

## GENETIC VARIATION IN MALE EFFECTS ON FEMALE REPRODUCTION AND THE GENETIC COVARIANCE BETWEEN THE SEXES

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**Abstract.**—Males of many insect species increase the fecundity and/or egg size of their mates through the amount or composition of their nuptial gifts or ejaculate. The genetic bases of such male effects on fecundity or egg size are generally unknown, and thus their ability to evolve remains speculative. Likewise, the genetic relationship between male and female investment into reproduction in dioecious species, which is expected to be positive if effects on fecundity are controlled by at least some of the same genes in males and females, is also unknown. Males of the seed beetle *Stator limbatus* contribute large ejaculates to females during mating, and the amount of donated ejaculate is positively correlated with male body mass. Females mated to large males lay more eggs in their lifetime than females mated to small males. We describe an experiment in which we quantify genetic variation in the number of eggs sired by males (mated to a single female) and found that a significant proportion of the phenotypic variance in the number of eggs sired by males was explained by their genotype. Additionally, the number of eggs sired by a male was highly positively genetically correlated with his body mass. The between-sex genetic correlation, that is, the genetic correlation between the number of eggs sired by males and the number of eggs laid by females, was highly positive when eggs were laid on *Acacia greggii* seeds. This indicates that males that sire many eggs have sisters that lay many eggs. Thus, some of the genes that control male ejaculate size (or some other fecundity-enhancing factor) when expressed in males appear to control fecundity when expressed in females. We found no significant interaction between male and female genotype on fecundity.

**Key words.**—Between-sex genetic correlation, ejaculate, fecundity, male effects, *Stator limbatus*.

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Males of many insect species contribute more to reproduction than just sperm. Large nuptial gifts, ejaculates, or spermatophores may be donated by males to their mates, and such donations are eaten or absorbed by females and used to improve female survival or increase the size and/or number of offspring (reviewed in Vahed 1998; Fox et al. 1995; Savalli and Fox 1998a; Savalli and Fox 1999a,b). Males may also stimulate females to lay eggs through components in their ejaculate (Park and Wolfner 1995; Moshitzky et al. 1996; Yi and Gillott 1999; Heifetz et al. 2000; Jin and Gong 2001) or secure their paternity by transferring accessory gland proteins that act as a plug (Bertram et al. 1996) or reduce the capacity of resident sperm to fertilize eggs (Prout and Clark 2000). In a few insect species, males exhibit mate-guarding behaviors (e.g., cricket *Gryllobates sigillatus*; Bateman et al. 2001) or paternal care of their offspring (e.g., burying beetle *Nicrophorus vespilloides*; Scott 1998). Relatively little is known about the amount of genetic variation in these nutritive, hormonal stimulant, or behavioral effects of males on the number of offspring they sire, and thus their evolutionary dynamics remain largely speculative in most taxa. Exceptions to this include the seed beetle *Callosobruchus maculatus*, in which ejaculate size is heritable ( $h^2 > 0.42$ ; Savalli and Fox 1998b; Savalli et al. 2000), and the cricket *Gryllobates supplicans*, in which spermatophylax size is heritable ( $h^2 = 0.47$ , Sakaluk and Smith 1988). Genetic variation in accessory gland substances that influence oogenesis or oviposition has been detected in *Drosophila melanogaster* (Rice 1996; Holland and Rice 1999) and the housefly *Musca domestica* (Andrés and Arnqvist 2001).

Species in which both the genotype of the female and the

genotype of her mate affect fecundity and/or offspring size have the potential for coevolution of male and female investment into reproduction. This coevolution is well studied in monoecious and hermaphroditic individuals of subdioecious plants, in which there is often a genetic trade-off between male and female reproduction (reviewed in Olson and Antonovics 2000; but see Mazer and Delesalle 1996; Campbell 1997). Yet in dioecious species there is no a priori reason to expect this trade-off because male and female functions are not produced in the same individual and thus are not constrained by the need to divide resources simultaneously between two functions. Instead, a positive genetic correlation between male and female traits is expected if genes are similarly expressed in both sexes, or no correlation is expected if traits are sex-limited in their expression (Lynch and Walsh 1998). However, little is known of the genetic relationship between male and female investment into reproduction in dioecious species. Here, we examine the genetic correlation between the number of eggs laid by females and the number of eggs sired by males in a dioecious species, a seed beetle, *Stator limbatus*.

