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# EVOLUTIONARY ECOLOGY OF EGG SIZE AND NUMBER IN A SEED BEETLE: GENETIC TRADE-OFF DIFFERS BETWEEN ENVIRONMENTS

MARY ELLEN CZESAK[1] AND CHARLES W. FOX
*Department of Entomology, University of Kentucky, Lexington, Kentucky 40546-0091*

*Abstract.*—In many organisms, large offspring have improved fitness over small offspring, and thus their size is under strong selection. However, due to a trade-off between offspring size and number, females producing larger offspring necessarily must produce fewer unless the total amount of reproductive effort is unlimited. Because differential gene expression among environments may affect genetic covariances among traits, it is important to consider environmental effects on the genetic relationships among traits. We compared the genetic relationships among egg size, lifetime fecundity, and female adult body mass (a trait linked to reproductive effort) in the seed beetle, *Stator limbatus*, between two environments (host-plant species *Acacia greggii* and *Cercidium floridum*). Genetic correlations among these traits were estimated through half-sib analysis, followed with artificial selection on egg size to observe the correlated responses of lifetime fecundity and female body mass. We found that the magnitude of the genetic trade-off between egg size and lifetime fecundity differed between environments—a strong trade-off was estimated when females laid eggs on *C. floridum* seeds, yet this trade-off was weak when females laid eggs on *A. greggii* seeds. Also differing between environments was the genetic correlation between egg size and female body mass—these traits were positively genetically correlated for egg size on *A. greggii* seeds, yet uncorrelated on *C. floridum* seeds. On *A. greggii* seeds, the evolution of egg size and traits linked to reproductive effort (such as female body mass) are not independent from each other as commonly assumed in life-history theory.

*Key words.*—Artificial selection, fecundity, genetic trade-off, offspring size, reproductive effort, *Stator limbatus*.

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Offspring size at birth, hatching, or germination is often under strong selection; large offspring frequently mature earlier, have improved ability to avoid or withstand predation or competition, or survive better in stressful environments compared to small offspring (e.g., low quality host plants; Fox et al. 1997, reviewed in Roff 1992; Carrière and Roff 1995; Azevedo et al. 1997; and Fox and Czesak 2000), although small offspring may have fitness advantages in some cases (e.g., hatch earlier; Fox 1997). Selection also generally favors high female fecundity, and it is generally assumed that the simultaneous evolution of large offspring and high fecundity is constrained by a trade-off between these two traits (e.g., Smith and Fretwell 1974; Parker and Begon 1986; McGinley et al. 1987; Winkler and Wallin 1987). Many studies have demonstrated a phenotypic correlation between offspring size and number (e.g., Sinervo and Licht 1991; Fox 1997; Vaughton and Ramsey 1998), but few studies have examined their underlying genetic relationship (but see Lynch 1984, 1985; Snyder 1991; Ebert 1993; Schwarzkopf et al. 1999).

A trade-off between offspring size and number may be obscured if total reproductive effort varies substantially among females, either through variation in the amount of resource stores or variation in acquisition of resources from the environment (van Noordwijk and de Jong 1986; de Laguerie et al. 1991). Generally, the evolution of offspring size and number and the evolution of total reproductive effort have been explored as separate problems (e.g., Schaffer 1974; Smith and Fretwell 1974; Parker and Begon 1986; McGinley et al. 1987). Reproductive effort is optimized with respect to trade-offs with the amount of resources allocated towards growth and/or somatic maintenance (survival), followed by allocation of this optimized reproductive effort among offspring of different sizes and numbers. Although a genetic relationship between offspring size and total reproductive effort or traits related to total reproductive effort (such as female size) has been considered in theory (Winkler and Wallin 1987), rarely has this relationship been examined experimentally (but see Schwarzkopf et al. 1999; Caley et al. 2001).

The pattern of selection on offspring size and reproductive effort may differ among environments (Fox 2000; Fox et al. 2001). Under conditions of poor juvenile survivorship, large offspring may have a selective advantage over small offspring (McGinley et al. 1987; Fox and Mousseau 1996; Fox et al. 1997, 2001). Many optimality models predict that females should expend more reproductive effort in oviposition sites of high quality compared to sites of low quality (e.g., Parker and Courtney 1984; Charnov and Skinner 1985). Among other traits, environmental differences in genetic (co)variances or genetic correlations have been detected (e.g., Via 1984; Service and Rose 1985; Scheiner et al. 1989; Holloway et al. 1990; Guntrip et al. 1997; Lazarević et al. 1998; Gu and Danthanarayana 2000; Bégin and Roff 2001), yet we lack research examining how evolutionary relationships among offspring size, number, and traits affecting total reproductive effort may differ among environments.

Here, we examine the genetic relationships among egg size, lifetime fecundity, and female body mass (a trait that affects total reproductive effort) in a seed beetle, *Stator limbatus*, that oviposits and develops within seeds of leguminous plants. These relationships were examined on two host-plant species (environments), *Acacia greggii* and *Cercidium floridum*, because *S. limbatus* exhibits very different reproductive strategies on seeds of these two species; females lay larger but fewer eggs on *C. floridum* seeds than on *A. greggii* seeds. This egg-size plasticity is an adaptation to host-related differences in the quality of seeds for larval survivorship (Fox et al. 1997, 1999). On *A. greggii* seeds, larval survivorship is high and not dependent on the size of eggs from which

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[1] Present address: Department of Biology, Vassar College, Poughkeepsie, New York 12604; E-mail: maczesak@vassar.edu.

larvae hatch, whereas on *C. floridum* seeds, larval survivorship is generally low (<50%), but improves for larvae that hatch from larger eggs (Fox 1997). We used half-sib analysis to estimate genetic correlations among egg size, lifetime fecundity, and female body mass on *A. greggii* and *C. floridum* seeds, followed with artificial selection on egg size on both host species to observe the correlated responses of lifetime fecundity and female body mass. Because larval survivorship on *C. floridum* seeds is generally low, we artificially selected for females laying large or small egg size when ovipositing on this host species, but bred the next generation on *A. greggii* seeds to avoid inadvertent natural selection on egg size. We found that the strength and direction of the genetic correlations among egg size, lifetime fecundity, and female body mass differed between the host-plant species on which egg size and fecundity were measured. Results were entirely consistent between genetic correlation estimates from half-sib analysis and realized genetic correlations from artificial selection on egg size.

## MATERIALS AND METHODS

### *Natural History of Stator limbatus*

*Stator limbatus* (Horn) (Coleoptera: Bruchidae) is a beetle that develops within seeds of more than 70 legume species and ranges from northern South America to the southwestern United States (Johnson and Kingsolver 1976; Johnson et al. 1989; Nilsson and Johnson 1993). Although a generalist over its wide geographical range, it is limited to one or a few host-plant species in any specific location. In central Arizona, *S. limbatus* commonly uses *Acacia greggii* (Fabaceae: Mimosoideae) and *Cercidium floridum* trees (Fabaceae: Caesalpinioideae) as host plants.

Females enter pods that have either dehisced or been damaged by other organisms and lay their eggs on the surface of seeds. Upon hatching, first instar larvae burrow through the seed coat directly underneath the egg and continue their development within the seed. Beetles emerge from seeds as adults and are the only dispersing stage; larvae are restricted to the seed upon which their mother oviposited. In the laboratory, mating and oviposition occur 24–48 hours after emergence from the seed. The resources inside a single seed provide all required nutrients for developing larvae. The short development time (approximately 23 days at 30°C) of *S. limbatus* and ease of laboratory rearing allow for large-scale laboratory experiments.

### *Study Population*

Beetles were collected in August 1998 along Mountainview Road in Apache Junction, Pinal County, Arizona, near the base of the Superstition Mountains (in central Arizona; 33° 48′ N, 111° 47′ W). Both *A. greggii* and *C. floridum* trees are present at this location and distributed between populations of *S. limbatus* (M. E. Czesak and C. W. Fox, pers. obs.). Mature pods were collected from *A. greggii* trees and examined for *S. limbatus* eggs on the seeds. These eggs were used to establish a laboratory colony, which was initiated with more than 300 individuals collected from more than 20 trees.

