THE EVOLUTIONARY GENETICS OF AN ADAPTIVE MATERNAL EFFECT: EGG SIZE PLASTICITY IN A SEED BEETLE

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Abstract.—In many organisms, a female's environment provides a reliable indicator of the environmental conditions that her progeny will encounter. In such cases, maternal effects may evolve as mechanisms for transgenerational phenotypic plasticity whereby, in response to a predictive environmental cue, a mother can change the type of eggs that she makes or can program a developmental switch in her offspring, which produces offspring prepared for the environmental conditions predicted by the cue. One potentially common mechanism by which females manipulate the phenotype of their progeny is egg size plasticity, in which females vary egg size in response to environmental cues. We describe an experiment in which we quantify genetic variation in egg size and egg size plasticity in a seed beetle, *Stator limbatus*, and measure the genetic constraints on the evolution of egg size plasticity, quantified as the genetic correlation between the size of eggs laid across host plants. We found that genetic variation is present within populations for the size of eggs laid on seeds of two host plants (*Acacia greggii* and *Cercidium floridum*; h^2 ranged between 0.217 and 0.908), and that the heritability of egg size differed between populations and hosts (higher on *A. greggii* than on *C. floridum*). We also found that the evolution of egg size plasticity (the maternal effect) is in part constrained by a high genetic correlation across host plants ($r_G > 0.6$). However, the cross-environment genetic correlation is less than 1.0, which indicates that the size of eggs laid on these two hosts can diverge in response to natural selection and that egg size plasticity is thus capable of evolving in response to natural selection.

Key words.—*Acacia greggii*, *Cercidium floridum*, genetic correlation, heritability, parental investment, phenotypic plasticity, *Stator limbatus*.

Variation in selection across space and time can result in the evolution of phenotypic plasticity, in which genotypes exhibit different phenotypes in different environments (Bradshaw 1965; Thompson 1991; Scheiner 1993; Via 1993, 1994; de Jong 1995; Gotthard and Nylin 1995). In many organisms, a female's environment may provide a reliable indicator of the environmental conditions that her progeny will encounter. In such cases, maternal effects may evolve as mechanisms for "transgenerational" phenotypic plasticity (Mousseau and Dingle 1991a,b; Fox and Mousseau 1998; referred to as "cross-generational" phenotypic plasticity by Rossiter 1996) whereby, in response to a predictive environmental cue (e.g., short or long photoperiod), a mother can change the type of eggs that she makes or can program a developmental switch in her offspring, which produces offspring prepared for the environmental conditions predicted by the cue (e.g., Fox et al. 1997; Donohue and Schmitt 1998). In other words, maternal environmental cues can stimulate phenotypic plasticity in progeny by mechanisms other than the transmission of nuclear genes.

Theoretical and empirical studies indicate that maternal effects can have dramatic effects on short-term evolutionary responses to selection (Riska 1991)—they may result in time lags in evolutionary responses and characters subject to large maternal effects may even respond to selection in a maladaptive direction (Riska et al. 1985; Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990). We now also know that maternal effects themselves are often genetically variable and can thus respond to selection (e.g., Wade 1998, papers in Mousseau and Fox 1998). However, few studies have quantitatively assessed genetic variation in maternal effects, particularly those that are presumed to have evolved as adaptations (Roach and Wulff 1987; Riska 1991; Byers et al. 1997; Shaw and Byers 1998). Because maternal effects can confound our interpretation of within and among population variation in morphological and life-history traits (Sinervo 1991; Fox 1994a, 1997a; Fox et al. 1995), researchers generally consider them in experiments only to avoid overestimating additive genetic variances and avoid confusing environmentally based maternal effects with genetic variation (Mazer 1989; Shaw and Byers 1998). Thus, little is understood about the evolutionary genetics of maternal effects such that the potential for them to respond to natural selection remains largely speculative.

Variation in egg size is the best studied and likely one of the most important sources of maternal effects because it is simultaneously a maternal and offspring character (Sinervo 1991); eggs are produced by mothers, but also determine initial offspring resources or size. Many studies suggest that growth and survival of progeny are influenced by the amount and quality of resources allocated to eggs by mothers. In animals, progeny developing from large eggs generally mature sooner and have higher survivorship than those developing from small eggs (reviews in Fleming and Gross 1990; Fox 1994b, 1997b; Bernardo 1996; Fox and Mousseau 1996), which favors the evolution of large eggs. However, mothers laying larger eggs must also lay fewer eggs due to a trade-off between egg size and egg number (Smith and Fretwell 1974; Fleming and Gross 1990; Berrigan 1991; Bernardo 1996; Fox et al. 1997), which results in the evolution of an egg size that is a balance between selection for large eggs and selection for many eggs.

