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Fitness consequences of host plant choice: a field experiment

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Determining the relative contributions of different ecological factors for herbivore fitness is one key to understanding the ecology and evolution of host plant choice by herbivores. Natural enemies are increasingly being recognized as an important factor: host plants of inferior quality for development may still be used by herbivores if they provide enemy-free space (EFS). Here we used the tobacco hornworm, *Manduca sexta*, to experimentally disentangle the effects of natural enemies from the potentially confounding factors of host plant quality, competition and microhabitat. We explored the consequences for both individual components of fitness and total fitness of *M. sexta* feeding on a typical high quality host plant, tobacco *Nicotiana tabacum* and a novel, low quality host plant, devil's claw *Proboscidea louisianica* in an experimental field environment in the presence of a parasitoid natural enemy, *Cotesia congregata*. Although early larval survival, development and growth rates, final body size and fecundity were all reduced for *M. sexta* feeding on devil's claw, a high rate of parasitism on tobacco and an absence of parasitism on devil's claw contributed to similar total fitness (net reproductive rate, R_0) across the two host plant species. Our results suggest *M. sexta* has adopted a novel host plant (devil's claw) outside its typical host range because this host plant provides enemy free space. In addition, oviposition behavior of adult female *M. sexta* appears to be well suited to exploiting the enemy-free space on devil's claw; oviposition by *M. sexta* on devil's claw appears to correspond with seasonal variation in parasitoid abundance.

Many physiological and ecological factors can affect the fitness of herbivores feeding on different host plants (Brower 1958, Hairston et al. 1960, Ehrlich and Raven 1964, Price et al. 1980, Mitter and Farrell 1991, Becerra 1997). Nutritional quality, secondary metabolites and phenology of host plants can all affect whether a particular host plant species is used by an herbivorous insect population or species (Scriber and Slansky 1981, Wood and Keese 1990, Cornell and Hawkins 2003). Microhabitat differences between host plants can alter plant growth, nutritional quality and phenology. The abundance and species diversity of herbivores may differ across host plants, altering the fitness of herbivores of particular interest. Differences in natural enemy attack on different host plants can potentially impact the success of an herbivore on those plants (Bernays and Graham 1988, Dicke 1994). Determining the relative contributions of these different factors for herbivore fitness is one key to understanding the ecology and evolution of host plant choice by herbivores (Thompson 1988a).

The interaction between natural enemy attack and host plant quality has recently been of particular interest, because of its potential for increasing the breadth of host plants use by herbivores. For example, host plants of inferior quality may still be used by some herbivores because they provide enemy free space (EFS), in which the habitat or other characteristics of a species reduces its vulnerability to important natural enemies (Jeffries and Lawton 1984). To assess the importance of EFS in determining host plant use, it is necessary to disentangle the effects of natural enemies from host plant quality, competition, microhabitat and other potentially confounding factors (Berdegue et al. 1996, Murphy 2004, Singer et al. 2004a, 2004b). In addition, it is important to quantify the impact of host plant use on total fitness (e.g. intrinsic rate of increase (r) or net reproductive rate (R_0)), not just for individual components of fitness (e.g. survival or development rate) (Berdegue et al. 1996). This is because there may be tradeoffs or interactions among fitness components, such that individual components may not be strongly correlated with total fitness (Thompson 1988b). Although these factors have been recognized as critical components in examining the role of EFS in host plant use (Berdegue et al. 1996), only a few previous studies have controlled for major confounding explanatory variables to EFS (Murphy 2004), and none have obtained direct estimates of total fitness (e.g. R_0).

The tobacco hornworm, *Manduca sexta*, has been an important study system for exploring insect herbivory and host plant use (Yamamoto and Fraenkel 1960, del Campo et al. 2001, Kester et al. 2002, Mira and Bernays 2002). This insect is largely restricted to feeding on host plants in the Solanaceae (Yamamoto and Fraenkel 1960). Recently, however, *M. sexta* was found to use a non-solanaceous host, devil's claw (*Proboscidea* spp., Martyniaceae), even when it co-occurs with solanaceous host plants, in the southwestern US (Mechaber and Hildebrand 2000, Mira and Bernays 2002).

Laboratory experiments and field observations with *M. sexta* suggest that survival and growth are significantly reduced on devil's claw, *Proboscidea* spp., compared with typical solanaceous host plant species (Mira and Bernays 2002, Diamond et al. unpubl.).

Why then is devil's claw used as a host plant given the negative performance consequences that result from feeding on this non-solanaceous host plant? An observational field study of southwestern US (Arizona) populations of *M. sexta* feeding on a typical solanaceous host plant species, *Datura wrightii,* and a species of devil's claw, *Proboscidea parviflora,* suggests the use of devil's claw may be driven by enemy escape, as fewer predators and parasites were found on this host plant (Mira and Bernays 2002). In the field, survival to the final larval instar was greater on *P. parviflora* in association with decreased natural enemy attack on this host, despite high mortality of eggs and early instar larvae not attributable to natural enemies. Observational field studies take advantage of the natural variation in environmental factors to identify important ecological patterns in the field, in this case the positive association between host plant quality and natural enemy attack (Mira and Bernays 2002). Field experiments can complement observational studies by disentangling environmental factors that may potentially covary in nature, and allow us to distinguish the effects of natural enemies and host plant quality from competition, microhabitat and other factors affecting host use.

