PARENTAL HOST PLANT AFFECTS OFFSPRING LIFE HISTORIES IN A SEED BEETLE[1]

CHARLES W. FOX, KIM J. WADDELL, AND TIMOTHY A. MOUSSEAU
Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208 USA

Abstract. Non-genetic effects of maternal environment often have large influences on offspring phenotypes. Some maternal effects may be adaptive in heterogeneous environments, whereby mothers can program a developmental switch in their offspring in response to a predictive environmental cue. For herbivorous insects, maternal rearing host (i.e., maternal environment) may have large effects on offspring, including adaptive effects on patterns of host suitability. For example, if maternal rearing host is predictive of local host availability, then it is advantageous for mothers reared on one host to produce offspring that are physiologically "acclimated" to that host.

Previous research has demonstrated that seed beetles, *Stator limbatus,* collected from *Cercidium floridum* survived better and developed faster on this host than beetles collected from *Acacia greggii* or *C. microphyllum* at either the same site or from a different locality. These results indicate either within- and among-population host-associated genetic differentiation or a non-genetic maternal host effect, in which offspring survive better and develop faster on the host their mother was reared on. We examine the influence of maternal and paternal rearing hosts on the survivorship, development time, and body size of offspring in *S. limbatus.* We find no evidence that beetles are acclimated to perform better on the host their mother was reared on via non-genetic maternal effects. However, maternal rearing host did affect offspring in other ways; mothers reared on *C. floridum* produced larger offspring that developed faster than offspring of mothers reared on *A. greggii,* due to a non-genetic maternal effect (paternal rearing host had no effect). In addition, both maternal and paternal host had a large effect on offspring survivorship; offspring of parents reared on *C. floridum* survived better than those of parents reared on *A. greggii.* The symmetry between the maternal and paternal host effects on survivorship suggests that they represent a response to intense selection during the experiment, and suggest the potential for rapid adaptation to this host. Alternatively, non-genetic effects of both maternal and paternal rearing host may simultaneously influence survivorship of *S. limbatus* offspring. Although paternal effects are generally disregarded as an important source of environmental variation, they are potentially important for *S. limbatus*; non-genetic paternal effects were detected in this experiment for development time of offspring: fathers reared on *C. floridum* produced offspring that developed *slower* on *C. floridum,* and offspring that developed *faster* on *A. greggii,* than fathers reared on *A. greggii.*

Key words: conditioning; diet breadth; host suitability; insect–plant interactions; maternal effects; Stator limbatus.

INTRODUCTION

Phenotypic variation in natural populations is influenced both by genetic variation among individuals and by environmental variation. One important source of environmental variation is the maternal effect: non-genetic effects of maternal phenotype or maternal environment on offspring phenotypes (Mousseau and Dingle 1991*a, b*). Although long-term evolutionary responses of a population to natural selection are largely determined by the available genetic variation and the direction and magnitude of selection, maternal effects can also have dramatic effects on evolutionary responses (Riska 1991). They may result in large time lags in an evolutionary response to selection, and characters subject to large maternal effects may even re-

spond to selection in a maladaptive direction (Riska et al. 1985, Kirkpatrick and Lande 1989, Lande and Kirkpatrick 1990). They also confound our interpretation of within- and among-population variation in morphological and life history traits (Sinervo 1991).

However, maternal effects can be adaptive for organisms, particularly in heterogenous environments. Adaptive maternal effects are likely common in insects (reviews in Saunders 1982, Danks 1987, Mousseau and Dingle 1991*a, b*), as well as other animals (Reznick 1991, Sinervo 1991) and plants (Roach and Wulff 1987, Lacey 1991). The overwhelming conclusion from these studies is that many maternal effects have evolved as a mechanism for "trans-generational phenotypic plasticity" (Mousseau and Dingle 1991*b*), whereby a mother that experiences a predictive environmental cue (e.g., high or low host density, short or long photoperiod), can program a developmental switch in her

offspring appropriate for the environmental conditions predicted by the cue.

For phytophagous insects, host plants represent heterogeneous resources due to temporal and spatial variability in host quality and availability. Studies examining the evolution of insect host-use patterns during the last decade have found tremendous amounts of genetic variation within and among populations of herbivorous insects for both host preference and host suitability (Futuyma and Peterson 1985, Via 1990). More recently, researchers have begun to recognize that *maternal* rearing host may also have dramatic effects on offspring phenotypes (e.g., Morris 1967, Rossiter 1991*a, b*), including effects on observed patterns of host suitability (Gould 1988, Via 1991). In some cases, these maternal host effects may be adaptive. For example, if a mother's (or a father's) rearing host is predictive of local or future host availability, then it is advantageous for parents reared on one host to produce offspring that are "acclimated" to that host (possibly via induction of enzymatic activity in the offspring). However, the only available test of the hypothesis that mothers produce offspring that are acclimated to the maternal rearing host due to non-genetic effects found no evidence of acclimation of larval performance; using replicate lineages of two aphid clones reared on both alfalfa and red clover, Via (1991) demonstrated that maternal host species had no effect on the relative performance of either clone on either host. However, generalizations cannot be made from a single study, so other systems need to be examined. Such effects, if demonstrated for any insect, could have profound implications for our understanding of host-use evolution of herbivores, host-race formation, and sympatric speciation. For example, host experience often influences oviposition preference of females (Papaj and Lewis 1993), and if host experience also influences larval performance on these hosts, then non-genetic correlations between oviposition preference and larval performance may be maintained in a randomly mating population through the effect of maternal host experience.

