# Environmentally Based Maternal Effects on Development Time in the Seed Beetle *Stator pruininus* (Coleoptera: Bruchidae): Consequences of Larval Density

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**ABSTRACT** In response to food limitation, many insects have evolved developmental plasticity in which larvae mature at a smaller body size. Here we investigate the consequences of this smaller body size for development of individuals in the next generation, after resource competition has been relaxed. Families of the seed beetle *Stator pruininus* (Horn) reared at high density (≈20 eggs per seed) matured at a substantially smaller adult body size than families reared at 1 egg per seed. Females emerging from high density seeds also laid smaller eggs than did females reared at low density (7–14% lighter). Progeny developing from these smaller eggs eventually attained the same adult body size as progeny developing from eggs laid by low-density mothers when all progeny were reared at low density (i.e., resource competition was relaxed). They achieved this by developing on average ≈0.5 d longer. Reciprocal crosses between high and low-density lines demonstrated that the differences among lines in development time was maternally inherited; only maternal lineage explained some of the variance in development time. Thus, larvae appear to compensate for the small eggs laid by their mothers by extending development time to mature at the same size as progeny from larger eggs.

**KEY WORDS** *Acacia*, *Stator pruininus*, body size, clutch size, egg size, larval density

MANY INSECTS LAY their eggs on discrete resource patches, such as other insects (parasitoids; Godfray et al. 1991) or seeds (bruchid beetles; Fox et al. 1996). Because larvae of these insects generally cannot move among patches of food, larval competition can be intense when multiple larvae must share a small resource patch. In response to this competition, many insects have evolved developmental plasticity in which they mature at a smaller body size in response to food stress (e.g., Fox et al. 1996, Fox and Savalli 1998).

Phenotypic plasticity in body size can have substantial consequences for the life history of individuals. In general, smaller individuals tend to lay smaller eggs. Progeny hatching from these smaller eggs often have lower survivorship (e.g., Fox and Mousseau 1996) and either mature smaller (Fox and Savalli 1998) or must extend development to compensate for their reduced size at hatching (Fox 1997). For example, in the seed beetle *Stator limbatus* (Horn) (Coleoptera: Bruchidae) females reared at high density mature substantially smaller than females reared at low density. These high density-reared females subsequently lay smaller eggs, but their progeny develop longer than progeny hatching from normal-sized eggs and eventually mature at a normal body size (Fox 1997). These results indicate that *S. limbatus* exhibits developmental plasticity in which they compensate for small egg size by extending development.

Thus, much of the variation in development time within populations of *S. limbatus* has been interpreted as the result of an environmentally based maternal effect—a nongenetic effect of maternal environment (in this case, maternal rearing density) on the phenotype of her progeny. However, this previous work does not demonstrate that variation in development time is solely maternally inherited, rather than also being paternally inherited. In both *S. limbatus* (Fox et al. 1995, Savalli and Fox 1998a) and *S. pruininus* (Horn) (unpublished data), male body size has been demonstrated to have large effects on female egg production. This is apparently mediated through male ejaculate size; large males produce larger ejaculates than small males (Fox et al. 1995; Savalli and Fox 1998a, b) and materials in these ejaculates are used during egg production (e.g., Huignard 1983, Boucher and Huignard 1987, Fox 1993). Thus, because larval density affects male body size, and ejaculate size is positively correlated with male body size, male rearing density may affect egg production in females, and possibly influence the development of progeny.

