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The ecology of diet expansion in a seed-feeding beetle: Pre-existing variation, rapid adaptation and maternal effects?

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Summary

Most studies of insect dietary evolution suffer from limited information on the history of host use patterns; historical patterns can only be inferred from modern patterns. We examine the ecology of a recent diet breadth expansion onto an introduced host by the seed beetle, *Stator limbatus*, to (1) determine if pre-existing variation for survival on *Chloroleucon ebano* is present in natural populations of *S. limbatus*, and (2) test whether natural selection has resulted in local adaptation to *C. ebano* in a population where this host is used. Our results indicate that variation in survival on *C. ebano* does exist in natural populations of *S. limbatus* that have historically never encountered this host, providing the variation necessary for adaptation to this host. However, we found no evidence that *S. limbatus* have locally adapted to *C. ebano*. Our most interesting discovery, however, was that the use of *C. ebano* by *S. limbatus* is facilitated by non-genetic effects of parental host plant on progeny survival; parents reared on *Cercidium floridum* produce progeny with substantially higher survivorship on *C. ebano* than parents reared on *Acacia greggii* due to an environmentally based parental effect. We argue that such host-plant-mediated maternal effects are likely to be common and thus important for our understanding of herbivorous insect evolution and population dynamics. This paper provides one example of how an understanding of environmentally based maternal effects can provide important information on the evolution of life-history patterns observed in nature.

Keywords: *Cercidium floridum; Chloroleucon ebano;* diet breadth; host range; *Stator limbatus*

Introduction

A large proportion of all interactions between species involve herbivorous insects and their host plants (Strong, 1988). Thus understanding the evolution and diversification of insect–plant associations is a key component in our broader attempt to understand species interactions and diversity, and understanding the evolution of insect diet breadths is of central importance to understanding the evolution and diversification of insect–plant relationships (Bowers *et al.*, 1992). Unfortunately, most studies of insect dietary evolution suffer from limited information on the history of host use patterns; historical patterns can only be inferred from modern patterns (Carroll and Boyd, 1992). However, recent introductions of non-native plants, and the subsequent expansion of herbivores onto these plants, provide a powerful tool for analyses of diet breadth evolution (Tabashnik, 1983; Bowers *et al.*, 1992; Carroll and Boyd, 1992; Fraser and Lawton, 1994; Leclaire and Brandl, 1994).

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The first step of incorporating a novel plant into the diet of an insect herbivore is the recognition of the plant as a potential host, and subsequent colonization of the plant (Bowers *et al.*, 1992). If genetic variation for host-plant-mediated life-history characters, or host-plant-mediated phenotypic plasticity, is present, then rapid adaptation to this new host generally follows (Bush, 1992; Leclaire and Brandl, 1994). This sometimes results in the formation of host-associated subpopulations (host races) (Feder *et al.*, 1988), permitting the refinement of host-associated adaptations, possibly followed by sympatric speciation (Bush, 1992). Expected genetic differences among host-associated populations have evolved in several insects, including fruit flies (Bush, 1969; Feder *et al.*, 1990a,b), treehoppers (Wood, 1980), moths (Phillips and Barnes, 1975; Pashley, 1986), beetles (Sturgeon and Mitton, 1986) and bugs (Carroll and Boyd, 1992). Alternatively, if genetic variation in host-associated life-history characters is not present, or if gene flow from the source population is sufficiently high, then local adaptation or genetic substructuring may not evolve.

In this study, we examine the ecology of a recent diet breadth expansion onto an introduced host by the seed beetle, *Stator limbatus* (Horn) (Coleoptera: Bruchidae). *S. limbatus* is a generalist seed parasite distributed throughout the southwestern United States and south into northern South America (Johnson and Kingsolver, 1976; Johnson *et al.*, 1989). *S. limbatus* is an unusual species in the genus *Stator* because it has been collected from more than 50 host species, in at least nine genera, throughout its large geographic range (13 species in the southwestern US). A recent cladistic analysis (C.D. Johnson, personal communication) shows that diet is evolutionarily conserved in the genus *Stator*; most species are restricted to one or a few host genera, usually within a single subfamily of the Fabaceae (subfamily Mimosoideae), and most sibling pairs or monophyletic groups feed on the same genus or genera of legumes.

