Proximate Mechanisms Influencing Egg Size Plasticity in the Seed Beetle *Stator limbatus* (Coleoptera: Bruchidae)

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ABSTRACT The seed beetle *Stator limbatus* adjusts egg size in response to the quality of the oviposition host, laying small eggs on the high quality host, *Acacia greggii* seeds, and larger eggs on the poor quality host, *Cercidium floridum* seeds. We examined various proximate mechanisms of this egg-size plasticity. Once exposed to a new host, it takes 24 to 48 h for females to change the size eggs they lay. Beetles that came into direct contact with seeds adjusted egg size to a much greater extent than beetles that only came in contact with the fruits or were physically separated from the seeds. Egg size was not affected by size of the seed. Both the host seed in which the larvae were reared as well as the host to which adults were subsequently exposed influenced egg size. The default egg size in the absence of a suitable host is slightly larger than the size egg laid on *A. greggii*. Thus, beetles increase egg size in response to *C. floridum* seeds and slightly decrease egg size in response to *A. greggii* seeds.

KEY WORDS *Stator limbatus*, *Acacia greggii*, *Cercidium floridum*, egg size, plasticity

EGG SIZE IS AN ESPECIALLY interesting life history trait because it is simultaneously a maternal and progeny character–mothers determine egg size and composition, but egg size and composition can have substantial fitness consequences for progeny. Variation in egg size can affect larval size, development, survivorship, and so forth, with larvae hatching from larger eggs generally having higher fitness (reviewed in Fox and Czesak 2000). Egg size also influences maternal fitness, as there is usually a tradeoff between egg size and fecundity in which females laying larger eggs must lay fewer eggs (Berrigan 1991, Fox and Czesak 2000).

The fitness consequences of variation in egg size can differ among environments, such that selection favors different size eggs in different environments. This can lead to the evolution of geographic variation in egg size if the environments vary across geographic scales. In environments that are heterogeneous on a smaller scale, selection may favor egg size plasticity, in which females change the size eggs they lay in response to predictive environmental cues (Mousseau & Dingle 1991, Kawecki 1995, Fox and Mousseau 1998).

The seed beetle *Stator limbatus* (Horn) exhibits differences in egg size among populations that differ in the host trees to which they are exposed. Beetles from populations where catclaw acacia, *Acacia greggii*, is the dominant host lay smaller eggs than beetles from populations dominated by blue paloverde, *Cercidium floridum* (Fox & Mousseau 1996, Fox et al. 2001). Furthermore, females of this species can adjust egg size in response to oviposition environment. When exposed to the seeds of *A. greggii*, females lay smaller eggs than when exposed to seeds of *C. floridum* (Fox et al. 1994, 1997b). Such facultative adjustment in egg size is adaptive (Fox et al. 1997b). Eggs laid on *A. greggii* have very high survivorship (>90%) regardless of the size of the egg or origin of the population. Thus, on *A. greggii*, small eggs are favored because females laying smaller eggs have greater fecundity (Fox et al. 1997b). Eggs laid on *C. floridum*, however, have much lower survivorship that varies with egg size: larvae hatching from larger eggs have substantially greater survival on *C. floridum* seeds that those hatching from smaller eggs. Thus, when laying on *C. floridum*, large eggs are favored (Fox and Mousseau 1996, Fox et al. 1997b).

These beetles do not always adjust egg size when it is advantageous to do so, however. When exposed to other hosts in which they have low survivorship, such as *Chloroleucon ebano* or *Parkinsonia aculeata*, *S. limbatus* do not adjust egg size even though increased egg size greatly increases survivorship on these hosts (Fox et al. 1996, 1997a, Fox and Savalli 2000). Thus, the cues that trigger the production of larger eggs on *Cercidium floridum* seeds do not appear to be present in other hosts.

Although the adaptive significance of egg size plasticity by *S. limbatus* in response to different hosts has been well studied (reviewed in Fox and Mousseau 1998, Fox 2000, Fox and Messina 2001), the proximate mechanisms involved have not been examined. It is not clear what cues are used to trigger changes in egg size (such as chemical cues, seed size, and so forth),

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or what the response is in the absence of cues. There is some evidence to suggest that the cues used to recognize *C. floridum* are different from whatever factors result in the increased mortality. For instance, among *C. floridum* × *C. microphyllum* hybrids, egg size, and larval survivorship varied considerably, but the suitability of the host (survivorship for particular-sized eggs) did not correlate with the degree to which females adjusted egg size (Fox et al. 1997c).

In this study we investigate the possible proximate factors that might influence egg size such as the size of the host seed, mated status of the beetles, and whether there needs to be physical contact with the host seed. We also examine the relative importance of rearing host (the host in which the larvae were reared) versus subsequent experience with hosts during egg laying in the determination of egg size.

Population Origin, Maintenance, and General Methods. *Stator limbatus* is a generalist seed parasite distributed from northern South America to the southwestern United States (Johnson and Kingsolver 1976, Johnson et al. 1989). Unlike other members of the genus, *S. limbatus* is a host generalist that has been collected from seeds of ≈70 plant species in at least nine genera throughout its large geographic range. In the southwestern United States *S. limbatus* is abundant on many species of *Acacia* (Fabaceae: Mimosoideae) and two species of palo verde (*Cercidium floridum* and *C. microphyllum;* Fabaceae: Caesalpiniodeae). *A. greggii* and *C. floridum* are widespread throughout this region; at some sites either *A. greggii* or *C. floridum* dominates (*A. greggii* tends to occur at higher elevations), but many sites contain an abundance of both species.

S. limbatus oviposits directly onto host seeds, and is thus restricted to 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. Adults are the only dispersing stage; larvae are restricted to the host their mother has chosen for them. Beetles require only a single seed to complete development and reproduce. Thus, neither food nor water supplementation was necessary for the following laboratory experiments.

