

INHERITANCE OF ENVIRONMENTAL VARIATION IN BODY SIZE: SUPERPARASITISM OF SEEDS AFFECTS PROGENY AND GRANDPROGENY BODY SIZE VIA A NONGENETIC MATERNAL EFFECT

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Abstract.—Maternal effects provide the most common mechanism by which environmental variation in one generation affects the phenotype of individuals in subsequent generations. In egg-laying animals, however, we typically observe that maternal effects can have large influences on early growth (egg size and early development), but these effects gradually disappear and become undetectable by the time progeny mature due to developmental plasticity in progeny. We describe a system in which an environmentally induced reduction in body size is inherited by progeny via a nongenetic maternal effect. The seed beetle, *Callosobruchus maculatus*, completes development inside a discrete resource package (a seed) selected by its mother. Due to superparasitism in response to low host availability, progeny frequently develop at high densities, resulting in intense larval competition and pupation at a smaller body size. Females reared at higher density (and thus emergently smaller) lay smaller eggs than females reared at lower density. Progeny from these smaller eggs mature at a smaller size than progeny reared from the larger eggs laid by females reared at lower density. Crosses between high and low density lines demonstrated that treatment differences in body size are maternally inherited, confirming that the inheritance of body size variation in part involves an environmentally based maternal effect.

Key words.—Bruchidae, *Callosobruchus maculatus*, egg size, maternal effect, phenotypic plasticity.

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Evolutionary biologists have built a theoretical framework within which to think about phenotypic variation and natural selection—we partition phenotypic variation into genetic and environmental components (and their interactions), with evolutionary responses to selection dependent only on the magnitude of selection and the amount of genetic variation present within populations. We rarely consider the inheritance of environmental variation and its influence on evolutionary responses to selection (Mousseau and Fox, in press). Most often, the inheritance of environmental variation is mediated by maternal effects (Falconer 1965; Kirkpatrick and Lande 1989)—nongenetic effects of maternal phenotype or environment on progeny phenotype, independent of progeny genotype (Mousseau and Dingle 1991; Riska 1991; Mousseau and Fox, in press). Maternal effects are a fundamental consequence of anisogamy; immediately following fertilization, an individual's phenotype reflects the size and composition of the egg or seed it developed from, and thus its mother's physiological state (mediated by her genotype and environment), rather than its own (or its father's) genotype (Wade, in press).

Environmentally based maternal effects can be both ecologically and evolutionarily important (Mousseau and Dingle 1991; Rossiter 1996). Ecologically, they have been hypothesized to be a source of time-lagged effects on population dynamics, in which the per capita rate of increase of a population is influenced by the environment experienced by a previous generation (e.g., Ginzburg and Taneyhill 1994; review in Rossiter 1996; Ginzburg, in press). These time lags may result in population cycles (including outbreaks) and population destabilization (and possibly extinction). Evolutionarily, maternal effects are generally considered in experiments only to avoid overestimating direct additive ge-

netic variances and avoid confusing environmentally based maternal effects with genetic variation (Shaw and Byers, in press). However, they may also accelerate or impede responses to selection, and early theoretical analyses have indicated that the evolutionary dynamics of characters influenced by maternal effects are unusual relative to standard theory; maternal effects can result in large time-lags in evolutionary responses to selection (delay of one or more generations), and populations may even continue to evolve after selection has been relaxed (Kirkpatrick and Lande 1989). Characters subject to maternal effects may even respond to selection in a maladaptive direction, away from fitness optima (Kirkpatrick and Lande 1989). Experimental studies support some of these theoretical predictions (e.g., Galloway 1995). Furthermore, maternal effects may themselves be genetically variable, and thus capable of responding to selection (Wade, in press), and in some cases provide mechanisms for adaptive transgenerational phenotypic plasticity in response to predictable environmental variation (Mousseau and Fox, in press).

Transgenerational effects of environmental variation on adult phenotypes, mediated by maternal effects, have been observed in some plants (Miao et al. 1991; Case et al. 1996; reviews in Roach and Wulff 1987; Bernardo 1996a) but are particularly well documented in animals in which progeny spend a substantial amount of time during development inside of their mother (e.g., mammals, many aphids) or in which parental care is provided after progeny are born or hatched (Riska et al. 1985; Cheverud and Moore 1994). However, the persistence of environmental variation across generations appears to be uncommon in animals that lay eggs immediately after fertilization, in which progeny complete development entirely outside of their mother, without parental care (i.e.,

most animals). With the notable exceptions of maternal control of major development switches that are initiated very early in development, such as progeny diapause, flight polymorphisms, and sexual versus asexual reproduction (review in Fox and Mousseau, in press), we typically find that maternal effects can have large influences on early growth of non-live bearing animals (egg size and early development), but that these effects gradually disappear and become undetectable by the time progeny mature (Mousseau and Dingle 1991; Fox 1994a; Bernardo 1996a) due to developmental plasticity in progeny. For example, as females of the seed beetle *Callosobruchus maculatus* age, they begin laying smaller eggs. However, progeny from these smaller eggs compensate for their small initial size by extending development and maturing at a normal adult size (Fox 1993; Fox and Dingle 1994).