Despite the extensive diet breadth of *S. limbatus*, it has never been reared from seeds of Texas ebony (*Chloroleucon ebano*; Fabaceae: Mimosoideae) within the natural distribution of this plant (Nilsson and Johnson, 1993). This is a particularly surprising observation because *C. ebano* is used as a host plant by a sister species of *S. limbatus*, that is *S. beali* (Johnson and Kingsolver, 1976). Recently, however, *C. ebano* has been introduced as an ornamental tree in the Phoenix metropolitan area of Arizona (introduced post-1972, Phoenix Botanical Garden records). *S. limbatus* has since colonized this plant (adults readily oviposit on *C. ebano* seeds both in nature and the lab), and seeds of *C. ebano* successfully produce reproductively mature *S. limbatus* adults in nature (Nilsson and Johnson, 1993).

In a series of laboratory experiments, we examined three features of the ecology and evolution of this diet expansion by *S. limbatus* onto *C. ebano* in central Arizona. First, we examined populations of *S. limbatus* that are not associated with *C. ebano* for evidence of pre-existing variation in the ability to survive on this host. Such variation, if genetic, provides the raw material for adaptation to this host once colonized by adults. Second, we examined one *C. ebano*-associated population in Papago Park, Arizona, for evidence that *S. limbatus* has adapted to this host in the short time since its introduction. Third, we examined the role of environmentally based maternal effects, mediated through the parental host plant, on the ability of progeny to survive on *C. ebano*.

The natural history of *S. limbatus*

Stator limbatus is a seed parasite that oviposits directly onto host seeds, and is thus restricted to seed pods that have either dehisced or been damaged by other organisms. Upon hatching, the larvae burrow into the seed, where they complete development, pupate and emerge as adults (Siemens *et al.*, 1992). Adults are the only dispersing stage; larvae are restricted to the host their mother has chosen for them. In the lab, mating and egg laying begin approximately 24–48 h postemergence. Beetles require only a single seed to complete development and reproduce. Thus neither food nor water supplementation is necessary for the following lab experiments.

In the United States, *S. limbatus* is abundant on many species of *Acacia* (Fabaceae: Mimosoideae) and two species of *Cercidium* (*C. floridum* and *C. microphyllum*; Palo Verdes; Fabaceae: Caesalpinioideae), although only one or a few hosts may be available in any single locality. In central Arizona, *S. limbatus* is restricted largely to *C. floridum*, *C. microphyllum* and *Acacia greggii* (the three most abundant host plants).

Field collection of *S. limbatus* populations

For these experiments, we examined four populations of *S. limbatus*:

1. *Papago Park population.* On 17 October 1994, *S. limbatus* eggs were collected from the seeds of seven *C. ebano* trees planted in 1974 at the main entrance to Phoenix Botanical Gardens, Papago Park, Maricopa County, Arizona, and from more than 15 *C. ebano* trees planted as a hedge at the entrance to the Phoenix Zoo in Pagago Park. Eggs were collected both from seeds on the ground and from infested pods within reach of a researcher standing on the ground. For the local adaptation experiment described below, we pooled these two collections and refer to them as the Papago Park population. We estimate that this laboratory population was initiated with approximately 150 individuals.
2. *Scottsdale population.* On 16 October 1994, *S. limbatus* eggs were collected from *C. floridum* along Scottsdale Highway, 1.7 miles north of Bell Road (behind the Scottsdale Well), in Scottsdale, Maricopa County, Arizona. Beetles and seed stock were collected by picking mature seed pods from more than 25 *C. floridum* plants. Mature pods were transferred to the lab, and seeds containing beetles were separated from uninfested seeds. Seeds containing entrance or emergence holes of other bruchids (such as *Mimosestes* sp.) were discarded. We estimate the laboratory population was initiated with 300–400 field-collected individuals.
3. *Apache Junction population.* On 17 October 1994, *S. limbatus* eggs were collected from *C. floridum* and *C. microphyllum* in numerous locations throughout Apache Junction, Pinal County, Arizona. Beetles were collected as in the Scottsdale population. We estimate that this laboratory population was initiated with 200–300 field-collected individuals.
4. *Van Horn population.* In late July 1994, *S. limbatus* eggs were collected from *A. greggii* approximately 10 miles south of Van Horn, Texas. Beetles and *A. greggii* seed stock were collected as described above for the Scottsdale population. We estimate that this laboratory population was initiated with 200–300 field-collected individuals.