The objectives of this article are 2-fold. First, we extend our previous work with *S. limbatus* (examining effects of parental rearing density on growth of progeny) to another seed beetle in the same genus, *S. pruininus*. Like *S. limbatus*, individuals of *S. pruininus* that are reared at high density mature at a smaller body size than do individuals reared at lower density

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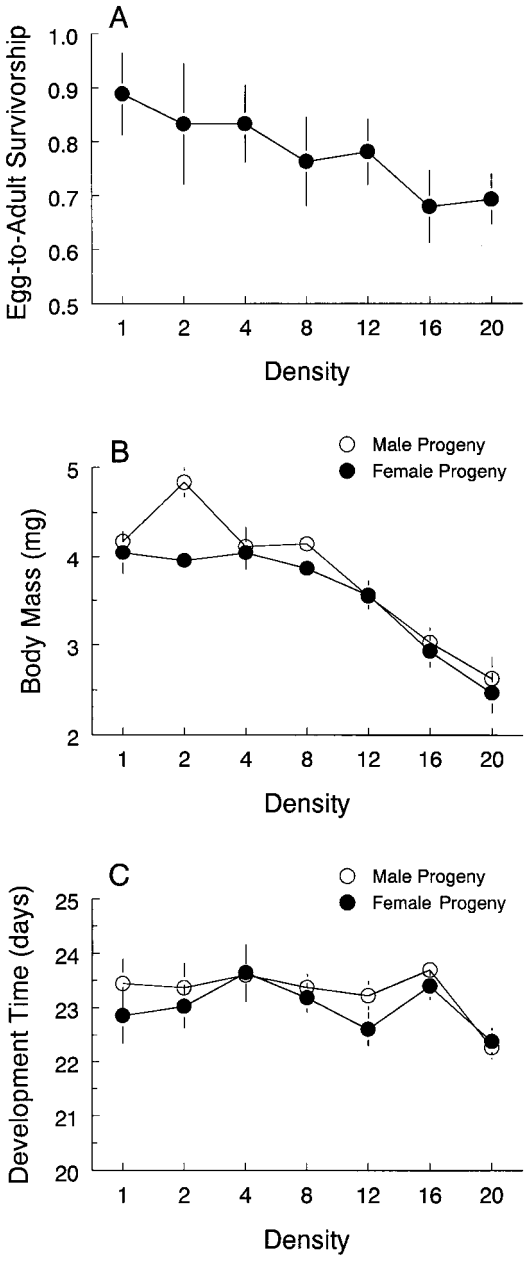


**Fig. 1.** The effect of *S. pruininus* larval rearing-density on (A) egg-to-adult survivorship, (B) egg-to-adult development time, and (C) body mass at adult emergence. All larvae were reared on seeds of *A. greggii* at 30°C and a photoperiod of 15:9 (L:D) h. Each data point represents the mean of family averages ± SEM, $N = 72$ families total.

(Fig. 1), presumably the result of resource competition among siblings within the seed. Here we test whether variation in parental rearing density, and the resulting variation in adult body size, affect progeny development time or body size at maturation. Second, we test whether this variation in development time and adult body size is inherited maternally, paternally, or through both parents. This hypothesis has not been tested previously.

## Materials and Methods

**Natural History and Study Populations.** *S. pruininus* is a generalist seed parasite distributed from northern South America (Venezuela) to the southwestern United States (Johnson and Kingsolver 1976, Johnson et al. 1989, Johnson and Siemens 1995). It has been reared from seeds of ≈55 plant species in ≈13 genera (C. D. Johnson, personal communication). In Arizona, *S. pruininus* is abundant on a few species of *Acacia* and *Mimosa* (Fabaceae: Mimosoideae), although only one or a few hosts may be available in any single locality. The population used in the experiment described below was collected on 7–8 August 1997 from seeds of *Acacia greggii* along Highway 95 between Blythe, CA, and Earp, CA, and along Highway 62 and Old Parker Dam Road near Earp, CA (all sites in San Bernardino County, CA).

Female *S. pruininus* 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 host their mother has chosen for them. In the laboratory, mating and egg laying generally begin <24 h after emergence. These seed beetles require only the resources inside of a single seed to complete development and reproduce; adults are facultatively aphagous. Thus, neither food nor water is necessary for adults in the following laboratory experiments.

**Experimental Design.** To examine how parental rearing density affects progeny of the next generation and to test whether the observed effects are inherited through 1 or both parents, we reared experimental lines at low density (1 egg per seed) or high density (≈20 eggs per seed) for 1 generation (generation 1), producing substantial variation in body size among lines, and then reared lines at a common density (1 egg per seed) for a single generation (generation 2) to examine how maternal and paternal rearing density affected the development time and size at adult emergence of their progeny after competition was relaxed.