Here, we examine the influence of maternal and paternal rearing host on the survivorship, development time, and body size of offspring in two populations of *Stator limbatus* (Horn) (Coleoptera: Bruchidae). This experiment was initially prompted by the large rearing host effects detected by Siemens and Johnson (1990), Siemens et al. (1991), and Fox et al. (1994). These experiments demonstrated that populations of *S. limbatus* collected from *Cercidium floridum* performed substantially better on this host than populations collected from *Acacia greggii* (measured as survival and development time), and that offspring of females reared from *C. floridum* survived better on this host than offspring of mothers reared from either *A. greggii* or *C. microphyllum,* within a single population. These results may be explained in two ways. First, beetles collected

from different hosts, either within the same population or from different populations, may differ genetically in their ability to use these hosts (representing either among-population genetic differentiation or host-associated genetic sub-structuring within populations). Alternatively, a non-genetic maternal rearing host effect, in which offspring survive better and develop faster on the host their mother was reared on, may produce the observed patterns (Siemens and Johnson 1990). In this manuscript, we examine the latter of these two hypotheses.

METHODS

Natural history of Stator limbatus (Coleoptera: Bruchidae)

S. limbatus is a generalist seed parasite distributed from northern South America to the southwestern United States (Johnson and Kingsolver 1976, Johnson et al. 1989). 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. *S. limbatus* is an unusual member of this genus because it has been collected from ≈50 host species, in at least nine genera, throughout its large geographic range (13 species in the southwestern U.S.). In the United States, and particularly in Arizona, *S. limbatus* is abundant on many species of *Acacia* (Fabaceae: Mimosoideae) and two species of *Cercidium* (*C. floridum* and *C. microphyllum* (palo verde); Fabaceae: Caesalpinioideae), although only one or a few hosts may be available in any single locality.

S. limbatus oviposits directly onto host seeds, and is thus restricted to seed pods that have either dehisced or been damaged by other organisms. For hosts that are indehiscent, such as *Cercidium floridum* and *Parkinsonia aculeata,* beetles attack seeds almost entirely through emergence holes of other bruchids, such as *Mimosestes* sp. (Coleoptera: Bruchidae), which oviposit on host legumes (fruits) rather than directly on seeds (Siemens et al. 1992). In dehiscent hosts, such as many *Acacia* species, beetles will attack both dehiscing pods and pods damaged by other insects (C. W. Fox, *personal observation*).

Upon hatching, the larvae burrow into the seed, where they complete development, pupate, and emerge as adults. Adults are the only dispersing stage; larvae are restricted to the host their mother has chosen for them. In the laboratory, mating and egg-laying begin ≈24–48 h post-emergence. Beetles require only a single seed to complete development and reproduce. Thus, neither food nor water supplementation is necessary for the following laboratory experiments.
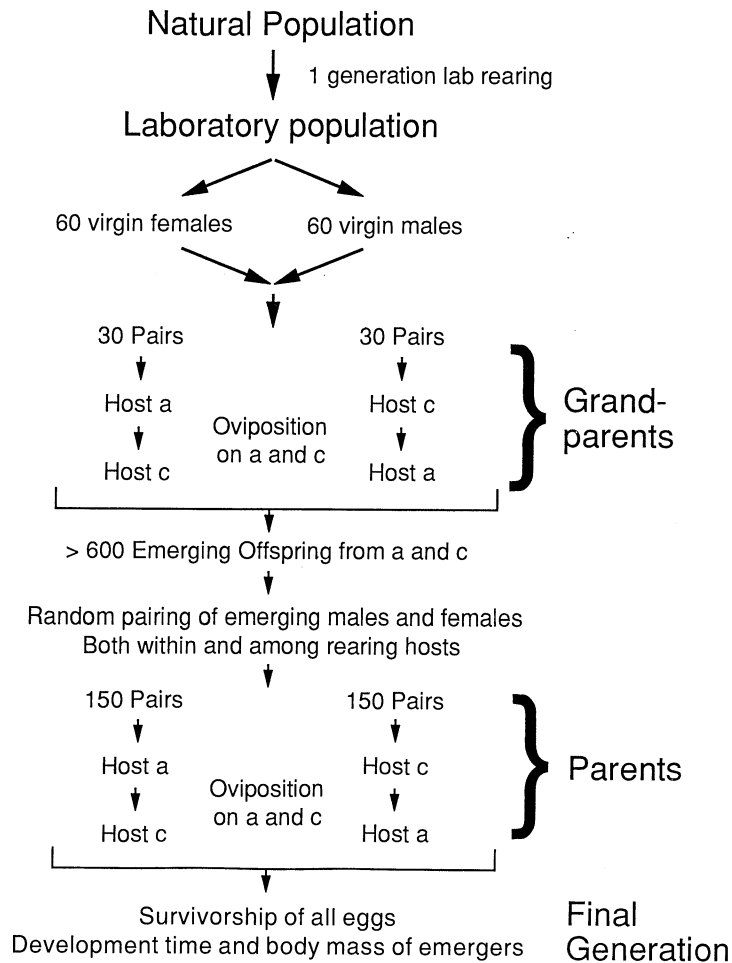
Natural Population

↓ 1 generation lab rearing

Laboratory population

60 virgin females   60 virgin males

30 Pairs		30 Pairs	
Host a	Oviposition	Host c	} Grand-parents
Host c	on a and c	Host a	

> 600 Emerging Offspring from a and c

↓

Random pairing of emerging males and females
Both within and among rearing hosts

↓

150 Pairs		150 Pairs	
Host a	Oviposition	Host c	} Parents
Host c	on a and c	Host a	

↓

Survivorship of all eggs   **Final**
Development time and body mass of emergers   **Generation**

FIG. 1. Outline of the experimental design. This design was replicated in two populations (Black Canyon City population and Scottsdale population). Host a = *Acacia greggii*; Host c = *Cercidium floridum*. Details are provided in *Methods*.