Beetles were collected at various localities in the vicinity of Phoenix, central Arizona, in August of 1995, 1996, 1998, and 2000. Collecting sites were <50 km from one another. Beetles were collected by picking mature seed pods from over 50 *Cercidium floridum, C. microphyllum,* or *Acacia greggii* plants in each year. Mature pods were transferred to the lab, and seeds containing beetles were separated from uninfested seeds. The laboratory populations were initiated with over 300 individuals collected from in the field from one or two of the hosts each year and reared on *A. greggii* seeds for 2 to 8 generations (varied among experiments), at 29 to 30°C, 16:8 h L:D before this study.

We initiated all experiments with virgin males and females collected from isolated seeds of *A. greggii,* generally within 12 h of their adult emergence. For all experiments beetles were reared at a density of one larva per seed by scraping off any excess eggs before hatching. It is not practical to weigh eggs because removing them from the seed is always destructive. Instead, we measured the length and width of eggs using an ocular micrometer on a stereomicroscope. Egg mass was calculated using the empirically derived equation $-0.035 + 0.086$ (egg length) $+0.022$ (egg width), $R^2 = 0.89$ (Fox and Savalli 2000). Results for egg length and width are qualitatively similar to the results for egg mass; for brevity we present only data for egg mass. For all experiments we measured 2 to 3 eggs per dish and calculated an average egg size per dish that was used in subsequent analyses.

We weighed beetles on an electronic balance to 0.1 mg precision. In all analyses, female mass was included as a covariate; in most cases its effect was not significant and is not reported here.

Experiment 1—Phenology of Egg Size Plasticity

Materials and Methods. This experiment was designed to determine the time course of egg size plasticity when exposed to a new host and to confirm earlier data that suggest that the response to a new host may be asymmetric (Fox et al. 1997b).

Virgin males and virgin females were collected from isolated seeds of *A. greggii* within 12 h of adult emergence, weighed and then paired with a single beetle of the opposite sex. 48 pairs where each confined in a 35 mm petri dish containing either eight *A. greggii* seeds or eight *C. floridum* seeds. Dishes were checked every 12 h until the female laid at least one egg. Half of the females from each host were randomly assigned to treatment or control. Treatment females were switched from seeds of *A. greggii* to seeds of *C. floridum* (Ag-Cf) or from *C. floridum* to *A. greggii* (Cf-Ag) after laying their first egg. Control females were left on their original host species (Ag-Ag or Cf-Cf) but switched to clean seeds. Every subsequent 12 h until they died, females were checked for oviposition. Any seeds bearing eggs were removed and replaced with clean seeds of the same species. Egg size was recorded for two haphazardly selected eggs from each 12-h interval.

Results

As in a previous study (Fox et al. 1997b), females initially exposed to *C. floridum* laid larger eggs than females initially exposed to *A. greggii* (Fig. 1, 0 h bars). Females that were continuously exposed to the same host (controls) gradually diverged in egg size, but females that were switched onto the other host began to adjust their egg size to the new host ≈24 to 36 h after the switch (Fig. 1; repeated measures analysis of variance (ANOVA), treatment-by-day interaction, $F_{12,104} = 5.19$, $P < 0.001$). After 48 h, there was no difference in egg size between the two treatments, and by 72 h females were laying eggs similar in size to those eggs laid by females when first starting to lay on the same host. Females begin egg laying 36 to 48 h after emerg-

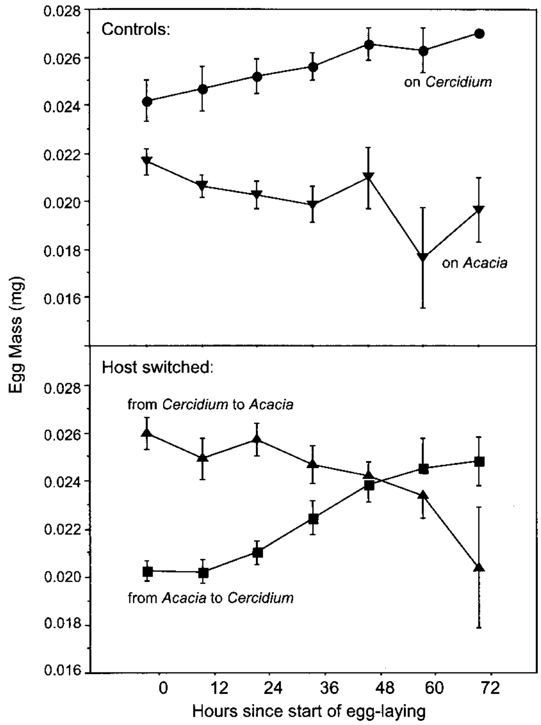

Fig. 1. The change in egg size after a switch to A) the same (controls) or B) new host species. Time zero represents those eggs present when beetles were switched onto new hosts and thus represent eggs laid before the host switch. Error bars represent ±1 SD.

ing from their natal seed (Fox et al. 1997b), which probably reflects the time needed to mature eggs. That females began to adjust egg size after a similar time period suggests that the delay in responding to a new host is because of the time it takes to mature eggs.

Although the time course for egg size plasticity is the same as reported in a previous study (Fox et al. 1997b), there is one notable difference. In this study, the response to a new host was symmetric, with Ag-Cf females increasing their egg size to a similar degree as Cf-Ag females decreased their egg size. This differs from the pattern described previously, in which Cf-Ag females showed only a slight decline in egg size while Ag-Cf females increased their egg size above that of females initially exposed to *C. floridum* (compare Fig. 1 with Fig. 3A of Fox et al. 1997b).

Experiment 2—Effect of Mated Status on Egg Size Plasticity

Methods. In experiment 1 we demonstrated that females require 24 to 36 h to adjust egg size. Egg maturation may be influenced by when females mate and fertilize the eggs. A delay in mating may retard egg maturation and thus influence subsequent egg size plasticity.

