1991). For example Hassel et al. (1991) found that parasitoid and caterpillar populations can persist if parasitoid density is sufficiently variable across space.

One of the more interesting aspects of the *L. melissa* system is the recent host shift to alfalfa, an inferior host for larval development and survival. The lack of any major differences in parasitism rates between native and exotic hosts in this study

suggests that parasitism is not a major influence in the ongoing host shift to alfalfa. However, one aspect of this host shift that has not been studied in *L. melissa* is how differences in plant chemistry between hosts may alter larval interactions with predators or parasitoids. For caterpillars that are able to sequester plant secondary metabolites, changes in host can profoundly change predation and parasitism dynamics (Fordyce 2001, Smilanich et al. 2009). Further studies using *L. melissa*, which probably does not sequester, could investigate how plant chemistry might change species interactions. Possibilities include changes in larval reward for ants, or caterpillar immune response when faced with a parasitoid.

LITERATURE CITED

- Axen, A. H. 2000. Variation in behavior of lycaenid larvae when attended by different ant species. *Evolutionary Ecology* 14:611-625.
- Burnham, K.P., and D. R. Anderson. 2002. *Model Selection and Multimodel inference: A practical information-theoretic approach*. Springer, New York, New York.
- Bush, G. L., and J. J. Smith. 1998. The genetics and ecology of sympatric speciation: A case study. *Researches on Population Ecology* 40:175-187.
- Devries, P. J. 1991. Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. *Biological Journal of the Linnean Society* 43:179-195.
- Dres, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society B-Biological Sciences* 357:471-492.
- Dyar, H. G. 1890. The number of molts of lepidopterous larvae. *Psyche* 5: 420-422.
- Ehrlich, P.R. and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586-608.
- Francoeur, A. 1973. Revision taxonomique des especes nearctiques du group fusca, genre Formica (Hymenoptera: Formicidae). *Memoires de la Société Entomologique du Québec* 3:1-316.
- Farrell, B. D., and C. Mitter. 1998. The timing of insect/plant diversification: might *Tetraopes* (Coleoptera : Cerambycidae) and *Asclepias* (Asclepiadaceae) have co-evolved? *Biological Journal of the Linnean Society* 63:553-577.

- Filchak, K. E., J. B. Roethele, and J. L. Feder. 2000. Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature* 407:739-742.
- Fisher, B. L., and S. P. Cover. 2007. *Ants of North America*. University of California Press, Berkeley and Los Angeles, California.
- Fordyce, J. A. 2001. The lethal plant defense paradox remains: inducible host-plant aristolochic acids and the growth and defense of the pipevine swallowtail. *Entomologia Experimentalis Et Applicata* 100:339-346.
- Forister, M. L., C. C. Nice, J. A. Fordyce, and Z. Gompert. 2009. Host range evolution is not driven by the optimization of larval performance: the case of *Lycaeides melissa* (Lepidoptera: Lycaenidae) and the colonization of alfalfa. *Oecologia* 160:551-561.
- Forister, M. L., Z. Gompert, C. C. Nice, G. W. Forister, and J. A. Fordyce. 2011. Ant association facilitates the evolution of diet breadth in a lycaenid butterfly. *Proceedings of the Royal Society B-Biological Sciences* 278:1539-1547.
- Forister, M. L., L. A. Dyer, M. S. Singer, J. O. Stireman, J. T. Lill. 2012. Revisiting the evolution of ecological specialization, with emphasis on insect-plant interactions. *Ecology*.
- Fraser, A. M., A. H. Axen, and N. E. Pierce. 2001. Assessing the quality of different ant species as partners of a myrmecophilous butterfly. *Oecologia* 129:452-460.
- Funk, D. J. 1998. Isolating a role for natural selection in speciation: Host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution* 52:1744-1759.
- Hassell, M. P., and R. M. May. 1974. Aggregation of predators and insect parasites and its effect on stability. *Journal of Animal Ecology* 43:567-594.