To provide seeds for the experiment, mature pods were collected from *A. greggii* and *C. floridum* trees. Undamaged pods were shipped to the laboratory where seeds were frozen until used in experiments. Within a species, seeds from all trees were thoroughly mixed to control for variation among trees in effects on beetle survival and life history (Fox et al. 2001).

Beetles were reared individually in petri dishes (35 mm) for three generations on *A. greggii* seeds before initiation of experiments (reared at 30°C, 16:8 L:D). Because larval survivorship is high on *A. greggii* seeds (>95%) yet low on *C. floridum* seeds (<50%), beetles were reared on *A. greggii* seeds to avoid inadvertent selection before and during the experiment.

### *Estimating Heritabilities of and Genetic Correlations among Egg Size, Lifetime Fecundity, and Female Body Mass*

#### *Experimental design*

A half-sib design (Falconer and Mackay 1996) was used to estimate additive genetic variation in and genetic correlations among adult body mass, egg size, and lifetime fecundity of females ovipositing on *A. greggii* and *C. floridum* seeds. Half-sib families were produced by mating each of 126 sires sequentially to five different dams. Males mated successfully to 3.2 females on average (range 2–5), creating 404 full-sib families. To create these families, virgin beetles were collected within 12 h of adult emergence and isolated in a petri dish (35 mm). Because adults do not emerge fully mature, they were not mated immediately after collection; maturation is complete approximately 24–48 h after adult emergence. Thus, before mating, males were aged 24 h (from time of collection) in an empty petri dish, and females were aged 24 h in a dish with three *A. greggii* seeds.

Seeds were checked every 12 h for eggs. After a female started laying eggs, she was transferred to a new petri dish with 20 *A. greggii* seeds. Every 12 h, seeds bearing eggs were removed and replaced with clean seeds. The first 20 eggs laid by each female on separate seeds were reared for the next generation at 30°C (16:8 L:D) and at a density of one beetle per seed (extra eggs were scraped from the seed surface). Offspring were collected within 12 h of adult emergence and weighed (0.1 mg precision).

To estimate egg size and lifetime fecundity of the daughters, half of the daughters from each full-sib family received eight *A. greggii* seeds and the other half received eight *C. floridum* seeds upon which to lay eggs. Adults were aged 24 h before mating, with females in contact with seeds during egg maturation. This contact is required for females to respond to host species and adjust egg size accordingly (Savalli and Fox 2002). Females and males were then paired randomly, and seeds were checked every 12 h for eggs. Average egg size (length and width) of each female was estimated by measuring three eggs laid during the first 12-h period of oviposition using an optical micrometer on a 55× dissecting scope (0.005 mm precision). Eggs were not weighed because they are glued to the seed surface and are destroyed if removed. After the first 12-h period of oviposition, females were transferred to a new petri dish containing either 30 *A. greggii* or 30 *C. floridum* seeds and allowed to lay eggs until death to estimate their lifetime fecundity.

In total, body mass data were collected from 2797 females, and egg size and lifetime fecundity were collected for 2667 females from 404 full-sib families (126 sires and 511 dams). Average egg-to-adult survivorship was very high (98.8%), and thus biases from inadvertent selection during the experiment should have been minimal.

### *Analyses*

Additive genetic variances ($V_A$) in egg size, lifetime fecundity, and female adult body mass and additive genetic correlations ($r_A$) among these traits were calculated from variance components using the restricted maximum likelihood method (SAS PROC VARCOMP, METHOD = REML; SAS Institute 1985). Additive genetic variances were estimated as $4V_S$ ($V_S$ is among sire component). Maternal effects variance ($V_M$) was calculated by assuming that dominance variance and epistatic interactions were zero (such that $V_M = V_D - V_A/4$, where $V_D$ refers to dam variance). Standard errors of heritabilities ($h^2$) and additive genetic correlations were estimated by jackknifing the estimates (using S-Plus, Insightful Corp. 2001; Roff and Preziosi 1994; Windig 1997). Genetic correlations were compared between environments using a test of homogeneity (Sokal and Rohlf 1995). Results for egg length and egg width were consistent for all analyses, so only results for egg length are reported.

## *Realized Heritabilities of and Genetic Correlations among Egg Size, Fecundity, and Body Mass*

### *Experimental design*

Artificial selection on egg length was imposed on two host species (*A. greggii* and *C. floridum* seeds), and the correlated responses of lifetime fecundity, female adult body mass, age at first reproduction, and adult lifespan were observed. Lines on the two host species are referred to as ''*Acacia lines*'' and ''*Cercidium lines*'' throughout this paper. ''Up lines'' refers to lines selected for increased egg size and ''down lines'' refers to lines selected for decreased egg size. Only the oviposition host differed between the *Acacia* lines and *Cercidium* lines because all lines were reared on *A. greggii* seeds.

The selected lines were maintained with offspring from females laying the largest eggs (top 20%, up lines) or smallest eggs (bottom 20%, down lines). Control lines were maintained with randomly selected offspring from every female, thereby imposing no artificial selection on egg length. There were two replicates of each line for a total of 12 lines (2 up lines, 2 down lines and 2 control lines on each host species).

*Parents of Acacia lines.*—To establish the *Acacia* lines, virgin beetles were collected from isolated seeds within 12 h of adult emergence, confined in a petri dish (35 mm), and weighed (0.1 mg precision). Adults were aged 24 h before mating, during which time females were confined with 15 *A. greggii* seeds. After this period, beetles were mated ($n = 40$ pairs in each of two replicates per line). Dishes were checked every 12 h for eggs, and the time females began ovipositing was recorded to estimate age at first reproduction (to the nearest 12 h). Average egg size (length and width) of each female was estimated by measuring three eggs laid during the first 12-h period of oviposition (as described above). After a female laid eggs on at least 10 different seeds, she and her mate were transferred to a new petri dish and confined with 30 *A. greggii* seeds until death to estimate her lifetime fecundity. Ten eggs from each female were reared for the next generation at 30°C (16:8 L:D) and at a density of one beetle per seed (extra eggs were scraped from the seed surface). Offspring developing from these eggs were used to establish the selected and control lines and consisted of 400 beetles per line.

*Parents of Cercidium lines.*—The *Cercidium* lines were established in a manner similar to the *Acacia* lines except that egg size (length and width) was measured on *C. floridum* seeds. Because larval survivorship is low on *C. floridum* seeds (often $<50\%$) and affected by egg size (Fox et al. 1997), rearing beetles on *C. floridum* seeds would result in selection on egg size during the experiment. Additionally, rearing beetles on *C. floridum* seeds would create a high risk of extinction of the lines selected for decreased egg size. Thus to establish the *Cercidium* lines, virgin beetles were collected and treated as described for the *Acacia* lines except that females were confined with eight *C. floridum* seeds during the egg maturation period. After mating, females were allowed to oviposit on *C. floridum* seeds until they laid at least three eggs, after which they (and their mates) were transferred to a new petri dish with 15 *A. greggii* seeds and allowed to continue ovipositing. Three of the eggs laid on *C. floridum* seeds were measured. Of the eggs laid on *A. greggii* seeds, 10 offspring per female were reared for the next generation (''generation 0'') and were used to establish the selected and control lines. Lifetime fecundity was not measured in the parental generation of the *Cercidium* lines because females were switched from *C. floridum* to *A. greggii* seeds during their oviposition period.