As in the previous experiment, virgin males and females were collected from *A. greggii* seeds and weighed. Females and males were either placed individually in separate petri dishes for 48 h before mating (delayed mating treatment) or were paired immediately and allowed to mate (immediate mating treatment) for 48 h without seeds present. Both sets of beetles were then placed on either *C. floridum* or *A. greggii* seeds and checked every 12 h until they laid at least three eggs. There were 17 to 19 pairs for each host/mating status combination. Three eggs were haphazardly selected from each dish for measurement.

Results

There was no direct effect of mating on egg mass ($F_{1,64} = 0.003$; $P = 0.95$), nor an effect of treatment on how beetles responded to the hosts (mating treatment*host interaction; $F_{1,64} = 0.112$; $P = 0.74$). Thus, whether or not beetles are given an opportunity to mate early does not affect egg maturation or egg size plasticity.

Experiment 3—The Effect of Exposure to Host Fruits on Egg Size Plasticity

Methods. All previous studies with this species have presented beetles with seeds that, for convenience, were removed from the fruits. In nature, females encounter the seeds within fruits, and the cues used by beetles to detect particular hosts may also be present within the fruits. It may be particularly advantageous to respond to fruits rather than seeds because females in search of oviposition sites will likely encounter many fruits within a single tree before finding one where the seeds are accessible (dehisced or damaged). By responding to cues from the fruits, females can begin adjusting egg size in advance of finding a suitable oviposition site.

Virgin males and females were collected from seeds of *A. greggii* within 12 h of emergence and paired with a single beetle of the opposite sex. Pairs where confined in 60-mm petri dishes containing fruits and/or seeds of either *A. greggii* or *C. floridum*. In one quarter of the dishes for each plant species, the fruits were intact (not damaged or dehisced), with the seeds present but inaccessible. This treatment restricts the beetles to the exterior of the fruit as they would encounter fruits in nature. In the second treatment, the fruits were split open and the seeds removed. Thus, the beetles had access to both the exterior and interior of the fruit but did not encounter any seeds. In the third treatment, beetles were exposed to dehisced fruits with the seeds present, exposing the beetles to both the fruit and the seed. The final treatment contained only seeds without any fruits. There were 42 pairs of beetles for each host-fruit treatment combination.

After 48 h (at which time females usually began laying eggs), beetles were removed from the fruits and/or seeds and transferred to 35-mm petri dishes containing 3 to 4 *A. greggii* seeds. These seeds were checked every 4 to 6 h. Once the beetles laid at least two eggs, usually within 12 h of transfer and thus

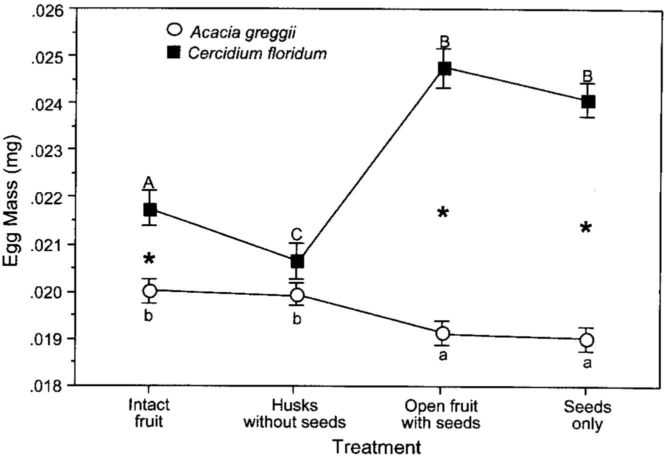

Fig. 2. The effect of fruit and seed exposure treatments on egg size (±1 SD) for two host species. Within a host, treatments indicated by different letters (uppercase for *C. floridum*, lowercase for *A. greggii*) are statistically significantly different from each other ($P < 0.05$; Fisher Protected least significant difference (LSD). Asterisks (*) indicate significant differences between hosts for a given treatment.

before egg size was adjusted in response to the *A. greggii* seeds, the beetles were removed from the seeds. Egg size was scored for two haphazardly chosen eggs from each dish.

Results. Beetles exposed to *C. floridum* seeds (either in opened fruits or in the seed-only treatment) laid larger eggs than those in contact with fruits only (either intact or as seedless husks) (Fig. 2). Similarly, beetles exposed to *A. greggii* seeds laid slightly but statistically significantly smaller eggs than those exposed to *A. greggii* fruits (ANOVA, treatment $F_{4,\ 310} = 10.27$, $P < 0.0001$; host*treatment interaction, $F_{3,\ 310} = 28.56$, $P < 0.0001$). Nonetheless the presence of seeds did appear to bleed through the intact fruits, as beetles exposed to intact *C. floridum* laid larger eggs than those exposed to either seedless husks of *C. floridum* or intact *A. greggii* fruits (Fig. 2). This indirect effect was much smaller than the effect of direct exposure to seeds.

Experiment 4—The Effect of Direct Contact with Seeds on Egg Size Plasticity

Methods. Females probably use chemical cues to recognize seeds. These chemicals may be volatiles or they may require direct contact with the seeds. To distinguish these possibilities we tested to see if females can adjust egg size both when in direct contact with seeds and when near but not in direct contact with seeds.

Virgin males and females were collected from seeds of *A. greggii* within 12 h of emergence and paired with a single beetle of the opposite sex. Pairs were confined in petri dishes containing ≈16 seeds of either *A. greggii* or *C. floridum*. In half of the dishes with each seed type, we allowed the beetles direct contact with the seeds. In the other half, ≈16 seeds of either *A. greggii* or *C. floridum* were placed in the bottom of a petri dish. A piece of screening was stretched over the dish, and a second petri dish was inverted over the first to produce a chamber twice as deep (18 mm) as a standard petri dish. The pair of beetles was placed in the upper petri dish and were separated by ≈5 mm from the seeds by the screen. Thus, these beetles were exposed to any volatile chemicals emitted from the seeds but were not in direct contact. There were 66 to 71 females for each host-exposure treatment combination. Beetles were left in these dishes for 36 h (and thus did not begin to lay eggs). After this time all beetles were transferred to *C. floridum* seeds and allowed to lay eggs for 24 h. The eggs were measured and then placed in an incubator. We scored hatching success for each egg.