- Hassell, M. P., R. M. May, S. W. Pacala, and P. L. Chesson. 1991. The persistence of host-parasitoid associations in patchy environments. 1. A general criterion. *American Naturalist* 138:568-583.
- Heard, S. B., J. O. Stireman, J. D. Nason, G. H. Cox, C. R. Kolacz, and J. M. Brown. 2006. On the elusiveness of enemy-free space: spatial, temporal, and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods. *Oecologia* 150:421-434.
- Janz, N. 2011. Ehrlich and Raven revisited: mechanisms underlying codiversification of plants and enemies. *Annual Review of Ecology, Evolution, and Systematics* 42:71-89.
- Michaud R., W. F. Lehman, and M. D. Rumbaugh. 1988. World distribution and historical developments. *Alfalfa and Alfalfa Improvement* (eds. A. A. Hanson, D. K. Barnes, and R. R. Hill), pp. 25-56. ASA-CSSA-SSSA, Madison, Wisconsin.
- Mira, A., and E. A. Bernays. 2002. Trade-offs in host use by *Manduca sexta*: plant characters vs. natural enemies. *Oikos* 97:387-397.
- Mitter, C., B. Farrell, and B. Wiegmann. 1988. The phylogenetic study of adaptive zones – has phytophagy promoted insect diversification. *American Naturalist* 132:107-128.
- Murphy, S. M. 2004. Enemy-free space maintains swallowtail butterfly host shift. *Proceedings of the National Academy of Sciences of the United States of America* 101:18048-18052.
- Nice, C. C., and A. M. Shapiro. 1999. Molecular and morphological divergence in the

- butterfly genus *Lycaeides* (Lepidoptera : Lycaenidae) in North America: evidence of recent speciation. *Journal of Evolutionary Biology* 12:936-950.
- Pacala, S. W., and M. P. Hassell. 1991. The persistence of host-parasitoid associations in patchy environments. 2. Evaluation of field data. *American Naturalist* 138:584-605.
- Palmer, T. M., and A. K. Brody. 2007. Mutualism as reciprocal exploitation: African plant-ants defend foliar but not reproductive structures. *Ecology* 88:3004-3011.
- Palmer, T. M., D. F. Doak, M. L. Stanton, J. L. Bronstein, E. T. Kiers, T. P. Young, J. R. Goheen, and R. M. Pringle. 2010. Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences of the United States of America* 107:17234-17239.
- Pierce, N. E., and P. S. Mead. 1981. Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. *Science* 211:1185-1187.
- Pierce, N. E., and S. Easteal. 1986. The selective advantage of attendant ants for the larvae of a lyceanid butterfly, *Glaucopsyche lygdamus*. *Journal of Animal Ecology* 55:451-462.
- Pierce, N. E., M. F. Braby, A. Heath, D. J. Lohman, J. Mathew, D. B. Rand, and M. A. Travassos. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology* 47:733-771.
- Rausher, M. D. 1984. Tradeoffs in performance on different hosts – evidence from within-site and between-site variation in the beetle *Deloyala guttata*. *Evolution* 38:582-595.
- Savignano, D. A. 1994. Benefits to Karner blue butterfly larvae from associations with

- ants. *Karner Blue Butterfly: a Symbol of a Vanishing Landscape* (eds. D. A. Andow, R. J. Baker and C. P. Lane), pp. 37-46. University of Minnesota Agricultural Experiment Station, St Paul, Minnesota.
- Scholl, C. F., C. C. Nice, J. A. Fordyce, Z. Gompert and M. L. Forister. 2012. Larval performance in the context of ecological diversification and speciation in *Lycaeides* butterflies. *International Journal of Ecology*. Article ID 242154. doi:10.1155/2012/242154
- Singer, M. S. and J. O. Stireman. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters* 8:1247-1255.
- Smilanich, A. M., L. A. Dyer, J. Q. Chambers, and M. D. Bowers. 2009. Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecology Letters* 12:612-621.
- Smilanich, A. M., P. A. Mason, L. Sprung, T. R. Chase, and M. S. Singer. 2011. Complex effects of parasitoids on pharmacophagy and diet choice of a polyphagous caterpillar. *Oecologia* 165:995-1005.
- Stireman, J. O., and M. S. Singer. 2002. Spatial and temporal variation in the parasitoid assemblage of an exophytic polyphagous caterpillar. *Ecological Entomology* 27:588-600.
- Wahl, D. B., and M. J. Sharkey. 1993. Superfamily Ichneumonoidea. *Hymenoptera of the World: An Identification Guide to Families* (eds. H. Goulet and J. T. Huber), pp. 358-509. Agriculture Canada, Ottawa.
- Weeks, J. A. 2003. Parasitism and ant protection alter the survival of the lycaenid

Hemiargus isola. Ecological Entomology 28:228-232.