All three experiments described below were initiated simultaneously, after beetles were reared in the lab for one generation at 27°C [light-to-dark (L:D) ratio = 15:9 h] on *A. greggii*, to standardize for non-genetic effects of parental rearing-host on subsequent host suitability (as in Fox *et al.*, 1995). The only exception to this was for the Van Horn population, which was reared in the lab for three generations at 27°C (L:D ratio = 15:9 h) on *A. greggii* prior to the start of these experiments.

Experiment 1: Pre-existing variation and local adaptation

This first experiment was designed (1) to determine if pre-existing variation for survival on *C. ebano* is present in natural populations of *S. limbatus*, and (2) to test whether natural selection has resulted in local adaptation to *C. ebano* in Papago Park.

Methods

Within each population, virgin females collected less than 24 h after emergence from an *A. greggii* seed were weighed and then randomly paired with a virgin mate from their own population (sample sizes presented in Table 1). Each pair was confined in a 60 mm Petri dish with 15 seeds of *C. ebano* and allowed to lay eggs. The dishes were checked at 24 h intervals, and seeds bearing eggs were replaced with clean seeds, until a female had laid an egg on more than 12 seeds. All eggs were reared to adults at densities of one beetle per seed (additional eggs were scraped from each seed) at 30°C (L:D ratio = 15:9 h). This experiment was replicated simultaneously with all four laboratory populations.

For comparison, laboratory estimates of survivorship on *C. ebano* were compared with field estimates of survivorship on this host. We examined survivorship on *C. ebano* at two sites within Papago Park, the Phoenix Botanical Garden and the Phoenix Zoo. Seeds of *C. ebano* were collected both on the ground and from infested pods within reach of a researcher standing on the ground. These seeds were brought to the lab, where the number of first instar larvae entering each seed and the number of adults emerging from each seed (estimated by counting emergence holes) were recorded. Although eggs occasionally fall off of the seed in the field, our estimates of the number of first instar larvae entering a seed are likely to be accurate; the entrance holes of first instar larvae are easily counted under a 10× dissecting scope.

Results

Beetles from all four populations of *S. limbatus* had very low egg-to-adult survivorship when reared on *C. ebano* in the lab (≤ 4.2% in each population; Table 1). Survivorship of Papago Park beetles on *C. ebano* was statistically higher than survivorship of beetles from Apache Junction, but did not differ from survivorship of Van Horn or Scottsdale beetles (Table 1). This pattern was identical for the proportion of families successfully producing at least one surviving adult beetle; more Papago Park families produced at least one surviving adult than did Apache Junction families, but there was no differences between the Papago Park and either of the Scottsdale and Van Horn populations (Table 1). This experiment thus demonstrates that variation in the ability to survive on *C. ebano* is present in populations of *S. limbatus* that do not have access to this host in nature. However, the Papago Park population has not locally adapted to *C. ebano*; although beetles from Apache Junction survived more poorly on *C. ebano* than beetles from Papago Park, the difference in survivorship between populations was very small (1.3%) and Scottsdale and Van Horn beetles survived as well as Papago Park beetles on *C. ebano*, despite having no access to this host.

Table 1. Egg-to-adult survivorship of four populations of *Stator limbatus* on *Chloroleucon ebano* (Texas ebony)

Population	Survivorship ± s.e.m. (*n*)	Percentage of families producing survivors
Papago Park	0.042 ± 0.007 (82)^{a}	36.7%^{a}
Scottsdale	0.038 ± 0.007 (155)^{a,b}	27.1%^{a,b}
Apache Junction	0.029 ± 0.005 (98)^{b}	20.4%^{b}
Van Horn	0.032 ± 0.006 (110)^{a,b}	25.5%^{a,b}

All parents of these families were reared on *A. greggii* to control for environmentally based maternal effects. *n* = number of families. Entries sharing a common superscript letter are not significantly different from each other (Mann-Whitney *U*-test).

The observed survivorship of Papago Park *S. limbatus* on *C. ebano* in the lab was substantially lower than the estimated survivorship on this host in nature; 13.5% (161 of 1195 eggs) and 10.2% (108 of 1056 eggs) of *S. limbatus* eggs collected on *C. ebano* in the field successfully produced an adult beetle (Phoenix Zoo and Phoenix Botanical Garden samples, respectively).