Here we describe a field experiment that examines the role of EFS in the adoption of a non-solanaceous host plant devil's claw, *P. louisianica* (a close relative of *P. parviflora*; Bretting 1981), by a southeastern US (North Carolina) population of *M. sexta* which typically feeds on solanaceous host plants such as tobacco. Our experimental design allowed us to exclude food limitation, herbivore competition and microhabitat variation as potential confounding factors, and to quantify the impact of host plant quality on herbivore success. In particular, we assessed both major fitness components (survival, growth rate, development rate, final body size and fecundity) and total fitness (net reproductive rate) for individuals reared on tobacco and devil's claw. Finally, to assess the ability of *M. sexta* to take advantage of a potential enemy free space on devil's claw, we monitored levels of oviposition by *M. sexta* on these host plants before and after our field parasitism experiment (oviposition could not be measured during the experiment).

The major goal of this study was to test whether the adoption of a novel host plant by an herbivore was driven by EFS. If the herbivore, *M. sexta* has adopted the novel host plant, devil's claw, as a result of EFS, we should expect: 1) natural enemies significantly affect fitness in *M. sexta*, demonstrating natural enemies are important agents of natural selection in this system; 2) total fitness and fitness components of *M. sexta* are lower on devil's claw relative to tobacco for individuals not attacked by natural enemies, demonstrating devil's claw is intrinsically inferior in diet quality; 3) total fitness of all *M. sexta* (those attacked and not attacked by natural enemies) is at least comparable across devil's claw and tobacco, demonstrating escape from natural enemy attack offsets the intrinsically inferior diet quality of devil's claw. Our results indicate that although *M. sexta* reared on devil's experience severe reductions in fitness not attributable to natural enemies, this novel host plant provides escape from natural enemies for *M. sexta*. The tradeoff between plant quality and natural enemy attack resulted in similar total fitness for *M. sexta* reared on tobacco and devil's claw, suggesting *M. sexta* has adopted devil's claw as a host plant because it provides enemy-free space.

Material and methods

Study organisms

Manduca sexta (Sphingidae) is distributed across tropical and temperature regions of the Nearctic (Rothschild and Jordan 1903). Larval feeding is generally restricted to plants in the Solanaceae, but recently *M. sexta* has been documented to use non-solanaceous host plants belonging to the family Martyniaceae, in the southwestern US (Mechaber and Hildebrand 2000, Mira and Bernays 2002). These plants (*Proboscidea* spp.) are commonly referred to as devil's claw, and are native to the southwest, but have been introduced to other locations across the US via contaminated crop seed and have escaped from gardens where they are grown as ornamentals. One species of devil's claw, *P. louisianica*, has been naturalized to the southeastern US (Small 1903). *Proboscidea louisianica* is patchily distributed across North Carolina, compared to the predominant local solanaceous host, tobacco (*Nicotiana tabacum*) which is widely cultivated across the state (Radford et al. 1968). Despite the limited distribution of devil's claw in North Carolina, our observations indicate field populations of *M. sexta* oviposit and feed on this host when grown adjacent to tobacco plants.

Here we consider devil's claw a novel host plant for *M. sexta* relative to tobacco. Although cultivated tobacco, *Nicotiana tabacum*, may be considered somewhat novel because of its likely hybrid origins (Ren and Timko 2001), wild *Nicotiana* spp. and many other members of the Solanaceae are typical host plants for *M. sexta* (Yamamoto and Fraenkel 1960). Devil's claw, a native species to the southwestern US, is a relatively recent introduction (approximately 100 years ago) to the southeastern US (Small 1903). In contrast, tobacco has been cultivated in the area for at least several hundred years, and wild *Nicotiana* is native to the region (Radford et al. 1968).

An important source of mortality for *M. sexta* is the braconid wasp *Cotesia congregata*, which in North Carolina can parasitize more than 90% of *M. sexta* larvae during their second generation (*M. sexta* is bivoltine in North Carolina; Rabb 1971). *Cotesia congregata* is a gregarious larval endoparasitoid of several species of sphingid moths including *M. sexta* (Krombein et al. 1979, Kester and Barbosa 1994). This parasitoid prefers to oviposit into third instar *M. sexta* larvae (Barbosa et al. 1991), although larvae at earlier and later developmental stages can also be parasitized (Alleyne and Beckage 1997). Like all parasitoids, *C. congregata* is lethal to its host; all successfully parasitized *M. sexta* larvae die before pupation.

Field experiment

We established a 12 × 20 m field plot at the Mason Farm Biological Reserve (Chapel Hill, NC). The plot was planted

with 30 tobacco and 30 devil's claw plants that were randomized to grid locations spaced 2 m apart, to control for microenvironmental variation. Tobacco and devil's claw plants were grown from seed in a greenhouse for three weeks before being transplanted to the field site in mid-July. Greenhouse plants were fertilized weekly with Peter's Pro Solution (15-16-17); no additional fertilizer was provided after being transferred to the field site. The plants were allowed to grow to a large size (approximately 1.25 m in height for tobacco and 0.75 m in height for devil's claw, as most of its growth is lateral) before *M. sexta* larvae were transferred to the plants. This was to ensure *M. sexta* larvae were not subject to food limitation or competition for food on either host plant species.