Wheeler, G. C., and J. N. Wheeler. 1986. The Ants of Nevada. Natural History Museum of Los Angeles County, Los Angeles, California.

Table 1: Locations of populations (see also Fig. 1) and host plants associated with the six populations studied.

Location	Latitude/longitude	Host
Silver Lake (SLA)	39.6497, -119.9263	<i>Astragalus canadensis</i>
Washoe Lake (WLA)	39.2331, -119.7795	<i>Astragalus canadensis</i>
Bishop (BHP)	37.3585, -118.3906	<i>Glycyrrhiza lepidota</i>
Beckwourth Pass (BWP)	39.7797, -120.0734	<i>Astragalus canadensis</i> and <i>Medicago sativa</i>
Verdi Crystal Peak (VCP)	39.5141, -119.9950	<i>Medicago sativa</i>
Verdi (VER)	39.5145, -119.9927	<i>Medicago sativa</i>

Table 2: Results from model comparisons across sites for 2010, with percent parasitism per plant as the response. Models are listed by order of decreasing ΔAIC_c values, which compare consecutively-ranked models.

Model	ΔAIC_c	w_i	No. Parameters
Site + Ant	0	0.46	7
Site	1.50	0.21	6
Site + Plant Size + Ant	1.59	0.21	8
Site + Plant Size	2.62	0.12	7
Plant Size + Ant	21.74	0	3
Plant Size	22.45	0	2
Ant	23.97	0	2
Null	25.69	0	1

Table 3: Results from model comparisons across sites for 2011, with parasitism of individual caterpillars as the response. Models are listed by order of increasing AICc values; delta AIC values compare consecutively-ranked models.

Model	Δ AIC_c	w_i	No. Parameters
Site	0	0.28	5
Site + Caterpillar Size	1.85	0.11	6
Site + Plant	2.01	0.10	6
Ant + Site	2.03	0.10	6
Site + Plant Size	2.07	0.10	6
Site + Plant + Caterpillar Size	3.81	0.04	7
Ant + Site + Caterpillar Size	3.89	0.04	7
Site + Caterpillar Size + Plant Size	3.97	0.04	7
Ant + Site + Plant	4.05	0.04	7
Site + Plant + Plant Size	4.09	0.04	7
Ant + Site + Plant Size	4.12	0.04	7
Ant + Site + Caterpillar Size + Plant	5.85	0.02	8
Site + Caterpillar Size + Plant + Plant	5.95	0.01	8
Ant + Site + Caterpillar Size + Plant	6.03	0.01	8
Ant + Site + Plant + Plant Size	6.14	0.01	8
Ant + Site + Caterpillar Size + Plant +	8.00	0.01	9
Ant + Caterpillar Size + Plant Size	43.10	0	4
Ant + Plant Size	43.80	0	3
Ant + Caterpillar Size + Plant + Plant	45.12	0	5
Ant + Plant + Plant Size	45.52	0	4
Ant + Caterpillar Size	46.04	0	3
Caterpillar + Plant Size	47.72	0	3
Ant + Caterpillar Size + Plant	48.04	0	4
Ant	48.96	0	2
Plant Size	49.41	0	2
Ant + Caterpillar Size + Plant Size	49.51	0	4
Caterpillar Size	49.96	0	2
Ant + Plant	50.45	0	3
Plant + Plant Size	50.76	0	3
Size Class + Plant	51.74	0	3
Null	54.03	0	1
Plant	55.22	0	2

Table 4: Results from logistic regression analyses of parasitism within sites for 2010. Data is analyzed by plant (the percent of caterpillars parasitized). For each term within each model, estimates of coefficients from logistic regressions are given, as well as standard errors around those estimates and associated *P* values. None of the factors reported below were significant predictors of parasitoid attack.

		Estimate	Std. Error	<i>P</i>
		2010		
WLA				
	Plant Size	-6.995e-6	1.531e-5	0.648
	Ant	0.7853	1.246	0.528
VCP				
	Plant Size	9.563e-7	1.130e-6	0.397
	Ant	0.1765	0.002584	0.995

Table 5: Results from logistic regression analyses of parasitism within sites for 2011. Data is analyzed by individual caterpillar (with the response variable being parasitized or not). For each term within each model, estimates of coefficients from logistic regressions are given, as well as standard errors around those estimates and associated *P* values. None of the factors reported below were significant predictors of parasitoid attack.