Results. As expected, females in direct contact with *A. greggii* laid smaller eggs that had lower hatching success (on *C. floridum*) than females in direct contact with *C. floridum* (ANOVA, effect of host on egg size, $F_{1,270} = 102.34$, $P < 0.0001$; effect of host on egg survivorship, $F_{1,270} = 229.16$, $P < 0.0001$; Fig. 3). The beetles that did not contact the seeds directly, regardless of the seed type, all laid small eggs that had low survivorship similar to those exposed directly to *A. greggii* (ANOVA, host*contact treatment interaction on egg size, $F_{1,270} = 101.37$, $P < 0.0001$; on egg survirvorship, $F_{1,270} = 162.10$, $P < 0.0001$). Thus, females do not appear to respond to volatile chemicals but instead require direct contact with the host to adjust egg size.

Experiment 5—Effect of Seed Size on Egg Size

Methods. Females may use either physical or chemical cues to recognize *Cercidium* seeds and consequently adjust egg size. The possibility that females may respond to physical cues has not been tested. The seeds of *C. floridum* are considerably larger than the seeds of *A. greggii* (229 ± 2 mg vs. 187 ± 5 mg ± SE; Fox et al. 1996); this size difference could provide a

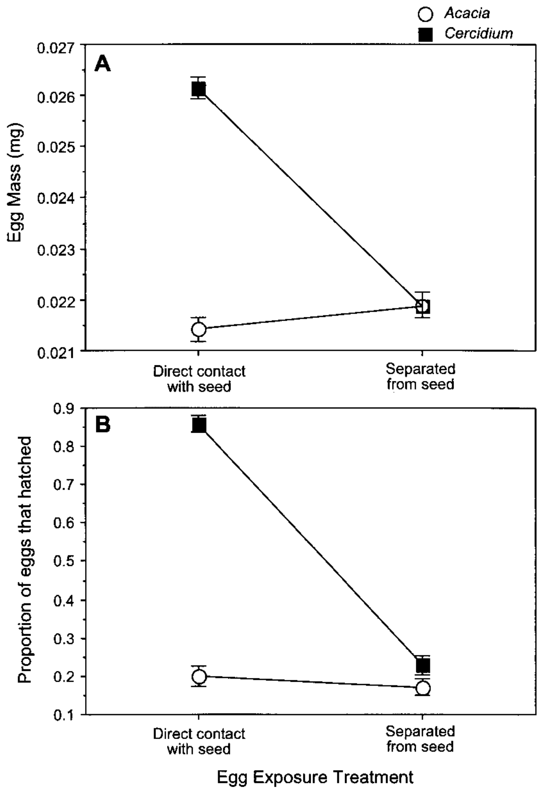

Fig. 3. The effect of host contact treatment (allowed direct contact with seeds versus separated from seeds by screening) on egg mass (A) and survivorship (B) (±1 SD).

cue that females use in adjusting egg size. Seed size also reflects the amount of available resources. Females might lay larger eggs on small seeds to compensate for the reduced resources available to her offspring. In this experiment we test if females respond to seed size as a cue.

As in previous experiments, virgin males and females were collected from isolated seeds of *A. greggii*, weighed and then paired with a single beetle of the opposite sex. Pairs were confined in single petri dishes containing ≈16 seeds and allowed to lay eggs. Beetles were reared from these seeds at a density of one larva per seed. Virgin adults were collected within 12 h of emergence from these seeds. The females emerging from each full-sib family were mated with a randomly selected male. Half of the females from each family were placed on eight large (≥230 mg) *C. floridum* seeds, while the other half were placed on eight small (≤160 mg) seeds. There 24 families with an average of 3.9 females (range, 1–9) per family and treatment. Dishes were checked every 12 h until at least three eggs were laid. We calculated the average egg size for each family in each treatment.

Results. Egg size (family means) did not differ between those eggs laid on large seeds and those laid on small seeds (repeated measures ANOVA, $F_{1,23} = 0.357$; $P = 0.36$). Similarly, there was no correlation between the average size of the eight seeds presented to females and the size of eggs laid by those females ($r = 0.132$, DF = 178, $P = 0.078$). Thus, females do not appear to use seed size a cue in determining egg size, although other physical cues, such as surface texture, cannot be ruled out.

Experiment 6—Effect of Host Exposure Delay on Egg Size Plasticity

Methods. In previous experiments in this study and in previous studies, beetles were usually exposed to one of their natural hosts. It is unclear, however, if the size of eggs laid on *A. greggii* represents the "default" egg size (that is, the size laid in the absence of any cues)—with females responding only to the cues provided by *C. floridum*—or if females actually adjust egg size downward in response to specific cues from *A. greggii* seeds. Females exposed to seeds not normally encountered in nature lay small eggs (Fox et al. 1997b), similar in size to those laid on *A. greggii*, suggesting that this may be the default egg size. However, some observations (e.g., Fig. 1A) suggest that females may decrease egg size in response to exposure to *A. greggii*.

To determine the default egg size that is produced in the absence of any cues, we compared egg size of females isolated from seeds for 48 h with egg size of females exposed to seeds immediately after hatching. As in previous experiments, virgin males and females were collected from *A. greggii* seeds and weighed. 109 females were assigned to one of three treatments: placed individually in dishes without seeds or placed in dishes containing either eight *C. floridum* or eight *A greggii* seeds. After 48 h, females were mated with randomly selected males. Half of the females from each treatment were presented with either *C. floridum* or *A. greggii* seeds until they laid at least three eggs. Three eggs were haphazardly selected from each dish for measurement.

