

Host range evolution is not driven by the optimization of larval performance: the case of *Lycaeides melissa* (Lepidoptera: Lycaenidae) and the colonization of alfalfa

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Abstract Herbivorous insects that have recently incorporated novel hosts into their diet provide unique opportunities for understanding factors that promote or constrain the evolution of niche breadth. *Lycaeides melissa* has colonized both cultivated and feral alfalfa (*Medicago sativa*) throughout much of North America within the past 200 years. We investigated the quality of the novel host as a resource for juvenile development, and asked if the novel host is a preferred host for oviposition relative to a native host (*Astragalus canadensis*). Larval-performance and oviposition-preference were examined using *L. melissa* individuals from a population associated with both *M. sativa* and *A. canadensis*, and oviposition-preference was also examined in another population associated exclusively with *M. sativa*. In addition, we investigated the effects of *M. sativa* and *A. canadensis* flowers on both preference and performance. Only one of the hosts, *M. sativa*, has flowers that are accessible to nectaring butterflies, and we hypothesized that the presence of flowers could affect female

behavior. We find that the novel host is a relatively poor larval resource: adults that were reared as larvae on *M. sativa* were roughly one-third the size of adults that were reared on the native host, *A. canadensis*. The native host, *Astragalus canadensis*, is the preferred host in choice experiments involving only foliage. However, when flowers were included in preference assays, the native and novel hosts received similar numbers of eggs. Thus, the presence of flowers on hosts in the field might influence the utilization of a novel and inferior larval resource. These results are consistent with a model in which host shifts are driven by adult behavior that does not directly optimize larval performance.

Keywords Niche breadth · Niche shift · Preference · Performance · Specialization

Introduction

A fundamental question concerning plant–herbivore interactions is: what determines the host range, or niche breadth, of an herbivorous insect (Brues 1924; Levins and MacArthur 1969)? Based on fossil evidence and patterns in contemporary plant–insect associations, we know that the niche breadth of phytophagous insects can be highly conserved, in that certain lineages of insects retain associations with certain lineages of plants for great spans of time (Ehrlich and Raven 1964; Labandeira et al. 1994; Futuyma and Mitter 1996). While associations are frequently conserved between insect and host lineages, the host range of individual herbivore species can be quite labile (e.g., Hsiao 1978; Singer et al. 1993; Carroll et al. 1997; Feder 1998; Groman and Pellmyr 2000). Shifts in host use most frequently involve closely related plants but can also involve the

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colonization of more distantly related species. For example, approximately one-third of the 236 butterfly species in California have been reported ovipositing on non-native species (Graves and Shapiro 2003), and most of these shifts in host use involve relatively closely related species of plants. However, others shifts are more dramatic, such as the colonization of *Citrus* trees (family Rutaceae) by the butterfly *Papilio zelicaon*, which historically fed on native hosts in the family Apiaceae (Shapiro 1980).

Research on host range evolution most frequently involves investigations of oviposition preference and larval performance (Dethier 1954; Thompson 1988; Jaenike and Holt 1991). At least for species with relatively immobile juveniles, the choices that females make regarding the placement of their eggs determines the environment that their offspring experience during development. A common expectation has been that female preference for different host plant species will evolve to optimize larval performance (Levins and MacArthur 1969; Thompson 1988; Courtney and Kibota 1990; Mayhew 1997). The hypothesis of optimal host choice has received equivocal support (Chew 1977; Courtney and Kibota 1990; Mayhew 2001; Berenbaum and Feeny 2008). Some authors have pointed out that performance has been too narrowly defined and that natural enemies must be included in models of host range evolution (Bernays and Graham 1988; Singer and Stireman 2005). Perspectives from optimal foraging have also emerged as alternatives to the traditional preference/performance framework, in particular the possibility that females make oviposition decisions to maximize their own realized fecundity rather than the performance of their offspring (Scheirs et al. 2000).

Considering these perspectives on host range, we propose three conceptual models with which to investigate host range evolution, specifically the phenomenon in which a novel host is incorporated into the diet of an insect herbivore.

1. In the first model, which we will refer to as the Chemical Similarity model, a novel plant is accepted by ovipositing females if the new host presents phytochemical stimuli that are sufficiently similar to an ancestral or primary host plant (i.e., females respond by laying eggs on the novel plant as if it were their primary host) (Fraenkel 1959; Ehrlich and Raven 1964; Jermy 1984; Murphy and Feeny 2006). Relative to a primary host, the novel plant in this scenario might be a comparable host for larval development, or it might be an inferior larval resource. In the latter scenario, in which females are ovipositing on a host that results in reduced performance relative to native hosts, we would either expect selection to favor larval adaptation to the new host or we would expect female preference to

evolve in such a way that the novel host is not accepted for oviposition (though this outcome would of course be contingent on a number of factors, including available genetic variation for behavior) (Chew 1977; Thompson 1988).

2. In a second model of host range expansion, the Novel Superiority model, a novel plant is initially not highly ranked by ovipositing females (relative to the ancestral host), but is a suitable or even superior host (relative to the ancestral host) for larval development. In this case, selection could lead to a change in preference, such that the novel host would become a preferred host (Thomas et al. 1987; Singer et al. 1993, 2008). Note that the quality of a host as a larval resource in this model (as in the first model) need not be considered narrowly, but could incorporate the presence of natural enemies, as well as competitors and mutualists (Bernays and Graham 1988; Mayhew 1997).
3. Finally, in the Adult Resources model, females oviposit on a novel plant simply because they spend time on or around the plant taking advantage of resources, such as the opportunity to nectar at flowers, not directly related to larval performance (Scheirs et al. 2000; Scheirs and De Bruyn 2002). In this model, a host might be utilized even if it is a relatively poor larval resource.