Body size is an ecologically important phenotypic trait that may, in some organisms, reflect the environmental conditions experienced by mothers. Because females produce eggs, egg size and composition are dependent on a female's physiological state and size, which are affected by the environmental conditions that she experiences. Egg size and composition can in turn affect progeny growth (reviews in Fleming and Gross 1990; Kaplan 1991; Reznick 1991; Fox 1994b; Bernardo 1996b; Fox and Mousseau 1996; Fox 1997a) and in some cases may affect progeny body size at maturation (but see Fox 1994a, in press). Thus, environmental conditions that affect a female's size or physiological state—and therefore, indirectly, the size or composition of her eggs—could also affect the size of her progeny.

Like many parasitic insects, larvae of the seed beetle *C. maculatus* (Coleoptera: Bruchidae) develop on discrete resource patches and are incapable of moving between patches; females lay their eggs on seeds of their host plants, and larvae subsequently complete larval development inside the seed selected by their mothers, emerging only after pupation (Mitchell 1975). When seeds are limiting, female *C. maculatus* superparasitize seeds such that individual seeds frequently must support multiple larvae (Smith and Lessels 1985; Wilson 1988; Møller et al. 1989; Messina, in press). Thus, larval competition is a frequent and important component of this insect's life history (Messina 1990, 1991, 1997). Larvae of some populations exhibit developmental plasticity in which they can mature at a smaller than normal size in response to larval competition (Wilson 1994). These smaller females subsequently lay smaller eggs than normal-sized females (see below). However, the consequences of larval competition-induced body size plasticity—and the resulting decrease in egg size—for growth of individuals in subsequent generations has never been explored. Here, we (1) quantify the consequences of larval density for the survivorship and growth of *C. maculatus* larvae, confirming the results of previous research that *C. maculatus* exhibits phenotypic plasticity in body size in response to larval density; (2) demonstrate that larval density has consequences not only for the growth of the stressed individuals, but also for the growth of individuals in subsequent generations (i.e., environmental variation in body size persists across generations); and (3) using crosses between high and low density lines, demonstrate that environmental variation in body size is ma-

ternally inherited, and thus represents an environmentally based maternal effect, likely mediated by egg size.

Natural History and Study Populations

Callosobruchus maculatus is a cosmopolitan pest of stored legumes (Fabaceae), particularly beans of the genus *Vigna*. Females cement their eggs to the surface of host seeds (Messina 1991). Approximately 4–5 days later (at 26–28°C), the eggs hatch and the first instar larvae burrow through the seed coat and into the seed. Larval development and pupation are completed entirely within a single seed. This beetle's short generation time and ease of laboratory rearing in a seminatural storage environment make it an excellent animal for life-history studies. All beetles used in these experiments were collected from infested pods of cowpea (*V. unguiculata*) in Niamey, Niger, at the University of Niamey Experiment Station (Messina 1993), and maintained in laboratory growth chambers since November of 1989, at > 1000 adults per generation, prior to this experiment. All experiments in this manuscript were executed between July 1996 and March 1997.

EXPERIMENT 1: LARVAL DENSITY AFFECTS BODY SIZE AT ADULT EMERGENCE

In this first experiment, we artificially manipulated larval density within seeds to confirm the results of previous researchers that *C. maculatus* is developmentally plastic in response to variation in density such that body size at adult emergence decreases with increasing larval density.

Methods

To manipulate larval density, virgin females were collected within 24 h of adult emergence and confined with a virgin male (also collected within 24 h of adult emergence) and a single seed of *V. unguiculata* in a 35-mm petri dish. All seeds were weighed prior to egg laying so that seed size could be used as a covariate in the final statistical analyses.

Females were allowed to lay eggs for 48 h. Each family (all eggs laid on a single seed are full-sibs) was then randomly assigned to a larval density of between one and the number of eggs laid on the seed. Excess eggs were scraped from the seed. Only eggs laid during this 48-h period were used in this experiment to control for maternal age effects on larval survivorship and development, in which later-laid offspring develop from smaller eggs, have lower survivorship, and take longer to develop (e.g., Wasserman and Asami 1985; Fox 1993; Fox and Dingle 1994). This method created 103 families varying in larval density from 1 to 21 eggs.