Results. Because females were allowed to mature for 48 h before being mated and placed on seeds, most females began laying eggs almost immediately. Consequently, females showed only a weak, nonsignificant response to the test host (ANOVA, $F_{1,102} = 3.16$; $P = 0.079$). Not surprisingly, there was a highly significant effect of treatment (initial exposure to *C. floridum*, *A. greggii*, or no seeds) ($F_{2,102} = 48.89$; $P < 0.0001$). Females not presented with seeds during egg maturation laid eggs that were only slightly (and not statistically significantly) larger than those of females exposed to *A. greggii* during egg maturation (Fig. 4; Student-Neuman-Keuls, $P > 0.05$ for *A. greggii* vs. none). This suggests that the size of eggs laid on *A. greggii* are at least close to the default egg size, though some additional adjustment may be involved. This result is also consistent with females responding to their natal host (in this case, *A. greggii*) and simply making no additional adjustments to egg size.

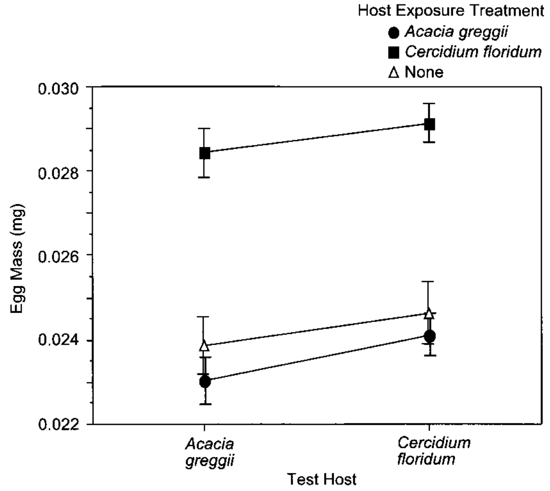

Fig. 4. The effect of previous exposure to *A. greggii*, *C. floridum*, or no host seeds on egg size plasticity when tested with either *A. greggii* or *C. floridum.* Error bars represent ±1 SD. As expected, beetles exposed to *C. floridum* laid significantly larger eggs, while beetles exposed to *A. greggii* laid eggs similar in size to those exposed to no seeds at all.

Experiment 7—The Effect of Natal Host and Early Adult Exposure on Egg Size

Methods. *S. limbatus* females reared on *C. floridum* lay larger eggs than females reared on *A. greggii.* Because seeds were checked for emerging beetles only every 12 h—thus, exposing beetles to their host seed for up to 12 h after emergence—these studies do not allow us to distinguish between the effects of rearing host and early postemergence exposure to those hosts. To distinguish these possibilities, we raised beetles on *A. greggii* or *C. floridum* and then allowed them to emerge into an environment containing only their host species or an environment dominated by seeds of the other species.

Virgin males and virgin females from two populations were collected from *A. greggii* seeds, paired, and confined in single petri dishes containing either ≈12 *A. greggii* seeds or ≈12 *C. floridum* seeds. The beetles were allowed to lay eggs for sufficient time to ensure that most of the seeds had at least one egg on them (typically around 60 h). Once laying was completed, excess eggs were scraped off the seeds such that each seed contained only a single haphazardly chosen egg. Half of the seeds from each dish were placed individually in dishes with 3 to 4 seeds of the same species. The rest of the seeds were placed individually in dishes containing ≈15 seeds of the alternate host. Thus, beetles would emerge and be exposed to either their natal host or else would be exposed predominantly to the new host. Once a beetle emerged, it was sexed and weighed. Females were immediately replaced in their dishes, while males were randomly paired with the females. The pairs were retained in the female's dish until the female laid at least two eggs (usually 36–48 h after emergence), at which point they were removed and the eggs measured. We performed two replicates of this experiment, using beetles collected from different areas in central Arizona.

Table 1. Repeated-measures ANOVAs for the effect of natal rearing host and the host experienced as an adult on the family means of egg mass

Factor	df	*F*	*P*
Apache junction			
Natal host	1	20.98	<0.0001
Subject (group)	34		
Adult experience	1	31.15	<0.0001
Experience*natal host	1	1.86	0.18
Experience*subject (group)			
Scottsdale			
Natal host	1	31.17	<0.0001
Subject (group)	72		
Adult experience	1	37.64	<0.0001
Experience*natal host	1	1.197	0.28
Experience*subject (group)	72		

Results. In initial analyses using individual male-female pairs as data points, we tested for the effects of both the female's and the male's families and natal hosts on the size eggs produced by a pair of beetles. Males had no effect on egg size (ANOVA; Apache Junction: male's natal host, $F_{1,25} = 0.0078$; $P = 0.93$; male's family [nested in male host] $F_{59,25} = 1.019$; $P = 0.50$; Scottsdale: male's natal host, $F_{1,91} = 0.00008$; $P = 0.99$; male's family [nested in male host] $F_{99,91} = 1.186$; $P = 0.21$). We thus focused only on females in subsequent analyses. We calculated family means for females in each treatment.

Both natal (rearing) host and the host that beetles encountered after emergence had significant effects on subsequent egg size in both populations, and these effects were of similar magnitude (Table 1; Fig. 5). The effects of natal and early hosts were additive; there were no significant interactions. These results suggest that both natal host and early experience can influence egg size. However, it is also possible that host effects were a result of natural selection. On *C. floridum,* larval survivorship is low (<50%) and positively correlated with egg size, while on *A. greggii,* survivorship is nearly 100% and independent of egg size (Fox & Mousseau 1996, Fox et al. 1997b). Because egg size is heritable (h^2 ranged from 0.2 to 0.9; Fox et al. 1999), it is possible that the increased egg size of *C. floridum*-raised beetles is because only those beetles that hatched from large eggs that survived to reproduce.