		Estimate 2011	Std. Error	<i>P</i>
BHP	Ant	0.6807	0.9699	0.483
	Caterpillar Size	-0.3171	0.4932	0.520
	Plant	-0.06083	0.07330	0.407
	Plant Size	-4.147e-9	9.969e-6	1.000
SLA	Ant	21.28	8821	0.998
	Caterpillar Size	-19.12	4706	0.997
	Plant	0.2795	0.2674	0.296
	Plant Size	-7.058e-6	4.802e-5	0.883
WLA	Ant	-16.17	2400	0.995
	Caterpillar Size	0.3071	0.4552	0.500
	Plant	0.02223	0.08699	0.798
	Plant Size	2.485e-5	2.013e-5	0.217
VCP	Ant	-16.86	3287	0.996
	Caterpillar Size	0.2731	0.7119	0.701
	Plant	0.03677	0.1942	0.850
	Plant Size	-8.336e-6	1.067e-5	0.435

Table 6: Parasitoid abundance across years for the sites with parasitoids. Numbers in parentheses refer to the percent parasitism by the given parasitoid within sites. All are subfamily groupings: Exoristinae (Tachinidae), Campopleginae (Ichneumonidae), Rogadinae (Braconidae) and Microgastrinae (Braconidae).

Site	Exoristinae	Campopleginae	Rogadinae	Microgastrinae
2010				
WLA		4 (15.4%)		
VCP	12 (23.1%)			
2011				
BHP	2 (3.2%)	4 (6.5%)		
SLA			3 (7.3%)	
WLA	6 (18.8%)	12 (37.5%)		3 (9.4%)
VCP	1 (2.9%)	1 (2.9%)		

Table 7: Abundance of ants found tending caterpillars across sites and years with totals and percent of all tending ants given at the bottom. For species names, *F. sp.* = *Formica sp.*

Site	<i>Camponotus vicinus</i>	<i>F. aerata</i>	<i>F. gnava</i>	<i>F. hewitti</i>	<i>F. lepida</i>	<i>F. neoclara</i>	<i>F. oreas</i>	<i>Myrmecina americana</i>	<i>Monomorium minimum</i>	<i>Tapinoma sessile</i>
2010										
BWP (Ac)				2	10					
BWP (Ms)				3	1		33			
SLA		2	2	2	4	3				1
VCP					8		34			
VER					2		15			
WLA				4	2		9		12	
2011										
BHP			2		19		48			
SLA		2	26		5					
VCP					2		11	2		
VER	1				3		17			
WLA			1							
Total across all sites and years										
	1	4	31	11	56	3	167	2	12	1
	0.3%	1.4%	10.8%	3.8%	19.4%	1.0%	60.0	0.7%	4.2%	0.3%
							%			

Figure legends

Figure 1: Map of populations sampled with symbols referring to host use. See Table 1 for exact coordinates of the given populations.

Figure 2: Parasitism rates for 2010 by site (a) and number of plants found with tending ants (b).

Figure 3: Parasitism rates for 2011 by site (a) and tending rates for 2011 (b).

Figure 4: Subfamily diversity of parasitoids across hosts (a) and diversity of ant species found tending across hosts (b). The ant species *Camponotus vicinus* was found on the exotic host and *Tapinoma sessile* was found on a native host.