Experiment 2: Rearing host affects larval survivorship

The results of Experiment 1 unexpectedly indicated that, despite evidence for phenotypic variation in the ability to survive on *C. ebano*, local adaptation to this host has not occurred in Papago Park. However, the observed survivorship of Papago Park beetles on *C. ebano* in our laboratory experiment (4.2%) was substantially lower than our estimates of survivorship in the field (> 10.2%), suggesting that our laboratory experiment did not accurately reflect survivorship in nature. One potential explanation for these differing estimates of survivorship concerns the choice of rearing hosts for parents of the test generation. Experiment 1 was intended to detect evidence for both genetic variation within populations and differences among populations. Thus to control for maternal effects (Fox *et al.*, 1995), each population was reared on *A. greggii* for one generation prior to initiating Experiment 1. This second experiment was designed to test the hypothesis that maternal host plant affects progeny survivorship on *C. ebano* via environmentally based maternal effects.

Methods

After a single generation of laboratory rearing on *A. greggii* (as above), virgin females collected less than 24 h after emergence from an *A. greggii* seed were weighed and then randomly paired with a virgin mate from their own population. Each pair was confined in a 60 mm Petri dish with 15 seeds of either *C. ebano*, *A. greggii* or *C. floridum*. As above, the dishes were checked at 24 h intervals, and seeds bearing eggs were replaced with clean seeds, until a female had laid eggs on more than 12 seeds. All eggs were reared to adults at densities of one beetle per seed at 30°C (L:D ration = 15:9 h).

Adult females emerging from the three hosts were collected within 24 h of emergence from their seed (up to two females per family) and paired with a single virgin male (non-sibling) that had been reared from the same host species. All pairs were confined in a 60 mm Petri dish with 15 seeds of *C. ebano*. Again, the dishes were checked for eggs at 24 h intervals, and seeds bearing eggs were replaced with clean seeds, until a female had laid eggs on more than 12 seeds. The eggs were then reared to adults at densities of one beetle per seed at 30°C (L:D ratio = 15:9 h). This experiment was replicated simultaneously with the Scottsdale, Apache Junction and Van Horn populations.

Results

When parents were reared on *A. greggii* for a single generation, survivorship on *C. ebano* was very low in all three populations (≤ 2.6%; Table 2), as observed in Experiment 1. However, when parents were reared for a single generation on *C. ebano*, survivorship of their progeny on this host was significantly higher than survivorship of progeny of *A. greggii*-reared parents (significant for all three populations; Table 2). Unexpectedly, survivorship of progeny of *C. floridum*-reared parents was also substantially higher than that of progeny from *A. greggii* parents (Table 2), and even higher than the survivorship of progeny of *C. ebano*-reared parents (survivorship of progeny of *C. floridum*- and *C. ebano*-reared parents differed significantly for the Van Horn population, and differed significantly when all three populations were pooled; Mann-Whitney *U*-test, $P < 0.001$).

Table 2. The effect of maternal host on survivorship of *Stator limbatus* on *Chloroleucon ebano*

	Maternal host			Probability (Mann-Whitney *U*-test)		
Population	*A. greggii*	*C. floridum*	*C. ebano*	AG = CF	AG = CE	CF = CE
Scottsdale	0.026 ± 0.008 (23%; n = 53)	0.182 ± 0.049 (56%; n = 18)	0.070 ± 0.021 (34%; n = 23)	***	*	N.S.
Apache Junction	0.024 ± 0.008 (23%; n = 47)	0.117 ± 0.028 (69%; n = 16)	0.078 ± 0.027 (64%; n = 14)	***	**	N.S.
Van Horn	0.012 ± 0.006 (10%; n = 62)	0.117 ± 0.033 (57%; n = 21)	0.042 ± 0.020 (33%; n = 13)	***	*	*

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; N.S., not significant.
The number in parentheses is the percentage of mothers that produced one or more surviving progeny; n is the number of families.

Experiment 3: Genetic *vs* non-genetic effects – a selection experiment

Experiment 2 demonstrated that parental rearing host affects the survivorship of progeny on *C. ebano*. In particular, mothers reared on *C. floridum* produced progeny that survived better on *C. ebano* (12–18%) than progeny of mothers reared on either *C. ebano* (4–8%) or *A. greggii* (1–3%). However, Experiment 2 did not provide information on the source of the observed effects (genetic responses to selection *vs* non-genetic effects of parental environment). That non-genetic effects of parental host may in part account for the observed increase in survivorship is suggested by the previous observation that maternal rearing host affects progeny body size and development time, mediated via non-genetic effects of maternal host (Fox *et al*., 1995). Alternatively, parental rearing on *C. floridum* may affect progeny survivorship on *C. ebano* via a correlated response to selection, in which selection for increased survivorship on *C. floridum* results in a correlated increase in survivorship on *C. ebano*. The following selection experiment was designed to disentangle the genetic and non-genetic effects of parental rearing host on progeny survivorship on *C. ebano*.