### Male Effects on Fecundity in *Stator limbatus*

*Stator limbatus* (Horn) (Coleoptera: Bruchidae) is a beetle that oviposits on and develops within seeds of leguminous trees and shrubs. Males contribute large ejaculates to their mates during mating, averaging ( $\pm$ SE)  $4.8 \pm 0.1\%$  of their body mass (Fox et al. 1995). Ejaculate size varies among males and is positively correlated with male body mass. Females gain direct fitness benefits from mating with larger males by laying larger and/or more eggs (Fox et al. 1995; Savalli and Fox 1998a). Females mated to larger males may incorporate more male-derived nutrients into egg production.

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Interestingly, males and females invest a similar amount of biomass into reproduction (Savalli and Fox 1998a).

Males of *S. limbatus* are larger than females (Fox et al. 1995; Savalli and Fox 1998a), which is unusual in insect species without male-male competition for mates. Fecundity selection favors the evolution of large males because females mated to larger males lay more eggs than females mated to smaller males (Fox et al. 1995; Savalli and Fox 1998a). Large males are also favored by sexual selection because females mated to small males are more likely to remate than females mated to large males (Savalli and Fox 1998a). Thus, it appears that the large ejaculate size of *S. limbatus* males has evolved as both a form of paternal investment, such that females receiving larger ejaculates are more fecund, and also as a form of mating effort, to increase the proportion of offspring sired by a male by reducing the likelihood of female remating. Although the importance of these male effects on female fecundity has been explored, their genetic basis and thus their evolutionary dynamics are unknown.

Because fecundity is affected by both a female's phenotype (larger females lay more eggs) and the phenotype of her mate (females mated to larger males lay more eggs; Fox et al. 1995; Savalli and Fox 1998a), fecundity can be treated as a trait of both parents; that is, fecundity is the number of eggs laid by a female and also the number of eggs sired by the male mated to that female. In this study, we quantify genetic variation in the number of eggs sired by males. We also estimate the amount of genetic variation in the size of eggs sired by males because ejaculate-derived nutrients are known to be used by females to increase egg size in other insect taxa (reviewed in Vahed 1998) and because egg size is genetically correlated with fecundity (Czesak and Fox 2003). To predict the coevolutionary response of male and female investment into reproduction, we quantify the genetic correlation between the number of eggs laid by females and the number of eggs sired by males. We found that a male's genotype explained a significant proportion of the phenotypic variance in the number of eggs he sired, and that the number of eggs he sired was genetically correlated to his body mass. The number of eggs laid by females and the number of eggs sired by males were positively genetically correlated, but this correlation differed between two host species on which females laid eggs.

## MATERIALS AND METHODS

### *Natural History of Stator limbatus*

*Stator limbatus* is a beetle that develops inside seeds of more than 70 legume species (Johnson and Kingsolver 1976; Johnson et al. 1989; Nilsson and Johnson 1993). Although a generalist over its wide geographical range (from northern South America to the southwestern United States), 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* (Fabaceae: Caesalpinioideae) as host plants.

Females enter pods that have either dehisced or have been damaged by other organisms and lay eggs on the surface of seeds. Upon hatching, first instar larvae burrow through the seed coat directly underneath the egg and continue their de-

velopment 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 to 48 hours after emergence. The resources inside a single seed provide all nutrients required for developing beetles. The short development time of *S. limbatus* (approximately 23 days at 30°C) 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). At this location, both *A. greggii* and *C. floridum* trees are abundant. Mature pods were picked 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 20 individual 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 they were 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 in the laboratory for three generations on *A. greggii* seeds before initiation of experiments (30°C, 16:8 L:D). Because larval survivorship is high on *A. greggii* seeds (>95%) but low on *C. floridum* seeds (<50%), beetles were reared on *A. greggii* seeds to avoid inadvertent natural selection before and during the experiment.

### *Experimental Design*

A half-sib design (Falconer and Mackay 1996) was used to estimate additive genetic variation in the number and size of eggs sired by males and to estimate the genetic correlation between the number of eggs laid by females and the number of eggs sired by males. The family data (sire and dam) of both parents were recorded. This allowed for separate analyses of the effect of a male's genotype on the number and size of eggs he sired and the effect of a female's genotype on the number and size of eggs she laid.