To create high and low density lines, virgin males and females were collected from isolated seeds of *A. greggii* within 24 h of adult emergence. Each beetle was weighed and then paired with a virgin beetle of the opposite sex. These beetles will subsequently be referred to as the parental generation. To create lines for generation 1, pairs were confined in a 35-mm petri dish (1 pair per dish) containing either 1 *A. greggii* seed (high density lines) or 15 *A. greggii* seeds (control lines). Females were allowed to lay eggs for 72 h and then removed from their dishes. Eggs in excess of 20 per seed (high density lines, $N = 30$ pairs in each of 2 replicates) or 1 per seed (low density lines, $N = 30$

pairs per replicate) were scraped from each seed. Eggs were allowed to hatch, and beetles in all lines were reared to adult at 30°C and a photoperiod of 15:9 (L:D) h.

All emerging progeny were collected and weighed on an electronic balance within 12 h of their emergence as adults from their rearing seed. To initiate generation 2 of the experiment, 2 female and 2 male emergers were haphazardly selected from each family within each line. From each family, 1 beetle of each sex was paired randomly with a nonsibling from the same line (within replicates only). The other individual of each sex was paired randomly with a beetle of the opposite sex from the other treatment (i.e., 1 female from each high density family was paired with a male from the low density line, and vice versa). This created 4 experimental lines; 1 line (LL) was produced by crossing low density-reared females with low density-reared males, HH was produced by crossing high density-reared females with high density-reared males, LH was low density-reared females crossed with high density-reared males, and HL was high density-reared females crossed to low density-reared males. The subsample of beetles chosen to be parents of generation 2 did not differ in size from the average size of all beetles emerged in their respective lines (*t*-tests comparing family-mean size with size of beetles chosen to be parents of generation 2, $P > 0.05$ for each sex, line and replicate).

Each pair was confined in a 35-mm petri dish with 10 *A. greggii* seeds. Dishes were checked every 12 h for eggs. All seeds bearing eggs were removed and replaced with clean seeds until the female had laid eggs on ≥10 seeds. After laying eggs on ≥10 seeds, females were transferred to a new 35-mm dish containing 20 seeds and allowed to lay eggs until she died. This allowed lifetime fecundity to be estimated. Egg size (egg length and width) was recorded for 2 haphazardly selected eggs laid by each female (chosen from the 1st dish only). Based on egg length and width, egg mass was estimated as egg mass = −0.035 + 0.0086 * egg length + 0.0022 * egg width. This equation was empirically derived (Fox and Mousseau 1996). It is not practical to directly weigh each egg in the experiment because they are glued to seeds and removing them is very time consuming and generally destructive. Larvae were reared to adult at 1 egg per seed (excess eggs were scraped from each seed) at 30°C and a photoperiod of 15:9 (L:D) h. All emerging progeny were collected and weighed within 12 h of their emergence as adults from the seed. Development time was recorded for all individuals as the time between when an egg was laid and when the individual emerged from the seed as an adult. Development time thus included embryonic, larval, and pupal development.

This experiment was replicated twice. The 2 replicates were executed sequentially (initiated ≈10 d apart) and intermixed within the same laboratory growth chamber. All lines were dispersed randomly on the same shelves of a single growth chamber.