Field collections of S. limbatus *and two host plants*

Beetles for these experiments were collected from two localities in central Arizona. On 5–6 September 1993, beetles were collected from *Cercidium floridum* (Fabaceae: Caesalpinioideae) along Scottsdale Highway, 2.7 km north of Bell Road (behind the Scottsdale Well), in Scottsdale, Arizona. On 7 September 1993, beetles were collected from *Acacia greggii* (Fabaceae: Mimosoideae) in numerous locations throughout Black Canyon City, Arizona. These two populations are ≈50 km apart, and differ in the host plants available to them. In Black Canyon City, *A. greggii* and *C. microphyllum* are each abundant, and both are attacked by *S. limbatus* (C. W. Fox, *personal observation*). In Scottsdale, *C. floridum* is abundant and *C. microphyllum* is present (though uncommon), but *A. greggii* is not found at this site. *Parkinsonia aculeata* (Fabaceae: Caesalpinioideae), which has recently been introduced as an ornamental in Arizona and is readily used by *S. limbatus*, is present, although uncommon, at both sites.

Beetles and seed stock were collected by picking mature seed pods from >25 *C. floridum* plants in Scottsdale and >25 *A. greggii* plants in Black Canyon City. Mature pods were transferred to the laboratory, 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 that both laboratory populations were initiated with 500–1000 field-collected individuals.

All beetles were reared in the laboratory for one generation, on the host they were collected from, at 26°C, 24 h light, before the experiments were initiated.

The effects of maternal rearing host on larval performance

A diagrammatic outline of the experimental protocol is presented in Fig. 1. Within each population 60 virgin females collected <24 h after emergence from the seed were randomly paired with a virgin mate from their own population (60 pairs from each population, total

of 120 pairs). These pairs served as the grandparents of the experimental generation. Each pair was confined in a 30-mm petri dish with 10 seeds of either *A. greggii* or *C. floridum* and allowed to lay eggs. When females had laid >10 eggs on this initial host, they were transferred to the other host (from *A. greggii* to *C. floridum,* or from *C. floridum* to *A. greggii*), and confined until a female had laid >10 eggs on this second host. Within each population, half of the females were presented first with *A. greggii* and then with *C. floridum,* while the other half were presented first with *C. floridum* and then with *A. greggii.*

All eggs were reared to adult at densities of one beetle per seed (additional eggs were scraped from each seed), 28°C, constant light. Thus, full-sib individuals were obtained on both *C. floridum* and *A. greggii*. These beetles were used as the parents of the experimental population. Adult beetles were randomly paired (male + female) within 24 h of adult emergence with a non-sibling mate from their own population; the beetles were randomly paired irrespective of rearing host, such that both within rearing host and between rearing host pairings were made. As above, each pair was confined in 30-mm petri dishes with 10 seeds of either *A. greggii* (30 pairs from each population) or *C. floridum* (30 pairs from each population), and allowed to lay eggs. When females had laid >10 eggs on this initial host, they were transferred to the other host, and confined until a female had laid >10 eggs on this second host.

As above, all offspring were reared to adult at densities of 1 egg per seed, 28°C, 24 h light. Development time, body mass, and survivorship were recorded for all offspring. Development time was estimated as the time between egg-laying and adult emergence, and thus includes embryonic, larval, and pupal development time. Emerging adults were weighed individually within 12 h of adult emergence on an electronic balance.

Sex, population, rearing host, maternal rearing host, and paternal rearing host effects on development time and body mass were examined using an analysis of variance (SAS GLM procedure, type III sums of squares; SAS 1985). Although we do not specifically examine genetic variation in these populations, maternal and paternal lineages are included in each analysis to control for non-independence among siblings. These are included in the model as maternal grandmother and paternal grandmother. However, we were unable to control for mother and father (nested within grandparents) because of the large number of families and the resulting zero degrees of freedom on the various host effects (due to an unbalanced design). Our presentation of partial models does not influence our results; regardless of the terms added into various partial models, all effects reported here as significant were always significant.

For all models, maternal and paternal lineage were treated as random effects, nested within population, while all other effects were treated as fixed. *F* statistics

were calculated following the procedure of Neter et al. (1990). No data transformations were necessary for body size data to satisfy the assumptions of the multivariate models. Development time data were transformed as 1/(development time) for all analyses. Nonparametric methods were used when analyzing survivorship data. All significance tests are at the 0.05 level.