All larvae were reared to adult at 26°C, constant light. Egg-to-adult development time and adult body size (mass) at emergence were recorded for all surviving progeny. Because we were interested in the effects of larval competition, the number of eggs that hatched, rather than the number of eggs laid on a seed, was used as our estimate of larval density for all statistical analyses (hatched eggs are easily identified because larvae are visible under a dissecting scope and can be observed entering the seed). For most females, all eggs hatched. However, some females laid one or more unfertilized

TABLE 1. The relationship between larval density of *Callosobruchus maculatus* and larval egg-to-adult survivorship, development time, and body weight at adult emergence (from experiment 1). For this analysis, each family mean was treated as a single data point. The sign on the partial correlation reflects the sign on the regression coefficient; n = the number of families.

	Partial r^2	t	n
Egg-to-adult survivorship			
Seed size	0.00	0.62 ns	99
Larval density	-0.61	-12.36***	99
Adult weight: female progeny			
Seed size	0.01	0.86 ns	87
Larval density	-0.26	-5.42***	87
Adult weight: male progeny			
Seed size	0.00	-0.25 ns	94
Larval density	-0.34	-6.84***	94
Development time: female progeny			
Seed size	0.01	1.12 ns	87
Larval density	0.01	1.03 ns	87
Development time: male progeny			
Seed size	0.00	0.65 ns	94
Larval density	0.00	0.17 ns	94

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

or nondeveloping eggs. Also, seed mass was included in all regression analyses to control for effects of variable seed size on progeny growth.

Results

Larval density had a large effect on egg-to-adult survivorship of *C. maculatus* larvae; high density seeds produced far fewer emerging adult progeny than lower density seeds (Table 1; Fig. 1). Adult body weight at emergence also decreased substantially with increasing larval density (Fig. 2a,b; Table 1), although development time was unaffected by increasing larval density (Fig. 2c,d; Table 1). Thus, larvae developing in high density seeds emerged at the same time as conspecific larvae reared at lower densities, but at a substantially smaller body size. The sex ratio of emerging adults (49% female) did not differ significantly from 1:1 (sign test, $P = 0.26$) and did not correlate with larval rearing density (Spearman rank correlation, $P = 0.33$).

Because eggs could not be moved among seeds without killing them (eggs are glued to seeds and are difficult to remove without damaging the embryo), families had to be assigned to larval densities of less than or equal to the number of eggs laid on the seed. Thus, larval densities are only artificially reduced (by scraping eggs) and not also artificially augmented (eggs added), potentially confounding our interpretation of the observed density effects. If some component of female oviposition behavior and/or female fecundity is correlated with progeny life history, then we may observe a correlation between larval density and progeny body size mediated by female behavior rather than competition among progeny. However, that this is not the case is indicated by an analysis in which progeny survivorship, body size, and development time are first regressed on the number of eggs laid on a seed (prior to the manipulation of larval density), and then the effect of larval density on the residuals is ex-

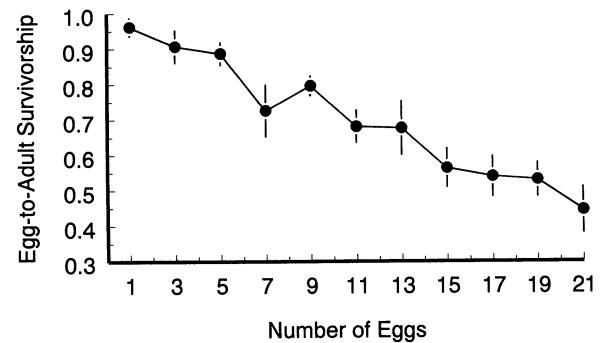


FIG. 1. The influence of larval density on egg-to-adult survivorship of *Callosobruchus maculatus* reared on *Vigna unguiculata* (from experiment 1).

amined. In these analyses, the effect of larval density was still highly significant for egg-to-adult survivorship ($P < 0.001$) and body size ($P = 0.008$ and $P < 0.001$ for females and males, respectively), and still nonsignificant for development time ($P = 0.90$ and $P = 0.16$ for females and males, respectively).

EXPERIMENT 2: ENVIRONMENTALLY BASED VARIATION IN BODY SIZE PERSISTS ACROSS GENERATIONS

Experiment 1 demonstrated that as larval density increases within a seed, the body size of emerging *C. maculatus* decreases, presumably as a result of resource competition among siblings within the seed. To test the hypothesis that this variation in body size generated by resource competition (caused by maternal superparasitism) persists into the next generation, after resource competition has been relaxed, we reared experimental lines at low (one egg per seed) or high density (≈ 20 – 25 eggs per seed) for one generation, producing substantial variation in body size among lines. All lines were then reared at a common density until they converged on a common average body size.