Theoretical models suggest that larger offspring should be produced when conditions for juvenile growth and survivorship are poor (Sibly and Calow 1983; Parker and Begon 1986). Empirical studies support this—the consequences of egg size variation depend on environmental conditions, with the fitness differences between large and small eggs generally greatest in adverse environments (Janzen 1977; Braby 1994; Fox and Mousseau 1996; review in Fox, unpubl. ms.). Thus, selection favors eggs of different sizes in different environments and potentially produces substantial geographic variation in egg size (Fleming and Gross 1990; Rowe 1994). In temporally and spatially heterogeneous environments, a single female may experience selection favoring large eggs or small eggs depending on the time and location of oviposition (Fox and Mousseau 1996). This favors the evolution of egg size plasticity (Fox et al. 1997; but see McGinley et al. 1987) whereby females, in response to predictive environmental cues, produce eggs of a size appropriate for the conditions predicted by the cue (Mousseau and Dingle 1991a).

Egg size plasticity in response to predictive environmental cues has been demonstrated in a handful of arthropods, including cladocerans (females respond to food stress by increasing egg size; Smith 1963; Perrin 1989; Gliwicz and Guisande 1992; Guisande and Gliwicz 1992; Boersma 1997), parasitoids (female *Nasonia* respond to egg density by increasing egg size: Niehaus and Lalonde, unpubl. data), and seed beetles (*Callosobruchus maculatus* females respond to adult density by increasing egg size: Kawecki 1995; female *Stator limbatus* females lay different size eggs on different hosts: Fox 1997b; Fox et al. 1997; Fox and Mousseau 1998). Yampolsky and Scheiner (1996) also suggest that the production of larger eggs at low temperature that is observed in many ectothermic organisms may also be an example of adaptive egg size plasticity. Here, we describe an experiment in which we quantify genetic variation in egg size and egg size plasticity in the seed beetle, *Stator limbatus,* and measure the genetic constraints on the evolution of egg size plasticity.

Egg Size Plasticity in Stator limbatus

Stator limbatus (Coleoptera: Bruchidae) is a widely distributed beetle that feeds inside the seeds of its host plants. Variation in egg size exists within and among populations of *S. limbatus.* This variation appears to in part represent an adaptation to host plant quality. In central Arizona *S. limbatus* uses primarily three host plants: *Cercidium floridum* (blue paloverde), *C. microphyllum* (small-leaf paloverde), and *Acacia greggii* (cat-claw acacia). Laboratory and field studies demonstrate that selection on egg size varies substantially among these hosts and favors large eggs on *C. floridum* (because larvae hatching from small eggs are incapable of penetrating the seed coat; Fox and Mousseau 1996, 1998) and smaller eggs on *C. microphyllum* and *A. greggii* (larvae hatching from small eggs survive very well on these hosts, but females laying small eggs can lay many more eggs due to a trade-off between egg size and number; Fox and Mousseau 1996, 1998; Fox 1997b; Fox et al. 1997).

When we compare populations of *S. limbatus* that use different host species we find evidence that egg size has evolved in response to these host-associated differences in selection;

populations of *S. limbatus* collected from *C. floridum* lay fewer and larger eggs than populations collected from either *C. microphyllum* or *A. greggii* (Fox and Mousseau 1998). More interestingly, when females have access to both hosts they exhibit egg size plasticity by laying larger eggs on *C. floridum* than on either *C. microphyllum* or *A. greggii* (Fox et al. 1997a; Fox and Mousseau 1998). Females are capable of adjusting egg size in response to early host experience because they delay oviposition for more than 24 h after emergence. During this time they mature eggs, generally while in contact with their oviposition substrate. Females can also change the size of the eggs that they lay during their lifetime if they encounter a new host.

Laboratory experiments demonstrate that this egg size plasticity influences maternal fitness; failure to exhibit egg size plasticity comes at a substantial fitness cost to females—they either produce progeny with low survivorship if they encounter *C. floridum* or they have low fecundity if they encounter *A. greggii* (Fox et al. 1997; Fox and Mousseau 1998). Thus, egg size plasticity appears to be an adaptive strategy by which females increase progeny survivorship when progeny develop on a "poor" host and increase maternal fecundity when progeny develop on a "good" host. We thus interpret egg size plasticity as the mechanism of inheritance of a environmentally based maternal effect on progeny survivorship (Fox et al. 1997). Here, we describe an experiment in which we quantify genetic variation in egg size and egg size plasticity in *S. limbatus* and measure the genetic constraints on the evolution of egg size plasticity, quantified as the genetic correlation between the size of eggs laid on *A. greggii* and the size of eggs laid on *C. floridum* (the cross-environment genetic correlation; Fry 1992). Few other studies have quantitatively assessed genetic variation in maternal effects and the genetic constraints on their evolution, such that the potential for maternal effects to respond to natural selection remains largely speculative (Roach and Wulff 1987; Riska 1991; Byers et al. 1997; Shaw and Byers 1998).