*Establishing and maintaining selected and control lines.*—Upon adult emergence of generation 0, females were mated and allowed to lay eggs in the same manner as the parental generation except that adults were not weighed and lifetime fecundity and age at first reproduction were not recorded. Because 400 beetles were reared per line, there were 200 mated pairs per line. Average egg length was measured for each female. Control lines were established with two randomly selected offspring from each of 100 randomly selected females, thereby imposing no artificial selection on egg length. The up lines were established with 10 offspring from each of the 40 females that laid the longest eggs (top 20%), and the down lines were established with 10 offspring from each of the 40 females that laid the shortest eggs (bottom 20%). Thus, the up, down, and control lines within each replicate were initiated from a single group of 400 beetles.

*Sample sizes.*—The selected lines were maintained at 400 beetles per generation (40 selected females, 10 eggs per female) and the control lines at 200 beetles per generation (100 females, two eggs per female). Thus, 4000 beetles emerged each generation from a total of 12 lines (two replicates for each of the three *Acacia* lines [up, down and control], and two replicates for each of the three *Cercidium* lines). Because beetle survival was slightly less than 100% (average survival was 98%) and sex ratios deviated randomly from 1:1 each generation, the sample size of the control lines dropped below 200 beetles in certain generations. In these cases, for a single generation, three offspring per female were reared to boost the number of beetles in the next generation, after which two offspring per female were reared. This was not an issue for the selected lines because each line was maintained with 40 females and 10 offspring per female each generation. The sample sizes listed above are approximate—actual sample sizes varied slightly due beetle mortality (although mortality was very low, 2% on average).

*After selection.*—Artificial selection on egg length was imposed for nine generations, with mean egg length measured each generation. After these nine generations and prior to scoring the correlated responses to selection, beetles were raised for one generation without artificial selection on egg length. Thus, to create the 10th generation, three offspring were reared from all females in each line (i.e., selection was not imposed; $n = 600$ beetles per selected line, 300 beetles per control line). Upon emergence of the 10th generation, adults were aged 24 h mated, and confined with 10 *A. greggii* seeds. Six eggs per female were reared for the next (11th) generation.

Upon emergence of the 11th generation, adults were collected and weighed ($n = 1800$ beetles in each selected line, 900 beetles in each control line). Females were given either eight *A. greggii* seeds (*Acacia* lines) or eight *C. floridum* seeds (*Cercidium* lines) on which to oviposit. Average egg length and width, lifetime fecundity, and age at first reproduction were recorded for each female. In the 12th generation, adult lifespan was estimated for virgin females to determine whether selection on egg size led to a change in the amount of resources allocated toward or away from somatic maintenance, which we tried to detect as a change in adult lifespan. Virgin females were isolated in a petri dish without seeds until death. The time of death was recorded within 12 h.

Although selection was imposed only on egg length, egg width should evolve along with egg length because these traits are positively genetically correlated (result from half-sib analysis). Egg width was measured in the parental generation (prior to generation 0) and at the end of selection (11th generation) to ensure that overall egg size was evolving along with selection on egg length.

To summarize, prior to artificial selection on egg length, female adult body mass, average egg length and width, lifetime fecundity (*Acacia* lines only), and age at first reproduction were recorded. After selection, correlated responses of the following life-history traits were measured: female adult body mass, lifetime fecundity, age at first reproduction, and female adult lifespan (all traits measured at 11th generation except for adult lifespan, which was measured at the 12th generation). Initial means of these traits (before selection) were compared with means in the control lines after selection to ensure that significant changes in trait means occurred only in the selected lines (although female adult lifespan was not estimated prior to selection).

### *Analyses*

Mean egg length (±SE) was calculated each generation for each line. From these means, the response to selection on egg length was calculated each generation as the difference between mean egg length of generation $n + 1$ and mean egg length of generation $n$. Selection differentials were calculated each generation as the difference between mean egg length of females whose offspring were selected for the next generation and mean egg length of all females in that line. Realized heritability of egg length on both host species was estimated from the regression slope of the cumulative response to selection and the cumulative selection differential forced through the origin (using SAS PROC REG; SAS Institute 1985; Roff 1997). The response and correlated responses of the up, down, and control lines at the end of the experiment were compared, and Tukey's test was used to test whether both directions of selection deviated from the control lines. Realized genetic correlations were estimated from the following equation:

$$r_A = CR_Y/[0.5(h_X h_Y)(i)(\mathrm{stdev}_Y)],$$

where $CR_Y$ is the correlated response of trait Y, $h_X h_Y$ is the product of the square roots of the narrow-sense heritabilities of each trait, $i$ is the selection intensity, and $\mathrm{stdev}_Y$ is the standard deviation of the distribution in trait Y (Falconer and Mackay 1996). This equation contains ''0.5'' because selection was applied to one sex only (egg length is a trait of females). Heritabilities used in this equation were obtained from half-sib analysis (conducted two generations prior to the parental generation of the artificial selection experiment). Realized genetic correlations were compared between environments using a test of homogeneity (Sokal and Rohlf 1995). Realized genetic correlations could not be compared with half-sib estimates because such correlations are not independent (realized $r_A$ was calculated using heritabilities from half-sib analysis). The direction of mean observed and predicted correlated responses were compared. Standard errors for mean observed correlated responses were obtained by averaging the correlated responses between replicates. Predicted correlated responses were calculated from heritability and genetic correlation pseudovalues obtained by jackknifing the estimates, and the resulting predicted correlated responses were averaged.

## RESULTS

### *Host Species Effect on Egg Size and Fecundity*

Females ovipositing on *C. floridum* seeds laid larger eggs and had lower lifetime fecundity than females ovipositing on *A. greggii* seeds (significant host effect in Table 1; data from half-sib analysis; mean [±SE] egg length on *C. floridum*: 0.594 ± 0.001 mm, on *A. greggii*: 0.537 ± 0.001 mm; mean fecundity on *C. floridum*: 22.3 ± 0.1 eggs, on *A. greggii*: 36.7 ± 0.1 eggs, averaged across females within families and then across families).

### *Heritability of Egg Size and Lifetime Fecundity*

#### *Parameter estimates from half-sib analysis*

The genotype of a female affected the length of her eggs (Table 2) and her lifetime fecundity (Table 3) on both *A. greggii* and *C. floridum* seeds, as indicated by significant sire effects on these traits. These large sire effects on egg length and fecundity lead to highly positive heritability estimates for these traits (Tables 2 and 3).

Because a female's egg size and fecundity were correlated

TABLE 1. Analysis of variance of egg length and lifetime fecundity for *Stator limbatus* females ovipositing on seeds of *Acacia greggii* or *Cercidium floridum.* Note the significant host, sire, and dam effects. Type III sums of squares were computed using the general linear model of SAS (PROC GLM, with RANDOM statement and TEST option; SAS Institute 1985).

| | Analysis of variance | | | |
|---|---|---|---|---|
| | df | MS | $F$ | $P$ |
| Egg length (mm) | | | | |
| Host | 1 | 1.617249 | 4858.11 | <0.001 |
| Sire | 126 | 0.001626 | 2.36 | <0.001 |
| Dam (sire) | 265 | 0.000657 | 2.19 | <0.001 |
| Sire × host | 126 | 0.000366 | 1.27 | 0.027 |
| Dam × host (sire) | 250 | 0.000309 | 1.13 | 0.095 |
| Lifetime fecundity | | | | |
| Host | 1 | 100836.8 | 2413.20 | <0.001 |
| Sire | 126 | 80.7 | 1.40 | 0.027 |
| Dam (sire) | 264 | 45.1 | 1.54 | <0.001 |
| Sire × host | 126 | 43.0 | 1.47 | 0.005 |
| Dam × host (sire) | 247 | 29.1 | 0.88 | 0.897 |

with her body mass (results presented below), genetic variances in these traits could in large part be due to genetic variation in female body mass. Thus, residuals from regressions of egg length or fecundity versus female body mass were also analyzed to remove the effects of body mass on these traits. Large sire effects and highly positive heritability estimates were also found for these residuals (for egg length residuals: $h^2$ ± SE on *A. greggii*, 0.64 ± 0.14; on *C. floridum*, 0.50 ± 0.12; for fecundity residuals: $h^2$ on *A. greggii*, 0.40 ± 0.12; on *C. floridum*, 0.29 ± 0.09), indicating that the genetic variation in egg length and fecundity is not due entirely to genetic variation in female body mass.