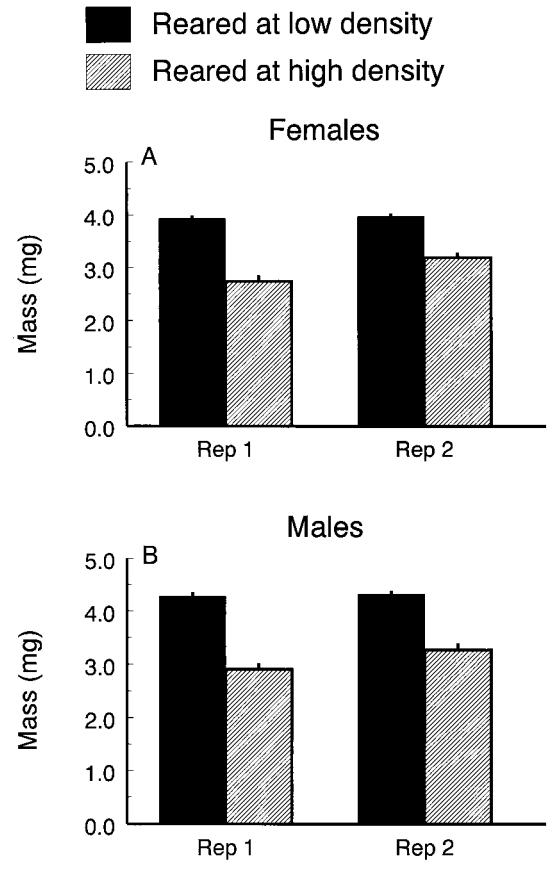


**Fig. 2.** Effect of larval density on the body size of *S. limbatus* at adult emergence. Each point represents the mean of family averages + SEM.

## Results

In both replicates, *S. pruininus* families reared at high density (≈20 eggs per seed) emerged at substantially smaller adult body sizes than families reared at 1 egg per seed (generation 1; Fig. 2; analysis of variance [ANOVA] including replicate, family [nested within replicate], and treatment; treatment $F = 132.1$, $P < 0.001$ for female progeny; $F = 175.9$, $P < 0.001$ for male progeny; replicate and family effects were also significant for both sexes at $P < 0.001$). These females emerging from high density seeds laid smaller eggs than females reared at low density (Fig. 3 A and C; Table 1). However, this decrease in egg size was small, high density-reared females laid eggs that were on average 14 and 7% lighter (replicates 1 and 2, respectively) than low density females (Fig. 3 A and C). Females emerging from high density seeds had lower fecundity than females reared at low density (Fig. 3 B and D; Table 1). Female fecundity was also affected by the line of her mate; females mated to low density males laid more eggs than females mated to high density males (Table 1). Egg size was not affected by male line (Table 1).