MATERIALS AND METHODS

Natural History of Stator limbatus

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; Nilsson and Johnson 1993; Johnson 1995). Throughout its large geographic range, it has been reared from seeds of more than 70 plant species in at least nine genera. In the United States, and particularly in Arizona, it is abundant on many species of *Acacia* (Fabaceae: Mimosoideae) and two species of *Cercidium* (*C. floridum* and *C. microphyllum*; Paloverdes; Fabaceae: Caesalpinioideae), although only one or a few hosts may be available in any single locality.

Females oviposit directly onto host seeds in fruits 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 seed that their mother has chosen for them. In the laboratory, mating and egg laying begin approximately 24–48 h post-

emergence. Beetles require only the resources inside of a single seed to complete development and reproduce. Thus, neither food nor water supplementation is necessary for the following laboratory experiments.

Study Populations

Beetles for this experiment were collected from one *S. limbatus* population in central Arizona and one in western Texas. On June 25, 1995, beetles were collected from *Acacia berlandieri* (Fabaceae: Mimosoideae) along an approximately 1-km stretch of Highway 277, about 15 km south of Del Rio, Val Verde County, Texas (Del Rio population). This 1-km stretch is densely populated with *A. berlanderii,* but is surrounded by agricultural fields. On August 20, 1995, beetles were collected from *Cercidium floridum* and *C. microphyllum* along an approximately 2-km stretch of Mountainview Road in Apache Junction, Pinal County, Arizona (Apache Junction population). This region is at the base of the Superstition Mountains and is populated primarily with *C. floridum* at the lowest elevations and *C. microphyllum* at higher elevations. We collected beetles from both hosts.

Beetles and seed stock were collected by picking mature seed pods from more than 25 plants at each site. Mature pods were transferred to the laboratory and seeds bearing beetle eggs were separated from uninfested seeds. Seeds containing entrance or emergence holes of other bruchids (such as *Mimosestes* sp.) were discarded. We estimate that both laboratory populations were initiated with at least 200-field collected individuals. The populations were reared in the laboratory for two (Apache Junction populations) or four (Del Rio population) generations on seeds of *A. greggii,* before the experiment was initiated. All beetles (parents and progeny) were reared on seeds of *A. greggii* because larval mortality is very low on this species, which minimizes the risk of natural selection occurring during the experiment.

Experimental Design

A traditional half-sib design (Falconer 1989; Roff 1997) was used to estimate genetic variation in egg size and egg size plasticity, and the genetic correlation between the size of eggs laid on *C. floridum* and the size of eggs laid on *A. greggii.* To create half-sib families, virgin male beetles (59 in the Del Rio population and 61 in the Apache Junction population) were mated sequentially to two to four different females, creating 189 and 168 full-sib families (Del Rio and Apache Junction, respectively). To create half-sib families, each virgin male was collected within 12 h of emergence from an isolated *A. greggii* seed and then confined with a virgin female in a 35-mm petri dish containing 12 *A. greggii* seeds and allowed to copulate. Pairs were confined until the female laid at least one egg, after which the male was transferred to another 35-mm petri dish containing a new virgin female, and again confined until his mate laid at least one egg. This procedure was repeated until males either successfully fertilized four females or died. Dishes containing egg laying females were checked for eggs at 12-h intervals. Seeds bearing eggs were replaced with clean seeds until a female had laid eggs on at least 10 seeds.

The length and width of eggs laid by each female were measured using an optical micrometer on a 55× dissecting scope. Because eggs are glued to seeds, removing them for weighing is destructive and very time consuming. Egg mass is positively correlated with both egg length ($R^2 = 0.88$) and egg width ($R^2 = 0.61$) (Fox and Mousseau 1996) and was estimated from the equation: egg mass = −0.035 + (egg length × 0.0086) + (egg width × 0.0022), where egg mass is estimated in milligrams and egg dimensions are measured in mm/10. This equation was derived empirically in the laboratory and did not differ among the egg-laying substrates (hosts) that were examined.