Other herbivores were removed from the plants every 1–4 days to maintain ample leaf material for the experimental *M. sexta* larvae and to reduce the production of induced defenses that would reduce leaf quality prior to the start of the experiment (Baldwin 2001). Naturally occurring *M. sexta* females laid eggs on the plants to be used in the experiment; we took advantage of this to explore oviposition preferences for tobacco versus devil's claw in the field. We counted the number of *M. sexta* eggs laid on each host plant species and then removed them every 1–4 days beginning in mid-July and ending in mid-September, exclusive of the time during the parasitism experiment when the plants were covered with netting, preventing oviposition by *M. sexta*. No pesticides were ever applied to the plants while in the greenhouse or at the field site.

We collected *M. sexta* eggs in early August from a field of tobacco plants in Clayton, NC. The eggs were brought into the lab and randomly assigned to cut leaves from greenhouse-grown tobacco and devil's claw plants; leaf water content was maintained by placing leaves in water picks. Eggs were maintained in growth chambers (25°C, 16L:8D cycle) on their assigned host plant species until hatching. First instar larvae were transferred to new leaves and offered leaf material ad libitum. After molting into 2nd instar, larvae were brought out to the experimental field plot. Larvae were randomly assigned to plants in the field corresponding to the same host plant species on which they were reared in the lab. Three larvae were placed on each plant.

After *M. sexta* larvae were transferred to the field plants, the plants were covered with small gauge nylon netting. This allowed access by naturally occurring *C. congregata* parasitoids to the experimental *M. sexta* larvae, but prevented access by larger predators including birds, vespid wasps and predatory bugs. The netting also prevented potential competition between *M. sexta* larvae and other herbivores present at the field site.

The *M. sexta* larvae were checked daily to assess survival, developmental stage and whether they were parasitized. Larvae were allowed to feed on their host plants at the field site until: 1) death, 2) egression of larval parasitoids from their *M. sexta* hosts, or 3) mid-5th instar (a mass of approximately 3–4 g) for surviving non-parasitized *M. sexta* larvae. Upon any one of these three events, *M. sexta* larvae were returned to the lab. Dead and parasitized *M. sexta* larvae were immediately frozen (–80°C). Surviving non-parasitized *M. sexta* larvae had to be returned to the lab during 5th instar because at the end of the larval stage (the wandering stage), larvae purge their guts and burrow underground to pupate where they would be effectively lost from the experiment. The larvae returned to the laboratory were reared individually in plastic enclosures with screened lids. Larvae were fed on leaves cut from their host plant, and leaf water content was maintained by keeping the leaf material in water picks. The enclosures containing the larvae were maintained in growth chambers under standard rearing conditions (25°C, 16L:8D). Upon wandering, they were placed individually in wooden pupation chambers at room temperature (~25°C) until pupation. Pupae were transferred to plastic cups lined with soil and remained there until eclosion. Adults were frozen at –80°C within five hours of eclosion.

Mass and development time were recorded for each individual larva at wandering, pupation and at the adult stage within five hours of eclosion. For adult females, estimates of potential fecundity were obtained by dissecting out the ovarioles into Ringer's solution. The number of follicles at stage 6 (S6) and all subsequent stages of development (Yamauchi and Yoshitake 1984) were counted with the aid of a dissecting microscope.

Larvae without external signs of parasitism that died prior to reaching 5th instar were dissected to search for internal indications of parasitism (e.g. punctures in the cuticle, *C. congregata* eggs or early-stage larvae). We were able to recover all *M. sexta* larvae (alive, dead or parasitized) from each host plant species, although four of the dead *M. sexta* larvae were severely desiccated and only a portion of each of these larvae were recovered from the field.

As a metric of total fitness, female net reproductive rate (R_0) was calculated as the product of survival to eclosion and potential fecundity. Females that survived to eclosion were scored as 1, and females that died prior to eclosion where no estimate of fecundity could be obtained, were scored as 0. Survival (1 or 0) was multiplied by potential fecundity (total number of follicles for an individual female that survived to eclosion, or 0 for females that died prior to eclosion) for each female to obtain R_0. We estimated the number of females that died prematurely because larvae that died in early developmental stages (either due to plant effects or parasitism) could not be sexed. Sex ratios for individuals surviving to eclosion were similar across host plant species ($\chi^2 = 0.033$, DF = 1, $p = 0.8549$), so the total number of males and females surviving to eclosion were pooled. The overall ratio of adult males to females was statistically indistinguishable from unity ($\chi^2 = 0.373$, DF = 1, $p = 0.5416$). Assuming the adult sex ratio reflects that of earlier developmental stages, i.e. that half of the larvae that died prematurely were male, we removed half of the total number of individuals that died prematurely on devil's claw and did the same for tobacco.