These three models address a change in host breadth, but do not address specialization on the novel host (i.e., a rejection of the ancestral or primary host after an initial expansion of host range), which requires additional mechanisms, such as trade-offs in performance on the two hosts (Joshi and Thompson 1995) or simply geographic isolation on the novel host followed by a loss of traits associated with the ancestral host. Distinguishing among these three possible models of host range expansion can be most effectively done with an insect species for which a sequence of host colonization can be identified with some certainty.

In this study, we focused on the butterfly *Lycaeides melissa* (W.H. Edwards 1873), which has recently undergone an expansion in niche breadth, such that it can be found throughout much of North America associated with both cultivated and feral alfalfa, *Medicago sativa*, which was introduced to North America approximately 200 years ago (Michaud et al. 1988). Not only has *L. melissa* adopted alfalfa on a large geographic scale, it has done so in many places to the exclusion of native hosts that are still locally available (these include leguminous hosts in the genera *Astragalus*, *Glycyrrhiza*, and *Lupinus*) (Nice and Shapiro 1999). Using a population of *L. melissa* where both *M. sativa* and a native host, *Astragalus canadensis*, are present (our focal population), and a secondary population associated only with *M. sativa*, we asked the following

questions. First, where does the novel host rank in relation to the native host in the preference hierarchies of ovipositing females? Second, how does larval performance on the novel host, *M. sativa*, compare to performance on the native host, *A. canadensis*? The combination of these two questions addresses different facets of the three models proposed above. For example, if the novel host has a high ranking in the preference hierarchies of ovipositing females, but is a poor larval resource, this would be consistent with the Chemical Similarity model. We also tested for the presence of genetic variation in performance, which similarly informs the models described above with respect to the possibility of larval adaptation to a novel host. Finally, we asked: does the presence of flowers on the two host plants at our focal population affect either adult behavior or larval performance? This last question was motivated by field observations: *L. melissa* adults cannot extract nectar from the long corolla of the native host, *A. canadensis*, but readily nectar at the novel host, raising the possibility that the availability of adult resources (as opposed to oviposition sites and larval resources) could influence adult behavior (Grossmueller and Lederhouse 1987; Karban 1997; Brommer and Fred 1999; Adler and Bronstein 2004; Janz 2005).

Materials and methods

Our focal location for this study is in California, on the western edge of the Great Basin, just east of Beckwourth Pass. We hereafter refer to this location (39.78°N, 120.07°W) simply as Beckwourth Pass. Two hosts of *L. melissa* occur at this location: a native host, *Astragalus canadensis*, and feral alfalfa, *Medicago sativa* (the nearest cultivated *M. sativa* is approximately 10 km to the west). The two species occur in close proximity at Beckwourth Pass, easily within the cruising range of *L. melissa*: *Astragalus* is the most abundant host, while isolated patches of *M. sativa* grow commonly within a few meters of the *Astragalus*, and in some cases the plants are immediate neighbors with interdigitated branches. *L. melissa* females have been observed ovipositing on both hosts (authors, personal observations; A.M. Shapiro, personal communication). This *L. melissa* population is somewhat unusual, as it is the only location that we know of where observations have confirmed the use of both the novel host, *M. sativa*, and a sympatric native host.

Collections and experiments were conducted over the course of 2 years: experiments in the first year (2007) addressed the first two experimental questions relating to preference and performance (described above), and experiments in the second year (2008) addressed the effect of flowers on preference and performance. In both years,

females at Beckwourth Pass were collected throughout July and the first 2 weeks of August. Females were collected in the vicinity of both host plants, but a majority (between approximately 50 and 75%) was taken on or near *M. sativa* (where adults are more abundant, nectaring at *M. sativa* flowers). The second population examined was at Verdi, Nevada, approximately 17 km south of Beckwourth Pass. *L. melissa* occurs in Verdi along train tracks in association with feral alfalfa. No known native hosts are present at this location. Females were collected at Verdi for use in preference experiments during the first 2 weeks in August 2007.

Preference experiments

To evaluate the preferences of ovipositing females for the novel host relative to the native host, females from the focal population at Beckwourth Pass, and from Verdi (where only *M. sativa* is present) were exposed to oviposition choice tests. The comparison between the two populations is of interest because it should be informative as to whether or not a population isolated on the novel host (as at Verdi) becomes more specialized in preference for that host (i.e., have Verdi females lost a preference for *A. canadensis*?), as could happen if there were a cost to having a preference for a host that is not present (Rosenheim et al. 2008).

Wild-caught females were confined in arenas consisting of large, opaque, plastic drinking cups covered with bridal veil and containing sprigs of plants, the cut ends of which reached through holes in the bottoms of the cups and into second cups containing water. Gatorade® (fruit punch flavor) was used as an artificial nectar source, applied directly to the bridal veil. Each arena contained three plants: *Astragalus canadensis*, *Medicago sativa*, and *Lotus nevadensis*; the latter plant is the host of a *Lycaeides idas* population on the west slope of the Sierra Nevada, and is used here as a negative control. Plants were stored in a refrigerator at 4°C prior to use in arenas, and plant collections in cold storage were discarded and replaced approximately every 8–10 days while preference experiments were conducted. Preference experiments lasted 48 h, after which time the number of eggs on each host was counted as a measure of preference.