The logical basis for this experiment was as follows: If observed effects of parental rearing on *C. floridum* represent genetic responses to selection, then the differences between *C. floridum* and *A. greggii* lines should persist in the population after selection has been relaxed for a single generation. However, if observed effects of parental rearing on *C. floridum* represent non-genetic effects of parental environment, then a single generation of rearing on a common host species should reduce or even eliminate these differences.

Methods

The first generation of the experiment was initiated with virgin females and males collected within 24 h of emergence from an *A. greggii* seed after a single generation of rearing in the lab. All beetles were paired and confined in a 30 mm Petri dish with 15 seeds of *C. floridum*. The dishes were checked for eggs every 24 h until a female had laid an egg on more than 12 seeds. Half of these pairs were then switched to dishes containing 10 *A. greggii* seeds and allowed to lay eggs for 24 h. The remaining pairs were discarded. This resulted in twice as many families being initiated on *C. floridum* as on *A. greggii*, which was necessary because mortality on *C. floridum* is very high, and resulted in the extinction of numerous families each generation. The order of host presentation (*C. floridum* first and *A. greggii* second) was necessary to control for the effects of maternal experience on egg size; females first exposed to *A. greggii* lay small eggs that have nearly 100% mortality on *C. floridum* (Fox *et al*., 1997), while females that first encounter *C. floridum* lay large eggs that survive well on both *C. floridum* and *A. greggii* (Fox and Mousseau, 1996). The design of this experiment, with the same families used to initiate the first generation of both the *C. floridum* and *A. greggii* lines, ensured that the genetic starting conditions of each line were similar.

All eggs were reared to adults at 30°C (L:D ratio = 15:9 h). From each family, the first emerging female and the first emerging male were used to initiate the second generation. Beetles were paired within lines (excluding sib pairings) and confined with 10 seeds of either *C. floridum* (selected line) or *A. greggii* (control line), and allowed to lay eggs. The dishes were checked at 24 h intervals, with egg-laden seeds replaced with clean seeds, until a female had laid an egg on more than eight seeds. These beetles were again reared to adults as above, and the process repeated.

This procedure was followed for four generations (lines on *C. floridum*) or five generations (lines on *A. greggii*). This difference in the number of generations between lines resulted from differences in development time on the two hosts – larvae develop substantially faster on *A. greggii* than on *C. floridum* (Fox *et al*., 1994, 1995). The final generation (generation 5 on *C. floridum* or 6 on *A. greggii*) was initiated identically to the previous lines except that all lines were transferred onto *A. greggii*. Mortality in each line was < 5% during this generation on *A. greggii*, such that selection was relaxed (but not reversed) and the non-genetic parental effects of being reared on *C. floridum* were removed.

Finally, survivorship of each line on *C. ebano* was quantified by pairing one virgin female from each family with a virgin male from another family in the same line and confining them in a 60 mm Petri dish with 15 seeds of *C. ebano*. The dishes were again checked for eggs as in previous experiments until a female had laid an egg on more than 12 seeds. The eggs were reared to adults at 30°C (L:D ratio = 15:9 h).

Three populations were used for this experiment: the Scottsdale population, the Apache Junction population and a 'mixed' population. The 'mixed' population was created during the first generation of the experiment by pairing a female derived from either the Scottsdale or Apache Junction population with a male derived from the remaining population. Final sample sizes (numbers of families) are presented in Table 3. Final sample sizes varied from the original number of families due to the high mortality on *C. floridum* that resulted in the extinction of families, the failure of some females to lay eggs and a laboratory accident in which some families were lost from the experiment.