Data processing and statistical analyses

Parasitism and survival

We first assessed how host plant use of tobacco or devil's claw affected rates of parasitism by *C. congregata* or overall survival of *M. sexta*. Parasitism was considered a binomial variable and modeled using analysis of deviance with host plant species as a fixed effect. Because multiple larvae inhabited the

same plant, we included the plant identification number as a random effect in the model to account for potential correlation among the results for a given plant.

Survival to pupation was treated as a binomial variable and modeled using analysis of deviance with host plant species (tobacco or devil's claw) and plant identification number as a random effect.

We also examined the covariate of final larval density of *M. sexta* (the number of larvae on a given plant at mid-5th instar, just prior to being returned to the laboratory) in the survival and parasitism models. Although starting densities of *M. sexta* larvae were equivalent, mortality varied across plant species and individual plants over the course of the experiment. We examined final *M. sexta* larval density and its interaction with the fixed effect of host plant species (where applicable) in these models to test for potential density-mediated survival and parasitism.

Body size, development time, fecundity and fitness

We assessed how host plant use impacted size, development time and fecundity of *M. sexta* that survived to adulthood. Development time and mass at pupation were modeled using mixed-model ANOVA (REML) with host plant species, sex and the interaction between host plant species and sex as fixed effects. Plant identification number was included as a random effect. We focused on performance at the pupal stage for comparison with previous results for performance on devil's claw and tobacco in the laboratory environment. We include only those individuals that survived to eclosion in our analyses.

Potential fecundity was modeled using mixed-model ANCOVA (REML) with host plant species as a fixed effect and plant identification number as a random effect. Because body size has important effects on fecundity in *M. sexta* and other insects (Davidowitz et al. 2004, 2005), ln(adult mass) and the interaction of ln(adult mass) with host plant species were included as fixed effects in this model.

Here again, we also examined the covariate of final larval density of *M. sexta* and its interaction with the fixed effects of host plant species and sex (where applicable) in the models for body mass, development time and potential fecundity to test for potential density-mediated effects on *M. sexta* performance. We used a Mann-Whitney–Wilcoxon test to examine the consequences of host plant use for total fitness of *M. sexta*.

Patterns of oviposition

Finally, to assess *M. sexta*'s ability to take advantage of a potential enemy free space on devil's claw, we recorded egg counts on both host plant species over a total of 33 days prior to and following the time during the parasitism experiment when plants were netted and unavailable for oviposition by *M. sexta*. We divided this 33 day period into three smaller time intervals (A, B and C). The dates corresponding to each interval and the number of samples of eggs taken during each time interval were as follows: A (20–31 July; four samples), B (1–11 August; seven samples), and C (8–17 September; four samples). Oviposition patterns of naturally occurring *M. sexta* were modeled using analysis of deviance. Whether an egg was laid on tobacco or devil's claw was considered a binomial response variable. Time interval was included as a fixed effect, and the sample was included as a random effect. All statistical analyses were performed using R (ver. 2.6.0).

Results

Parasitism and survival

Analysis of deviance detected a significant effect of host plant species ($\chi^2 = 31.619$, $p < 0.0001$) on the probability of being parasitized. Parasitism exceeded 40% on tobacco (Fig. 1), and all parasitized *M. sexta* larvae died prior to metamorphosis. Parasitism on tobacco had a significant effect on survival to pupation ($\chi^2 = 94.47$, $p < 0.0001$). In contrast, no larvae were successfully parasitized on devil's claw (Fig. 1); specifically, no devil's claw-reared *M. sexta* larvae had *C. congregata* parasitoid larvae egress from them. Although unsuccessful parasitism (injection of parasitoid eggs that fail to develop) of *M. sexta* larvae on devil's claw is possible, post-mortem dissections of *M. sexta* larvae that died prematurely revealed no evidence of parasitism (parasitoid eggs or puncture wounds).

Survival of non-parasitized *M. sexta* larvae was significantly lower on devil's claw compared to tobacco (Fig. 1). Analysis of deviance detected a significant effect of host plant species on the probability of survival to pupation for non-parasitized individuals ($\chi^2 = 15.466$, $p < 0.0001$). Results were qualitatively similar for the probability of survival to eclosion for non-parasitized individuals.

Analysis of deviance detected a marginal, but non-significant effect of host plant species (tobacco or devil's claw) ($\chi^2 = 2.786$, $p = 0.0951$) on the overall probability of survival to pupation, when survival of both parasitized and non-parasitized individuals was combined. Results were qualitatively similar for the probability of survival to eclosion ($\chi^2 = 2.304$, $p = 0.1290$). Although there were significant differences in daily survival on the two host plant species at earlier stages of development (e.g. at wandering, $\chi^2 = 4.417$, $p = 0.0356$), there was no significant difference in survival through metamorphosis (Fig. 2).