In experimental design, this first set of preference experiments followed previous oviposition-preference experiments in this system (Nice et al. 2002; Gompert et al. 2006), including the exclusion of flowers from experimental arenas. The rationale behind the exclusion of flowers has been that oviposition in the wild is most frequently observed on foliage (authors, personal observations). Furthermore, flowers are ephemeral, not necessarily available simultaneously on different hosts, and thus difficult to standardize in arenas containing multiple hosts.

Data from the three-way choice arenas was analyzed with the Quade test, a rank-based randomized block

analysis of variance (ANOVA) which is appropriate for preference tests in which data points on different hosts are not independent, and is more powerful than the more commonly used Friedman test when the number of treatments is less than five (Conover 1999). Quade tests and subsequent post-hoc comparisons were computed as described in Conover (1999).

Performance experiments

Larvae were reared to address multiple questions, chief among them being: how does larval performance on the novel host, *M. sativa*, compare to larval performance on the native host, *A. canadensis*? Larval rearing experiments also allowed us to investigate both the existence of genetic variation for the ability to use the two hosts and potential trade-offs in larval performance. As with the initial preference experiments, described above, these performance experiments did not include flowers as larval food. To initiate performance experiments, eggs were collected from the oviposition-preference arenas described above, and were stored in Petri dishes until hatching. Family groups (the offspring from individual mothers) were kept separate, and larvae from each family were assigned alternately to the two host plants as they hatched. Neonate larvae were enclosed individually in 1.5-ml microcentrifuge tubes and held in vertical racks at room temperature on a laboratory bench. Standard 100-W bulbs were suspended above the larval rearing area, and kept on a day/night cycle mimicking natural conditions at the time of the experiment. Larvae in microcentrifuge tubes were checked daily, and given new leaves ad libitum (which was roughly every other day in most cases). *A. canadensis* foliage was collected from Beckwourth Pass, but *M. sativa* foliage was taken from an abandoned alfalfa field on the University of Nevada, Reno, campus. *M. sativa* foliage was not collected from the focal location because *M. sativa* is relatively scarce at Beckwourth Pass and we did not want to deplete the population with the collections necessary to support this rearing experiment. *M. sativa* from Beckwourth Pass was used in a second rearing experiment (described below) that used less foliage because larvae were not reared individually.

After approximately 10–14 days, when the larvae were too large for the microcentrifuge tubes, they were transferred to Petri dishes (100 × 15 mm) and given sprigs of plants with the cut ends wrapped in wet Kimtech tissues. Larval weight was recorded at 17 days (on a Mettler Toledo microbalance to the nearest hundredth of a milligram), which is approximately mid-way between hatching and eclosion. When larvae had pupated, they were moved to larger Petri dishes (150 × 25 mm). Upon eclosion, fresh adult weight was recorded to the nearest hundredth of a milligram. Data recorded from rearing also included

development time (number of days from egg hatch to adult eclosion) and survival (fraction of larvae surviving to eclosion from each family). Adult longevity was measured as an additional response variable to test the effect of larval diet on an adult trait. Newly emerged adults were confined to small, clear plastic drinking cups covered with bridal veil, and placed in a climate-controlled greenhouse set to 30°C. Butterflies were fed with Gatorade spread across the bridal veil and dripped onto folded strips of Kimtech tissues draped down the inside of the cups. The number of days that adults survived was recorded as a measure of potential adult longevity.

Data from performance experiments was analyzed with ANOVA using restricted maximum likelihood (REML) mixed models. REML models are more powerful than traditional ANOVAs when data are not balanced, as often happens in experiments with family structure (since survival is rarely homogenous across families) (Littell et al. 1996). In our models, family (all the progeny from one female) and interactions with family were treated as random factors, and fixed factors included host (plant on which larvae were reared) and sex. The family by host interaction was not included in models analyzing survival, as each family was only represented by a single value (the fraction surviving) on each host. JMP-IN software, version 7.0. (SAS Institute 2007), was used for these analyses.

In studies of larval performance, the assumption is typically made, but not tested, that adult size is a correlate of fitness, with the expectation being that larger adults will live longer, have greater mating success, and lay more eggs (Awmack and Leather 2002). We have addressed two aspects of adult performance. First, we measured longevity in the experiment described above. Second, we addressed the relationship between adult size and fecundity, by studying the relationship between the size of wild-caught females and the number of eggs laid in the 48-h preference arenas. As a measure of adult size, we used abdomen length, which has a significant linear relationship to fresh adult weight, one of the response variables in our performance experiments. The Pearson product-moment correlation between abdomen length and fresh adult weight is 0.85 ($n = 77$, $P < 0.0001$), a relationship based on measurements from 51 laboratory-reared females. We also studied, for a subset of females, the relationship between the number of eggs laid and egg size (diameter, measured to the nearest hundredth of a millimeter under a dissecting microscope).

Preference and performance with and without flowers

Only one of the hosts, *M. sativa*, has flowers that are accessible to *L. melissa* adults. Because nectar availability has been shown to influence herbivory and oviposition in other systems (e.g., Adler and Bronstein 2004), we wished to

address the possibility that the presence of flowers could affect both adult behavior and larval performance. To this end, larvae were reared both with and without flowers on both hosts, and female preferences were assayed both with and without flowers on both hosts. For the preference tests, similar arenas as described above were used, with the exception that this round of tests consisted of four-way, no-choice arenas. These were simultaneous, no-choice tests, in which a group of females collected from the field was divided evenly into one of four treatments: *A. canadensis* with flowers, *A. canadensis* without flowers, *M. sativa* with flowers, and *M. sativa* without flowers. A roughly similar biomass of plant was included in each arena (arenas with flowers, therefore, had proportionally less foliage than arenas without flowers). The number of eggs after 48 h was recorded as the oviposition response in each cage. No-choice tests were used primarily because we felt that the interdigitation of stems in a choice arena would not be an effective way to compare the same host species with and without flowers. No-choice tests also provide an informative comparison with choice tests (Singer et al. 1992).