Half-sib families were produced by mating each of 126 sires sequentially to five different dams. Sires mated successfully to 3.2 dams on average (range = 2–5), creating 404 full-sib families. To create these families, virgin beetles were collected within 12 hours of adult emergence and isolated in a 35-mm petri dish. Because adults do not emerge fully mature, they were not mated immediately after collection; maturation is complete approximately 24 to 48 hours after adult emergence. Thus, males were aged 24 hours (from time of collection) in an empty petri dish before mating. Females were also aged 24 hours before mating, during which they were provided with three *A. greggii* seeds. After 24 hours, the male was paired with his first mate. Dishes were checked every 12 hours for eggs on the seeds. After a female began laying eggs, she was transferred to a new petri dish with 20

*A. greggii* seeds. Every 12 hours, seeds bearing eggs were removed and replaced with clean seeds. Twenty eggs from each female were reared for the next generation.

#### Data on offspring

Larvae were reared to adult at a density of one beetle per seed (30°C, 16:8 L:D); extra eggs were scraped from the seed surface. Offspring were collected within 12 hours of adult emergence and weighed (0.1 mg precision).

To estimate lifetime fecundity and the size of eggs laid by all female offspring, half of the daughters from each full-sib family were confined with eight *A. greggii* seeds and the other half were confined with eight *C. floridum* seeds upon which to lay eggs. Lifetime fecundity and egg size were measured on these two host species because significant differences between these host species have been detected previously in the genetic relationships among egg size, fecundity, and female body size (Czesak and Fox 2003). Adults were aged 24 hours before mating, with females in contact with seeds during egg maturation. Males and females were then paired randomly. Seeds were checked every 12 hours for eggs. After the first 12-hour period of oviposition, male and female pairs were transferred to a new petri dish containing either 30 *A. greggii* or 30 *C. floridum* seeds (the same host species as in the first dish) and allowed to lay eggs until death to estimate lifetime fecundity. The size of eggs (length and width) laid by each female was recorded by measuring three of the eggs laid during the first 12-hour period of oviposition using an optical micrometer on a 55× dissecting scope (0.005 mm precision). Eggs laid later in a female's oviposition period were not measured because egg size declines with female age as in other seed beetle species (e.g., *Callosobruchus maculatus*, Fox 1993a). Eggs were not weighed because they are glued to the seed surface, and removing them is destructive. Results for egg length and egg width were consistent for all analyses, so only results for egg length are reported.

#### Analyses

Additive genetic variances ( $V_A$ ) in the number and size of eggs sired by males were computed from the family data (sire and dam) of males using the restricted maximum likelihood method of variance component analysis (SAS PROC VARCOMP, METHOD = REML, SAS Institute 1985), where  $V_A = 4V_S$  ( $V_S$  = among sire component), and  $h^2 = V_A/V_P$  ( $h^2$  = heritability,  $V_P$  = total phenotypic variance). Additive genetic correlations ( $r_A$ ) between male body mass and (1) the number of eggs sired by males and (2) the size of eggs sired by males were calculated using variances and covariances estimated from variance component analysis (SAS PROC VARCOMP, METHOD = REML, SAS Institute 1985). Sire variance and covariance components were used to avoid bias due to maternal effects.

The additive genetic correlation ( $r_A$ ) between the number of eggs sired by males and the number of eggs laid by their sisters was calculated using variances and covariances estimated from variance component analysis (SAS PROC VARCOMP, METHOD = REML, SAS Institute 1985). This between-sex genetic correlation was estimated as  $r_A = \sigma_{\text{sire-both sexes}}^2 / \sigma_f \sigma_m$ , where  $\sigma_{\text{sire-both sexes}}^2$  is the covariance be-

tween sexes and estimated as the sire variance component from a complete mixed model analysis of variance (not separated by sex; Fry 1992). Reduced models, one for each sex, provided estimates of  $\sigma_m$  and  $\sigma_f$ , the square root of the additive genetic variance within each sex ( $m$  = male,  $f$  = female). This analysis treats the two sexes as two environments for gene expression. Note that this is not the correlation between the number of eggs sired by males and the number of eggs laid by their mates, but rather the correlation between the number of eggs sired by males and the number of eggs laid by their sisters that are mated to unrelated males.