All eggs were reared to adult on *A. greggii* seeds at densities of one beetle per seed (excess eggs were scraped off), 29°C, 15:9 L:D. *Acacia greggii* seeds were used for rearing all beetles because survivorship is very high on this host (generally > 95%), which minimizes the influence of selection during the experiment on parameter estimates. Emerging adults were collected and weighed individually within 12 h of adult emergence. In total, 1633 and 1263 females were reared from the Del Rio and Apache Junction populations, respectively.

The size of eggs laid by all female offspring was estimated by pairing emerging female progeny with a haphazardly chosen virgin male (excluding siblings) and confining them in a 35-mm petri dish containing eight seeds of either *A. greggii* or *C. floridum.* Half of the females emerging from each family were confined with *A. greggii* and half with *C. floridum.* Dishes were checked approximately daily for eggs. Females were discarded when their dish was found to contain two or more eggs. Eggs were stored at room temperature until it was convenient to measure them. Because eggs contain only dry frass (excrement) after larvae burrow into a seed, long-term storage does not distort egg shape or size (C. W. Fox, unpubl. data). Two or three haphazardly selected eggs from each female were measured using an optical micrometer in a 55× dissecting scope.

Analyses

Because egg size appears to increase isometrically across hosts (analysis not shown) and the untransformed egg size data fit the assumptions of the subsequent statistical analyses, untransformed data were used in all analyses.

Genetic and maternal influences on the size of eggs laid by female *S. limbatus* were examined using the restricted maximum-likelihood variance-component estimation procedure of SAS (SAS VARCOMP method = REML; SAS Institute 1985). The size of eggs was initially examined separately for each host species. The proportion of phenotypic variance explained by additive genetic effects was estimated as $4V_S$ (the among-sire variance component). Standard errors for the proportion of V_P explained by V_A (i.e., the heritability, h^2) were calculated following Becker (1992). The maternal effects variance (V_M) was calculated assuming that the dominance variance and higher order interactions (e.g., V_{AA}, V_{AAA}) were zero. See Fox (1994b, 1998) for other examples of these procedures. Note that for these analyses, we treat egg size as a maternal character (because eggs are produced by mothers) and not as a progeny character, although egg size determines progeny size at egg hatch. This is different

than the treatment of seed size in recent studies by some plant evolutionary biologists (e.g., Byers et al. 1997). Because we treat eggs as maternal characters, the effect of a mother's phenotype or environment on the size of eggs that her daughters lay is a maternal effect on egg size (estimated as V_M in the analysis described above). (Note also that, because we treat eggs as maternal characters, egg size plasticity is not a maternal effect, but is the mechanism by which larval phenotype is manipulated by mothers in response to environmental variation [i.e., the mechanism producing the maternal effect]).

Heritabilities of egg size were also calculated via parent-offspring regression, in which the mean size of the eggs laid on *A. greggii* by full-sib sisters was regressed against the mean size of the eggs that their mother laid on *A. greggii* (Roff 1997). These estimates are only available for *A. greggii* because all progeny were reared on *A. greggii*, and thus all mothers laid eggs only on *A. greggii*.

Additive genetic correlations (r_G) between the size of eggs laid on *A. greggii* and the size of eggs laid on *C. floridum* (cross-environment genetic correlations) were calculated from the maximum-likelihood variance components (Yamada 1962; Fry 1992), as $r_G = \sigma^2_{\text{sire-mixed}}/\sigma_{\text{sire-}Acacia}\sigma_{\text{sire-}Cercidium}$, where $\sigma^2_{\text{sire-mixed}}$ is the estimated sire main effect variance component from the complete mixed model analysis of variance (the covariance across environments) and $\sigma_{\text{sire-}Acacia}$ and $\sigma_{\text{sire-}Cercidium}$ are the square roots of the estimated sire main effect variance components from the two reduced models (one for each host; the variance within environments). We used only the sire (co)variance components to avoid possible bias due to maternal effects. Standard errors for r_G were estimated by jackknifing the estimate of r_G from the VAR-COMP procedure outlined above (Roff and Simons 1997; Windig 1997). The hypothesis that $r_G < 1$ was tested using a one-sample t-test (Knapp et al. 1989). Ratio estimators are frequently not normally distributed and so we additionally performed the following test. The test for $r_G = 1$ can be written as Cov/Prod $= 1$, where Cov is the cross-environment covariance ($\sigma^2_{\text{sire-mixed}}$) and Prod is the product of $\sigma_{\text{sire-}Acacia}$ and $\sigma_{\text{sire-}Cercidium}$. Rearranging the equation we obtain: Cov/Prod $= 1$, Cov $=$ Prod, Cov $-$ Prod $= 0$. Letting X $=$ Cov $-$ Prod, we can estimate X for the original sample and then estimate its standard error using the jackknife procedure, as above. We can now test the hypothesis that $r_G < 1$ indirectly by testing the hypothesis that X < 0.