Estimating maternal effects variance from half-sib data assumes that dominance variance and epistatic interaction variance are zero (Falconer and MacKay 1996). Maternal effects variance explained none or very little of the phenotypic variance in egg length or lifetime fecundity on either host species ($V_M$ in Tables 2 and 3). Thus, a mother's environment or phenotype did not significantly influence the egg size or fecundity of her daughters.

### *Response to selection on egg length*

Egg length responded strongly to artificial selection on both host species (Fig. 1; *Acacia* lines: $F_{2,3} = 530.87$, $P = 0.001$; *Cercidium* lines: $F_{2,3} = 138.59$, $P = 0.001$). The response was symmetric on both host species (*Acacia* lines: 8.4 ± 0.8% [±SE, averaged between replicates] and 8.8 ± 0.2% for up and down lines, respectively; *Cercidium* lines: 9.3 ± 0.9% and 8.5 ± 0.2%). On both host species, there was significant variation between replicate lines (*Acacia* lines: $F_{3,808} = 3.72$, $P = 0.011$; *Cercidium* lines: $F_{3,768} = 9.97$, $P < 0.001$). The *Acacia* control lines did not change significantly in mean egg length from the start to the end of the selection experiment (Fig. 1; $F_{1,1} = 18.40$, $P = 0.146$). For the *Cercidium* control lines, there was a significant interaction between replicate and generation ($F_{1,301} = 9.38$, $P = 0.002$): from the start to the end of the experiment, mean egg length of replicate 1 decreased slightly more than mean egg length of replicate 2 (1.9% vs. 1.2%; Fig. 1).

Because selection was imposed on egg length, egg width was measured before and after selection was imposed to ensure that overall egg size was evolving along with egg length. From half-sib analysis, egg width and egg length were genetically correlated on both host species (on *A. greggii*: $r_A$ ± SE = 0.60 ± 0.10; on *C. floridum*: $r_A = 0.53 \pm 0.12$; not significantly different from each other at $\chi^2_1 = 0.65$, $P = 0.419$). As predicted, egg width evolved in response to selection on egg length. At the end of selection, mean egg width

TABLE 2. Nested analysis of variance and variance component analysis of egg length for *Stator limbatus* females ovipositing on seeds of *Acacia greggii* or *Cercidium floridum.* Type III sums of squares were computed using the general linear model of SAS (PROC GLM, with RANDOM statement and TEST option). $V_s$, among sire variance component; $V_D$, among dam variance component; $V_E$, error variance component; $V_P$, phenotypic (total) variance; $V_A$, additive genetic variance; $V_M$, maternal effects variance; $V_e$, environmental variance.

| | Analysis of variance | | | Observational variance component | Genetic | |
|---|---|---|---|---|---|---|
| Source | df | MS ($\times 10^{-3}$) | $F$ | ($\times 10^{-4}$) | Variance component | Proportion of $V_P$ explained |
| *Acacia greggii* | | | | | | |
| Sire | 126 | 0.80 | 2.45* | $V_S = 0.485$ | $V_A = 1.938$ | 67 ± 13% |
| Dam (sire) | 255 | 0.35 | 1.83* | $V_D = 0.486$ | $V_M = 0.001$ | 0% |
| Error | 955 | 0.19 | | $V_E = 1.920$ | $V_e = 0.952$ | 32% |
| $r^2 = 0.52$ | | | | $V_P = 2.891$ | | |
| *Cercidium floridum* | | | | | | |
| Sire | 126 | 1.19 | 2.07* | $V_S = 0.627$ | $V_A = 2.506$ | 50 ± 12% |
| Dam (sire) | 260 | 0.63 | 1.75* | $V_D = 0.831$ | $V_M = 0.205$ | 4% |
| Error | 943 | 0.36 | | $V_E = 3.575$ | $V_e = 2.322$ | 46% |
| $r^2 = 0.48$ | | | | $V_P = 5.033$ | | |

* $P < 0.001$.

TABLE 3. Nested analysis of variance and variance component analysis of lifetime fecundity for *Stator limbatus* females ovipositing on seeds of *Acacia greggii* or *Cercidium floridum.* Statistics as in Table 2.

| Source | Analysis of variance: df | Analysis of variance: MS | Analysis of variance: $F$ | Observational variance component | Genetic: Variance component | Genetic: Proportion of $V_P$ explained |
|---|---|---|---|---|---|---|
| *Acacia greggii* | | | | | | |
| Sire | 126 | 89.46 | 1.75* | $V_S$ = 4.143 | $V_A$ = 16.570 | 32 ± 10% |
| Dam (sire) | 252 | 51.56 | 1.14 ns | $V_D$ = 2.345 | $V_M$ = 0 | 0% |
| Error | 922 | 45.05 | | $V_E$ = 44.774 | $V_e$ = 34.692 | 68% |
| $r^2$ = 0.38 | | | | $V_P$ = 51.262 | | |
| *Cercidium floridum* | | | | | | |
| Sire | 126 | 36.81 | 1.57* | $V_S$ = 1.592 | $V_A$ = 6.369 | 27 ± 10% |
| Dam (sire) | 259 | 23.66 | 1.13 ns | $V_D$ = 1.270 | $V_M$ = 0 | 0% |
| Error | 920 | 20.93 | | $V_E$ = 20.729 | $V_e$ = 17.222 | 73% |
| $r^2$ = 0.48 | | | | $V_P$ = 23.591 | | |

* $P < 0.001$; ns, not significant ($P > 0.05$).

in the up lines was 5.4 ± 0.2% (±SE, averaged between replicates; *Acacia* lines) or 6.5 ± 1.1% (*Cercidium* lines) wider than eggs in the control lines, while eggs in the down lines were 5.0 ± 0.1% (*Acacia* lines) or 5.1 ± 0.5% (*Cercidium* lines) more narrow than eggs in the control lines (*Acacia* lines: $F_{2,3} = 76.95$, $P = 0.003$; *Cercidium* lines: $F_{2,3} = 111.53$, $P = 0.002$; up and down lines significantly different from control lines at $P < 0.001$). On both host species, there was significant variation between replicate lines in mean egg width (*Acacia* lines: $F_{3,1218} = 8.03$, $P < 0.001$; *Cercidium* lines: $F_{3,700} = 3.37$, $P = 0.018$).

The realized heritability of egg length ranged from 0.36 to 0.55, as estimated from the regression slope of the cumulative response to selection and the cumulative selection differential (*Acacia* lines: $h^2 \pm$ SE = 0.46 ± 0.01 or 0.55 ± 0.01, up and down lines, respectively; *Cercidium* lines: $h^2$ = 0.36 ± 0.02 or 0.46 ± 0.01). These estimates were lower than heritability estimates from half-sib analysis (Table 2).

### *Phenotypic and Genetic Correlations with Body Mass*

#### *Correlations from half-sib analysis*

Larger females laid larger eggs on both host species than did smaller females ($r_p$ in Table 4; estimates not significantly different from each other at $\chi^2_1 = 0.52$, $P = 0.469$). Female body mass was heritable ($h^2 \pm$ SE = 0.24 ± 0.08) and positively genetically correlated with the length of eggs laid on *A. greggii* seeds, but female body mass was not genetically correlated with the length of eggs laid on *C. floridum* seeds (Estimated $r_A$ in Table 4). Genetic correlations between female body mass and egg length on the two host species were significantly different from each other ($\chi^2_1 = 9.93$, $P = 0.002$).