Standard errors of heritabilities ( $h^2$ ), phenotypic correlations ( $r_P$ ), and additive genetic correlations ( $r_A$ ) were estimated by jackknifing the estimates (using S-Plus, Insightful Corp. 2001; Roff and Preziosi 1994; Windig 1997). Genetic correlations were compared between sexes 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.

In total, body mass data were collected from 5643 progeny (2797 females and 2846 males), and both the number and size of eggs sired by males and the number and size of eggs laid by females were measured for 2667 pairs from 404 full-sib families (126 sires and 511 dams). Biases from inadvertent selection during the experiment should have been minimal because egg-to-adult survivorship of progeny was very high (98.8%).

## RESULTS

### *Genotypic Effect of a Male on the Number and Size of Eggs*

The effect of a male on the number of eggs he sired was at least partially genetically based ( $V_A$  in Table 1A). The number of eggs sired by males was heritable when females laid eggs on *A. greggii* and *C. floridum* seeds (Table 2A). The heritability of the number of eggs laid by females was higher than the heritability of the number of eggs sired by males (Table 2B). None of the phenotypic variance in the size of eggs sired by males was heritable (Tables 1B and 2A), whereas the size of eggs laid by females was highly heritable (Table 2B).

### *Effect of Body Mass on Number and Size of Eggs*

Large females laid more eggs than small females on both host species ( $r_P$  column in Table 3A). Large males also sired more eggs than small males on both host species on which females laid eggs ( $r_P$  column in Table 3A; Fig. 1). From multiple regression, there were large partial effects of both male and female body mass on the number of eggs laid on both host species (Table 4). Large females laid larger eggs than small females on both host species ( $r_P$  column in Table 3B). On *C. floridum* seeds, large males sired smaller eggs, contrary to our prediction, but male size had no effect on egg size when eggs were laid on *A. greggii* seeds ( $r_P$  column in Table 3B). From multiple regression, there were large partial effects of both male and female body mass on the size of eggs laid on *C. floridum* seeds, but only female body mass had a significant effect on the size of eggs laid on *A. greggii* seeds (Table 4).

TABLE 1. Nested analysis of variance and variance component analysis examining the effect of male's sire and dam on the phenotypic variance in the (A) number of eggs he sired and (B) length of eggs he sired for *Stator limbatus*. Maternal effects variances ( $V_M$ ) were calculated assuming that dominance variance and epistatic interactions were zero. Proportion of  $V_P$  explained by  $V_A$  is heritability.  $V_S$ , 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.

Source	Analysis of variance			Observational variance component	Genetic	
	df	MS	F		Variance component	Proportion of $V_P$ explained
<b>(A) Number of eggs sired</b>						
<i>Acacia greggii</i> (mean $\pm$ SE = 36.7 $\pm$ 0.2 eggs)						
Sire	126	83.20	1.59**	$V_S = 2.688$	$V_A = 10.71$	21 $\pm$ 8.6%
Dam (sire)	254	52.31	1.14 ns	$V_D = 1.758$	$V_M = 0$	0%
Error	914	46.49		$V_E = 46.889$	$V_e = 41.513$	81%
$r^2 = 0.31$				$V_P = 51.334$		
<i>Cercidium floridum</i> (mean $\pm$ SE = 22.3 $\pm$ 0.1 eggs)						
Sire	127	33.78	1.37*	$V_S = 0.811$	$V_A = 3.246$	19 $\pm$ 5.0%
Dam (sire)	243	24.78	1.11 ns	$V_D = 0$	$V_M = 0$	0%
Error	931	22.25		$V_E = 22.753$	$V_e = 21.130$	90%
$r^2 = 0.29$				$V_P = 23.564$		
<b>(B) Length of eggs sired (mm) (<math>\times 10^{-3}</math>)</b>						
<i>Acacia greggii</i> (mean $\pm$ SE = 0.5369 $\pm$ 0.0004 mm)						
Sire	126	0.28	0.98 ns	$V_S = 0$	$V_A = 0$	0%
Dam (sire)	255	0.31	0.12 ns	$V_D = 0.127$	$V_M = 0.127$	4%
Error	955	0.28		$V_E = 2.723$	$V_e = 2.723$	96%
$r^2 = 0.31$				$V_P = 2.850$		
<i>Cercidium floridum</i> (mean $\pm$ SE = 0.5935 $\pm$ 0.0006 mm)						
Sire	127	0.48	0.99 ns	$V_S = 0$	$V_A = 0$	0%
Dam (sire)	244	0.53	1.07 ns	$V_D = 0.057$	$V_M = 0.057$	1%
Error	954	0.49		$V_E = 4.930$	$V_e = 4.930$	99%
$r^2 = 0.29$				$V_P = 4.987$		