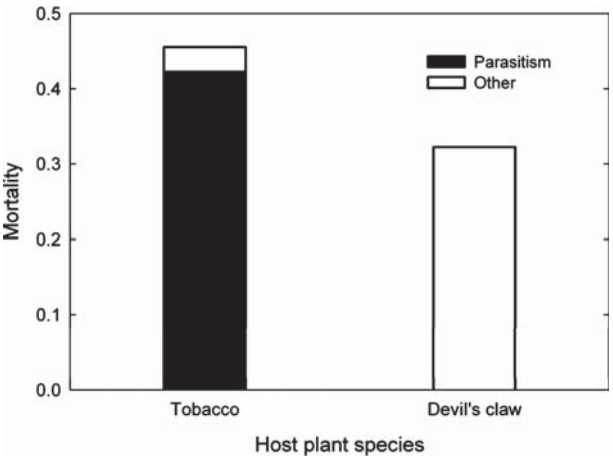

Figure 1. Cumulative mortality from 2nd instar to pupation due to parasitism (solid bar) and other non-parasitoid causes (open bar) for *Manduca sexta* feeding on tobacco and devil's claw in the field.

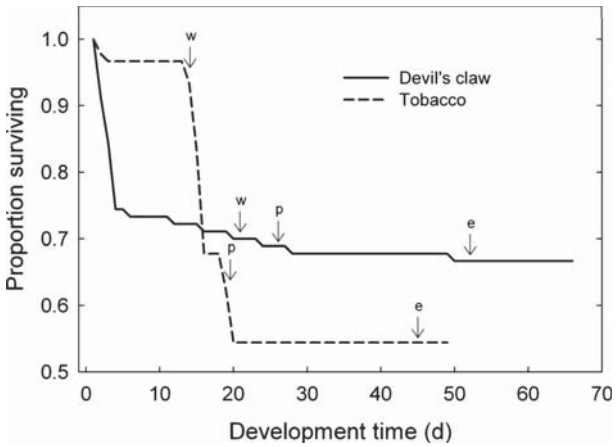

Figure 2. Mean daily survival from 2nd instar to eclosion for *Manduca sexta* feeding on tobacco (dashed line) and devil's claw (solid line); data are for survival until eclosion of the last individuals on tobacco and devil's claw. Mean times to reach wandering (w), pupation (p) and eclosion (e) are indicated for each of the two hosts.

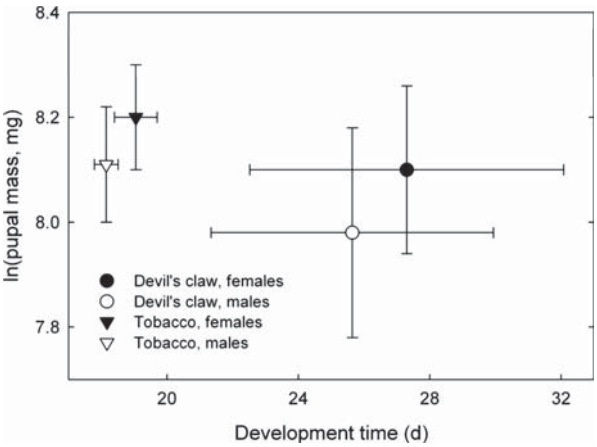

Figure 3. Body mass (in ln(mg)) at pupation as a function of development time (d) from 2nd instar to pupation for female (closed symbols) and male (open symbols) *M. sexta* feeding on tobacco (triangles) and devil's claw (circles). Mean ± 1 SD is indicated. Results for supernumerary instars are included with those for 5 larval instars.

The main effect of larval density and its interaction with host plant species (where applicable) were not significant in all parasitism and survival models (mean 5th instar larval density ± 1 SE, on tobacco: 2.90 ± 0.06, on devil's claw: 2.13 ± 0.06).

Development time, size, fecundity and total fitness

ANOVA detected significant effects of host plant species ($F_{1, 52}$ = 125.885, p < 0.0001), and sex ($F_{1, 53}$ = 5.265, p = 0.0258) on time to pupation, but no significant interaction was found between host plant species and sex ($F_{1, 53}$ = 0.629, p = 0.4312). Development time to pupation was faster for *M. sexta* feeding on tobacco relative to devil's claw, and for males relative to females (Fig. 3). Results for development time to eclosion were qualitatively similar.

ANOVA also detected significant effects of host plant species ($F_{1, 52}$ = 14.260, p = 0.0004) and sex ($F_{1, 53}$ = 14.369, p = 0.0004) on mass at pupation, but no significant interaction was found between host plant species and sex ($F_{1, 53}$ = 0.026, p = 0.8725). Body mass at pupation was greater for *M. sexta* feeding on tobacco relative to devil's claw; female pupal mass was greater than male pupal mass, reflecting sexual dimorphism in this species (Madden and Chamberlin 1945) (Fig. 3). Results for body mass at eclosion were qualitatively similar.

ANCOVA revealed significant effects of host plant species ($F_{1, 36}$ = 80.114, p < 0.0001) and adult body mass ($F_{1, 9}$ = 17.919, p = 0.0022) on the number of follicles in the ovarioles (Fig. 4). No significant interaction between host plant species and adult body mass was detected ($F_{1, 9}$ = 2.561, p = 0.1440). Larger females were more fecund than smaller females, and females reared on tobacco were significantly more fecund than females reared on devil's claw.

Again, the main effect of larval density and its interaction with the fixed effects of host plant species and sex (where applicable) were not significant in the models for body mass, development time and potential fecundity.