Experiment 8—Natal-Host Effects on Female Egg Size

Methods. In the previous experiment we demonstrated that both rearing host and early exposure can influence egg size. However, the rearing host effect could be a result of evolution in response to natural selection. Females that lay larger eggs are more likely to produce surviving offspring that will also lay larger eggs. To distinguish between a plastic response to rearing host and selection for large egg size, we per-

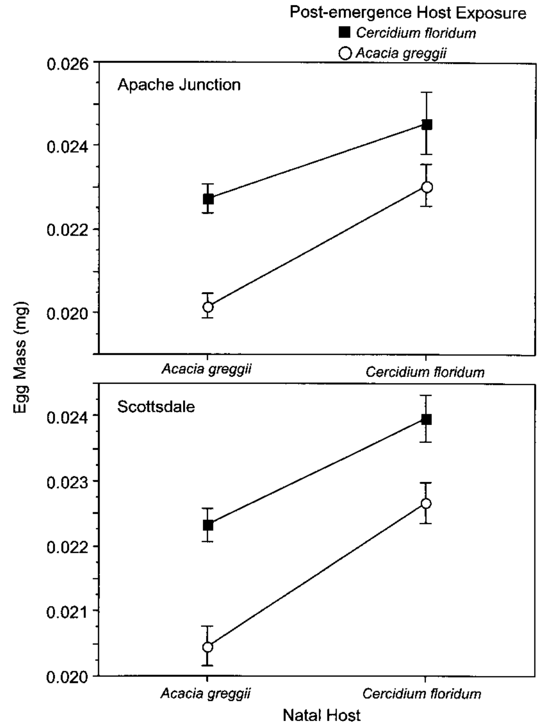

Fig. 5. Both natal host (the host upon which the larva fed) and the host seed experienced after emergence affected egg mass (±1 SD). See Table 1 for statistics.

formed a two-generation experiment, such that we could compare the egg size of female lines that experienced similar levels of selection (one generation on *C. floridum*), but differed in their most recent host (Fig 6). If the effect of rearing host is due entirely to selection for large eggs when reared on *C. floridum*, we expect that egg size should follow the patterns of Cf-Cf > Cf-Ag = Ag-Cf > Ag-Ag (where Cf and Ag represent, in sequence, the first and second generation hosts), because the Cf-Cf treatment will experience two generations of selection on *Cercidium* while the CF-Ag and Ag-Cf treatments will each experience only one generation. However, if egg size is a facultative response to natal host, then we expect an egg size pattern of Cf-Cf = Ag-Cf > Cf-Ag = Ag-Ag.

Furthermore, instead of allowing females to emerge into an environment dominated by the natal or alternate host, we instead elected to reduce early host experience by removing beetles from their dish within an hour of emergence from their rearing seed. Therefore, we also tested for the influence of immediate exposure to a new host versus delaying without the presence of any host.

Virgin males and virgin females were collected from *A. greggii* seeds, paired, and confined in single 30 mm petri dishes containing either ≈12 *A. greggii* seeds or ≈12 *C. floridum* seeds. Excess eggs were scraped off the seeds such that each seed contained only a single egg. Virgin adults emerging from these treatments (henceforth referred to as generation 1) were mated and placed on ≈12 seeds of either *A. greggii* or *C. floridum.* Beetles emerging from these seeds thus represent full-sib families (generation 2). We checked for emerging beetles at 1-h intervals throughout the lights-on phase of the incubator L:D cycle. Any beetles that emerged during the dark phase (≈1%) were discarded when beetles were first checked in the morning. Beetles were weighed and mated within 1 to 2 h of emergence (occasionally slightly older [< 12 h old] males were used when needed for mates). Half of the beetles from each family were assigned to one of two seed-exposure treatments. For the zero-hour delay treatment, mated pairs were immediately placed on 3 to 4 *A. greggii* seeds, while for the 48-h delay treatment, mated pairs were placed in dishes without seeds for 48 h before being given seeds. Seeds were checked for eggs every 4 to 6 h during the day. Beetles were removed from the seeds once they laid at least two eggs. Egg size was scored for two haphazardly selected eggs from each dish. Family means of egg size were calculated for each delay-treatment/host-family combination and analyzed with delay treatment as a repeated-measure. We performed two experiments, using beetles collected from each of two areas in central Arizona.

Results. As in the previous experiment, we detected no significant effects of either male rearing host or male family on the mass of eggs for either generation of males (ANOVA; Apache Junction: male's natal host, $F_{1,42} = 0.0599$; $P = 0.81$; male's family [nested in male host] $F_{42,42} = 1.840$; $P = 0.026$, male's father's host, $F_{1,42} = 0.015$, $P = 0.90$; Scottsdale: male's natal host, $F_{1,81} = 0.140$; $P = 0.71$; male's family [nested in male host] $F_{82,81} = 0.697$; $P = 0.95$, male's father's host, $F_{1,81} = 0.0532$, $P = 0.82$). Thus, male contributions do not influence female egg size plasticity and all subsequent analyses present data for female families.

As predicted by the plastic response hypothesis and contrary to the natural selection hypothesis, only the second generation host, and not the first, influenced female egg size in the Scottsdale population (Fig. 7A, Table 2). There was a significant effect of generation one maternal host for the Apache Junction population, although this effect was much weaker than the generation two-host effect (Fig. 7B, Table 2).

Females from both hosts exposed to *A. greggii* immediately after emerging laid smaller eggs than females kept from seeds for 48 h, but in the Scottsdale population the effect was more pronounced for females initially exposed to *C. floridum* (as indicated by the significant treatment-host interaction term; Table 2). There was no significant interaction for the Apache junction population, possibly attributable to the smaller sample size for this population. These results indicate that, in the absence of cues from a new host, females lay the size eggs determined by their natal host.