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

There was a genetic basis for the positive phenotypic correlation between the number of eggs sired by males and their body mass. Male body mass was heritable ( $h^2 = 0.34 \pm 0.09$ ) and was positively genetically correlated with the number of eggs that they sired on both host species ( $r_A$  column in Table 3A). On *A. greggii* seeds, the genetic correlation between the number of eggs sired by males and male body mass was significantly higher than the genetic correlation between the number of eggs laid by females and female body mass ( $r_A$  column in Table 3A;  $\chi^2_1 = 22.10$ ,  $P < 0.001$ ), but these genetic correlations were not significantly different when eggs were laid on *C. floridum* seeds ( $\chi^2_1 = 2.23$ ,  $P = 0.136$ ).

Because the number of eggs sired by males was genetically

correlated with their body mass, genetic variation among males in the number of eggs they sired could in large part be caused by genetic variation in male body mass. Thus, we also analyzed the residuals from a regression of the number of eggs sired versus male body mass. These residuals were heritable ( $h^2$  on *A. greggii* =  $0.20 \pm 0.08$ ;  $h^2$  on *C. floridum* =  $0.08 \pm 0.05$ ), indicating that the genetic variation in the number of eggs sired by males was not caused entirely by genetic variation in male body mass.

*Interaction between Male and Female Genotypes on Number of Eggs*

Male and female genotypes may interact with each other to affect reproductive traits (Andrés and Arnqvist 2001). For *S. limbatus*, this interaction between male and female genotypes on the number of eggs was not significant on either host species (nonsignificant male's sire  $\times$  female's sire interaction; on *A. greggii*: proportion of phenotypic variance explained by variance due to interaction =  $4.00 \pm 6.39\%$ ; on *C. floridum*: proportion = 0). Thus, male and female effects on fecundity appear to be additive.

*Genetic Correlation between Male and Female Effects on Fecundity*

A male's genotype affected the number of eggs he sired, and a female's genotype significantly affected the number of eggs she laid (Tables 1A; and 2A, B). To test whether at

TABLE 2. Heritability of the number and length of eggs sired by males and the number and length of eggs laid by females on either *Acacia greggii* or *Cercidium floridum* seeds for *Stator limbatus*. Additive genetic variances for heritability estimates were computed from variance component analyses using (A) the family (sire and dam) of males and (B) the family of females.

	$h^2$	$h^2$
	<i>A. greggii</i>	<i>C. floridum</i>
<b>(A) Males' family</b>		
Number of eggs	0.21 $\pm$ 0.09	0.19 $\pm$ 0.05
Length of eggs	0	0
<b>(B) Females' family</b>		
Number of eggs	0.32 $\pm$ 0.10	0.27 $\pm$ 0.10
Length of eggs	0.67 $\pm$ 0.13	0.50 $\pm$ 0.12

TABLE 3. Mean phenotypic ( $r_p$ ) and additive genetic correlations ( $r_A$ ) between body mass of *Stator limbatus* and (A) number of eggs and (B) length of eggs. Females oviposited on either *Acacia greggii* or *Cercidium floridum* seeds.

Host		$r_p$	$r_A$
(A) Number of eggs and body mass			
Female body mass	<i>A. greggii</i>	0.46 ± 0.03	0.28 ± 0.32
	<i>C. floridum</i>	0.45 ± 0.03	0.44 ± 0.30
Male body mass	<i>A. greggii</i>	0.40 ± 0.03	0.71 ± 0.22
	<i>C. floridum</i>	0.36 ± 0.03	0.58 ± 0.22
(B) Length of eggs and body mass			
Female body mass	<i>A. greggii</i>	0.20 ± 0.03	0.32 ± 0.19
	<i>C. floridum</i>	0.11 ± 0.03	-0.07 ± 0.25
Male body mass	<i>A. greggii</i>	-0.01 ± 0.03	0
	<i>C. floridum</i>	-0.08 ± 0.03	0

least some of the same genes in females that influence the number of eggs laid are the same genes or linked to genes in males that affect the number of eggs sired, we estimated the between-sex genetic correlation for number of eggs. To do this, we treated number of eggs as a trait of both sexes (as before) and estimated the genetic correlation between the number of eggs sired by males and the number of eggs laid by females. A positive between-sex genetic correlation would indicate that males that sired many eggs had sisters that laid many eggs, and conversely, males that sired few eggs had sisters that laid few eggs. This is not the correlation between the number of eggs sired by males and the number of eggs laid by their mates. There was a positive between-sex genetic correlation for number of eggs laid on *A. greggii* seeds ( $r_A = 0.75 \pm 0.11$ ) but not for the number of eggs laid on *C.*