Methods

To create high and low density lines, virgin males and females were collected from isolated seeds of *V. unguiculata* 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 (one pair per dish) containing either one *V. unguiculata* seed (high density lines) or 15 *V. unguiculata* seeds (control lines). Females were allowed to lay eggs for 48 h and then were removed from their dishes. Eggs in excess of 20–25 per seed (high-density lines, $n = 30$ pairs in each of two replicates) or one per seed (low-density lines, $n = 30$ pairs per replicate) were scraped from each seed. Larvae in all lines were reared to adult at 26°C, constant light.

All emerging progeny were collected and weighed on an electronic balance within 24 h of their emergence as adults from their rearing seed. To initiate generation 2 of the experiment, two female and two male emergers were haphazardly selected from each family within each line, and ran-

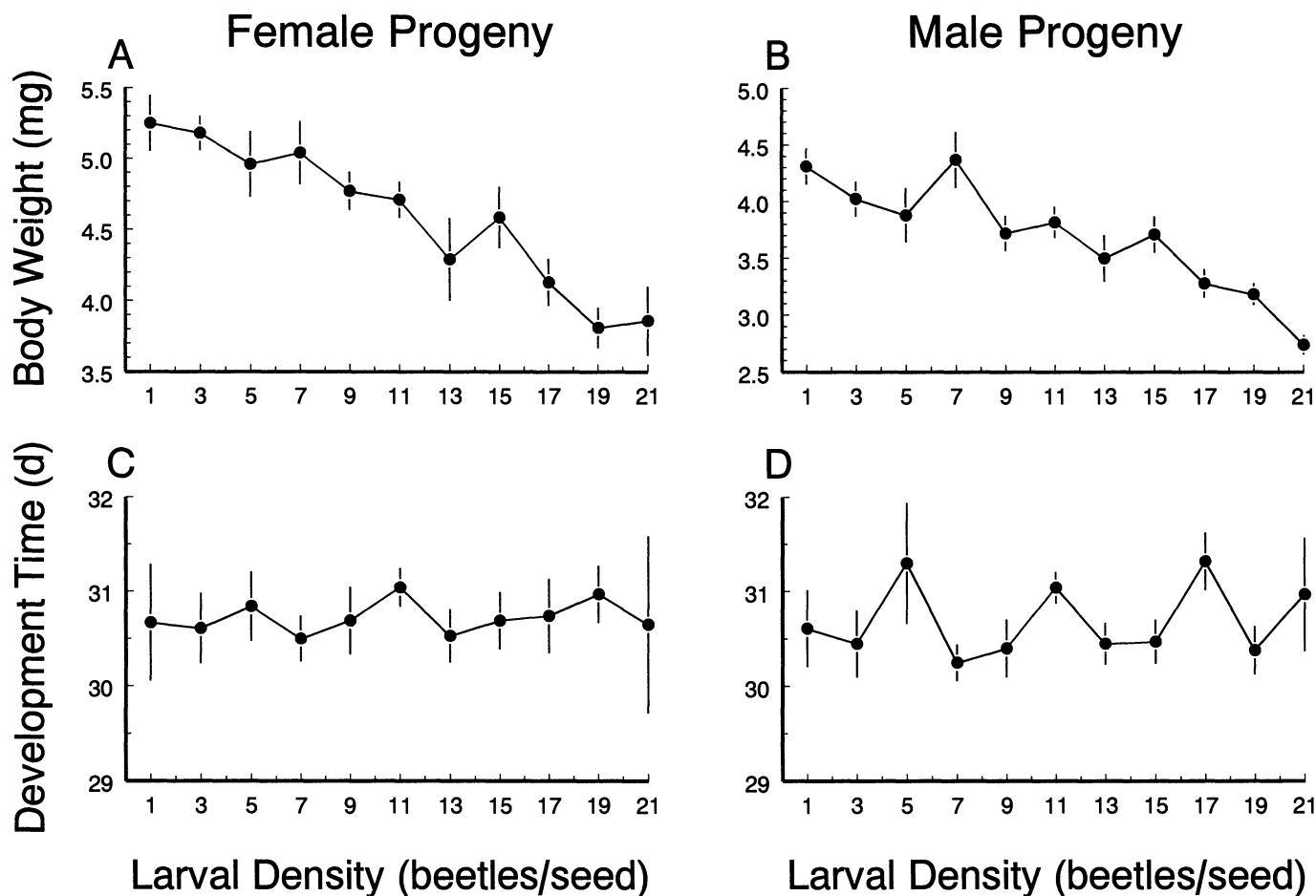


FIG. 2. The relationship between larval density (within seeds) and (a,b) body weight at adult emergence or (c,d) egg-to-adult development time in the seed beetle, *Callosobruchus maculatus* reared on *Vigna unguiculata* (from experiment 1). For clarity of presentation, each point is the mean (\pm SEM) across all families reared at each larval density, where larval density 1 = one or two larvae/seed, larval density 3 = three or four larvae/seed, and so on. Statistical analyses on unpooled family means are presented in Table 1.