A related experiment, in which beetles were selected for increased survivorship on *C. ebano*, was also established simultaneously with this experiment to test whether parental rearing on *C. ebano*

Table 3. The effect of four generations of rearing on *Cercidium floridum* on survivorship of *Stator limbatus* on *Chloroleucon ebano* (mean egg-to-adult survivorship ± S.E.; Mann-Whitney *U*-test)

	Survivorship (egg-to-adult)		
Population	Control line	Selected line	Probability
Scottsdale	0.012 ± 0.007	0.006 ± 0.004	N.S.
	(16%; *n* = 31)	(13%; *n* = 23)	N.S.
Apache Junction	0.024 ± 0.007	0.033 ± 0.014	N.S.
	(28%; *n* = 50)	(32%; *n* = 22)	N.S.
Mixed	0.032 ± 0.014	0.020 ± 0.005	N.S.
	(20%; *n* = 35)	(28%; *n* = 46)	N.S.

The number in parentheses is the percentage of mothers that produced one or more surviving progeny; *n* is the number of families. Probabilities calculated with a Mann-Whitney *U*-test.

affected survivorship of progeny on this host due to environmentally mediated maternal effects. However, due to the very high mortality of beetles on *C. ebano,* the selected lines quickly became extinct, and thus no data are available from this experiment.

Results

The results of our four-generation selection experiment indicate that non-genetic effects are largely responsible for the parental host effects observed in Experiment 2; in all three experimental populations, the lines selected for increased survivorship on *C. floridum* did not differ from the control lines in survivorship on *C. ebano* after a single generation of rearing on *A. greggii* (Table 3). Also, the observed survivorship in each line was of similar magnitude to survivorship of progeny from *A. greggii*-reared parents in the preceding two experiments (≤ 3.2%; Table 3). Thus although we saw large effects of parental rearing host on survivorship on *C. ebano* in Experiment 2 (in three of three replicates; Table 2), these large effects disappeared when each line was reared for a single generation on *A. greggii* (in three of three replicates; Table 3), indicating that the parental host effects are non-genetic effects. If the parental effects observed in the preceding experiment represented genetic effects, then the selected lines in this experiment should have survived substantially better on *C. ebano*, regardless of the single generation of rearing on *A. greggii*, due to genetic differences between the treatments built up during four generations on *C. floridum*.

Discussion

The original aims of the experiments presented here were (1) to determine if pre-existing variation for survival on *C. ebano* is present in natural populations of *S. limbatus*, and (2) to test whether natural selection has resulted in local adaptation to *C. ebano* in Papago Park. Our results indicate that variation in survival on *C. ebano* does exist in natural populations of *S. limbatus* that have historically never encountered this host, providing the variation necessary for adaptation to this host. However, we found no evidence that *S. limbatus* from Papago Park have locally adapted to *C. ebano*. This lack of adaptation may imply that the observed variation in survival on *C. ebano* does not reflect underlying genetic variation, such that the intense selection associated with high mortality does not result in an evolutionary improvement in the ability of *S. limbatus* to survive on this host. However, we suspect that this is not the case. Instead, we interpret this lack of adaptation to *C. ebano* as one of two lines of evidence that gene flow from the ancestral host onto the introduced host is swamping out any genetic changes associated with using *C. ebano*. Our maternal effects data provide the second line of evidence; the estimated survivorship on *C. ebano* in nature is similar to the expected survivorship if parents had been reared on *C. floridum*, but higher than expected if parents had been reared on *C. ebano* (Table 2), suggesting that many of the families collected on *C. ebano* in nature were produced by parents reared from *C. floridum*.

Although these experiments were originally designed to examine local adaptation in *S. limbatus*, our most interesting discovery was that the use of *C. ebano* by *S. limbatus* is facilitated by non-genetic effects of parental host plant on progeny survival; parents reared on *C. floridum* produce progeny with substantially higher survivorship on *C. ebano* than parents reared on *A. greggii* due to an environmentally based parental effect, rather than a genetic response to natural selection during the experiment. Host-plant-mediated maternal effects have been reported for only a few insects (e.g. Morris, 1967; Leonard, 1970; Rossiter, 1991a,b and references therein) but are likely to be common (Fox *et al.*, 1995). In *S. limbatus*, host-plant-mediated maternal effects have previously been shown to affect offspring development time and body size (Fox *et al.*, 1995); mothers reared on *C. floridum* produced larger offspring that developed faster than progeny of mothers reared on *A. greggii*, regardless of progeny host plant. The results of this study further demonstrate that parental rearing on *C. floridum* has large non-genetic effects on progeny life-history, and that these non-genetic effects can mediate the incorporation of a new host into the diet of the insect.