*floridum* seeds ( $r_A = 0.07 \pm 0.29$ ). To remove the effects of body mass on these between-sex genetic correlation estimates, residuals from a regression of number of eggs sired versus male body mass and from a regression of number of eggs versus female body mass were analyzed. This analysis revealed a positive between-sex genetic correlation for number of eggs on *A. greggii* seeds ( $r_A = 0.51 \pm 0.16$ ) but not for fecundity on *C. floridum* seeds ( $r_A = 0$ ), as consistent with the previous analysis. Thus, male and female effects on fecundity, defined as the number of eggs laid by females or the number of eggs sired by males, were genetically correlated, but only when eggs were laid on *A. greggii* seeds.

DISCUSSION

In *Stator limbatus*, both male and female genotype affected the number of eggs produced by a pair of beetles. The heritability of the number of eggs sired by a male was similar, though slightly lower, than the heritability of the number of eggs laid by a female (Table 2A, B). Additionally, the number of eggs sired by males was positively genetically correlated with their body mass (Table 3A, B). There was a high between-sex genetic correlation for number of eggs when females laid eggs on *A. greggii* seeds, indicating that males

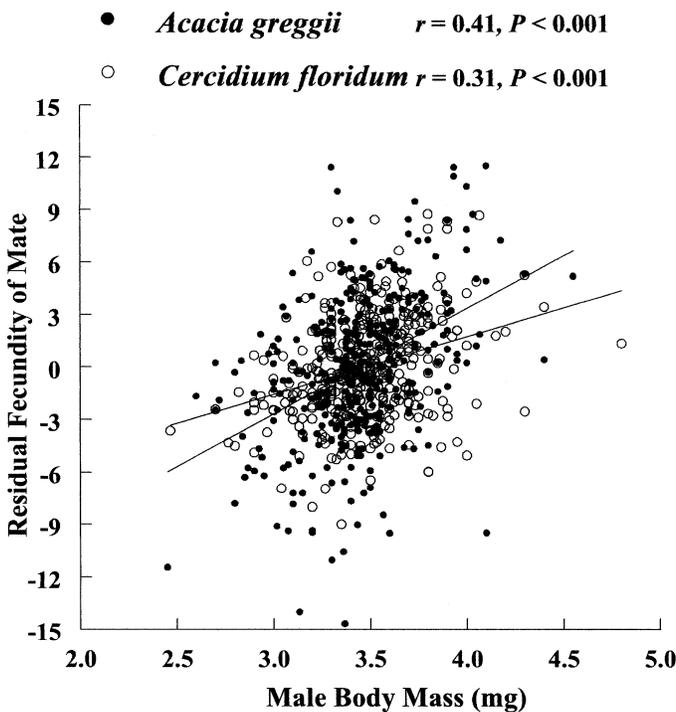


FIG. 1. Relationship between the fecundity of a male's mate (residual fecundity, to remove effects of female body mass) and male body mass for *Stator limbatus*. Fecundity residuals were obtained from the relationship between female body mass and her fecundity on two host species (*Acacia greggii* and *Cercidium floridum* seeds).

TABLE 4. The relationship between the number and length of eggs sired by males and their body mass (male body mass) and the number and length of eggs laid by females and their body mass (female body mass) for *Stator limbatus*, of which females oviposited on either *Acacia greggii* or *Cercidium floridum* seeds. Data are for offspring of half-sib families. Coefficients of determination were calculated from a multiple regression using the general linear model of SAS (PROC GLM). Squared partial correlations and *P*-values were calculated using SAS PROC STEPWISE (SAS Institute 1985).