A performance experiment involving flowers was similarly conducted with four treatments, both hosts, with and without flowers. For these trials, larvae were reared without family structure in groups of five (i.e. larvae from multiple females were pooled). Larvae in the flower treatments were kept for their first 10 days only on the foliage of their respective plants (for example, the larvae assigned to the *M. sativa* + flowers treatment were reared for their first 10 days on *M. sativa* foliage before being fed flowers). This was a practical necessity, since the earliest instars are difficult to retrieve from flowers when the plant material in a Petri dish needs to be changed. Subsequently, larvae in flower treatments were fed only flowers with no foliage included in dishes. Being restricted to flowers is not an unrealistic scenario, as larvae on both hosts will readily consume flowers in favor of foliage in a rearing dish containing both (*M. Forister*, personal observations). As before, survival, days to pupation, and fresh adult weight were recorded as measures of performance (values from each rearing group were averaged prior to analysis). *A. canadensis* and *M. sativa* foliage and flowers for these experiments (preference and performance) were collected from the field at our focal location.

Both preference and performance experiments involving plants with and without flowers were analyzed using ANOVA with two fully-crossed factors: flowers (present or absent) and host (*A. canadensis* or *M. sativa*). This analysis allowed us to explicitly test for an interaction between the presence of flowers and patterns of preference and performance. Prior to analysis, a square root transformation was used on the preference data (the number of eggs laid in no-choice arenas), and survival data was arcsine transformed. JMP-IN software, version 7.0. (SAS Institute 2007), was

used for these analyses. Data from the no-choice preference arenas involving flowers were further analyzed with Wilcoxon signed-rank tests (the distribution of eggs for this test was not appropriate for a parametric test) to ask if a greater number of eggs were laid on flowers or on foliage when both were available on the same stem of plant in a single arena (the location of eggs was noted when recording data from these trials).

Results

Preference

Forty-three females from Beckwourth Pass were confined individually to preference arenas containing foliage of both hosts and a negative control, and 37 females laid eggs. A clear preference hierarchy was evident, with *A. canadensis* ranked first, *M. sativa* second, and *L. nevadensis* third (Fig. 1a). Twenty-four females from the alfalfa-only population at Verdi were confined in arenas, and all 24 laid eggs. These females exhibited a qualitatively identical preference hierarchy to Beckwourth Pass females (Fig. 1b), preferring *A. canadensis* to *M. sativa*, which is in turn ranked higher than *L. nevadensis*, the negative control. We assume that the oviposition preferences we observed are heritable, as has been most frequently observed in other systems, and not directly influenced by larval experience (Jaenike 1990; Thompson and Pellmyr 1991; but see Barron 2001). We did test the preferences of seven laboratory-reared females, four of which were reared on *M. sativa*, and the other three on *A. canadensis*. *A. canadensis* was the preferred host in all cases (*M. Forister*, unpublished data), as with wild-caught females.

Performance

A total of 270 larvae from 16 wild-caught females were reared on foliage of the two experimental host plants, *M. sativa* and *A. canadensis*. A total of 106 individuals were reared successfully to eclosion as adults. The generally low survival was due primarily to high mortality in the first instar. If larvae wander off of the host plants within the first few days after hatching, they are often found dead in the rearing container, even in the small microcentrifuge tubes used in this experiment. The host on which larvae were reared had a significant effect on two aspects of performance: survival and adult weight (Fig. 2; Tables 1 and 2). Survival on *M. sativa* in the first half of development was an average of 42% higher than survival on *A. canadensis* (Fig. 2a; Table 1a). This difference in survival was, however, reversed in the second half of development, when survival on *M. sativa* was significantly lower than on