RESULTS

Genetic and Maternal Influences on Egg Size

In both populations, females laid larger eggs on seeds of *C. floridum* than on seeds of *A. greggii*, which confirms that egg size plasticity is present in both of these populations (Fig. 1; Table 1). There was no evidence of differences between populations in the average size (mass) of eggs laid on either host nor in the average magnitude of egg size plasticity exhibited by either population (non-significant population and population*host effects in the combined population ANOVA presented in Table 1).

In the Apache Junction population, there were large sire and dam (nested within sire) effects on both the size of eggs
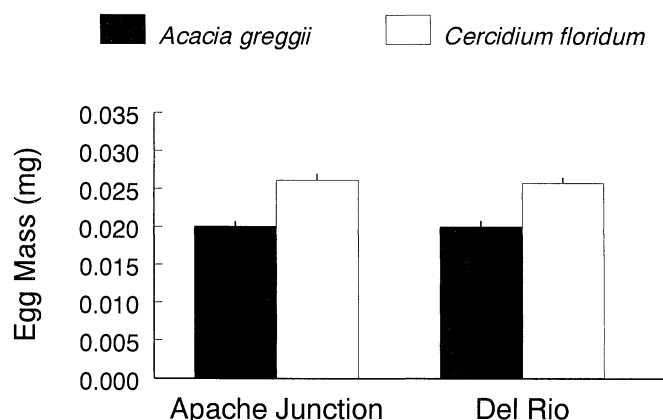

■ *Acacia greggii* □ *Cercidium floridum*

FIG. 1. The effects of host species on the size of eggs laid by female *Stator limbatus* (mean ± SEM). Note that for both populations eggs laid on *Cercidium floridum* are ≈ 30% larger than eggs laid on *Acacia greggii*. Means were calculated by first calculating the average egg size for each full-sib family and then averaging across these family means. Statistics are in Table 1.

laid on *A. greggii* and on the size of eggs laid on *C. floridum* (separate analyses for each host; Table 2). In the Del Rio population, sire effects on egg size were detected only for eggs laid on *A. greggii* (Table 2), whereas dam (nested within sire) effects were detected on both hosts. These sire effects translated into significantly higher heritabilities in the Apache Junction population ($h^2 = 0.909$ and 0.664 for the mass of eggs laid on *A. greggii* and *C. floridum*, respectively) than in the Del Rio population (Table 2; $h^2 = 0.437$ and 0.217; between-population difference is statistically significant). There was also a trend for the heritability of the size of eggs that were laid on *C. floridum* to be lower than the heritability of the size of eggs that were laid on *A. greggii*, which corresponds to an increase in the estimated environmental variance on *C. floridum*, but this was not statistically significant for either population ($P > 0.05$). The estimates of the heritability of egg mass obtained from the parent-offspring regressions did not differ significantly from estimates obtained from the half-sib analyses (estimates available for *A. greggii* only; $h^2 = 0.582 \pm 0.124$ and 0.442 ± 0.100 for the Apache Junction and Del Rio populations, respectively). As with the half-sib analysis, the estimate of h^2 was lower for the Del Rio population than for the Apache Junction population, but not significantly so.

There appeared to be no influence of a mother's phenotype or environment on the size of the eggs that her daughters laid, other than via the genes that she contributed to them—the proportion of the variance in egg size explained by maternal effects was zero for three analyses and only 5.8% for the fourth (Table 2). Thus, the size of eggs that a female lays is influenced by both her genotype and the host that she oviposits on (Table 1), but not by a nongenetic effect of her mother's phenotype or environment.

Genotype-Environment Interactions and Genetic Correlations across Hosts

The estimated genetic correlations (r_G; Table 3) between the size of eggs laid on *A. greggii* and the size of eggs laid

TABLE 1. Analysis of variance demonstrating large effects of oviposition host, but small host-family interactions, on the size of eggs laid by female *Stator limbatus*. Results are presented for egg mass only. Results for egg length and egg width are similar. The analysis was performed using SAS PROC GLM, Type III sums of squares (SAS Institute 1985). F-statistics were calculated using the "random" statement (with the "/ test" option).