Non-parametric analysis indicated median net reproductive rate for non-parasitized individuals was significantly greater on tobacco relative to devil's claw (W = 100.5, p < 0.0001). In contrast, non-parametric analysis indicated median net reproductive rate was not significantly different across host plant species when both parasitized and non-parasitized individuals are considered (W = 757, p = 0.1834). Mean net reproductive rates were similar on the two host plant species (mean ± SE: tobacco, 181.093 ± 27.735, n = 43; devil's claw, 150.238 ± 19.079, n = 42). As a result, when effects of both host quality and parasitism are considered, total fitness of *M. sexta* was similar on the two hosts.

Patterns of oviposition

Analysis of deviance revealed a significant effect of time interval on the probability of laying eggs on tobacco (χ^2 = 18.111, p = 0.0001). Post-hoc analyses indicated the probability of laying eggs on tobacco was significantly

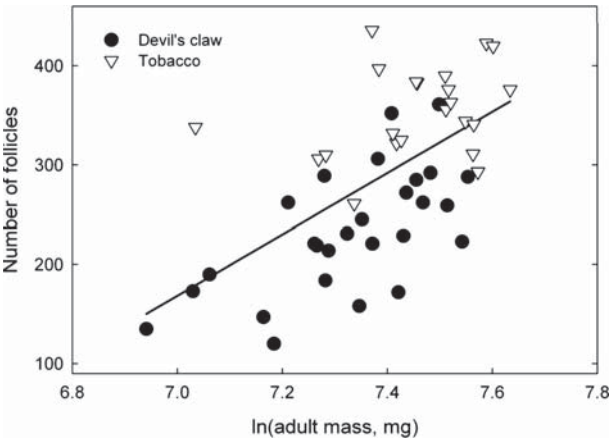

Figure 4. Potential fecundity (number of follicles) as a function of body mass (in ln(mg)) for adult females reared on devil's claw (closed circles) and tobacco (open triangles). The regression of fecundity on ln(adult mass, mg) is shown (y = 309.02x – 1994.82; r^2 = 0.37).

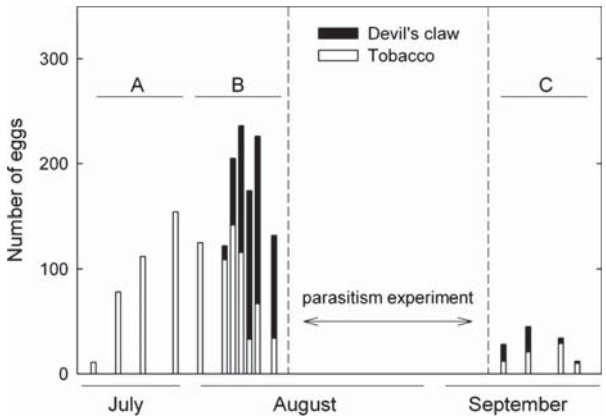

Figure 5. The number of eggs laid by naturally occurring *Manduca sexta* females on the randomized tobacco and devil's claw plants used in the parasitism experiment prior to and following the experiment. The three time intervals used in the statistical analyses are indicated: A (20–31 July), B (1–11 August), and C (8–17 September).

different between time intervals A and B (χ^2 = 12.909, p = 0.0003) and A and C (χ^2 = 16.761, p < 0.0001), but not B and C (χ^2 = 0.011, p = 0.9166). Patterns of oviposition at the field plot used in the parasitism experiment (Material and methods) changed substantially during the period before and after the experiment (Fig. 5). Earlier in the season (mid to late June) during time interval A, females almost exclusively laid eggs on tobacco. However, as the season progressed into July (time interval B), females began to lay eggs on devil's claw. Just prior to the parasitism experiment in mid July, females laid over twice as many eggs on devil's claw as on tobacco (Fig. 5).

Discussion

Evaluating host plant use and enemy free space in *Manduca*

The primary goal of this study was to test whether the adoption of a novel host plant by an herbivore was driven by enemy free space (EFS). To accomplish this, we experimentally disentangled the effects of natural enemies from the potentially confounding factors of host plant quality, competition, and microhabitat. Here we present evidence which strongly suggests the tobacco hornworm, *Manduca sexta*, has adopted a novel host plant outside its typical host range (devil's claw) because this host plant provides enemy free space from a parasitoid natural enemy, *Cotesia congregata*.

First, we found a striking pattern of differential parasitism of *M. sexta* across the two host plant species. Greater than 40% of the *M. sexta* larvae on tobacco were parasitized, all of which died prior to metamorphosis; however, no larvae were parasitized on devil's claw. This high rate of parasitism on tobacco and absence of parasitism on devil's claw (Fig. 1) offset the high initial mortality on devil's claw through the first three larval instars (Fig. 2). As a result, overall survival through metamorphosis of both parasitized and non-parasitized individuals combined was similar across the two host-plant species. Importantly, parasitism contributed to significantly increased levels of mortality on tobacco, relative to the levels of background mortality of non-parasitized larvae (Fig. 1). Together, these results demonstrate that the parasitoid, *C. congregata*, is an important agent of natural selection in this system, and that devil's claw can provide enemy free space from this parasitoid for *M. sexta*.