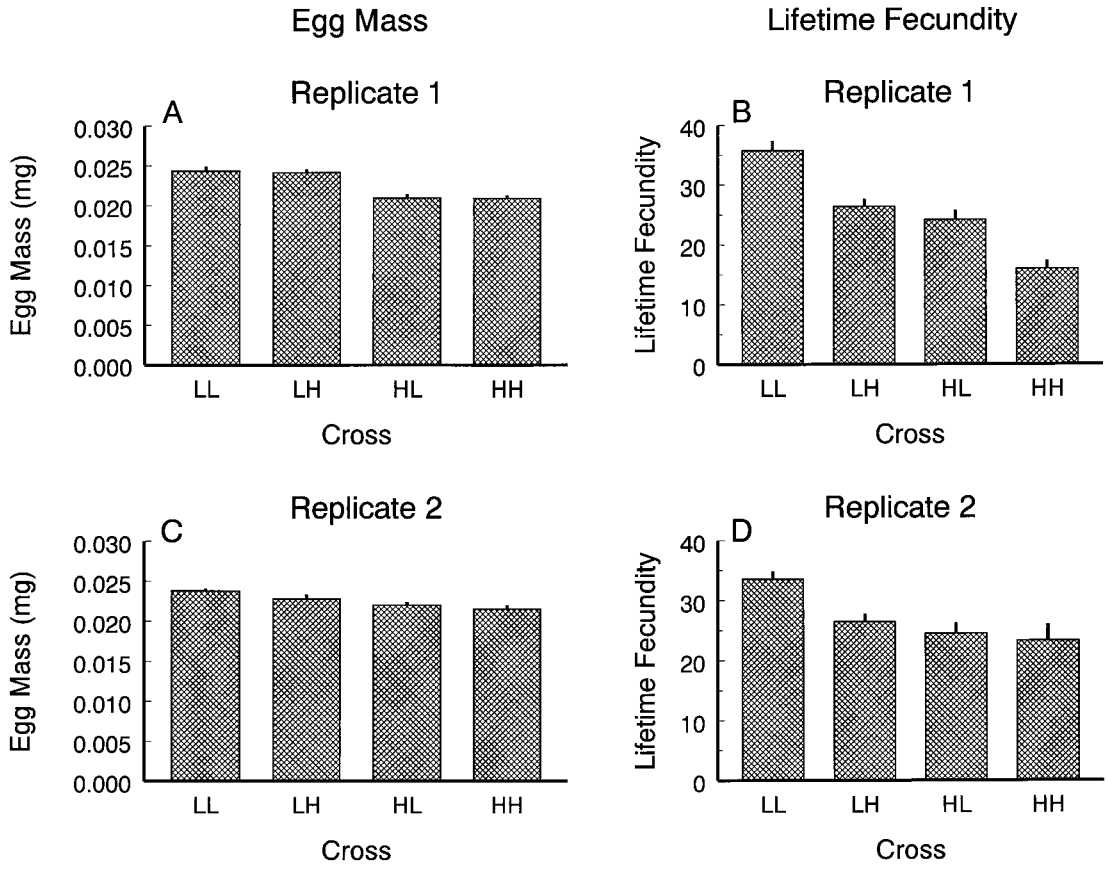


**Fig. 3.** The (A, C) size of eggs laid by, and (B, D) lifetime fecundity of, females reared at either high (≈20 eggs per seed) or low (1 egg per seed) density (+SEM). LL = low density-reared females each mated to a low density-reared male; LH = low density female × high density male; HL = high density female × low density male; HH = high density female × high density male. Note that female density affects the size of her eggs, but the density that her mate was reared at does not, whereas both female and male density affect a female's lifetime fecundity (see also Table 1).

When all larvae were reared at low density (generation 2) those progeny developing from the smaller eggs laid by high density-reared mothers (Fig. 3) eventually attained the same adult body size as progeny developing from eggs laid by low density-reared mothers (Fig. 4) in each line of both replicates (Table 2). They achieved this by developing on average ≈0.5 d longer (Fig. 5). The experimental crosses demonstrated that the differences among lines in development time was maternally inherited; in an ANOVA, only maternal lineage explained some of the variance in development time (Table 2; LL progeny resembled LH progeny, and HH progeny resembled HL progeny; note that, in addition to being nonsignificant, the $F$ ratio for the paternal density effect was small relative to the maternal density effect). Thus, larvae appear to have exhibited developmental plasticity in which they compensated for the small eggs laid by their mothers by extending development time to eventually mature at the same size as progeny from the larger eggs laid in other lines.

**Table 1. ANOVA demonstrating that females reared at high density (≈20 eggs per seed) produce smaller eggs and have lower fecundity than females reared at low density (1 egg per seed)**

| | df | $F$ ratio |
|---|---|---|
| Egg size | | |
| Replicate | 1 | 0.20 NS |
| Female density | 1 | 56.26*** |
| Male density | 1 | 2.02 NS |
| Female*Male density | 1 | 0.10 NS |
| $R^2 = 0.22$ | | |
| Lifetime fecundity | | |
| Replicate | 1 | 0.61 NS |
| Female density | 1 | 65.93*** |
| Male density | 1 | 39.76*** |
| Female*Male density | 1 | 0.80 NS |
| $R^2 = 0.38$ | | |

NS, not significant ($P > 0.05$); ***, $P < 0.001$. The analysis is on the beetles chosen to be parents of generation 2 (which includes both intra- and interdensity crosses) of experiment 2. See Figs. 3 and 4.