RESULTS

In total, 4582 offspring were reared to adult for the final offspring generation (Scottsdale: 1121 males and 1182 females from 295 families; Black Canyon City: 1169 males and 1110 females from 291 families). The sex ratio of emerging beetles did not differ from 1:1 for either population on either host (sign test, $P > 0.05$ for each).

Maternal rearing host had a large effect on all three estimates of offspring survivorship (egg-to-adult, entering seeds, and within seeds); offspring of mothers reared on *Cercidium floridum* survived better on *C. floridum* than offspring of mothers reared on *Acacia greggii* (Table 1A). Paternal rearing host had an almost identical affect on offspring survivorship (Table 1B), suggesting that the improvement in survivorship on *C. floridum* is a result of intense selection during the parental generation. Egg-to-adult survivorship of parents on *C. floridum* was only 38% for Black Canyon City beetles and 48% for Scottsdale beetles, compared to 95 and 99% on *A. greggii,* respectively (Table 2).

There was no evidence for a maternal host × offspring host interaction for survivorship (Table 1). Although offspring of mothers reared on *C. floridum* survived better on this host than offspring of mothers reared on *A. greggii,* offspring of mothers reared on *A. greggii* did not survive better on *A. greggii* than offspring of mothers reared on *C. floridum* (Table 1).

Maternal rearing host had a large effect on both offspring development time (Fig. 2; Table 3) and body size (Fig. 3; Table 4); mothers reared on *C. floridum* produced larger offspring that developed faster than offspring from mothers reared on *A. greggii.* Two lines of evidence indicate that this is a maternal effect, and not a result of selection during the experiment. First, mothers reared on *C. floridum* were actually smaller than mothers reared on *A. greggii,* rather than larger (Table 5), while surviving offspring of these *C. floridum* mothers were larger, regardless of rearing host, than offspring of *A. greggii*-reared mothers (due to the large host effects on development time, this comparison cannot be made for development time). Second, while the maternal rearing host effect was significant for both development time and body size, there was no evidence of a paternal rearing host effect for either character.

Contrary to our expectations at the start of this experiment, there was no interaction between maternal rearing host and offspring rearing host for either development time or body size (Tables 3 and 4). Thus,

TABLE 1.　The effect of rearing host, maternal host, and paternal host on survivorshp in two populations of *S. limbatus*. (A) The effect of maternal rearing host. (B) The effect of paternal rearing host.*

		A. Maternal host effects					
		Survivorship (proportion surviving)					
		Egg to adult		Entering† seed		Within‡ seed	
Rearing host	Maternal host	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
		Black Canyon City					
Cercidium floridum	*Cercidium floridum*	0.58ᴬ	0.04	0.70ᴬ	0.04	0.82ᴬ	0.03
Cercidium floridum	*Acacia greggii*	0.32ᴮ	0.02	0.40ᴮ	0.03	0.72ᴮ	0.03
Acacia greggii	*Cercidium floridum*	0.98ᴬ	0.01	0.99	0.01	0.99	0.01
Acacia greggii	*Acacia greggii*	0.96ᴮ	0.01	0.98	0.01	0.97	0.01
		Scottsdale					
Cercidium floridum	*Cercidium floridum*	0.53ᴬ	0.04	0.62ᴬ	0.04	0.81ᴬ	0.03
Cercidium floridum	*Acacia greggii*	0.34ᴮ	0.03	0.43ᴮ	0.03	0.72ᴮ	0.03
Acacia greggii	*Cercidium floridum*	0.95	0.02	0.98	0.01	0.96	0.01
Acacia greggii	*Acacia greggii*	0.95	0.01	0.98	0.01	0.97	0.01

		B. Paternal host effects					
		Survivorship (proportion surviving)					
		Egg to adult		Entering† seed		Within‡ seed	
Rearing host	Paternal host	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
		Black Canyon City					
Cercidium floridum	*Cercidium floridum*	0.51ᴬ	0.05	0.61ᴬ	0.05	0.78	0.04
Cercidium floridum	*Acacia greggii*	0.34ᴮ	0.03	0.43ᴮ	0.03	0.73	0.03
Acacia greggii	*Cercidium floridum*	0.98	0.01	1.00	0.00	0.98	0.01
Acacia greggii	*Acacia greggii*	0.96	0.01	0.98	0.01	0.98	0.01
		Scottsdale					
Cercidium floridum	*Cercidium floridum*	0.52ᴬ	0.04	0.58ᴬ	0.04	0.85ᴬ	0.03
Cercidium floridum	*Acacia greggii*	0.37ᴮ	0.03	0.46ᴮ	0.03	0.71ᴮ	0.04
Acacia greggii	*Cercidium floridum*	0.95	0.02	0.98	0.01	0.96	0.02
Acacia greggii	*Acacia greggii*	0.93	0.02	0.98	0.00	0.94	0.02

　* For analyses of maternal host effects, paternal host types are lumped together. Likewise, for analyses of paternal host effect, maternal host types are lumped together. Males and females are also lumped because early instars could not be sexed. All means are averages of family means. For calculation of means, each rearing dish was treated as a single observation within a family, and each family a single observation within a grand-maternal lineage. In both populations, all three estimates of survivorship were lower on *Cercidium floridum* than on *Acacia greggii*, regardless of maternal or paternal host (Wilcoxon signed-rank tests, $P < 0.05$). Within each population, column, and rearing host, significant maternal and paternal host effects are indicated by different letters. (Mann-Whitney U tests on means for each grandparental lineage, $P < 0.05$; Wilkinson 1990).