		Analysis of variance		
	df	MS ($\times 10^{-6}$)	F	P
Apache Junction population				
Host	1	6917.23	1592.36	< 0.001
Sire	60	17.80	2.43	< 0.001
Dam (sire)	99	6.31	1.80	0.002
Sire × host	59	4.77	1.37	0.086
Dam × host (sire)	88	3.53	1.40	0.012
Del Rio population				
Host	1	9338.13	1340.34	< 0.001
Sire	58	16.36	1.67	0.026
Dam (sire)	123	8.44	1.51	0.012
Sire × host	58	7.20	1.30	0.115
Dam × host (sire)	120	5.61	1.27	0.032
Combined populations				
Host	1	15783.18	2814.91	< 0.001
Population	1	17.92	1.21	0.273
Host × population	1	8.13	1.45	0.231
Sire (population)	118	17.09	2.01	< 0.001
Dam (sire × population)	222	7.49	1.59	< 0.001
Sire × host (population)	118	5.97	1.28	0.060
Dam × host (sire, population)	208	4.73	1.32	0.003

TABLE 2. Nested analysis of variance and estimated variance components for the mass of eggs laid by female *Stator limbatus* on *Acacia greggii* and *Cercidium floridum*. Analyses for egg length and egg width are qualitatively the same, and thus not presented here. Type III sums of squares were calculated using SAS GLM (SAS Institute 1985). Variance components were estimated using the restricted maximum-likelihood method of SAS VARCOMP. The maternal effects variance (V_M) was calculated assuming that the dominance variance and higher order interactions (e.g., V_{AA}, V_{AAA}) were zero. Standard errors for the percentage of V_P explained by V_A ($= h^2$) were calculated following Becker (1992). V_S, among-sire variance component; V_D, among-dam variance component; V_E, error variance in ANOVA; V_P, total phenotypic variance; V_A, additive genetic variance; V_M, maternal variance; V_e, environmental variance.

					Variance components		
	Analysis of variance					Genetic ($\times 10^{-5}$)	
Source	df	MS ($\times 10^{-5}$)	F	Observational ($\times 10^{-5}$)		Variance component	Proportion of V_P explained
Apache Junction population							
Acacia greggii							
Sire	60	0.95	2.60***	$V_S = 0.069$		$V_A = 0.276$	90.8 ± 22.9%
Dam (sire)	99	0.37	1.98***	$V_D = 0.048$		$V_M = 0$	0%
Error	421	0.19		$V_E = 0.187$		$V_e = 0.028$	9.2%
$R^2 = 0.56$				$V_P = 0.304$			
Cercidium floridum							
Sire	59	1.45	2.19***	$V_S = 0.081$		$V_A = 0.324$	66.4 ± 20.0%
Dam (sire)	88	0.66	2.02***	$V_D = 0.058$		$V_M = 0$	0%
Error	378	0.33		$V_E = 0.349$		$V_e = 0.164$	33.6%
$R^2 = 0.53$				$V_P = 0.488$			
Del Rio population							
Acacia greggii							
Sire	58	1.35	2.11***	$V_S = 0.055$		$V_A = 0.220$	43.7 ± 15.2%
Dam (sire)	122	0.64	1.64***	$V_D = 0.048$		$V_M = 0$	0%
Error	567	0.39		$V_E = 0.400$		$V_e = 0.283$	56.3%
$R^2 = 0.41$				$V_P = 0.503$			
Cercidium floridum							
Sire	58	1.06	1.35 ns	$V_S = 0.033$		$V_A = 0.132$	21.7 ± 12.5%
Dam (sire)	121	0.79	1.57***	$V_D = 0.068$		$V_M = 0.035$	5.8%
Error	487	0.50		$V_E = 0.506$		$V_e = 0.440$	72.5%
$R^2 = 0.39$				$V_P = 0.607$			

*** $P < 0.001$; ns, not significant.

TABLE 3. The genetic correlations (r_G) between the size of eggs laid on *Acacia greggii* and the size of eggs laid on *Cercidium floridum*. Standard errors were calculated by jackknifing the estimates.