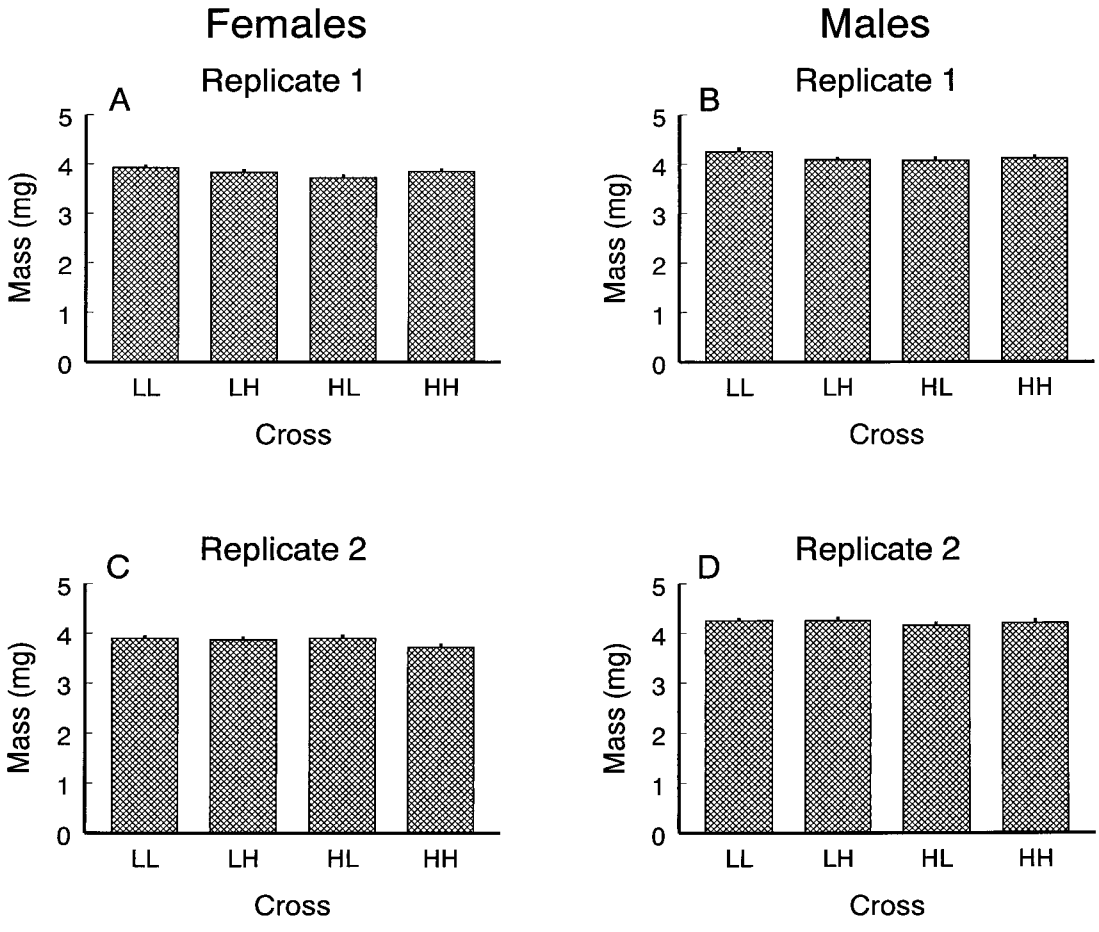


**Fig. 4.** The affect of maternal and paternal rearing-density on the body size of their progeny at adult emergence (+SEM). Crosses are as in Fig. 3 (these are beetles reared from the eggs described in Fig. 3). Note that there is no statistically significant effect of either maternal or paternal rearing-density on the body size of their progeny (see Table 2).

## Discussion

When *S. pruininus* larvae are reared at high larval density they exhibit developmental plasticity in which they mature at a smaller body size. Females of these smaller beetles subsequently lay smaller eggs than do females that had been reared at low density. However, larvae hatching from these smaller eggs apparently compensate for their small size at hatching by extending larval development to mature at an adult body size similar to that of adults reared at 1 egg per seed. These results are consistent with those previously obtained for *S. limbatus* (Fox et al. 1996, Fox 1997). However, in this previous experiment with *S. limbatus* we did not test whether the observed difference in development time between high and low density treatments was maternally or paternally inherited. Here, we have extended this previous study to demonstrate that variation in development time among treatments is inherited via an environmentally based maternal effect (i.e., inherited nongenetically through the female parent).