On both host species, larger females also laid more eggs than did smaller females ($r_p$ in Table 4; estimates not significantly different from each other at $\chi^2_1 = 0.01$, $P = 0.921$). These positive phenotypic correlations translated into positive genetic correlations, but the standard errors of estimates are large (Estimated $r_A$ in Table 4; estimates not significantly different from each other at $\chi^2_1 = 2.09$, $P = 0.148$).

#### *Correlated response to artificial selection on egg length*

In the control lines, mean female body mass did not evolve during the artificial selection experiment for the *Acacia* or *Cercidium* lines (Table 5A; *Acacia* control lines: $F_{1,1} = 0.002$, $P = 0.962$; *Cercidium* control lines: $F_{1,1} = 25.23$, $P = 0.125$).

Based on genetic correlation estimates from half-sib analysis, selection on egg size on *A. greggii* seeds should lead to the correlated evolution of female body mass ($r_A \pm$ SE = 0.32 ± 0.19) whereas this is not expected for selection on egg size on *C. floridum* seeds ($r_A = -0.07 \pm 0.25$; Estimated $r_A$ in Table 4). Female body mass evolved as predicted. In

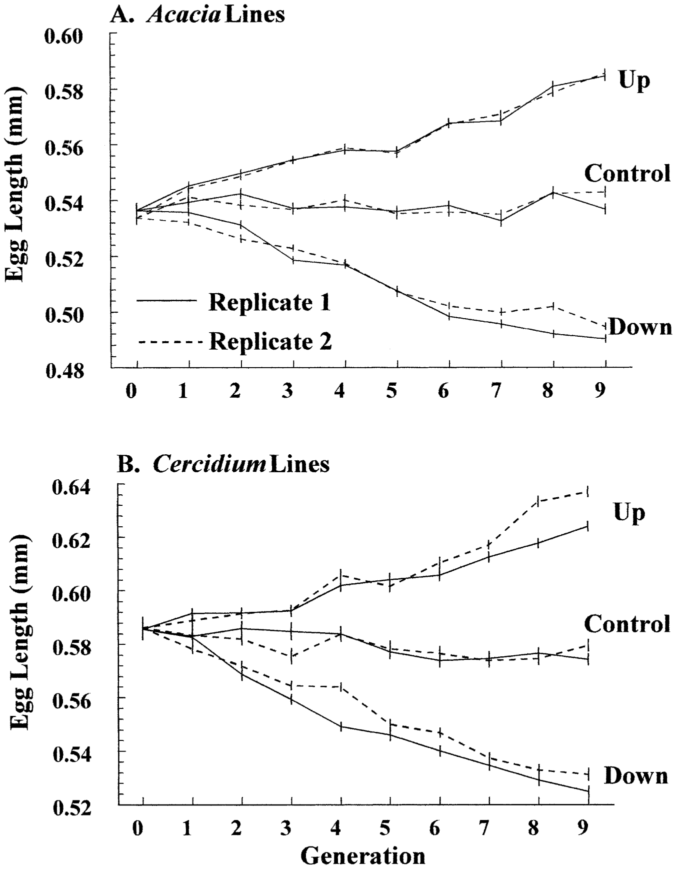

FIG. 1. Response to artificial selection on egg length (mean ± SE) for *Stator limbatus* females ovipositing on seeds of (A) *Acacia greggii* (*Acacia* lines) or (B) *Cercidium floridum* (*Cercidium* lines) over nine generations of selection.

TABLE 4. Mean phenotypic ($r_P$) and additive genetic correlations ($r_A$; ± SE) between female body mass and egg traits for *Stator limbatus* females ovipositing on either *Acacia greggii* or *Cercidium floridum* seeds. Note the positive genetic correlation (estimated and realized $r_A$) between female body mass and egg length on *A. greggii* seeds, yet a correlation generally near zero on *C. floridum* seeds. rep, replicate. Realized genetic correlations were calculated from correlated responses to artificial selection on egg length as described in text: $h^2$ of egg length in Table 2, $h^2$ of female body mass = 0.24 ± 0.08. Average $i$ (± SE; averaged over nine generations within each line) = 1.3 ± 0.02 and 1.3 ± 0.02 (*Acacia* up lines, replicates 1 and 2 respectively); −1.2 ± 0.02 and −1.2 ± 0.04 (*Acacia* down); 1.1 ± 0.03 and 1.1 ± 0.02 (*Cercidium* up); −1.2 ± 0.03 and −1.2 ± 0.02 (*Cercidium* down). Standard deviation of female body mass from half-sib analysis = 0.39 mg.

| Correlated traits | $r_P$ | Estimated $r_A$ | Realized $r_A$ | |
|---|---|---|---|---|
| | | | Up lines | Down lines |
| Body mass and egg length | | | | |
| *Acacia greggii* | 0.20 ± 0.03 | 0.32 ± 0.19 | 0.21 (rep 1) | 0.24 (rep 1) |
| | | | 0.27 (rep 2) | 0.17 (rep 2) |
| *Cercidium floridum* | 0.11 ± 0.03 | −0.07 ± 0.25 | 0.23 (rep l) | −0.08 (rep 1) |
| | | | 0.05 (rep 2) | −0.15 (rep 2) |
| Body mass and fecundity | | | | |
| *Acacia greggii* | 0.46 ± 0.03 | 0.28 ± 0.32 | — | — |
| *Cercidium floridum* | 0.45 ± 0.03 | 0.44 ± 0.30 | — | — |

the *Acacia* lines, selection for large eggs led to the correlated evolution of larger females (compared with control line, $P < 0.001$) and conversely, selection for small eggs led to the correlated evolution of smaller females (Fig. 2; $P < 0.001$). There was significant variation between replicate lines in mean female body mass ($F_{3,2115} = 20.85$, $P < 0.001$). From these correlated responses, positive realized genetic correlations between female body mass and egg length were estimated that ranged between 0.17 and 0.27 (Realized $r_A$ in Table 4; estimates among *Acacia* lines not significantly different at $\chi^2_3 = 0.731$, $P = 0.866$). The mean observed correlated responses of the *Acacia* lines were in the predicted direction (Fig. 3A). Among the *Cercidium* lines, no consistent correlated response was detected for female body mass (Fig. 2; $F_{2,3} = 0.80$, $P = 0.526$), and there was significant variation among replicate lines ($F_{3,2005} = 9.86$, $P < 0.001$). Realized genetic correlations of the *Cercidium* lines were opposite in direction with regard to the direction of selection (Realized $r_A$ in Table 4; $r_A$ among *Cercidium* lines significantly different from each other at $\chi^2_3 = 10.33$, $P = 0.016$). The mean observed correlated response of the *Cercidium* down lines was in the predicted direction, but the mean observed correlated response of the *Cercidium* up lines was not in the predicted direction (Fig. 3A).

## *Genetic Correlation between Egg Size and Fecundity*

### *Estimated correlations from half-sib analysis*

There was a phenotypic trade-off between egg length and lifetime fecundity on both host species ($r_P$ in Table 6; estimates significantly different from each other at $\chi^2_1 = 4.04$, $P = 0.044$). This trade-off had a genetic basis when females laid eggs on *C. floridum* seeds, but not when females laid eggs on *A. greggii* seeds (Estimated $r_A$ in Table 6). Genetic correlations on the two host species were significantly different from each other ($\chi^2_1 = 11.48$, $P < 0.001$). Removing the effects of female body mass on egg length and fecundity did not change this pattern; phenotypic and genetic correlations of residual and nonresidual traits were not significantly different from each other (Table 6; $r_p$ on *A. greggii*: $\chi^2_1 = 0.79$, $P = 0.373$; $r_p$ on *C. floridum*: $\chi^2_1 = 0.71$, $P = 0.398$; $r_A$ on *A. greggii*: $\chi^2_1 = 1.13$, $P = 0.288$; $r_A$ on *C. floridum*: $\chi^2_1 = 0.05$, $P = 0.830$). Genetic correlations of residual egg length and residual fecundity on the two host