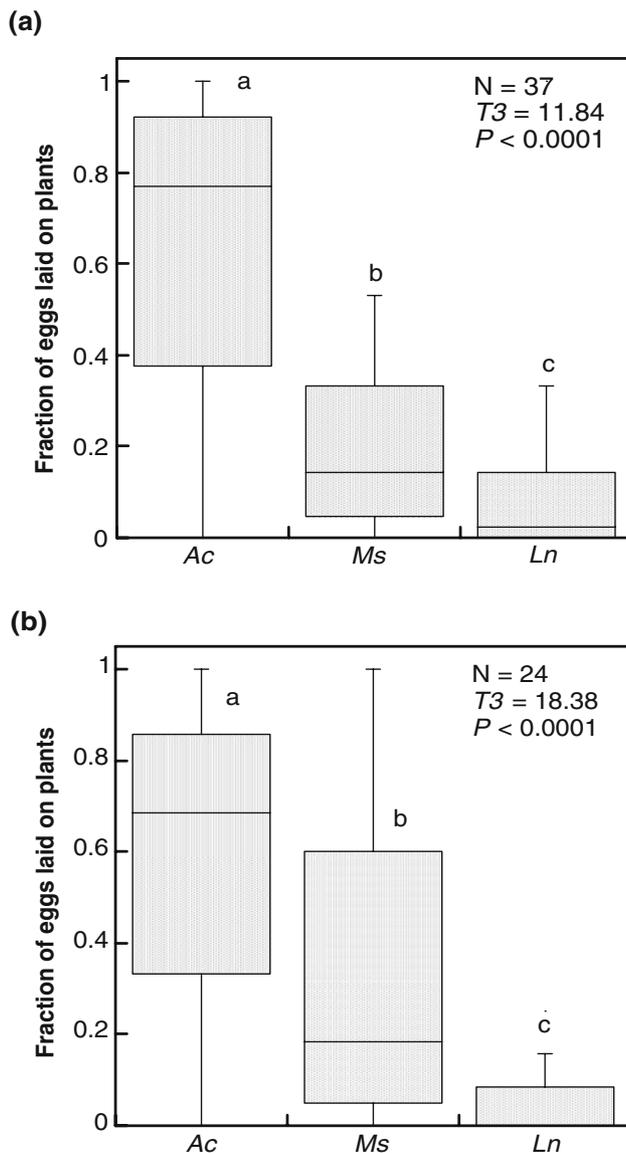


Fig. 1 Oviposition preferences expressed by females in three-way choice experiments. Box plots show the median number of eggs laid on three hosts: *Ac* (*Astragalus canadensis*), *Ms* (*Medicago sativa*), and *Ln* (*Lotus nevadensis*). Results reported in the upper-right corner of each graph are from nonparametric Quade tests, and lower case letters above bars indicate independent contrasts significant at $P < 0.05$. **a** Preferences of focal population (Beckwourth Pass) females; **b** preferences of females from a population (Verdi, NV) associated with a single host, *M. sativa*

A. canadensis (Table 1b). When survival was measured across both these intervals, from hatching to eclosion, no difference in survival was detected between the two hosts (Fig. 2b; Table 1c). For individuals that did survive to eclosion, *A. canadensis* was the superior host: individuals reared on *A. canadensis* were on average almost three times the weight of individuals reared on *M. sativa* (Fig. 2d). This variation in performance, expressed as adult weight, could have a direct relationship to fecundity, as we found a

positive relationship between the size of wild-caught females and the number of eggs laid in 48-h preference assays: (Spearman's $\rho = 0.40$, $P = 0.014$, $n = 37$). At least in the short span of a preference assay, larger females do lay more eggs. The greater number of eggs laid by larger females was not associated with a reduction in egg size (Spearman's ρ between egg diameter and the number of eggs laid = -0.04 , $P = 0.85$, $n = 21$).

We detected no effect of host plant on either development time (Fig. 2c; Table 2) or longevity ($F_{1,7.6} = 0.35$, $P = 0.57$). Results from an analysis of larval weights taken mid-development (on the 17th day) were qualitatively similar to results from fresh adult weights compared across hosts: the average mid-development weight on *A. canadensis* was $9.8 \text{ mg} \pm 1.3 \text{ SE}$, and on *M. sativa* the average weight was $6.7 \text{ mg} \pm 0.78$ ($F_{1,8} = 6.0$, $P = 0.04$). Significant differences among families were observed in mid-development survival, but not in survival to eclosion, development time, or adult weight (Tables 1 and 2). We found no significant host by family interactions, which is consistent with an absence of trade-offs in the ability of different families to utilize the two hosts (i.e. variation among families was in overall performance that was not host-specific) (Fry 1993).

The effect of flowers on preference and performance

The presence of flowers on both *A. canadensis* and *M. sativa* had a large influence on adult preference and larval performance under experimental conditions. A total of 64 wild-caught females from Beckwourth Pass were confined in no-choice preference arenas. As seen in Fig. 3a, the two hosts received similar numbers of eggs in no-choice arenas with flowers. The significant interaction between host type (*A. canadensis* or *M. sativa*) and the presence or absence of flowers is reported in the legend for Fig. 3, and the individual effects are as follows: without flowers, the difference in the number of eggs laid on the two hosts is significant ($F_{1,30} = 27.26$, $P < 0.0001$), which is consistent with results from foliage-only choice tests reported above (Fig. 1); with flowers present, the number of eggs did not differ on the two hosts ($F_{1,30} = 0.084$, $P = 0.77$).

The effect of flowers on the oviposition behavior of females could be explained by a number of factors, including the presence of deterrent compounds in *A. canadensis* flowers or the possibility that *A. canadensis* flowers do not have the same phytochemical cues as the foliage on which they will readily oviposit. These possibilities are at least partly supported by the distribution of eggs within the no-choice arenas involving flowers. In cages with *M. sativa* containing flowers, eggs were laid on both flowers and foliage in similar numbers: a mean of $8.06 (\pm 2.6 \text{ SE})$ eggs on flowers, and a mean of $5.88 (\pm 1.7)$ eggs on foliage

Fig. 2 Aspects of performance for individuals reared on the two hosts. *Ac* (*Astragalus canadensis*), *Ms* (*Medicago sativa*). See Tables 1 and 2 for ANOVA results. Error bars represent standard errors. A single asterisk corresponds to significance at $P < 0.05$; three asterisks correspond to $P < 0.0001$