**Table 2. ANOVA (SAS type III sums of squares; SAS Institute 1985) examining the effects of maternal and paternal density on (A) body size of their adult progeny, and (B) egg-to-adult development time of these progeny**

| | df | *F* |
|---|---|---|
| Mass | | |
| Sex | 1 | 78.71*** |
| Replicate | 1 | 1.94 NS |
| Maternal treatment | 1 | 3.49* |
| Paternal treatment | 1 | 0.25 NS |
| Maternal*Paternal treatment | 1 | 1.34 NS |
| Development time | | |
| Sex | 1 | 17.07*** |
| Replicate | 1 | 0.06 NS |
| Maternal treatment | 1 | 17.37*** |
| Paternal treatment | 1 | 0.01 NS |
| Maternal*Paternal treatment | 1 | 1.29 NS |

NS, $P > 0.05$ (nonsignificant); *, $P < 0.10$; ***, $P < 0.001$

The analysis is on the family means (average of all progeny of each sex within each family were treated as one data point) for generation 2.

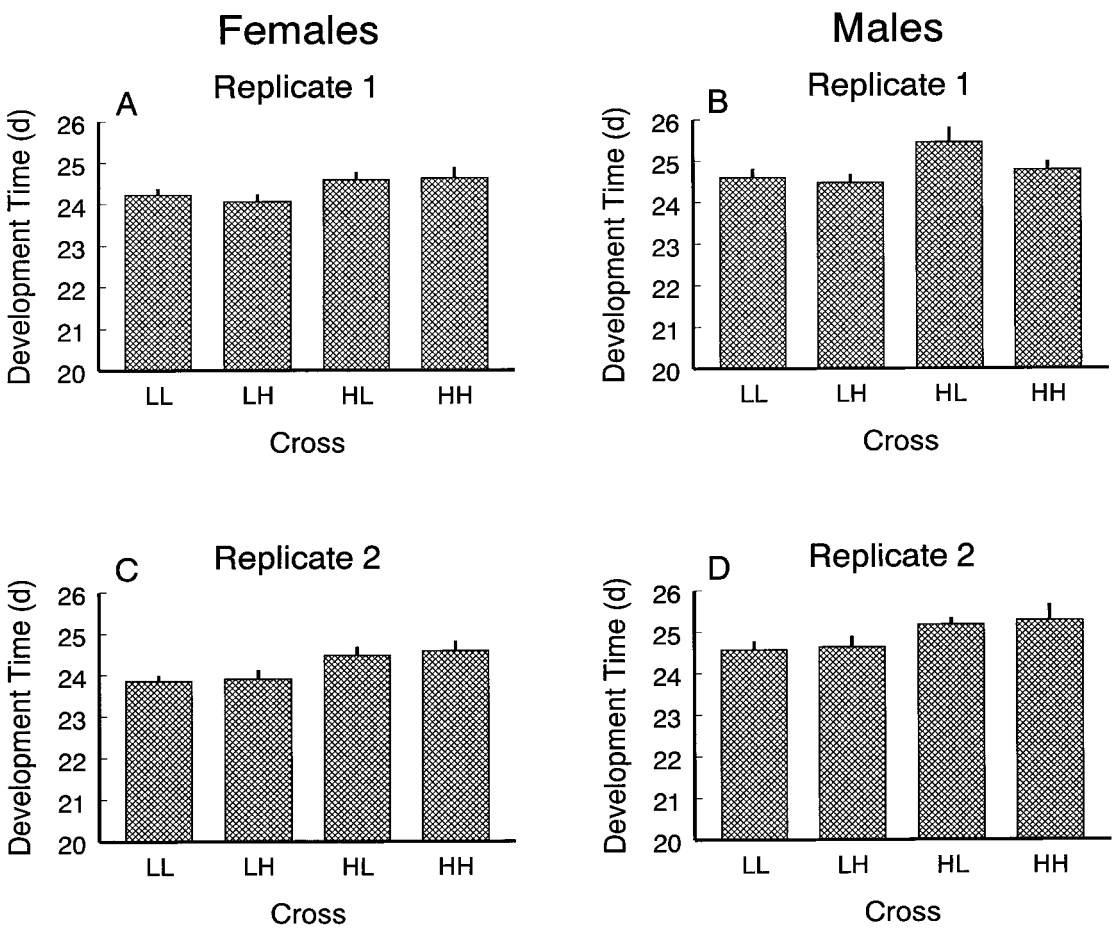


**Fig. 5.** The affect of maternal and paternal rearing-density on the development time of their progeny (egg-to-adult development time, including embryonic, larval, and pupal development; +SEM). Crosses are as in Fig. 3 (these are beetles reared from the eggs described in Fig. 3). Note that maternal rearing-density affects the development time of her kids, but paternal rearing density does not (see Table 2).

We expected maternal inheritance of development time because females produce eggs and egg size is dependent on a female's body size and physiological state. In *Stator*, females reared at high density lay smaller than normal eggs (Fig. 3; Fox 1997), and thus hatchlings from these eggs are necessarily smaller than normal. These hatchlings must then either extend development or mature at a smaller than normal size (assuming they are developing at the maximum possible rate). Thus, we expect female rearing-density to affect either progeny development time or size at maturation. However, paternal effects on development time were also plausible in *Stator* because male seed beetles contribute large ejaculates to females during mating (Fox 1993; Savalli and Fox 1998a, b), materials in these ejaculates are incorporated into eggs (Huignard 1983, Boucher and Huignard 1987). However, we found no evidence that male rearing-density affects female egg size or the development of his progeny—variation in development time among treatments was maternally, and not paternally, inherited. Instead, females mating to high density males laid fewer eggs, consistent with previous observations that females mating with small males have lower lifetime fecundity (M.E.C. and C.W.F., unpublished data for *S. pruininus*; Fox et al. 1995, Savalli and Fox 1998a for *S. limbatus*).