TABLE 5. Initial and final estimates of (A) mean female adult body mass, (B) mean lifetime fecundity, and (C) mean age at first reproduction for control lines of *Stator limbatus* females. rep, replicate.

| | | Parental generation | Control line 11th generation |
|---|---|---|---|
| A. Female body mass (mg) | | | |
| *Acacia* control lines | rep 1 | 3.3 ± 0.08 | 3.4 ± 0.02 |
| | rep 2 | 3.3 ± 0.07 | 3.2 ± 0.03 |
| *Cercidium* control lines | rep 1 | 3.4 ± 0.05 | 3.3 ± 0.03 |
| | rep 2 | 3.3 ± 0.06 | 3.3 ± 0.03 |
| B. Lifetime fecundity | | | |
| *Acacia* control lines | rep 1 | 29 ± 1.0 | 29 ± 0.7 |
| | rep 2 | 30 ± 1.2 | 28 ± 1.0 |
| *Cercidium* control lines | rep 1 | 22 ± 0.1 | 22 ± 0.8 |
| | rep 2 | 22 ± 0.1 | 21 ± 0.5 |
| C. Age at first reproduction (days) | | | |
| *Acacia* control lines | rep 1 | 1.7 ± 0.04 | 1.8 ± 0.03 |
| | rep 2 | 1.7 ± 0.07 | 1.7 ± 0.04 |
| *Cercidium* control lines | rep 1 | 1.9 ± 0.06 | 1.9 ± 0.05 |
| | rep 2 | 1.9 ± 0.06 | 2.0 ± 0.04 |

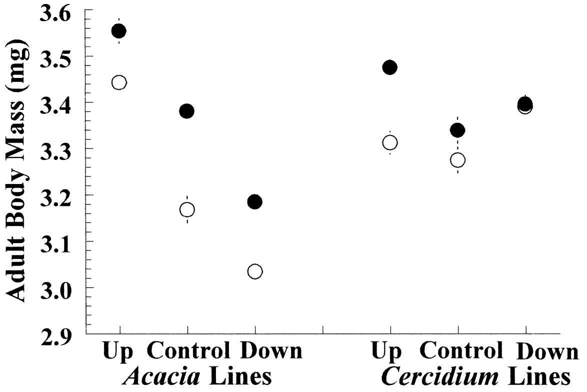

FIG. 2. Correlated response of female adult body mass (mg) of *Stator limbatus* following nine generations of artificial selection on egg length. Each circle represents mean adult body mass (±SE) for females within lines for two replicates (filled and open circles). Mean body mass of both replicates of the *Cercidium* down lines are almost equivalent.

species were significantly different ($\chi^2_1 = 7.63$, $P < 0.001$). Thus, a strong genetic trade-off between egg length and fecundity was detected when these traits were measured on *C. floridum* seeds, but not when these traits were measured on *A. greggii* seeds.

*Correlated response to artificial selection on egg length*

Mean lifetime fecundity did not change during the selection experiment for the *Acacia* or *Cercidium* control lines (Table 5B; *Acacia* control lines: $F_{1,1} = 0.82$, $P = 0.532$; *Cercidium* control lines: $F_{1,1} = 3.14$, $P = 0.327$).

Due to a genetically based trade-off between egg length and lifetime fecundity on *C. floridum* seeds ($r_A \pm \text{SE} = -0.51 \pm 0.18$; Estimated $r_A$ in Table 6), selection for large eggs on this host species should lead to reduced fecundity, and selection for small eggs should lead to increased fecundity. These traits were not genetically correlated on *A. greggii* seeds ($r_A = -0.13 \pm 0.21$), and so fecundity should not respond to selection on egg length on this host species. Fecundity evolved exactly as predicted. Selection for large eggs on *C. floridum* seeds led to the correlated evolution of lower fecundity compared to the control lines (Fig. 4A; compared with control line, $P < 0.001$), and conversely, selection for small eggs led to the correlated evolution of increased lifetime fecundity ($P < 0.001$). There was significant variation between *Cercidium* replicate lines in mean lifetime fecundity ($F_{3,496} = 6.07$, $P < 0.001$). Realized genetic correlations between egg length and fecundity were negative for all *Cercidium* lines and did not differ significantly from each other (Realized $r_A$ in Table 6; $\chi^2_3 = 2.33$, $P = 0.507$), and the mean observed correlated responses were in the predicted direction (Fig. 3B). In the *Acacia* lines, fecundity did not evolve strongly in response to selection on egg length (Fig. 4A) mean fecundities of the selected and control lines were not significantly different ($P > 0.060$). There was significant variation between *Acacia* replicate lines in mean lifetime fecundity ($F_{3,586} = 2.88$, $P = 0.035$). Realized genetic correlations of all *Acacia* lines were near zero and did not differ significantly from each other (Realized $r_A$ in Table 6; $\chi^2_3 = 1.31$, $P = 0.727$). The mean observed correlated response of the *Acacia* up lines was in the predicted direction, but the mean observed correlated response of the *Acacia* down lines was not in the predicted direction (Fig. 3B). After controlling for female body mass, mean lifetime fecundity in the *Acacia* lines responded more strongly to selection on egg length (Fig. 4B): selection for large eggs on *A. greggii* seeds led to a decrease in mean fecundity per unit female body mass compared to the control lines ($P \leq 0.001$), yet selection for small eggs did not lead to a significant increase in fecundity ($P = 0.104$).

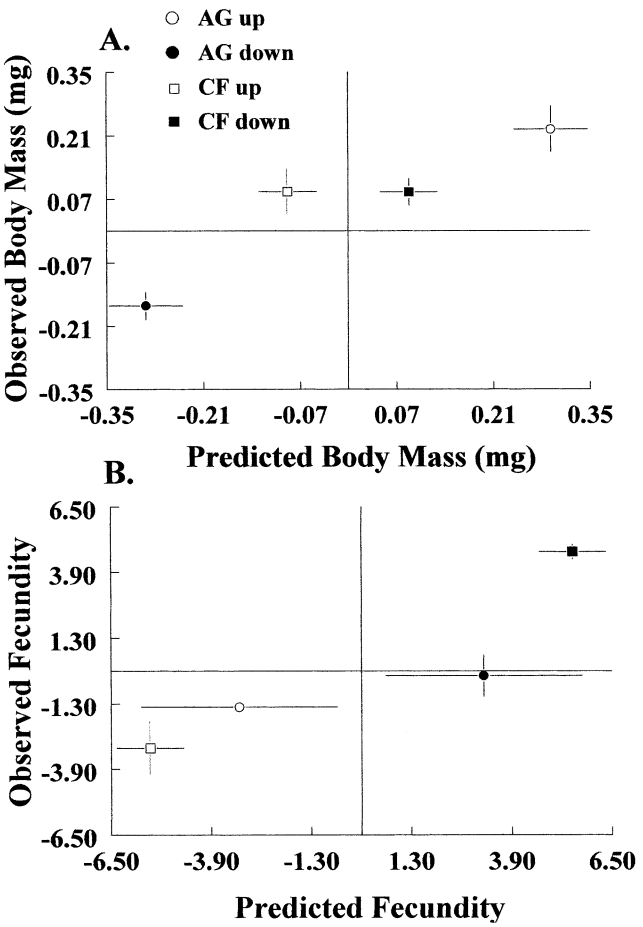

FIG. 3. Mean (±SE) observed and predicted correlated responses of (A) female adult body mass and (B) lifetime fecundity to artificial selection on egg length for *Stator limbatus*. Circles represent selection for increased and decreased egg length on *Acacia greggii* seeds (AG up and down), and squares represent selection for increased and decreased egg length on *Cercidium floridum* seeds (CF up and down). Some standard error bars are smaller than the points.