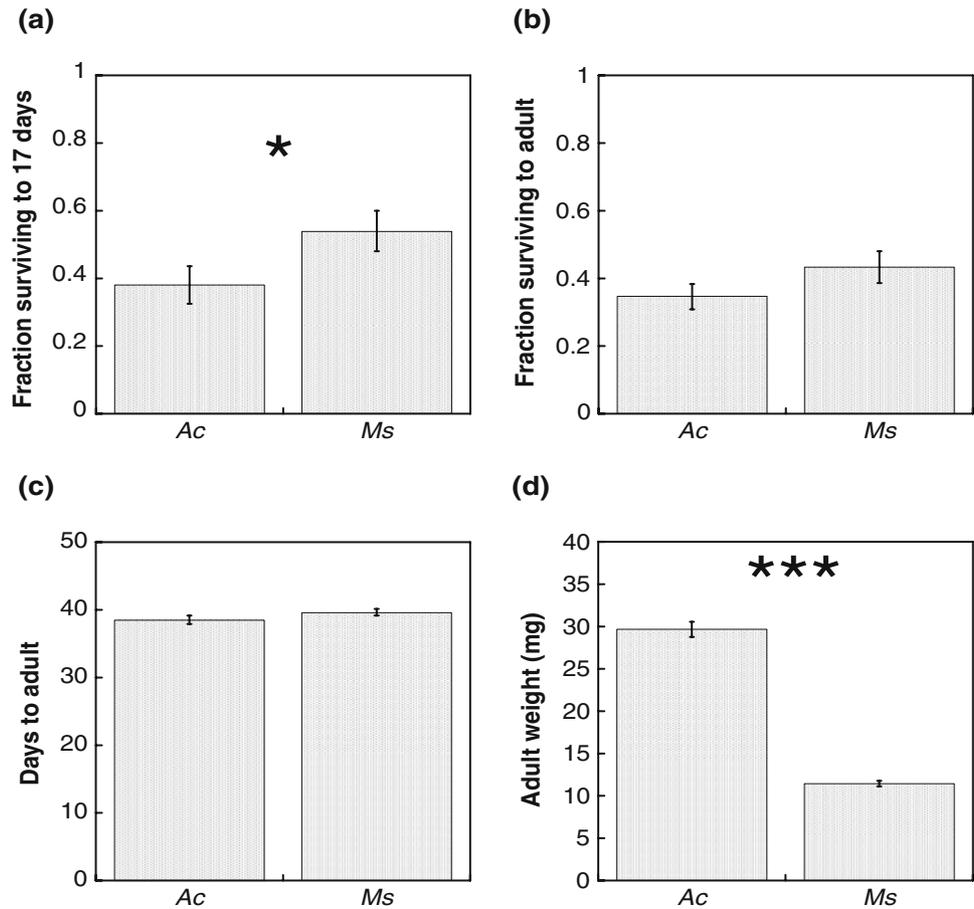


Table 1 Results from analyses of survival at three time intervals

Source	df	F	P
(a) 17-day survival			
Host	1, 15	8.43	0.011
	Covariance	SE	P
Family	0.057	0.028	0.0002
(b) Survival from 17 days to adult			
Host	1, 12.16	5.26	0.043
	Covariance	SE	P
Family	0	0.09	0.24
(c) Survival to adult			
Host	1, 15	0.016	0.9
	Covariance	SE	P
Family	0.064	0.032	0.12

The fraction of larvae surviving from each family was arcsine transformed. Degrees of freedom and *F* ratios are reported for fixed effects, covariance estimates and standard errors for random effects. Significant *P* values are shown in bold text

Table 2 Results from analyses of development time (number of days to adult) and adult weights, which were log transformed

Source	df	F	P
(a) Development time			
Host	1, 9.10	0.44	0.52
Sex	1, 84.28	2.19	0.14
	Covariance	SE	P
Family	0.97	1.31	0.15
Family × host	0	1.1	0.49
(b) Adult weight			
Host	1, 8.59	444.76	<0.0001
Sex	1, 96.03	7.53	0.0073
	Covariance	SE	P
Family	0.0021	0.0038	0.099
Family × host	0.0020	0.0049	0.31

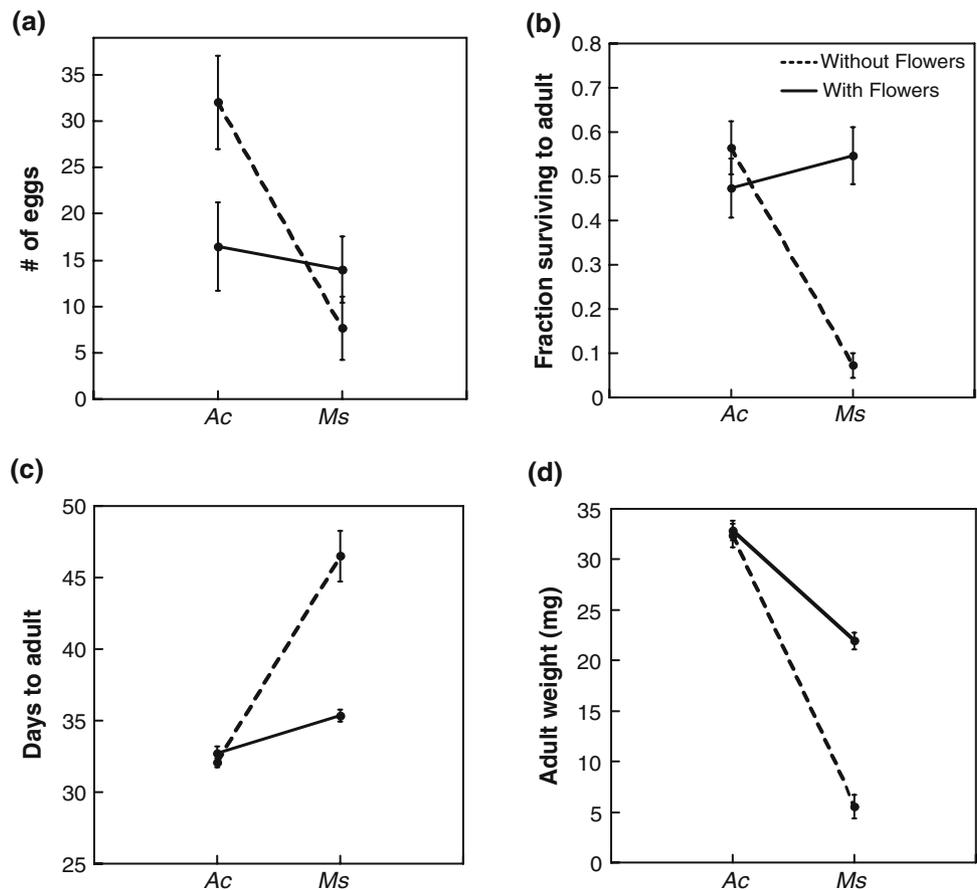
Degrees of freedom and *F* ratios are reported for fixed effects, covariance estimates and standard errors for random effects. Significant *P* values are shown in bold text