	Genetic correlations	t-test for $r_G = 1.0$
	r_G	t
Apache Junction population	0.701 ± 0.177	−2.25*
Del Rio population	0.612 ± 0.293	−1.16 ns

ns, $P > 0.05$; * $P < 0.05$.

on *C. floridum* were significantly greater than zero for both populations of *S. limbatus* (for egg mass, r_G ± SEM = 0.701 ± 0.177 and 0.612 ± 0.293 for the Apache Junction and Del Rio populations, respectively; $P < 0.001$ and $P < 0.05$). For egg size to be capable of evolving toward different optima on the two host plants, these cross-environment genetic correlations must be less than 1.0. The marginally significant sire-host ($P = 0.06$) and significant dam-host ($P = 0.003$) interactions in the full analysis of variance suggest that the true genetic correlations are less than 1.0 (Table 1). However, this interpretation assumes that the among-family variance in egg size is equal in both environments (on the two host plants). That this may not be appropriate is suggested by the variance component estimates in Table 2, which vary fairly substantially among hosts. Thus, we tested whether $r_G < 1.0$ by testing the null hypothesis of Cov − Prod = 0 using a one-tailed t-test (see Materials and Methods). Using this procedure, r_G for egg size was significantly less than 1.0 in the Apache Junction population, but not in the Del Rio population (Table 3).

DISCUSSION

Although we know that maternal effects are often genetically variable, few studies have quantitatively assessed genetic variation in maternal effects (or the mechanisms by which females produce maternal effects) or the genetic constraints on their evolution, particularly for maternal effects that are presumed to have evolved as adaptations. Thus, our primary objectives in this project were to quantify genetic variation in egg size and egg size plasticity in *S. limbatus* and to measure the genetic constraints on the evolution of egg size and egg size plasticity. We found that within *S. limbatus* populations genetic variation was present for the size of eggs laid on seeds of both host plants (*A. greggii* and *C. floridum*). We also found that the evolution of egg size plasticity (the mechanism of the adaptive maternal effect) is in part constrained by a high genetic correlation across host plants ($r_G > 0.6$). However, this genetic correlation across host plants is less than 1.0, which indicates that the size of eggs laid on seeds of these two hosts can diverge in response to natural selection and that egg size plasticity is thus capable of evolving in response to natural selection.

Genetic Variation in Egg Size

Propagule size has been demonstrated to be heritable in a variety of organisms, including many vertebrates (e.g., Larsson and Forslund 1992; Potti 1993; Sinervo and Doughty 1996), arthropods (e.g., Mashiko 1992; Fox 1994a), and

plants (e.g., Byers et al. 1997). The heritability of egg size ranged between 0.217 and 0.908 for *S. limbatus*, varying between populations (significantly higher in the Apache Junction than in the Del Rio population) and hosts (higher on *A. greggii* than on *C. floridum*). That the estimates of the heritability of egg size on *C. floridum* were generally lower than the heritability of egg size on *A. greggii* corresponded to an increase in the estimated environmental variance on *C. floridum*. However, we expect that this difference between hosts may be an artifact of our experimental design. Because all females were reared on *A. greggii*, even when they were eventually tested on *C. floridum* they did not encounter *C. floridum* for a period up to 12 h postemergence because they emerged into a dish containing the *A. greggii* seed within which they developed. Thus, the size of eggs laid on *C. floridum* may have been influenced by the delay between emergence and exposure to *C. floridum*, which ranged among females from 0 h to 12 h, resulting in an increase in V_E relative to the variance we should have observed had females encountered *C. floridum* immediately following emergence. Females tested on *A. greggii* had the opportunity to begin maturing *Acacia*-sized eggs immediately following emergence because they emerged into a dish containing this host, so there should be no increase in V_E attributable to a delay in encountering this host.

The motivation for estimating the heritability of egg size in the laboratory is to extrapolate these values to understand the evolution of egg size in nature. However, because h^2 represents the ratio of additive genetic variance to total phenotypic variance (which includes environmental variance), if one or both of these variables differ between the laboratory and the field, then estimates of h^2 from the lab may not reflect h^2 in nature (Riska et al. 1989; but see Weigensberg and Roff 1996). We suspect that our laboratory estimates of h^2 may overestimate the true heritability of egg size in nature. Because females change egg size in response to host encounters, we expect within-female variation to often be high in nature relative to our laboratory study. For example, if females emerge from an *A. greggii* seed in nature and then do not encounter a *C. floridum* seed until 48 h later, they should begin laying small eggs and gradually shift to laying larger eggs during the next 24–48 h (Fox et al. 1997a), which would result in high variance in the size of eggs laid on *C. floridum* and high V_E on this host. Similarly, when a female moves from *C. floridum* to *A. greggii* she will begin responding to the new host and thus gradually decrease the size of her eggs. In our experiment we exposed females to host seeds within 12 h of their emergence from the seed within which they pupated. They thus had access to their oviposition host during most of their period of egg maturation and were not subsequently switched among hosts. Thus, environmental variation in egg size should be lower in the laboratory than in nature.