Second, we were able to eliminate host plant quality as a confounding factor to EFS in *M. sexta*'s use of devil's claw. Consistent with our results for *M. sexta* reared on tobacco and devil's claw in the laboratory where natural enemies were absent (Diamond et al. unpubl.), the results of our field experiment demonstrate survival, growth, development time, and potential fecundity through metamorphosis (non-parasitized individuals) are significantly lower on devil's claw compared to tobacco (Fig. 2–4). This is important, as host plant quality and EFS could both conceivably promote an herbivore's use of a novel host plant if there were no reduction in food quality on the novel host plant. In this case, it would be difficult to attribute the adoption of a novel host plant to either factor. Because we clearly show that devil's claw is of inferior quality for *M. sexta*, our results indicate that EFS is more important than host plant quality in *M. sexta*'s adoption of this novel host plant. Our experimental approach also allowed us to control for herbivore competition, food limitation, and microhabitat variation as additional alternatives to EFS for *M. sexta*'s use of devil's claw.

Third, to demonstrate that EFS on devil's claw is not an artifact of potential tradeoffs or interactions among fitness components (Thompson 1988b), we examined total fitness (R_0) of *M. sexta* on tobacco and devil's claw. Our result that R_0 of non-parasitized *M. sexta* is greater on tobacco compared to devil's claw is consistent with our results for individual components of fitness. This reinforces the conclusion that devil's claw is of inferior host plant quality for *M. sexta*. Similarly, our result that R_0 of both parasitized and non-parasitized individuals combined is comparable across host plant species is consistent with our result for survival through metamorphosis. Again, this strongly suggests EFS plays a major role in *M. sexta*'s adoption of the novel host plant, devil's claw.

Importantly, *M. sexta* appears to be well suited to take advantage of enemy free space on devil's claw. Our monitoring of oviposition patterns in the experimental garden before and after the field experiment indicate that females laid more eggs on tobacco earlier in the season when parasitoid abundance was relatively low, but began to lay over twice as many eggs on devil's claw as tobacco later in the season (Fig. 5). Peak abundance of the parasitoid, *C. congregata*, typically occurs from mid-July, into early August for populations in the southeastern US (Rabb 1971, Kester and Barbosa 1994), which corresponds well with *M. sexta*'s increased oviposition on devil's claw. This pattern appears to be common: we have observed increased oviposition of *M. sexta* on devil's claw at our field site in a subsequent field season (the site layout was comparable to one in the study described here, except that the plants remained free of netting for the entire field season; Diamond unpubl.).

Selection of oviposition sites by adult females is a critical determinant of larval success in *M. sexta*. Naïve *M. sexta*

larvae will accept several non-solanaceous host plant species (Yamamoto and Fraenkel 1960). However, solanaceous-reared *M. sexta* larvae have been shown to refuse non-solanaceous host plants, which arises from larvae developing a dependence on solanaceous host plant chemical to initiate and continue feeding (del Campo and Renwick 1999). Preliminary results indicate *M. sexta* larvae (instars 2–4) will not survive if switched from tobacco to devil's claw, and vice versa (Diamond unpubl.). Because there are significant costs to switching host plant species, oviposition by *M. sexta* females is an important component of being able to take advantage of the enemy free space on devil's claw.

Most likely, these oviposition patterns across devil's claw and tobacco do not reflect the formation of host races in *M. sexta*. Laboratory experiments have shown that individual females lay eggs on both devil's claw and tobacco when given a choice between these two host plant species (Diamond unpubl.). In addition, *M. sexta* adults are powerful fliers and highly dispersive, and allozyme data suggest little population differentiation within the southeastern US (H.A. Woods pers. comm.).

Differences in plant phenology can also affect seasonal patterns of oviposition in the field. In our experiment, however, plants of each species were germinated at the same time, and our measurements in the field took place during a time where both plant species were well-established and flushing new leaves. Thus, although phenological variation in host plant species could be an important driver of oviposition (and subsequently EFS), it was unlikely a major factor in our experiment. Interestingly, a similar pattern to the one documented here (increased oviposition on devil's claw later in the season) was found for *M. sexta* laying eggs on wild solanaceous plants, *Datura wrightii* and a species of devil's claw, *P. parviflora* in Arizona (Mechaber and Hildebrand 2000), suggesting this pattern may be relatively robust.

Our field experiments demonstrate that EFS is a critical determinant of *M. sexta*'s use of devil's claw in North Carolina. A previous field observational study with *M. sexta* in Arizona also reported reduced larval mortality due to natural enemies by use of devil's claw (*Proboscidea* spp.) (Mira and Bernays 2002). The differences between these two *M. sexta* systems are instructive. In Arizona, *M. sexta* experienced fewer natural enemies on endemic *Proboscidea* spp., relative to a native solanaceous host plant (*Datura wrightii*). In North Carolina, *M. sexta* achieved EFS on *P. louisianica*, a naturalized plant species from a relatively recent introduction (Small 1903), relative to a hybrid solanaceous host plant, cultivated tobacco, *Nictotiana tabacum* (Ren and Timko 2001). In our study, we focused on a single parasitoid natural enemy of major importance in North Carolina, and experimentally excluded social wasps, birds and other larger predators; Mira and Bernays (2002) considered the impacts of a diverse assemblage of natural enemies. These two complementary studies suggest escape from natural enemies by use of devil's claw, and perhaps other non-solanaceous host plants, may be geographically widespread in *M. sexta*.