domly paired with a nonsibling from the same line (within replicates only). This 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 eight *V. unguiculata* seeds. Females were allowed to lay eggs for ≈ 12 h. Females that laid less than eight eggs in their first 12 h period were transferred to a new dish containing 8 *V. unguiculata* seeds and allowed to lay for another 12 h. To control for maternal age effects, in which egg size decreases with increasing maternal age, females were not allowed to lay eggs after the first 24 h. Egg size (egg length and width) was recorded for two haphazardly selected eggs laid by each female. Larvae were reared to adult at one egg/seed (excess eggs were scraped from each seed), 26°C, constant light. As above, all emerging progeny were collected and weighed within 24 h of their emergence as adults from the seed.

Generation 3 was initiated identically to generation 2, except that only one male and one female were randomly selected from each family to be parents of generation 3. Again,

egg size (length and width) was recorded for two haphazardly selected eggs laid by each female and larvae were reared to adult at one egg/seed, 26°C, constant light. Generation 4 was initiated in the same manner as generation 2 and 3, but, as will be noted below, it was not necessary to rear larvae to the adult stage.

The two replicates of this experiment were executed sequentially (initiated approximately one month apart) in the same laboratory growth chamber.

Results

In both replicates, *C. maculatus* families reared at high density (≈ 20 eggs/seed) emerged at substantially smaller adult body sizes than families reared at one egg/seed (generation 1 in Fig. 3 and Table 2). These females emerging from high density seeds also laid smaller eggs than females reared at low density (generation 1 emergers in Table 3), as expected due to a positive relationship between maternal body size and egg size (Fox 1993). However, this decrease in egg size was very small—high-density-reared females laid eggs that were only 3.2% and 3.3% shorter (egg length in