($T = 1.2, P = 0.23$). In contrast, in *A. canadensis* cages with flowers, significantly more eggs were laid on foliage: a mean of 2.68 (± 1.1) eggs on flowers, and a mean of 13.8 (± 4.6) eggs on foliage ($T = -2.18, P = 0.029$). *L. melissa*

females in oviposition cages will both walk and fly between the plants and the walls and the mesh at the top of the cages. Females in cages with *A. canadensis* flowers were observed to spend more time at the top of the cages and

Fig. 3 Patterns of preference and performance both with flowers (solid lines) and without flowers (dashed lines) on the two hosts. The interaction between host type and the presence or absence of flowers is significant in each case, as follows:

- a** $F_{1,60} = 9.14$, $P = 0.0037$;
b $F_{1,76} = 24.93$, $P < 0.0001$;
c $F_{1,57} = 87.96$, $P < 0.0001$;
d $F_{1,58} = 42.3$, $P < 0.0001$



away from the plants (*M. Forister*, personal observation). In many instances, females were observed landing on *A. canadensis* flowers and then flying back to the tops of their cages without spending any time on the plants. In contrast, when females in cages with *M. sativa* flowers landed on the flowers, they were often observed to probe flowers in search of nectar and then proceed to walk around the flowers and down onto the foliage of the plant.

Flowers also affected the performance of larvae. A total of 168 larvae, the offspring of approximately 25 females from preference tests, were reared in groups of five (each group consisted of the offspring of multiple females) on both hosts with and without flowers. The interaction between host type (*A. canadensis* or *M. sativa*) and the presence or absence of flowers was significant for all aspects of performance measured (survival to adult, development time, and adult weight) (see Fig. 3 legend for statistical details from interactions). As shown in Fig. 3b–d, the interaction between host and the presence or absence of flowers was driven by *M. sativa*, specifically the ameliorating effect of *M. sativa* flowers on the performance of larvae reared on that host. Larvae reared on *M. sativa* flowers had higher survival, shorter development time, and greater adult weight than larvae reared on *M. sativa* foliage. Larvae reared on *A. canadensis* flowers did not have significantly

different performance than larvae reared on *A. canadensis* foliage. For two of the three aspects of performance, development time and adult weight, the mean values for larvae reared on *A. canadensis* foliage and *A. canadensis* flowers were nearly identical; survival was slightly (but not significantly) lower for larvae reared on *A. canadensis* flowers as compared to *A. canadensis* foliage ($F_{1,36} = 1.01$, $P = 0.32$).

Despite the improved performance of larvae on *M. sativa* flowers compared to larvae on *M. sativa* foliage, *A. canadensis* was still the superior host in terms of development time and adult weight (Fig. 3). Comparing the performance of larvae reared on *M. sativa* flowers and *A. canadensis* flowers, survival was not significantly different ($F_{1,37} = 1.43$, $P = 0.24$), but development was significantly slower ($F_{1,35} = 15.1$, $P = 0.0004$), and adult weight was significantly less for larvae reared on *M. sativa* flowers ($F_{1,35} = 71.57$, $P < 0.0001$).

Considering only performance on foliage, it is worth noting the difference between the results shown in Figs. 2 and 3. As in the first experiment (Fig. 2d), average adult weight in the second experiment was less for individuals reared on *M. sativa* foliage relative to *A. canadensis* foliage (Fig. 3d). However, in the second experiment, individuals reared on *M. sativa* also had longer development times and considerably lower survival relative to individ-

uals reared on *A. canadensis*. Survival to adult for larvae reared on *M. sativa* foliage as shown in Fig. 3b is a mean (across rearing groups) of 7.3% ($\pm 2.7\%$ SE); compare with Fig. 2b where survival on *M. sativa* (averaged across families) is 43.3% ($\pm 4.6\%$). These two experiments differ in that they were conducted in different years, and larvae were reared in groups in the second experiment. In addition, *M. sativa* foliage for the second experiment (Fig. 3) was collected from the field at our focal location.

Discussion

For species in which adults and juveniles consume different resources, changes in resource or habitat use might result from changes in behavior that maximize the performance of either adults, juveniles, or both (Futuyma and Moreno 1988; Wackers et al. 2007). For herbivorous insects where females choose the plant that their offspring will consume, much empirical and conceptual research has focused on the possibility that changes in niche breadth are driven by optimal host choice that maximizes larval performance (Castillo-Chavez et al. 1988; Jaenike 1990; Mayhew 1997; Berenbaum and Feeny 2008). An alternative hypothesis is that host choice might be better explained by optimal foraging rather than optimal host choice; in other words, host choice is affected by resources (such as nectar) utilized directly by adults, even at the expense of offspring performance (Scheirs et al. 2000; Mayhew 2001; Scheirs and De Bruyn 2002). The utilization of *M. sativa* by *L. melissa* does not appear to be consistent with the hypothesis that host choice evolves to optimize larval performance. *M. sativa* is a poor resource for larvae relative to the native host *A. canadensis*. Individuals reared on the novel host reach considerably smaller adult weights than individuals reared on the native host (Fig. 2d), and adult weight appears to be correlated with potential fecundity, at least with the wild-caught females tested in preference arenas. When larvae were fed *M. sativa* foliage collected from Beckwourth Pass, survival was reduced by 87% relative to survival on *A. canadensis* foliage (Fig. 3b). Furthermore, we did not find any host-specific differences in the performance of different families (Table 2), suggesting that (at least for the pool of full-sibling, family variation assayed here) genetic variation for improved performance on the novel host is not detectable in our focal population.