Discussion. Female *S. limbatus* adjust egg size in response to environmental variation. Females lay small eggs on a high quality host, *A. greggii,* thereby maximizing the number of eggs that they can lay. On the poor quality host *C. floridum,* however, females lay

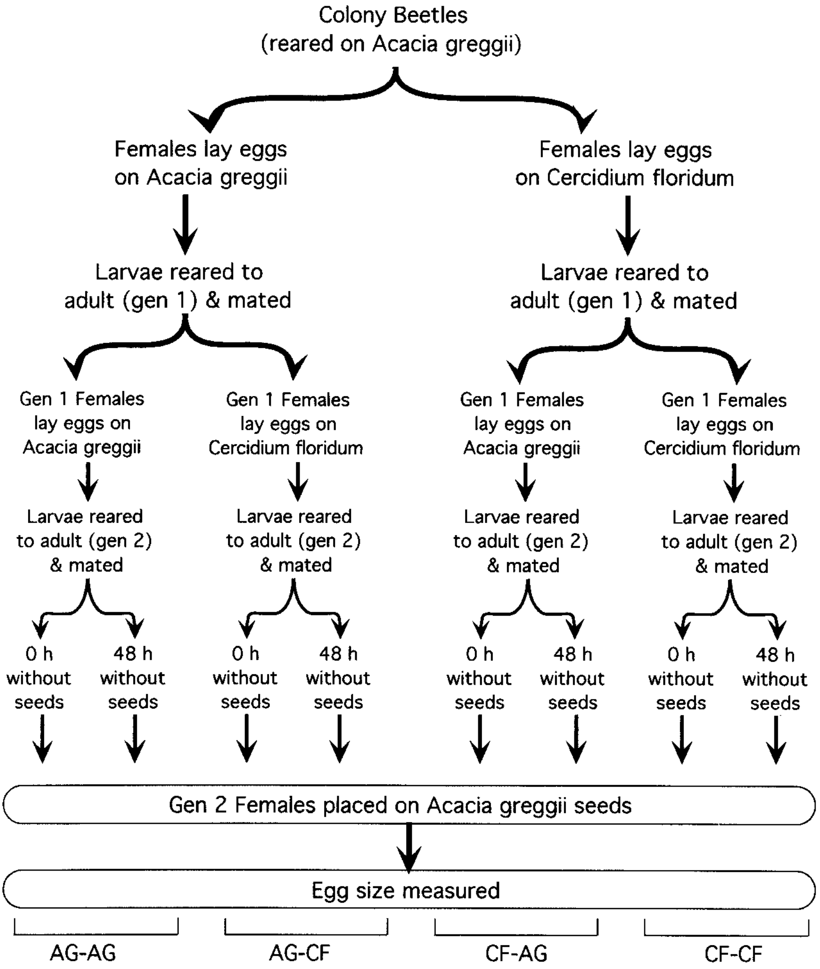

Fig. 6. Experimental procedure for experiment 8.

larger eggs that increase larval survivorship on that host. However, until now we have had no understanding of the mechanisms by which females identify and respond to seeds, and whether *S. limbatus* respond only to *C. floridum* or respond to both host species.

Although females are able to adjust egg size, there are constraints on their ability to do so. Specifically, females cannot adjust egg size immediately in response to a particular host. Instead, it takes females ≈36 to 48 h to respond to a host (Fig. 1). Although this delay has been demonstrated in other studies (Fox et al. 1997b), the previous study suggested that the response may be asymmetric, with the increase after a switch from *A. greggii* to *C. floridum* being greater than the decrease after switching from *C. floridum* to *A. greggii.* In this study, no such asymmetry was apparent: beetles adjusted egg size to a similar degree regardless of the direction of the switch (Fig. 1B). This difference could reflect population differences. The population in this study was collected from a mix of *A. greggii* and *C. floridum* (Apache Jct., AZ), while the population studied in Fox et al. (1997b) was collected about ≈50 km away (Scottsdale, AZ) in an area dominated by *C. floridum.* The weaker response to *A. greggii* shown by the Scottsdale population could be due to the absence of *A. greggii* at this site. It is also possible, however, that these differences simply reflect differences in laboratory conditions or seed quality, because the two studies were done at different times. Suitable host species are found throughout the intervening desert regions, and even beetle populations far from any *C. floridum* populations (southern Texas) show similar magnitudes of egg-size plasticity, suggesting there may be substantial gene flow among populations (Fox et al. 1994, 1999).

The delay in response after detecting a host has a number of implications for the life history of these beetles. If beetles frequently move among trees, they may encounter new hosts and begin laying before they have completed adjusting egg size. In previous experiments we found no evidence that females delay egg laying in response to novel hosts (Fox et al. 1994, 1997b). Females that encounter multiple species of hosts in short succession thus may lay intermediate-

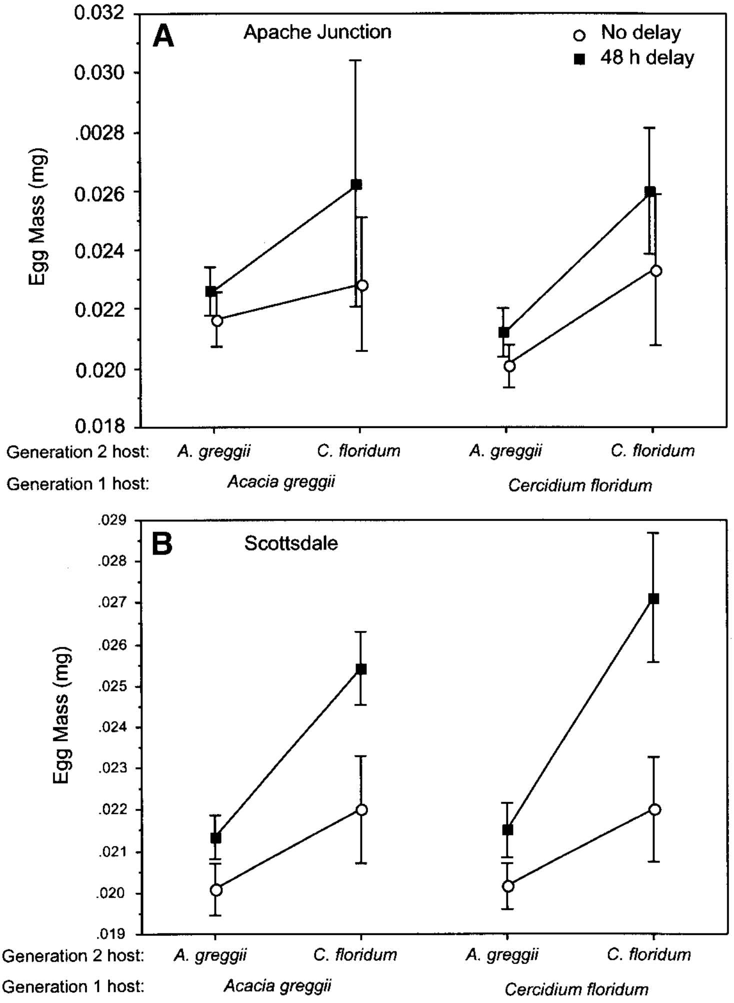

Fig. 7. The results of the two-generation host-effect experiment. There is no difference in egg mass (±1 SD) between those whose grandparents were raised on *A. greggii* versus *C. floridum*, suggesting no selection. There is, however, a significant effect of their parent's host.