　† Survivorship: Entering seed, is the proportion of offspring that survived until no body parts were protruding from the seed.

　‡ Survivorship: Within seed, is the survivorship of all larvae that successfully entered the seed (i.e., larvae that died entering the seed are not included in this estimate).

mothers did not produce offspring that had been "acclimated" to perform better on the maternal host. However, development time was influenced by an interaction between paternal rearing host and offspring rearing host (Table 3; Fig. 4). This effect is consistent in both populations and both sexes (Fig. 4), suggesting that it is not a spurious result. However, fathers reared on *C. floridum* produced *slower* developing offspring on *C. floridum*, and *faster* developing offspring on *A. greggii*, than fathers reared on *A. greggii*.

DISCUSSION

　The main goal of this experiment was to test the hypothesis that offspring perform better when reared on the *same* host as their mother due to a non-genetic maternal effect (i.e., "acclimation" of larval perfor-

mance). We found no evidence to support this hypothesis (e.g., maternal host × offspring host interactions were not significant; Tables 3 and 4). Our experiment did, however, indicate a large maternal host effect on offspring body size and development time in which mothers reared on *C. floridum* produced larger offspring that developed faster than offspring of mothers reared on *A. greggii*, regardless of offspring host. We also detected an effect of both maternal and paternal rearing hosts on offspring survivorship (offspring of parents reared on *C. floridum* survived better than offspring of parents reared on *A. greggii*), and an effect of paternal host on offspring development time (fathers reared on *C. floridum* produced *slower* developing offspring on *C. floridum*, and *faster* developing offspring on *A. greggii*, than fathers reared on *A. greggii*).

TABLE 2. Egg-to-adult survivorship of *Stator limbatus* on *Cercidium floridum* and *Acacia greggii* during the parental generation. These beetles served as parents for the beetles in Table 1. Note the very high mortality of beetles on *C. floridum*.

Population	Number of families	Egg to adult survivorship*	
		$\bar{X}$	SE
Acacia greggii			
Black Canyon City	56	0.95	0.02
Scottsdale	53	0.99	0.01
Cercidium floridum			
Black Canyon City	57	0.38	0.05
Scottsdale	55	0.48	0.05

* All values are significantly different from each other (host effects within populations, Wilcoxon signed-rank test, $P < 0.05$; population effects within hosts, Mann-Whitney U test, $P < 0.05$).

The maternal host effect on offspring body size and development time observed in this experiment is likely an environmentally based maternal effect, rather than a genetic response to natural selection during the experiment. Because all emerging beetles from the first generation were mated and used as parents of the experimental generation, there was no direct selection on development time or body size in our experiment other than through effects on mortality. The maternal effect is not likely a correlated response to selection on survivorship because there was no evidence of a paternal host effect on either character. Such a paternal effect would be expected if there was selection on both parents. Selection could potentially have produced a correlated response only in mothers, but this is unlikely because mortality of males and females was approximately the same (the sex ratio did not differ from 1:1 for either population), and thus any correlated response to selection is expected to be approximately the same, whether estimated from mothers or fathers.

Maternal host effects on offspring life histories have been reported for only a few insects (e.g., Morris 1967, Leonard 1970, Rossiter 1991*a, b* and references therein). For example, maternal diet (e.g., red vs. black oak) influenced development time of offspring in *Lymantria dispar* (Rossiter 1991*a*). *Heliothis virescens* larvae fed quercetin-containing diets (a commonly occurring plant compound) produced offspring that developed more slowly on quercetin-containing diets than did larvae from mothers reared on unaltered diets (Gould 1988). These maternal host effects are important for our understanding of herbivorous insect population dynamics because 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). Also, attempts to predict population dynamics in a multi-host environment will be impeded if maternal
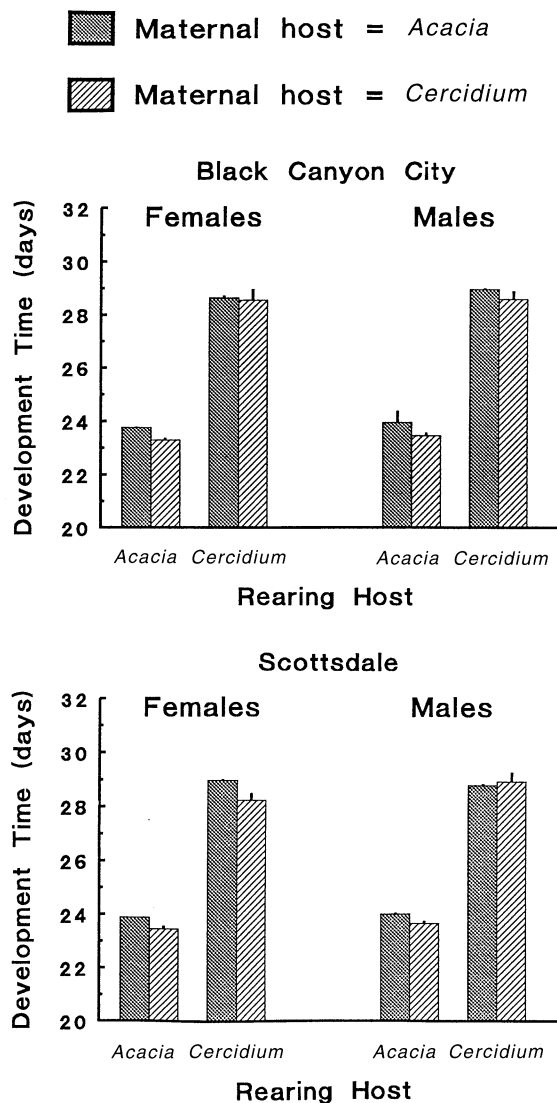

FIG. 2. The effect of maternal host on offspring development time. Note that for both sexes of both populations, mothers reared on *C. floridum* produced faster developing offspring than mothers reared on *A. greggii*, regardless of offspring host. Statistics are presented in Table 3.

host effects are excluded from models (Rossiter 1991*a*).