### *Correlated Response of Age at First Reproduction to Artificial Selection on Egg Length*

In the control lines, mean age at first reproduction (i.e., age at which females began to oviposit) did not evolve during the selection experiment for the *Acacia* lines or *Cercidium* lines (Table 5C; *Acacia* lines: $F_{1,1} = 59.36$, $P = 0.082$; *Cercidium* lines: $F_{1,1} = 1.81$, $P = 0.407$).

The correlated response of age at first reproduction to selection on egg length differed between the *Acacia* and *Cer-*

TABLE 6. Mean phenotypic ($r_P$) and additive genetic correlations ($r_A$; ± SE) between egg length and lifetime fecundity for *Stator limbatus* females ovipositing on either *Acacia greggii* or *Cercidium floridum* seeds. Correlations between egg length and fecundity and correlations between residuals of these traits (residuals from a regression of egg length or fecundity vs. female body mass) are reported. Note the strong negative genetic correlation (estimated and realized $r_A$) between egg length and fecundity on *C. floridum* seeds, yet no genetic correlation between these traits on *A. greggii* seeds. Statistics as in Table 4. rep, replicate. Realized $r_A$ calculated as described in text: $h^2$ of egg length and fecundity in Tables 2 and 3, respectively. For average *i*, see Table 4. Standard deviation of fecundity from half-sib analysis: 7.2 eggs (on *A. greggii*) and 4.9 eggs (on *C. floridum*).

| Correlated traits | Estimated $r_P$ | Estimated $r_A$ | Realized $r_A$ | |
|---|---|---|---|---|
| | | | Up lines | Down lines |
| Egg length and fecundity | | | | |
| *Acacia greggii* | −0.12 ± 0.03 | −0.13 ± 0.21 | −0.09 (rep 1)<br>−0.09 (rep 2) | −0.03 (rep 1)<br>0.04 (rep 2) |
| *Cercidium floridum* | −0.36 ± 0.03 | −0.51 ± 0.18 | −0.28 (rep 1)<br>−0.44 (rep 2) | −0.40 (rep 1)<br>−0.40 (rep 2) |
| Residuals of egg length and fecundity | | | | |
| *Acacia greggii* | −0.23 ± 0.03 | −0.25 ± 0.18 | — | — |
| *Cercidium floridum* | −0.46 ± 0.03 | −0.55 ± 0.15 | — | — |

*cidium* lines and was often asymmetrical (Fig. 5). For the *Acacia* lines, females of the down lines delayed oviposition compared to the control lines ($P < 0.001$), yet females of the up lines did not oviposit at a younger age compared to the control lines ($P = 0.552$). The opposite pattern was seen in the *Cercidium* lines—females of the up lines delayed oviposition ($P < 0.001$), but females of the down lines did not oviposit at a significantly younger age ($P = 0.230$).

### *Correlated Response of Female Adult Lifespan to Artificial Selection on Egg Length*

Adult lifespan was measured for females to detect a possible shift in resource allocation between egg production and somatic maintenance. There was no consistent pattern in the correlated response of female adult lifespan (as virgins) to selection on egg length. Mean female adult lifespan (±SE) ranged between 9.4 ± 0.2 and 11.9 ± 0.2 days for the *Acacia* lines and between 10.3 ± 0.3 and 11.1 ± 0.2 days for the *Cercidium* lines. In the *Acacia* lines and *Cercidium* lines, there was a nonsignificant effect of direction of selection on mean female adult lifespan (*Acacia* lines: $F_{2,3} = 2.73$, $P = 0.210$; *Cercidium* lines: $F_{2,3} = 0.75$, $P = 0.542$). There was also significant variation between *Acacia* replicate lines ($F_{3,812} = 9.90$, $P < 0.001$). Thus, there was no evidence of a correlation

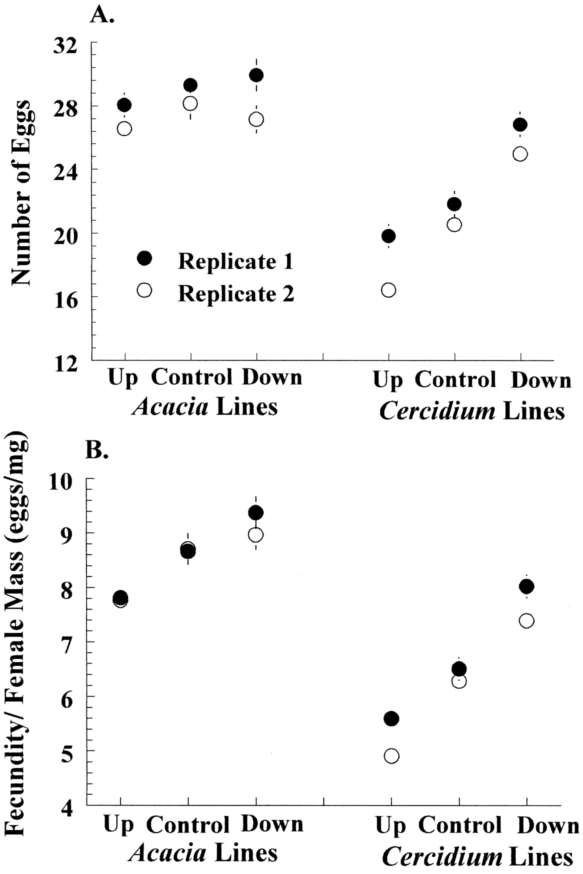

FIG. 4. Evolution of (A) lifetime fecundity and (B) lifetime fecundity per unit of female body mass for *Stator limbatus* females ovipositing on *Acacia greggii* seeds (*Acacia* lines) and *Cercidium floridum* seeds (*Cercidium* lines) following nine generations of artificial selection on egg length. Circles represent means (±SE; some standard error bars are smaller than the circles) within lines for two replicates (filled and open circles).

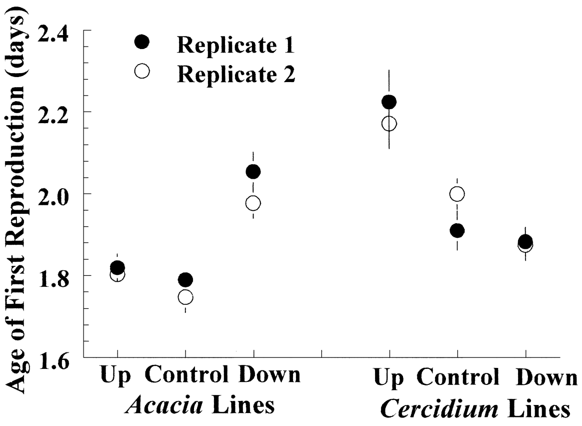

FIG. 5. Correlated response of age at first reproduction of *Stator limbatus* females ovipositing on *Acacia greggii* seeds (*Acacia* lines) and *Cercidium floridum* seeds (*Cercidium* lines) following nine generations of artificial selection on egg length. Circles represent mean age at first reproduction (±SE; some standard error bars are smaller than the circles) within lines for two replicates (filled and open circles).

between selection on egg size and changes in resource allocation toward or away from somatic maintenance.

## DISCUSSION

For *Stator limbatus*, the genetically based trade-off between egg size and lifetime fecundity differs between two of the common host species (environments) on which females lay eggs. The trade-off is much stronger when females lay eggs on *C. floridum* seeds than when females lay eggs on *A. greggii* seeds (Table 6), and the correlated response of fecundity observed in response to artificial selection on egg length was much stronger in the *Cercidium* lines than in the *Acacia* lines (Fig. 4A). In fact, the correlated evolution of fecundity to selection on egg size was detectable in the *Acacia* lines only after controlling for female body size (Fig. 4B). When selection was imposed on the size of eggs laid on *A. greggii* seeds, adult body size, and thus apparently total reproductive effort, evolved without a change in total fecundity (Figs. 2 and 4A): egg length and female body mass are positively genetically correlated when eggs are laid on *A. greggii* seeds (Table 4), but egg size is not genetically correlated to fecundity on this host (Table 6). The evolution of body size in response to selection on egg size was not observed when selection was imposed on the size of eggs laid on *C. floridum* seeds (Fig. 2) because female body size is not genetically correlated with egg size when eggs are laid on this host (Table 4). There was no evidence that selection on egg length led to changes in allocation between egg production and somatic maintenance when measured as a change in adult lifespan.