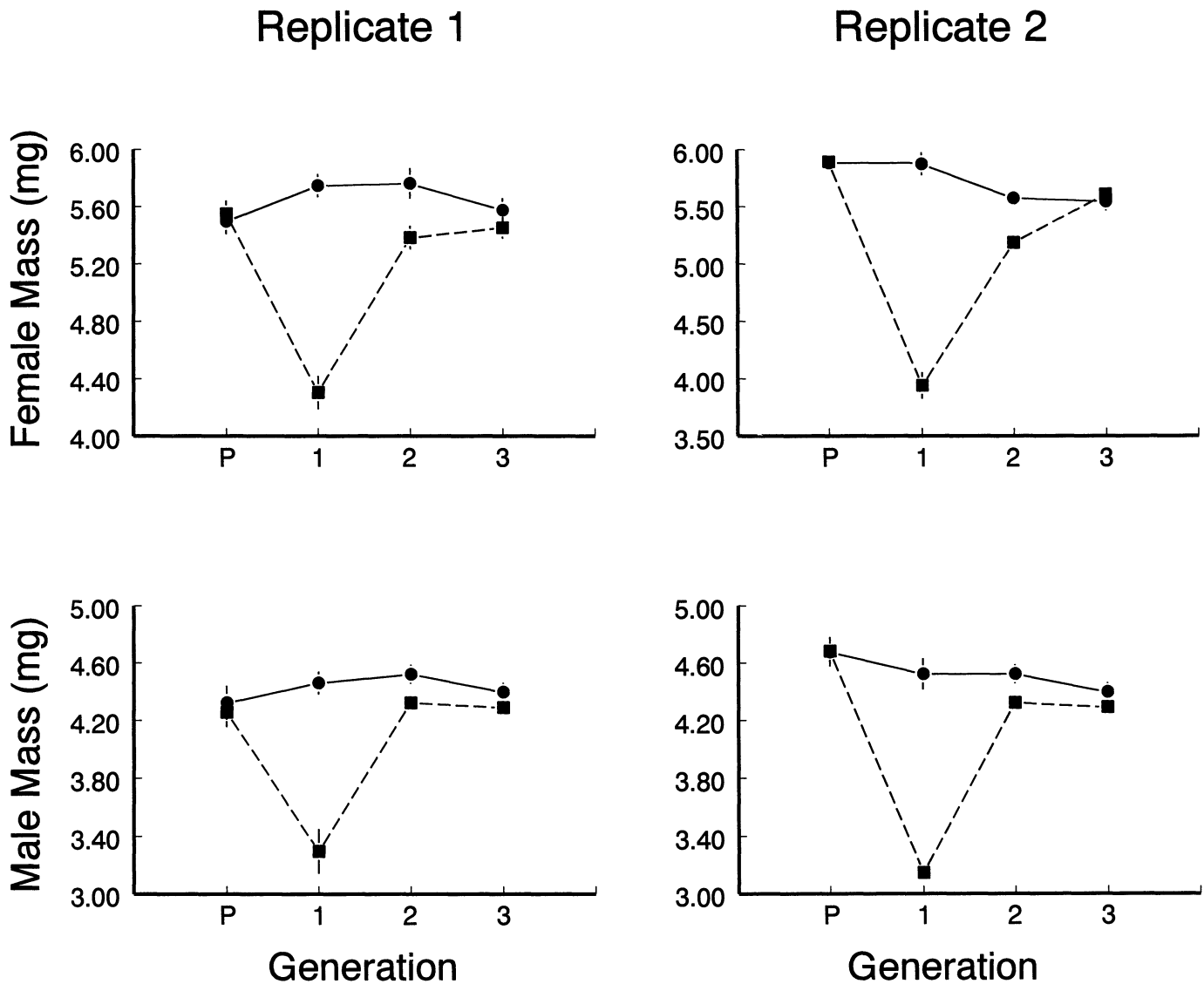


FIG. 3. Persistence of environmental variation in adult body size across generations in the seed beetle, *Callosobruchus maculatus* reared on *Vigna unguiculata* (from experiment 2). The dashed lines are beetles reared at high density during generation 1, and then low density for generations 2 and 3. The solid line are beetles reared at low density for all three generations (control line). Statistics are in Table 4. Generation P is the parents of generation 1.

replicates 1 and 2, respectively) than low density females (Table 3; a 3% difference in egg length represents an \approx 8–10 % difference in egg mass when mass is estimated from dimensions using the models in Fox 1997a).

Progeny developing from these smaller eggs laid by high-density-reared mothers developed for approximately the same length of time (generation 2 in Table 4) as progeny developing from the larger eggs laid by low-density-reared mothers. These generation 2 progeny of high-density mothers started smaller (developed from smaller eggs), but did not take longer to develop to adult. Thus, assuming that they cannot change their growth rate, we expect them to emerge at a smaller body size than progeny developing from eggs laid by low-density mothers, even though they were all reared at low density. This is in fact what we observed—beetles in the high-density lines emerged as smaller adults in generation

2 than beetles in the low-density lines (generation 2 in Fig. 3 and Table 2). In other words, the environmentally based reduction in body size generated by intense larval competition persisted in the population for an additional generation after larval competition was relaxed.

Although the larval density-induced reduction in body size persisted into generation 2, it did not persist into generation 3 (Fig. 3, Table 2). Instead, daughters of high-density-reared mothers did not lay eggs that were detectably smaller than those laid by daughters of low-density-reared mothers (generation 2 emergers in Table 4), and their progeny (generation 3) emerged at approximately the same size as those in the low density lines (generation 3 in Fig. 3 and Table 2). This convergence among lines suggests that the treatment differences in body size observed in generation 2 represents environmental variation persisting across generations rather

TABLE 2. Analysis of variance for the effects of larval density on *Callosobruchus maculatus* body size (SAS Proc GLM, Type III sums-of-squares). Generation 1 beetles were reared at either high (20 eggs/seed) or low (one egg/seed) density. Generation 2 and 3 beetles were all reared at densities of one egg/seed. Family, nested within the replicate*treatment interaction, was included in each analysis because siblings are not independent of each other. *F*-ratios were calculated with the MS[Family(Rep*Treat)] in the denominator. See Figure 3 for treatment means.

	Generation 1		Generation 2		Generation 3	
	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
Female progeny						
Replicate	1	1.63 ns	1	4.58*	1	1.23 ns
Treatment	1	169.05***	1	18.59***	1	0.48 ns
Replicate*treatment	1	4.13*	1	0.00 ns	1	2.16 ns
Family (rep*treat)	108	1.75***	212	2.76***	212	2.73***
<i>R</i> ² =		0.63		0.56		0.52
Male progeny						
Replicate	1	0.15 ns	1	5.33*	1	0.09 ns
Treatment	1	125.09***	1	6.72*	1	0.48 ns
Replicate*treatment	1	0.87 ns	1	0.12 ns	1	1.07 ns
Family (rep*treat)	109	2.88***	212	2.35***	212	2.95***
<i>R</i> ² =		0.69		0.50		0.52

* *P* < 0.05; *** *P* < 0.001; ns = not significant.

than a response to selection—genetic differences between lines should persist indefinitely in the population (barring both other sources of selection and genetic drift), whereas environmental effects should decrease in magnitude each generation, unless the population is again perturbed.