Fig. 1

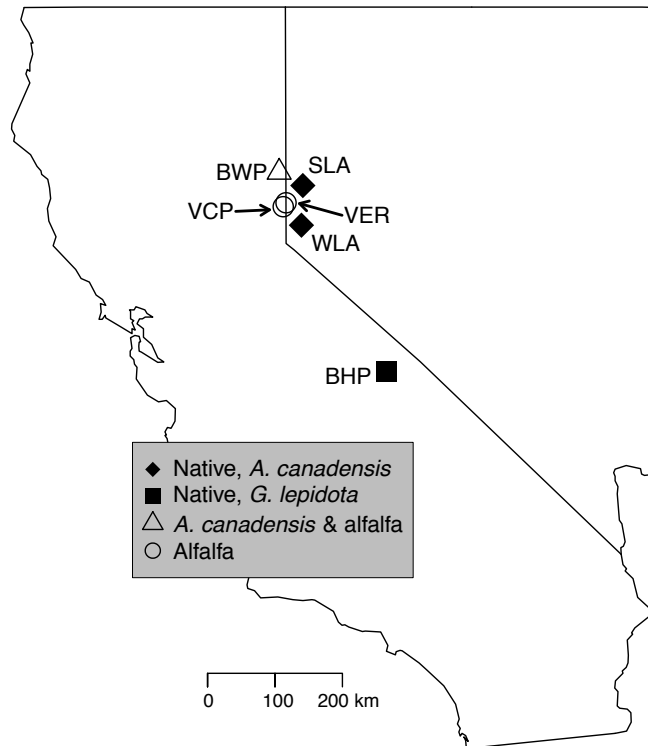


Fig. 2

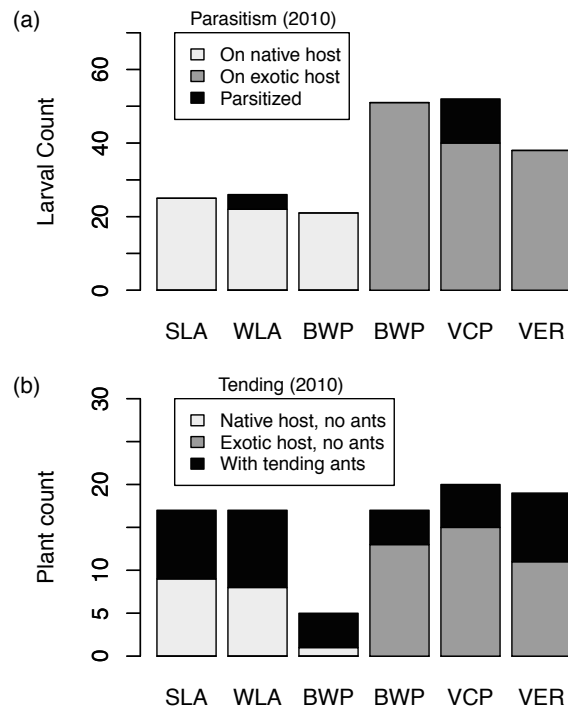


Fig. 3

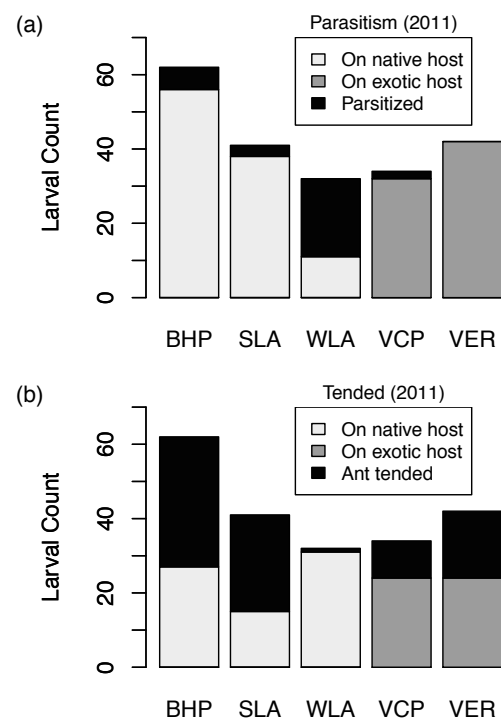
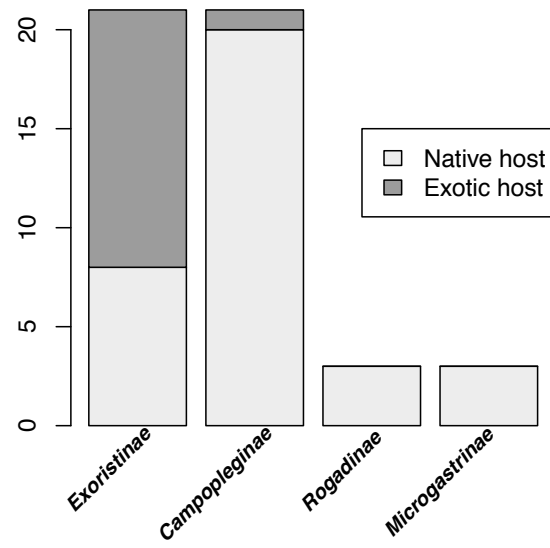


Fig. 4

(a)



(b)