An understanding of maternal host effects is also critical for the interpretation of within- and among-population patterns of life history variation and host adaptation of herbivorous insects. For example, population differences in body size and development time detected in a common garden experiment would typically be interpreted as genetic differentiation among populations. However, if these populations were collected from, and mothers were reared on, different host species, the observed patterns might represent maternal host effects rather than genetic differentiation. Thus, the use of common gardens experiments to detect pop-

TABLE 3. Analyses of variance for development time of two populations of *S. limbatus* reared on two host species (*Cercidium floridum* and *Acacia greggii*). $R^2 = 0.63$.

Source†	df	MS $(\times 10^{-5})$	F
Sex	1	8.45	12.17***
Population	1	0.09	0.07 NS
Maternal lineage (population)	110	1.52	2.19***
Paternal lineage (population)	110	1.16	1.67***
Rearing host	1	372.01	5355.74***
Sex × host	1	0.00	0.09 NS
Population × host	1	0.00	0.00 NS
Maternal host	1	4.71	6.78**
Paternal host	1	0.00	0.00 NS
Maternal host × paternal host	1	0.81	1.17 NS
Maternal host × rearing host	1	0.03	0.05 NS
Paternal host × rearing host	1	11.57	16.66***
Error	4171	0.70	

** $P < 0.01$; *** $P < 0.001$.

† The following interactions were not significant, and thus were deleted from the model: Population × maternal host; population × paternal host; population × host × maternal host; population × host × paternal host; population × maternal host × paternal host; host × maternal host × paternal host; population × host × maternal host × paternal host.

ulation differentiation should involve at least two generations of rearing in a common environment.

In *Stator limbatus,* maternal host likely affects offspring performance via changes in egg size and/or quality. For example, mothers reared on *C. floridum* produce distinctly yellow eggs, while mothers reared on *A. greggii* produce clear-white eggs, suggesting host effects on egg composition (C. W. Fox, *personal observation*). In addition, beetles collected from *C. floridum* in nature lay larger eggs in the laboratory than beetles collected from *A. greggii,* which may reflect host-induced egg-size variation (C. W. Fox, *personal observation*). In other insects, both egg size and egg composition have been demonstrated to have detectable effects on offspring life histories (Rossiter 1991*b*; reviewed in Fox 1994*a, b*), and this is likely the case for *S. limbatus* (C. W. Fox and T. A. Mousseau, *unpublished manuscript*). At this time, however, the sources of the maternal host effect are unclear for *S. limbatus.*

While maternal host (but not paternal host) affected development time and body mass of *S. limbatus* offspring, both maternal and paternal host had large effects on survivorship of offspring (Table 1). Because the effect of paternal host was of the same magnitude, and in the same direction, as the effect of maternal host, these effects likely represent a response to intense selection during a single generation of rearing on *C. floridum,* and suggest the potential for rapid adaptation to this host by *S. limbatus.* Such rapid evolutionary responses over a single generation indicate a serious complication that arises in laboratory studies of maternal effects: rapid adaptation to a laboratory host or laboratory conditions may be interpreted as a maternal effect when in reality it represents a response to selection. Maternal host effects on larval host suitability/
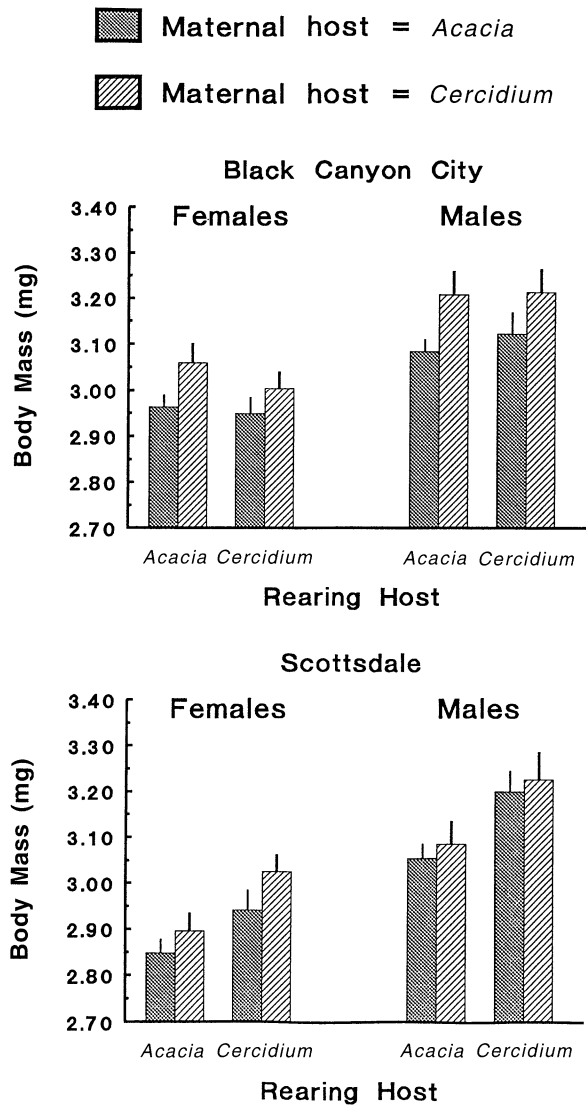