Because of the complexity and lability of multitrophic interactions (Thompson 1988a), the potential benefit of EFS that *M. sexta* gains on devil's claw is likely contingent on a number of environmental factors. For example, host plant abundance likely differs in nature, e.g. tobacco is often planted as a large monoculture (Radford et al. 1968), which may be particularly important if *M. sexta* females incur searching costs for devil's claw (Singer 1983). In addition, *M. sexta* is confronted with multiple natural enemies across several taxa that vary in importance and diversity across spatial and temporal scales (see Mira and Bernays 2002 for an extensive field survey). Clearly these factors could affect the overall suitability of devil's claw as a host plant for *M. sexta*, and are worth further study. More generally, spatial and temporal dynamics of plant–herbivore interactions have been shown to substantially impact EFS in some systems; the degree to which EFS may be characterized by a spatially and temporally varying mosaic is an interesting, but unresolved issue (Heard et al. 2006).

The mechanism by which *M. sexta* evade parasitism on devil's claw also deserves further study. We suspect either one or both of the following mechanisms may be involved: 1) the parasitoid wasps have a search image for caterpillar hosts on tobacco, a tall plant with broad, oval-shaped leaves, but cannot 'see' caterpillar hosts on devil's claw, a relatively short plant approximately half the height of tobacco with small, round-shaped leaves, and 2) the volatile compounds present in devil's claw are quite different from those in tobacco (see Sisson and Saunders 1982, and Riffle et al. 1990 for the biochemical profiles of these host plants) which may interfere with the parasitoid's attraction to or detection of caterpillar hosts on devil's claw.

Alternatively, *M. sexta* may not be entirely evading parasitism on devil's claw. Rather, the devil's claw allelochemicals may impair (or kill) the parasitoids, allowing the caterpillar hosts to survive without presenting symptoms of parasitism when feeding on devil's claw (see Singer and Stireman 2003 for an example of this mechanism). However, laboratory experiments in which *M. sexta* were reared on devil's claw and subsequently exposed to mated adult *C. congregata* females, revealed that all *M. sexta* larvae died within days of being parasitized (Diamond unpubl.), suggesting this pharmacological mechanism is unlikely to occur in our system. Importantly, however, this highlights the fact that multiple mechanisms (e.g. resistance versus evasion of natural enemies, among several others; see Berdegue et al. 1996 for a complete list) underlie the production of enemy-free space, suggesting enemy-free space may be more likely to evolve.

Host use and enemy free space in insect–plant systems

EFS can provide a mechanism by which insect herbivores can expand the set of host plants utilized to include host plants of inferior intrinsic quality. There are three necessary conditions for this to occur. First, there must be differences in attack rate by natural enemies across different host plants (Jeffries and Lawton 1984, Scheirs and de Bruyn 2002). Several reviews have found evidence for this condition in more than 80% of studies with insect–plant systems (Berdegue et al 1996, Heard et al. 2006). In most cases, however, these studies do not rule out alternative factors such as competition, host plant quality or microhabitat variation as drivers of host plant use by herbivores, which may require experimental manipulation (Mulatu et al. 2004).

Second, there must be a fitness cost to using the alternative host plant when natural enemies are excluded. Exclusion studies to demonstrate fitness costs have become more common in the past two decades (Denno et al. 1990, Ohsaki and Sato 1994, Gratton and Welter 1999, Ballabeni et al. 2001, Zangerl et al. 2002, Zvereva and Rank 2003, Mulatu et al. 2004, Murphy 2004, Singer et al. 2004a, 2004, this study), and have detected significant costs in about half of these systems.

Interestingly, several of the studies showing positive support for EFS are in experimentally generated (Gratton and Welter 1999) or recent natural host shifts (this study, Mulatu et al. 2004, Murphy 2004). Negative fitness consequences (not due to natural enemies) associated with using a novel host plant may only be detectable during ongoing host range shifts or recent expansions because these costs may disappear following physiological adaptation to the novel host plant. This may make it more difficult to document EFS in extant plant-herbivore systems with relatively long histories of association.

The third condition is that fitness costs of using an inferior host plant are balanced by fitness gains via escape from natural enemies, such that total fitness is similar on the different host plants. This quantitative assessment is rarely done: often, only one fitness component (typically survival) is assessed. Even when multiple fitness components are measured, they are not always concordant (Denno et al. 1990, Ohsaki and Sato 1994). In the absence of estimates of total fitness, it is difficult to tell whether reductions in some fitness components but not others are indicative of a biologically relevant fitness cost, and therefore whether EFS is the predominant factor driving host plant use in that system. The results of our study underscore the importance of assessing both individual components of herbivore fitness and total fitness, to fully evaluate the role of EFS in host plant use.

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