EXPERIMENT 3: MATERNAL EFFECTS AND THE INHERITANCE OF ENVIRONMENTAL VARIATION IN BODY SIZE

Experiment 2 demonstrated that the variation in body size generated by larval resource competition persists in a population for a generation after resource competition among larvae has been relaxed. To test the hypothesis that variation in body size generated by larval resource competition is maternally inherited, and thus persists into the next generation due to an environmentally based maternal effect, we performed crosses among high-density and low-density lines (with high-density females mated to low-density males, and vice versa) and examined the body size of the progeny developing from these crosses. The logic of this design is that, if the observed body size differences between lines is maternally inherited, then hybrid lines should resemble the control line from which their mothers were derived, rather than the control line from which their fathers were derived.

Methods

This last experiment was nearly identical in design to experiment 2, except that (1) 60 pairs each were used to initiate generation 1 of the high- and low-density lines; (2) one male and one female from each family were randomly paired with a nonsibling from the same line to initiate generation 2; and (3) one male and one female from each family were randomly paired with a nonsibling beetle of the opposite sex from the alternate line (i.e., one female from each high density family was paired with a male from the low density line, and vice versa). As in experiment 2, the subsample of beetles haphazardly chosen to be parents of generation 2 did not differ in size from the average size of all beetles emerging in their

respective lines (*t*-tests comparing family-mean size with the size of beetles chosen to be parents of generation 2, *P* > 0.05 for each sex and line). Pairs were confined in 35-mm petri dishes containing eight *V. unguiculata* seeds and allowed to lay eggs as in experiment 2. Egg size was measured and larvae were reared to adult as in experiment 2. No generation 3 was initiated in this experiment.

Results

As in experiment 2, families reared at high density emerged at substantially smaller adult body sizes than families reared at one egg/seed (mean ± SE [number of families]; female progeny, low density 5.28 ± 0.06 mg [63], high density 3.66 ± 0.09 [63], treatment *F* = 193.4, *P* < 0.001; male progeny, low density 4.19 ± 0.04 [63], high density 2.97 ± 0.06 [62], *F* = 243.9, *P* < 0.001). Females reared at high density laid smaller eggs than females reared at low density, but the density at which her mate was reared had no effect on the size of her eggs (two-way analysis of variance, female density effect: *F* = 41.2, *P* < 0.001 for egg length and *F* = 57.9, *P* < 0.001 for egg width; male density effect: *F* = 0.1, *P* = 0.74 for egg length and *F* = 1.92, *P* = 0.17 for egg width). Also, as in the previous experiment, progeny produced by mothers reared at high density were significantly smaller than progeny produced by mothers reared at low density (Fig. 4).

The experimental crosses between the high- and low-density lines indicated that the inheritance of the treatment difference in body size was nongenetic—the average body size of progeny (across families) emerging in the two hybrid lines (H-L and L-H in Fig. 4) resembled that of their maternal line (H-L resembled H-H and L-H resembled L-L; Fig. 4), but not their paternal line (Table 5). In addition to being non-significant, the *F*-ratio for the paternal density effect was very small relative to the *F*-ratio for the maternal density effect.

Contrary to the results of the previous experiment, in which there was no detectable effect of maternal density on progeny development time (Table 4), progeny produced by mothers

TABLE 3. The size of eggs (mean \pm SEM [n = number of females]) laid by females from high- and low-density lines of *Callosobruchus maculatus* in experiment 2.

A. Generation 1 emergers = parents of generation 2				Analysis of variance	
	Replicate 1	Replicate 2	Source	df	F
Egg length (mm)			Replicate	1	1.76 ns
Low density	0.623 \pm 0.003 (55)	0.628 \pm 0.004 (46)	Treatment	1	30.59***
High density	0.603 \pm 0.003 (55)	0.607 \pm 0.004 (63)	Replicate*treatment	1	0.00 ns
Egg width (mm)			Replicate	1	0.09 ns
Low density	0.393 \pm 0.002 (55)	0.388 \pm 0.005 (46)	Treatment	1	20.93***
High density	0.373 \pm 0.002 (55)	0.377 \pm 0.004 (63)	Replicate*treatment	1	1.83 ns