FIG. 3. The effect of maternal host on offspring body size. Note that for both sexes of both populations, mothers reared on *C. floridum* produced larger offspring than mothers reared on *A. greggii,* regardless of offspring host. Statistics are presented in Table 4.

larval performance are most likely selected for when host-associated mortality is high, and thus maternal "acclimation" can have a large effect on fitness. However, when mortality is high, laboratory experiments are complicated by responses to selection during the experiment, dramatically reducing our ability to detect these maternal effects. Comparison of maternal and paternal effects, as employed here, may help to disentangle the effects of selection from the effects of maternal environment.

Alternatively, rather than being a genetic response to selection, the maternal host effect on survivorship of *S. limbatus* offspring may result from simultaneous non-genetic effects of both maternal and paternal rear-

TABLE 4. Analyses of variance for body mass of two populations of *S. limbatus* reared on two host species (*Cercidium floridum* and *Acacia greggii*). $R^2 = 0.18$.

Source†	df	MS	F
Sex	1	41.73	213.00***
Population	1	0.04	0.07 NS
Maternal lineage (pop)	110	0.54	2.91***
Paternal lineage (pop)	110	0.42	2.28***
Rearing host	1	2.18	11.68***
Sex × host	1	1.08	5.82*
Population × host	1	2.05	11.04***
Maternal host	1	1.25	6.69**
Paternal host	1	0.11	0.45 NS
Maternal host × paternal host	1	0.10	0.52 NS
Maternal host × rearing host	1	0.20	1.09 NS
Paternal host × rearing host	1	0.41	2.20 NS
Error	4154	0.19	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† The following interactions were not significant, and thus were deleted from the model: Population × maternal host; population × paternal host; population × host × maternal host; population × host × paternal host; population × maternal host × paternal host; host × maternal host × paternal host; population × host × maternal host × paternal host.

ing host. While maternal effects are widely recognized as important contributors to offspring phenotypes, nongenetic paternal effects are generally ignored in ecological and genetic analyses. Because maternal effects are most commonly observed between adult mothers and the early developmental stages of their offspring (Mousseau and Dingle 1991*b*), probably due to the influence of cytoplasmic factors transmitted in their eggs, it is not surprising that environmentally induced paternal effects have rarely been observed. Paternal contributions to offspring are usually assumed to extend little beyond the limited materials contained within the sperm, and paternal effects are thus likely to be smaller and harder to detect than maternal effects. However, in many insects males transfer large spermatophores or ejaculates to females during mating. These ejaculates often contain a diversity of substances that are used by the recipient female for egg production (Boggs and

TABLE 5. Body mass of *Stator limbatus* reared on *Cercidium floridum* and *Acacia greggii* (means and standard errors). The beetles in this table served as parents to the experimental beetles of Tables 3 and 4.

Population	Body mass (mg)*	
	$\bar{X}$	SE
Black Canyon City		
Acacia greggii	2.97ᴬ	0.04
Cercidium floridum	2.88ᴮ	0.07
Scottsdale		
Acacia greggii	3.07ᶜ	0.03
Cercidium floridum	2.91ᴮ	0.05

* Values sharing a common letter are not significantly different from each other (host effects within populations, Wilcoxon signed-rank test, $P < 0.05$; population effects within hosts, Mann-Whitney U test, $P < 0.05$).