Why is *M. sativa*, the novel host, utilized by ovipositing females in the field at Beckwourth Pass? When exposed to the foliage of both the novel and native hosts in choice tests, females have a clear preference hierarchy which places the native host above *M. sativa*, and this was true

both for females from our focal population where both hosts are present (Fig. 1a) and for females collected from a second location where only *M. sativa* is present (Fig. 1b). The preferences of ovipositing females are, however, influenced by the presence of flowers. In no-choice cages with and without flowers, both the native and the novel hosts received roughly equal numbers of eggs when flowers were present (Fig. 3a). In part, females in oviposition arenas might spend more time on *M. sativa* plants when flowers are present (they were observed to probe *M. sativa* flowers for nectar and then investigate foliage for oviposition). However, the interaction shown in Fig. 3a appears to be primarily driven not by increased oviposition on *M. sativa* in the presence of flowers but by reduced oviposition in cages with *A. canadensis* flowers. Although these behaviors were expressed in small arenas, these results suggest that the presence of flowers in the field has the potential to affect adult behavior. At Beckwourth Pass, *M. sativa* tends to flower earlier in the spring, and then continuously throughout the summer and into the early fall. *A. canadensis* begins flowering later in the spring and supports a dense cover of flowers throughout the summer. With the exception of a brief window in early spring when foliage for both hosts is available but only *M. sativa* is flowering (M. Forister, personal observation), the flowering phenology for the two hosts largely overlaps.

We have suggested three models that might be useful for investigating expansions of host range: Chemical Similarity (a novel plant is accepted by ovipositing females if the plant presents phytochemical stimuli similar to an ancestral or native host), Novel Superiority (a novel plant is a suitable or superior larval resource and oviposition behavior evolves to encompass this host), and Adult Resources (females oviposit on a novel plant for reasons not directly related to larval performance). We can reject the Novel Superiority model for the expansion of *L. melissa* host range at Beckwourth Pass. The Chemical Similarity model can probably be rejected as well, at least when considering foliage (which, at least for the native host, is the primary target for oviposition). Our results are consistent with the third model, Adult Resources, although the mechanism involved in the reduction of oviposition in the presence of *A. canadensis* flowers is unknown. Possible explanations might include a lack of oviposition-associated phytochemical cues in *A. canadensis* flowers, or the presence of deterrent compounds. In any event, the reduced oviposition in the presence of *A. canadensis* flowers does not appear to involve an optimization of larval performance, which is similar for larvae reared on *A. canadensis* foliage and flowers (Fig. 3).

An interesting comparison to our findings is work by Tabashnik (1983), who studied the colonization of *M. sativa* by the butterfly *Colias philodice*, and discovered

a pattern of preference and performance which was more consistent with the Chemical Similarity model of host range expansion. Both *C. philodice* populations associated with *M. sativa* and populations associated with a native host readily oviposited on the novel host. The populations differed in that *M. sativa*-associated populations showed evidence of larval adaptation to the novel host. Karowe (1990), using the same butterfly, *C. philodice*, investigated barriers to the colonization of another novel host, *Coronilla varia*, which the butterfly encounters but is not known to utilize in the field. The novel host was found to be a suitable and even superior larval resource, relative to the primary host. Karowe (1990) hypothesized that variation for oviposition preference could be favored in the field, leading to an expansion of host range which would correspond to our Novel Superiority model.

There are a number of potential ecological facets to the expansion of *L. melissa* host range that have yet to be investigated, including competition for oviposition sites, the presence of mutualistic ants, and escape from predation or parasitism (Jeffries and Lawton 1984; Mayhew 1997; Murphy 2004; Moon and Stiling 2006). Competition for oviposition sites is an unlikely explanation for the use of the novel host, as *A. canadensis* plants are quite numerous compared to *M. sativa* plants at Beckwourth Pass (though in many other locations, of course, cultivated alfalfa is an abundant host). The presence of mutualistic ants is an interesting possibility. Ants at Beckwourth Pass tend not only *L. melissa* larvae on *M. sativa*, but also aphids and leafhoppers. Thus, it is possible that a concentration of resources draws ants to alfalfa, and so makes it a more suitable host for *L. melissa* (Axen and Pierce 1998). The presence of beneficial attending ants has been shown to affect the oviposition behavior of other Lycaenid butterflies (Atsatt 1981; Fraser et al. 2002; but see Thomas and Elmes 2001). We have no insight at this time into the possibility that escape from predation or parasitism affects host use at Beckwourth Pass, though these mechanisms have been observed in other herbivorous insects (Fox and Eisenbach 1992; Gratton and Welter 1999; Murphy 2004). In summary, a careful consideration of larval performance and adult behavior in this system can tentatively rule out certain models of host range expansion while raising many questions and highlighting potential ecological interactions that are not frequently studied within the context of host range evolution.

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