Host	Number of eggs		Length of eggs	
	Squared partial correlation	<i>P</i>	Squared partial correlation	<i>P</i>
<i>A. greggii</i>				
	<i>n</i> = 1295		<i>n</i> = 1331	
Female body mass	0.1984	<0.0001	0.0376	<0.0001
Male body mass	0.1540	<0.0001	0.0002	0.6014
	$r^2 = 0.35$		$r^2 = 0.04$	
<i>C. floridum</i>				
	<i>n</i> = 1302		<i>n</i> = 1326	
Female body mass	0.2068	<0.0001	0.0126	<0.0001
Male body mass	0.1496	<0.0001	0.0037	0.0256
	$r^2 = 0.36$		$r^2 = 0.02$	

that sired many eggs (when mated to a random unrelated female) had sisters that laid many eggs (when mated to a random unrelated male) and vice versa. This genetic correlation was near zero when females laid eggs on *C. floridum* seeds.

In *S. limbatus*, both male size and male genotype (independent of size) affected the fecundity of his mate. We hypothesize that the number of eggs sired by a male is affected by the size or composition of his ejaculate (as in other insects; reviewed in Vahed 1998). In other seed beetle species, females incorporate nutrients from male ejaculates into their eggs (Huignard 1983; Boucher and Huignard 1987). In *S. limbatus*, ejaculate size is positively correlated with male body mass, and females mated to large males lay more eggs than females mated to small males (Fox et al. 1995; Savalli and Fox 1998a). It is thus likely that females mated to large males incorporate more male-derived nutrients into eggs than females mated to small males. Males may also induce behavioral and physiological changes in their mates upon transfer of ejaculate, resulting in increased egg laying by females and thus enhanced reproductive success of males. In *S. limbatus*, females mated to large males are less likely to remate than females mated to small males, and the likelihood of remating by a female may be determined by the amount or composition of ejaculate received from her first mate (Savalli and Fox 1998a). Male-induced changes in female egg-laying behavior have also been reported in other insect species. Female *Drosophila melanogaster* dramatically increase oviposition rate and become less likely to remate after receiving male accessory gland proteins (Wolfner 1997 and references therein; Heifetz et al. 2001). In other insect species, females exhibit cryptic choice by increasing reproductive effort after mating with a preferred male (Wedell 1996). For example, after mating with a large male, females of the water strider *Gerris lateralis* increase egg production independently of the amount of ejaculate they receive (Arnqvist and Danielsson 1999). Females may also differentially use sperm from different males by expelling unwanted sperm or shunting sperm to specialized compartments (Eberhard 1996; Sakaluk and Eggert 1996). We currently cannot distinguish whether female *S. limbatus* exhibit either of these alternative mechanisms in addition to incorporating ejaculate-derived nutrients into egg production and exhibiting changes in their remating behavior.

Relatively little is known about the genetic basis of male effects on the fecundity and/or mating behavior of their mates. Ejaculate size is heritable and genetically variable among populations of another seed beetle species, *Callosobruchus maculatus* (Savalli and Fox 1998b; Savalli et al. 2000) and positively affects female fecundity as a form of paternal investment (Fox 1993b; Savalli and Fox 1999a,b). In the cricket *Grylodes supplicans*, the size of the spermatophylax, which is eaten by females and used as a source of nutrients for egg production, is heritable ( $h^2 = 0.47$ ; Sakaluk and Smith 1988). Genetic variation in accessory gland substances that influence oogenesis or oviposition has been detected in *Drosophila melanogaster* (Rice 1996; Holland and Rice 1999) and the housefly *Musca domestica* (Andrés and Arnqvist 2001).

Also little studied is the genetic relationship between male

and female investment into reproduction, with the exception of monoecious and hermaphroditic individuals of subdioecious plant species. When both sexes are expressed within a single individual, a common resource pool must be divided between male and female reproduction, such that selection for increased allocation to male reproduction necessarily leads to either the correlated evolution of decreased allocation to female reproduction (and vice versa) or the evolution of increased total allocation. This has been demonstrated in many studies (reviewed in Olson and Antonovics 2000; but see Mazer and Delesalle 1996; Campbell 1997). For example, Olson and Antonovics (2000) detected a negative genetic correlation between stamen and fruit production in hermaphroditic individuals of the subdioecious herb, *Astilbe bi-ternata*. Similarly, Mazer et al. (1999) selected on ovule production and anther production (per flower) in the hermaphroditic plant, *Spergularia marina* (wild radish), and found a negative genetic correlation between these traits, although the correlated responses to selection were asymmetrical (the genetic trade-off was absent when selecting for low ovules per flower and high anthers per flower). A few studies have detected a positive genetic correlation between male and female reproduction, which constrains the independent evolution of male and female reproductive characters (e.g., *Lythrum salicaria*, O'Neil and Schmitt 1993; *Begonia semiovata*, Ågren and Schemske 1995; *Silene latifolia*, Campbell 1997).