sized eggs that represent an optimal strategy to maximize fitness (Roff 2001). Moving between host trees may also be important in allowing females to colonize other low-quality hosts that they do not respond to directly. Females exposed to *C. floridum* lay larger eggs and when subsequently exposed to *C. ebano*, offspring of these females have much higher survivorship on this introduced host than offspring of those females not exposed to *C. floridum* (Fox & Savalli 2000).

The delay in responding to host seeds would also be alleviated if females can begin to adjust egg size without having to be in direct contact with the seed. In nature, females probably need to examine numerous fruits before finding one that is accessible (females need to enter a dehisced or damaged fruit to lay eggs directly on the seed). Thus, being able to respond to the fruits themselves—or to volatile chemicals produced by the fruits or seeds—would enable females to

Table 2. Repeated-measures ANOVAs for the effect of the exposure delay treatment (no delay versus 48 h) and the generation 1 (females' mother's) and generation 2 (females') natal hosts on the family means of egg mass

Factor	df	*F*	*P*
Apache junction			
Female's host	1	28.58	<0.0001
Female's mother's host	1	8.82	0.0053
Subject (group)	36		
Delay treatment	1	12.99	0.0009
Treatment*female's host	1	2.04	0.16
Treatment*mother's host	1	0.51	0.48
Treatment*subject (group)	36		
Scottsdale			
Female's host	1	93.10	<0.0001
Female's mother's host	1	0.41	0.52
Subject (group)	69		
Delay treatment	1	79.44	<0.0001
Treatment*female's host	1	25.04	<0.0001
Treatment*mother's host	1	0.46	0.50
Treatment*subject (group)	69		

begin adjusting egg size even before they begin to lay eggs on a suitable seed. We found that females needed direct contact with seeds to fully adjust egg size. Females that we separated from the seeds by an intact fruit made only very slight adjustments in egg size (experiment 3). Either fruits can transmit only small quantities of whatever substances beetles use to distinguish hosts from the seeds (c.f. intact fruit v. husks for *C. floridum* in Fig. 2) or larger amounts are present but beetles selectively ignore information coming only from fruits. The latter strategy could be adaptive if a large proportion of the fruits are inaccessible and beetles need to visit multiple trees (and thus, potentially multiple species) before finding seeds that are accessible; contact with fruits may not be a good predictor of the eventual host.

Females also do not respond to volatile chemicals produced by seeds, and instead must be in direct contact (experiment 4). Furthermore, seed size does not trigger egg size plasticity, and although we cannot rule out more subtle features such as seed texture or color, it seems likely that beetles are responding to chemical cues. Interestingly, those cues do not seem to be the same chemical factors that lead to high larval mortality. In a study of hybrid *C. floridum* × *microphyllum* trees (*C. microphyllum* is a high quality host with high larval survivorship; beetles exposed to this host lay small eggs), larval mortality for a given tree did not correlate with the size egg laid on that tree (Fox et al. 1997c). This suggests that the seed factors that beetles respond to are not the same as the factors that cause larval mortality. Seed coat extraction experiments succeeded in extracting and transferring the *C. floridum* mortality factor to *A. greggii* seeds, but did not affect the size eggs laid by females (M. E. Csezak, and C.W.F., unpublished data).

Not surprisingly, the host experienced during the first 48 h after emergence has a substantial impact on egg size. In addition, the host from which the female emerged also affects the size of her eggs. Indeed, the magnitudes of these effects were similar (Fig. 5). Natural selection favors larger eggs (which result in greater larval survivorship) when using *C. floridum* and could thus account for any observed rearing-host effects. However, a two-generation experiment (experiment 8) demonstrated that natural selection is not sufficient to account for the observed patterns of egg size.

To fully understand the evolution of egg size plasticity, we need to understand the default egg size; that is, the size egg laid in the absence of any cues. Previous studies have shown that when exposed to other potential hosts, *S. limbatus* tend to lay small eggs (as they do on *A. greggii*), even if the host is of low quality (Fox et al. 1996, 1997b, Fox & Savalli 2000). This suggests that the size eggs laid on *A. greggii* is the default size, adjusted only when females encounter specific cues from *C. floridum.* Unfortunately, this is difficult to interpret because beetles are always exposed to host seeds during their development. Nonetheless, several clues emerge. In the phenology experiment (experiment 1), egg size continued to decline even in the controls that were always on *A. greggii* seeds. Although this could be attributed to a general decline because of aging, it should be noticed that the controls exposed to *C. floridum* continued to increase in egg size during this period. This pattern is consistent with females continuing to adjust eggs in response to cues from both *C. floridum* and *A. greggii.* Further evidence suggesting that females respond to *A. greggii* by reducing egg size comes from experiments 3, 4, 6, and 8. In all cases, females that were not in direct contact with seeds laid larger eggs than those in direct contact with *A. greggii* seeds. Although not all of these results were statistically significant, the pattern was consistent across all experiments. These results suggest that the default egg size, even for beetles reared on *A. greggii*, is slightly larger than the size eggs laid after sufficient exposure to *A. greggii* seeds. Females adjust egg size in response to cues from *A. greggii* as well as from *C. floridum.*

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