### *Environmental Difference in Genetic Relationships among Egg Size, Lifetime Fecundity, and Female Body Mass*

The environmental difference in the strength of the genetic relationships among egg size, lifetime fecundity and female body mass may result from host-related differences in the amount of genetic variation in reproductive effort among females, perhaps through differential gene expression on the different host species (Hedrick et al. 1976). It is well known that selection on offspring size and total reproductive effort often differs among environments (e.g., Gadgil and Bossert 1970; Fox et al. 1997; Welham and Setter 1998), but it is generally not known whether the genetic relationships among offspring size, offspring number, and total reproductive effort also differ among environments. The genetic relationships among other traits vary among environments, although there is little empirical work on this topic (Bégin and Roff 2001). Much of the attention has been directed at the constancy of genetic (co)variance and genetic correlation matrices over phylogenetic time (e.g., Lofsvold 1986; Turelli 1988; Shaw et al. 1995) or over the course of artificial selection (e.g., Leroi et al. 1994). The empirical comparison of genetic (co)variance/correlation matrices among environments mostly compares novel and ancestral environments. For example, Guntrip et al. (1997) detected a higher amount of additive genetic variation in adult body weight of the seed beetle *Callosobruchus maculatus* when estimated in a novel environment versus an ancestral environment, perhaps as a result of the expression of new genes. Additionally, Service and Rose (1985) found that the genetic correlation between fecundity and starvation time in *Drosophila melanogaster* was disrupted in novel environments. Fewer studies have focused on differences among environments that organisms experience in nature. Gu and Danthanarayana (2000) found significant differences in the strength of negative genetic correlations between life-history traits of the light brown apple moth (*Epiphyas postvittana*) measured in two temperatures that moths experience in nature. Here we have demonstrated not only an environmental difference in the genetic correlation structure among life-history traits, but also a complete change in which genetic correlations were nonzero: there was a negative to zero switch in the genetic correlation between egg size and fecundity, and a zero to positive switch in the genetic correlation between egg size and female body size.

In our study, the difference between host species in the genetic correlation structure among egg size, fecundity, and female body size is not a result of differences in genetic variation in performance or oviposition preference between host species. In gypsy moths, the genetic correlation between pupal mass and an index of reproductive investment was positive only when larvae were reared on a marginally suitable host plant (locust tree), yet no correlation was detected when larvae were reared on a suitable host plant (oak; Lazarević et al. 1998). In a more stressful environment, larger female gypsy moths have a higher tolerance to stress (measured as reproductive index) than smaller females. Here, *S. limbatus* larvae were not reared in stressful versus suitable environments; all offspring were reared on *A. greggii* seeds on which larval survivorship was very high (98%). Females differed only in their oviposition substrate (seeds of either *A. greggii* or *C. floridum*), and females do not feed on seeds during oviposition.

### *Evolution of Egg Size and Fecundity along with Total Reproductive Effort*

Simple models on the evolution of optimal offspring size assume a trade-off between offspring size and number and fixed allocation of resources toward reproduction (e.g., Smith and Fretwell 1974). However, if reproductive effort varies among females in a population, both offspring size and number may increase or decrease together without an evident trade-off. In these cases, variation in reproductive effort obscures a trade-off, although a trade-off is generally present (Fox and Czesak 2000). On *A. greggii* seeds, a trade-off between egg size and number was obscured until female body mass was controlled (Fig. 4A and B). In some cases, a genetic trade-off may not be present if the genes that affect both offspring size and number do so by affecting the acquisition of resources rather than by affecting the distribution of those resources between offspring size and number (Houle 1991). In *S. limbatus*, we have not examined variation in acquisition of resources and how this affects the distribution of resources between egg size and number.

Patterns of acquisition and allocation of reproductive resources are rarely considered to be influenced by each other or to evolve together (but see Winkler and Wallin 1987; León and de Nóbrega 2000; Caley et al. 2001). Generally, reproductive effort is optimized with respect to trade-offs between reproductive effort and the amount of resources allocated towards growth and/or somatic maintenance (survival), followed by allocation of this optimized total reproductive effort among offspring of different sizes and numbers (Caley et al. 2001). Few studies have modeled or empirically investigated the simultaneous evolution of offspring size and number. Winkler and Wallin (1987) modeled the simultaneous optimization of offspring size and total reproductive effort and predicted an inverse relationship between these traits. In support of this prediction, Caley et al. (2001) detected a negative relationship between total reproductive effort and egg size in a comparative analysis among copepod families, which indicated an evolutionary link between these traits. Unfortunately for *S. limbatus*, we are not able to weigh eggs and obtain estimates of total reproductive effort because eggs are glued to seeds and removing them is destructive. We can, however, estimate reproductive effort by estimating egg mass from an approximate relationship between egg length and egg mass (Fox and Mousseau 1996) and multiplying egg mass by lifetime fecundity. In so doing, we find results consistent with Winkler and Wallin's prediction—females that lay eggs on *A. greggii* seeds, on which smaller eggs are laid, spend more reproductive effort on average (0.724 ± 0.004 number of eggs/mg [±SE]) than females that lay eggs on *C. floridum* seeds (0.568 ± 0.003 number of eggs/mg), on which larger eggs are laid.

The schedule of reproduction, rather than total reproductive effort, may be the subject of selection (Schaffer 1974; Orzack and Tuljapurkar 1989; Phillipi and Seger 1989; Tuljapurkar 1990; Charlesworth 1994; Candolin 1998; Ilmonen et al. 2000), or the schedule of reproduction may evolve along with selection on a correlated trait. For *S. limbatus*, age at first reproduction evolved with selection on egg size—selection for small eggs on *A. greggii* seeds and large eggs on *C. floridum* seeds led to a delay in the onset of oviposition (Fig. 5). We expected that selection for large eggs would lead to a delay in the onset of oviposition, as observed in the *Cercidium* lines, assuming that maturing large eggs requires more time than maturing small eggs (Fox 1997). In *S. limbatus*, a change in age at first reproduction is not related to the quality or availability of adult food, because adults do not require food for reproduction and were not fed in this experiment, nor is it likely related to a trade-off between current and future reproduction because *S. limbatus* females have a short reproductive lifespan (approximately 10 days). The delay in the onset of oviposition of females in the *Acacia* down lines may be related to body size—these females were the smallest of any of the selected lines (Fig. 2) but laid a similar number of eggs to that of females of the *Acacia* up lines (Fig. 4). Possibly the high ratio of female fecundity to female size resulted in complications during egg maturation, and thus a delay in oviposition. Explanation of the inconsistent correlated responses for age at first reproduction require a more detailed understanding of the physiology of egg maturation and egg-size plasticity in *Stator limbatus* than is currently available.

### *Conclusions*

This study demonstrates that a widely assumed genetic trade-off between two life-history traits depends on the environment in which traits are measured. On *C. floridum* seeds, egg size, and fecundity are negatively genetically correlated. However, on *A. greggii* seeds, this trade-off disappears because of a genetic correlation between egg size and female body mass, and we observed the correlated evolution of total reproductive effort in response to selection on egg size. Thus, simple theoretical models of the evolution of life-history traits are of limited value in complex environments because many traits covary and thus coevolve, and the complex of traits that coevolves varies with environmental conditions. Unfortunately, there is little understanding of how changes in environmental conditions affect patterns of resource allocation and the distribution of resources between somatic maintenance and reproduction. Thus, before major advances can be made in our understanding of the coevolution of egg size, egg number, and reproductive effort, we need to focus more attention on the underlying genetic and physiological mechanisms generating correlations among these traits.

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