In a previous study, Fox (1997a; see also Fox 1994a; Fox and Savalli 1998) tested the hypothesis that variation in egg size could in part be environmentally inherited—maternal size can affect egg size, which in turn can affect offspring performance (including offspring size), which can subsequently affect egg size again. As a result, the size of eggs that females lay may be affected by the phenotype of their mothers. Consistent with the results of Fox (1997a) for *S.*

limbatus, we found no evidence from our experiment that a female's phenotype or environment affected the size of eggs that her daughters laid, other than through the genes that she passed to them (three of the four estimates of V_M in Table 2 are zero). Instead, egg size is affected by a female's genotype (large estimate of V_A in Table 2) and environment (large host effect in Table 1), but not by her mother's phenotype or environment (low V_M is Table 2). The results presented here and elsewhere (Fox 1994a, 1997a, 1998; Fox and Savalli 1998) generally indicate that seed beetle progeny compensate for environmentally based differences among mothers and among eggs laid by the same mother by modifying development to attain a genetically targeted size (review in Fox and Savalli 1998). One interesting exception to this is the influence of maternal food stress (caused by high larval density) on progeny body size in another seed beetle, *Callosobruchus maculatus*—females reared at high density lay small eggs that produce smaller than normal progeny (Fox and Savalli 1998). However, these progeny of high-density-reared mothers are only slightly smaller (< 10%) and do not lay smaller than normal eggs.

Cross-Environment Genetic Correlations and Genetic Variation in Egg Size Plasticity

The evolution of *S. limbatus* egg sizes toward different optima on *A. greggi* and *C. floridum* requires that the cross-environment genetic correlations be less than 1.0. When the correlations are 1.0, an evolutionary change in egg size on one host will result in an identical change on the other host and egg size cannot diverge across hosts. Our estimates of the genetic correlations across hosts were 0.701 and 0.612 for egg mass, which are significantly greater than zero (both populations) and less than 1.0 (Apache Junction population). That the estimates are very high (> 0.6) indicates that the size of eggs laid on the two host plants cannot evolve entirely independently. Instead, selection for large eggs on *C. floridum* will result in a correlated increase in egg size on *A. greggii,* slowing the evolution of an adaptive reaction norm. This high genetic correlation is likely due to pleiotropic effects of genes (i.e., many of the same genes and alleles affect the size of eggs laid on each host), but possibly also due to linkage disequilibrium among alleles affecting egg size on each host. However, that the cross-environment genetic correlations are less than 1.0 indicates that egg size can evolve toward different optima on the different hosts because there is either variation in the environmental sensitivity of alleles at some loci that affect egg size or because some loci have environment-specific effects, which affect the size of eggs laid only in one environment (Via 1993, 1994).

That females from the Del Rio population exhibit egg size plasticity of similar magnitude to females of the Apache Junction population (Fig. 1) was unexpected because the Del Rio population does not have access to *C. floridum* in nature (it does have access to *Acacia berlandieri* and possibly *A. wrightii,* both of which are similar to *A. greggii*). We have since compared seven populations of *S. limbatus* (including two from Texas, where *C. floridum* does not occur) and find that egg size plasticity (in response to *A. greggii* and *C. floridum*) is present in all populations, although it differs in magnitude

among populations. This suggests that (1) substantial gene flow is occurring between populations as distant as Arizona and northern Texas, maintaining egg size plasticity in the Del Rio population; (2) egg size plasticity is an ancestral character that persists in populations that do not express it, possibly because there is no cost to being plastic (Scheiner and Berrigan 1998); or (3) females exhibit egg size plasticity in response to hosts other than *C. floridum*. We cannot currently distinguish among these hypotheses.

In this study we have focused on the evolutionary genetics of egg size and egg size plasticity. However, it is clear from recent data (C. W. Fox and U. M. Savalli, unpubl. ms.) that females also adjust egg composition in response to *A. greggii* and *C. floridum*. For some organisms (Bernardo 1996), including insects (Rossiter 1993), natural intraspecific variation in egg composition has been demonstrated to affect progeny growth and development. Differences in egg composition among our treatments (exposure to *A. greggii* or *C. floridum*) could potentially produce the treatment differences in larval survivorship observed in our previous studies (e.g., Fox 1997b; Fox et al. 1997). The amount of variation in egg composition, the consequences of this variation for progeny survivorship and growth, and the evolutionary genetics of egg composition are currently being explored (by M. E. Czesak and C. W. Fox).

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