The results of this experiment are consistent with results for most other animals that complete development external of their parents and without parental care. With the notable exceptions of maternal control of offspring diapause, flight polymorphisms, and sexual versus asexual reproduction (review in Fox and Mousseau 1998), we generally find that maternal environmental stresses have a large influence on progeny body size and growth immediately after hatching or birth, but that the magnitude of this maternal effect gradually decreases throughout development and often becomes undetectable by the time progeny mature (Mousseau and Dingle 1991, Fox 1994, Bernardo 1996a). However, there are interesting exceptions. For example, in another seed beetle, *Callosobruchus*

*maculatus* (F.), females reared at high density lay smaller eggs than females reared at low density, but, unlike *S. pruininus*, larvae hatching from these smaller eggs do not extend development time to eventually mature at normal body size (Fox and Savalli 1998). Instead, they mature smaller than normal such that variation in body size is maternally inherited via an environmentally based maternal effect.

Thus far we have assumed that the variation in development time among crosses is the result of differences in egg size between high and low density-reared females. However, this is not necessarily the case. Maternal environmental effects on egg composition have been demonstrated in other animals, including insects, and may provide an explanation for our observations. For example, in the gypsy moth, *Lymantria dispar* (L.), maternal diet affects progeny growth rate even after accounting for the effects of egg size, indicating that egg size is not necessarily an adequate measure of parental investment or egg quality (Rossiter 1991). Instead, female diet affects vitellogen (their primary yolk protein) concentrations in eggs, possibly explaining observed effects of maternal diet on progeny growth (Rossiter et al. 1993). Maternal nutritional status, which likely varies with larval density, may influence the concentration of proteins, lipids, or water within eggs, which may in turn affect progeny growth and development. However, much more research is needed in this area before broad generalizations can be made concerning the relative consequences of egg size versus egg content for progeny growth (Bernardo 1996b).

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