The mechanism by which parental rearing on *C. floridum* results in increased survivorship on *C. ebano* is unknown. However, we speculate on three potential mechanisms. First, mothers reared on *C. floridum* may lay larger eggs, or eggs of higher quality, than mothers reared on other hosts. *S. limbatus* has been demonstrated to exhibit phenotypically plastic egg size; females lay larger eggs on *C. floridum*, on which small eggs all die, and lay small eggs on *A. greggii* (and thus more eggs), on which small eggs survive well (Fox *et al.*, 1997). Possibly, mothers also respond to their rearing host by adjusting egg size, laying larger eggs if reared on *C. floridum* and smaller eggs when reared on *A. greggii*. If this egg size variation subsequently affects progeny survivorship on *C. ebano* (preliminary data suggest that it does; C.W. Fox, unpublished data), we would expect patterns of survivorship similar to those observed in these experiments.

Second, symbiotic microorganisms transmitted from mother to progeny may provide the ability to detoxify *C. floridum* allelochemicals, or may provide some essential nutrient not present in *C. floridum*. Almost all insects yet examined harbour both extracellular and intracellular microbial symbionts that provide essential nutrients and/or detoxify plant allelochemicals (Jones, 1984; Dowd, 1991). These symbionts are generally transmitted from parent to offspring through eggs, either by entering the oocytes directly or by being smeared on the outside of the egg as it is laid and subsequently being ingested by the hatching insect (Brooks, 1963). *S. limbatus* successfully surviving to adults on *C. floridum* may develop a dense microbial flora that assists in development on this host, and this flora may be transmitted to progeny providing a developmental advantage over progeny not inoculated with the appropriate symbionts, or progeny inoculated with an inadequate number of symbionts.

Third, enzyme induction by environmental cues may improve the ability of larvae to detoxify xenobiotic compounds (Nebert, 1979; Harshman *et al.*, 1991). Induction of detoxification enzymes after exposure to host plants has been reported for many herbivorous insects (e.g. Yu *et al.*, 1979; Farnsworth *et al.*, 1981). Interactions with highly toxic *C. floridum* seeds may result in the induction of detoxification enzymes, which may be metabolically expensive to produce when not needed (Dykhuizen, 1978). However, for enzyme induction to explain the parental effects observed in *S. limbatus*, enzymes induced in parents must be expressed in progeny. To our knowledge, such trans-generational expression of induced genes has not been reported in insects. However, we speculate that it may be common and worthy of further study.

Regardless of the mechanisms responsible, host-plant-mediated maternal effects are likely to be common and important in generating many phenotypic patterns observed in nature (Fox *et al.*, 1995). They are probably often important components of population responses to natural selection (e.g. they may result in large time lags in an evolutionary response to selection and characters subject to large maternal effects may even respond to selection in a maladaptive direction; Riska *et al.*, 1985; Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990), and evolutionary response to selection, particularly in heterogeneous environments, may even be manifested through the evolution of maternal effects (Fox *et al.*, 1997). Thus an understanding of host-plant-mediated maternal effects, and the mechanisms generating them, is necessary for us to understand herbivorous insect life-history evolution and population dynamics. For example, attempts to predict population dynamics in a multi-host environment will be impeded if maternal effects are excluded from models (Rossiter, 1991a, 1994; Ginzburg and Tanneyhill, 1994), and population patterns of life-history variation and host adaptation of herbivorous insects may be misinterpreted as genetically based characters if the influence of non-genetic effects is not considered.

However, despite their potential importance, host-plant-mediated maternal effects are rarely considered in ecological or evolutionary studies of insect–plant interactions. This study provides one example of how an understanding of environmentally based maternal effects can provide important information on the evolution of the life-history patterns observed in nature; patterns of survivorship initially interpreted as evidence for rapid adaptation to an introduced host instead result from host-plant-mediated maternal effects. This maternal effects interpretation has important consequences for predictions about the evolution of *S. limbatus* survivorship on *C. ebano*. For example, as *C. ebano* is more extensively planted throughout Arizona, and possibly even invades native plant communities, we may expect *S. limbatus* to either adapt to this host or evolve an aversion to ovipositing on it. However, the large maternal effects described in this paper indicate that selection for adaptation to *C. ebano* may be ineffective, because the observed variation in survival on this host is largely the result of an environmentally based maternal effect. Also, our results suggest repeated colonizations of *C. ebano* by *S. limbatus* each generation, prohibiting local adaptation to this host, and preventing the evolution of host races.

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