In dioecious plants and animals, in which male and female functions do not occur simultaneously within an individual, we have no a priori reason to expect a negative genetic correlation between male and female investment into reproduction. Instead, if the same genes affect reproduction in both sexes, we expect a positive genetic correlation between male and female traits (Lynch and Walsh 1998). Studies examining sexually dimorphic traits find a highly positive between-sex genetic correlation for many traits (e.g.,  $r_A$  ranges from 0.49 to 0.59 for tail length in the barn swallow, *Hirundo rustica*, Møller 1993;  $r_A$  ranges from 0.68 to 0.95 for calyx diameter in *Silene latifolia*, Meagher 1994;  $r_A = 1.037$  for tarsus length in the collar flycatcher, *Ficedula albicollis*, Merilä et al. 1998;  $r_A = 0.91$  for bill color of the zebra finch *Taeniopygia guttata*, Price and Burley 1993; but see Wilkinson 1993:  $r_A$  was positive but lower [ $= 0.29 \pm 0.05$ ] for eyespan in stalk-eyed fly *Cyrtodiopsis dalmanni*; reviewed in Fairbairn 1997 for sexual size dimorphism). However, there are no estimates of the between-sex correlation for reproductive investment in dioecious species. We found a positive genetic correlation in *S. limbatus* between the number of eggs sired by males and the number of eggs laid by females on *A. greggii* seeds, indicating that males that sired many eggs had sisters that laid many eggs (and vice versa); males that sired few eggs had sisters that laid few eggs). However, this between-sex genetic correlation was near zero when eggs were laid on *C. floridum* seeds.

Why this between-sex genetic correlation differed between the two host species is unknown. Fecundity and egg size are phenotypically plastic traits in *S. limbatus*, such that larger and fewer eggs are laid on *C. floridum* seeds than on *A. greggii* seeds. This egg size plasticity is adaptive; on *C. floridum* seeds, larvae hatching from larger eggs have increased survival compared to larvae hatching from small eggs, whereas

on *A. greggii* seeds, larvae hatching from either large or small eggs have equally high survival (Fox et al. 1997). Significant differences between these two host species have been detected for genetic correlations among egg size, fecundity, and female body size—egg size and fecundity are negatively genetically correlated when eggs are laid on *C. floridum* seeds, but not when eggs are laid on *A. greggii* seeds, and egg size and female body size are positively genetically correlated on *A. greggii* seeds, but not on *C. floridum* seeds (Czesak and Fox 2003). This difference between host species in the genetic correlations among egg size, fecundity, and female body size may result from differential gene expression of these traits in different environments, as may the difference between host species in the between-sex genetic correlation for male and female effects on fecundity. This hypothesis has yet to be tested.

The reproductive success of a male can depend not only on his genotype, but also on the genotype of his mate, such that there may be an interaction between male and female genotypes. Andrés and Arnqvist (2001) found a significant effect of a male's genotype on the oviposition rate of his mate in the housefly, *Musca domestica*, probably through genetic variation in the amounts of various proteins in male accessory gland fluid that stimulate oviposition. Female genotype also affected oviposition rate in this species, and there was a strong interaction between male and female genotype, suggesting genetic variation among females in their oviposition response to proteins in male accessory gland fluid. Similarly, an interaction between male and female genotype in the success of a male's sperm in sperm competition has been detected in *Drosophila melanogaster* (Clark et al. 1999). A significant interaction between male and female genotype on reproductive success may have implications for the evolution of female remating (polyandry) as a mechanism to ensure mating with a genetically compatible male (Jennions and Petrie 2000; but see Simmons 2001) or have implications on the evolution of reproductive isolation between populations (Brown and Eady 2001; Nilsson et al. 2002). However in this study with *S. limbatu*, fecundity was not affected by an interaction between male and female genotype. Instead, male and female effects appear to be additive, consistent with the prediction that the effect of a male on female fecundity is caused by the volume of ejaculate transferred.

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