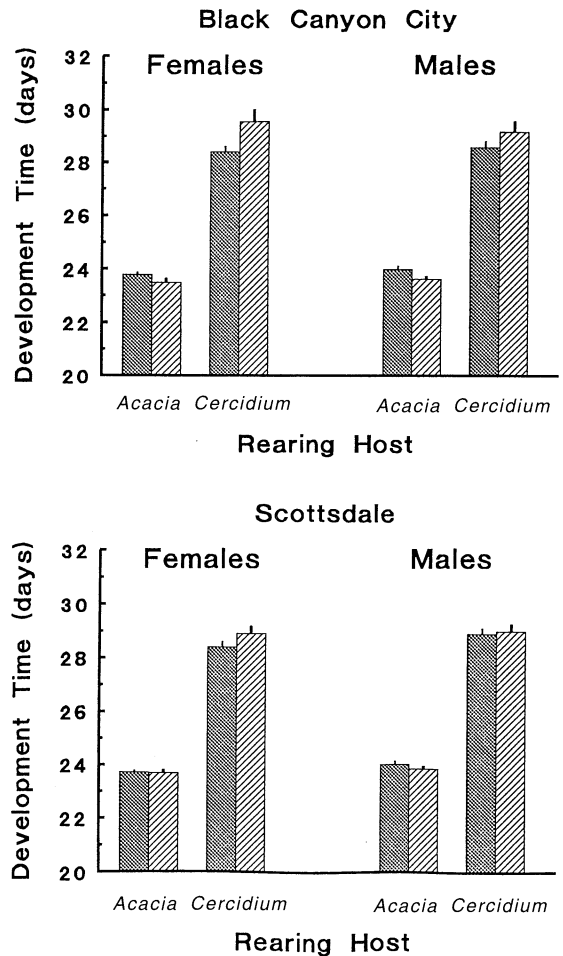

■ Paternal host = *Acacia*

▨ Paternal host = *Cercidium*

FIG. 4. The effect of paternal host on offspring development time. Note that fathers reared on *C. floridum* produced *slower* developing offspring on *C. floridum*, but *faster* developing offspring on *A. greggii*, than fathers reared on *A. greggii*. Statistics are presented in Table 3.

Gilbert 1979, Huignard 1983, Boucher and Huignard 1987), and may subsequently affect offspring life histories (review in Fox 1993). We have recently discovered that paternal body size affects female fecundity and egg size in *Stator limbatus*; females mated to large males produce both more and larger eggs than females mated to small males, likely due to variation among males in the size of extragametic contributions (C. W. Fox and T. A. Mousseau, *unpublished manuscript*). Therefore, factors that affect male body size (such as rearing host) can have an indirect effect on female egg size, and thus offspring phenotype, independent of any genetic effects.

Another difficulty in detecting paternal effects is that

most studies rarely employ a design of sufficient complexity to allow the decomposition of paternal genetic and paternal environmental influences on phenotypic variation in offspring. Exceptions can be found in the botanical literature where the ability to conduct complex diallel breeding designs permits the complete decomposition of variance components (e.g., Lacey 1991, Platencamp and Shaw 1993). In animal studies, one normally assumes that paternal contributions to offspring are limited to nuclear genetic effects. In fact, most quantitative genetics analyses assume that the sire component in a half-sib design will only contain the effects of additive genetic variance, V_A (Falconer 1989, Becker 1992), and maternal effects are estimated by subtracting sire from dam effects. In a parent–offspring design, the difference in coefficients between sire–offspring and dam–offspring regressions has been used to estimate m, the maternal effects coefficient (Lande and Price 1989). These approaches, like the one employed in this study, are probably reasonable given the infrequency and small contribution of paternal environmental effects to offspring, at least in animals.

Nonetheless, paternal effects on offspring life histories have been reported for a small number of insects (Futuyma et al. 1993). For example, the photoperiod experienced by fathers affects offspring development time in *Drosophila melanogaster*, independent of mother's photoperiod (Giesel 1988), most likely due to transgenerational changes in genome expression (Jablonka and Lamb 1989). An effect of both maternal and paternal host on genome expression in their offspring, and subsequently on the survivorship of larvae, could likewise explain the symmetry between the magnitude of paternal and maternal host effects on survivorship of *S. limbatus*.

Symmetrical non-genetic maternal and paternal rearing host effects would also explain the observed differences in survivorship between the parental generation and the final experimental generation (Fig. 1). In the parental generation, Scottsdale beetles survived substantially better on *C. floridum* than did Black Canyon City beetles (Table 2). However, these population effects disappeared by the final experimental generation (Table 1). Because Scottsdale grandparents (i.e., the parents of the parental generation; Fig. 1) were reared on *C. floridum* and Black Canyon City grandparents were reared on *A. greggii* (i.e., they were collected from these hosts), a parental effect (maternal or paternal) in which beetles reared on *C. floridum* produce better surviving offspring would create a population difference in survivorship. However, when parental host is controlled for by rearing parents from both populations on each host, the population effects should disappear, and parental host effects should be detectable (as we found in Table 1).

That paternal rearing host effects are possible, and even likely, in *S. limbatus* is also suggested by our analyses of development time; fathers reared on *C. flo-*

ridum produced *slower* developing offspring on *C. floridum*, but *faster* developing larvae on *A. greggii*, than fathers reared on *A. greggii*. This interaction possibly represents a response to selection in the paternal generation which is undetectable in the maternal host × offspring host interaction due to the large non-genetic maternal effect. However, whether a paternal effect or a response to selection, the effect is not in the direction expected; offspring developed slower on the host their father was reared on. Negative maternal effects, in which offspring express phenotypes the opposite of their mother's phenotype due to non-genetic effects, have been demonstrated in insects (Janssen et al. 1988, Rossiter 1991b) and vertebrates (e.g., Falconer 1965). For example, Gould (1988) demonstrated that offspring of mothers reared on quercetin-containing diets developed more slowly on quercetin-containing diets, the opposite from expected if offspring were "acclimated" to this host during maternal rearing. These results are similar to ours, except that our results were detected for fathers and so may represent negative paternal effects.

Our data indicate that parental rearing host has a large non-genetic influence on offspring life histories in the seed beetle, *Stator limbatus*. We also discovered paternal rearing host effects on offspring development time, although these effects were opposite in direction from what is expected if paternal effects were adaptations to a heterogeneous environment. Finally, our data demonstrate a difficulty in distinguishing non-genetic effects of parental environment from genetic effects using standard quantitative genetic analyses; when environmentally based parental effects are expressed through both mothers and fathers, they produce patterns of offspring phenotypes similar to those expected if selection was the causative agent of the phenotypic patterns.

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