B. Generation 2 emergers = parents of generation 3				Analysis of variance	
	Replicate 1	Replicate 2	Source	df	F
Egg length (mm)			Replicate	1	3.75 ns
Low density	0.676 \pm 0.004 (56)	0.688 \pm 0.004 (48)	Treatment	1	0.65 ns
High density	0.683 \pm 0.005 (56)	0.688 \pm 0.005 (61)	Replicate*treatment	1	0.47 ns
Egg width (mm)			Replicate	1	4.26*
Low density	0.427 \pm 0.003 (56)	0.430 \pm 0.003 (61)	Treatment	1	1.17 ns
High density	0.419 \pm 0.002 (56)	0.428 \pm 0.002 (48)	Replicate*treatment	1	3.87 ns

* $P < 0.05$; *** $P < 0.001$; ns = not significant.

reared at high density emerged later than those produced by mothers reared at low density (Fig. 5, Table 6; $P < 0.001$ for female progeny; $P = 0.08$ for male progeny), although the magnitude of the effect was small. This suggests that there is some compensatory growth in progeny, although not enough to alleviate the consequences of having a mother that was reared at high density.

DISCUSSION

Maternal effects provide a nongenetic mechanism by which environmental variation in the parental generation affects the

phenotype of their progeny (Riska et al. 1985). However, few studies have examined how long environmental variation, inherited via maternal effects, persists within populations. Environmental variation has been demonstrated to persist across generations in some plants, in live-bearing animals, and in animals with extensive parental care, but this appears to be relatively uncommon in other animals (Bernardo 1996a). The few studies that have examined the persistence of environmental variation in egg-laying animals have generally found that, although maternal effects often have large effects on early growth, these effects gradually disappear and become undetectable by the time progeny mature (Mousseau and Dingle 1991; Fox 1994a; Bernardo 1996a), presumably due to compensatory growth (developmental plasticity) by progeny. Here we described a system in which an environmentally induced reduction in body size was inherited by progeny via a nongenetic maternal effect. In fact, body size of progeny reflects two separate maternal effects in *C. maculatus*. First, a female's egg-laying decisions (whether to lay additional eggs on an already parasitized host) have consequences for the growth of her progeny—superparasitism results in increased larval density, which in turn forces the maturation of her progeny at a smaller size. Second, these smaller progeny produce smaller eggs, requiring the grandprogeny to either develop longer or mature at a smaller size. In *C. maculatus*, progeny choose primarily the latter option and emerge at a below-normal size. Thus, superparasitism of seeds by a female not only affects the size of her progeny, but also the size of her grandprogeny.

	Generation 2		Generation 3	
	df	F	df	F
Female progeny				
Replicate	1	17.76***	1	4.75*
Treatment	1	0.01 ns	1	0.01 ns
Replicate*treatment	1	4.18*	1	0.06 ns
Family(rep*treat)	212	2.95***	211	1.78***
$R^2 =$		0.59		0.42
Male progeny				
Replicate	1	30.19***	1	9.42**
Treatment	1	0.00 ns	1	2.04 ns
Replicate*treatment	1	3.20 ns	1	3.04 ns
Family(rep*treat)	212	3.14***	211	1.59***
$R^2 =$		0.59		0.39

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = not significant.

phenotype of their progeny (Riska et al. 1985). However, few studies have examined how long environmental variation, inherited via maternal effects, persists within populations. Environmental variation has been demonstrated to persist across generations in some plants, in live-bearing animals, and in animals with extensive parental care, but this appears to be relatively uncommon in other animals (Bernardo 1996a). The few studies that have examined the persistence of environmental variation in egg-laying animals have generally found that, although maternal effects often have large effects on early growth, these effects gradually disappear and become undetectable by the time progeny mature (Mousseau and Dingle 1991; Fox 1994a; Bernardo 1996a), presumably due to compensatory growth (developmental plasticity) by progeny. Here we described a system in which an environmentally induced reduction in body size was inherited by progeny via a nongenetic maternal effect. In fact, body size of progeny reflects two separate maternal effects in *C. maculatus*. First, a female's egg-laying decisions (whether to lay additional eggs on an already parasitized host) have consequences for the growth of her progeny—superparasitism results in increased larval density, which in turn forces the maturation of her progeny at a smaller size. Second, these smaller progeny produce smaller eggs, requiring the grandprogeny to either develop longer or mature at a smaller size. In *C. maculatus*, progeny choose primarily the latter option and emerge at a below-normal size. Thus, superparasitism of seeds by a female not only affects the size of her progeny, but also the size of her grandprogeny.

A common concern of most laboratory studies examining the sources of phenotypic variation is that inadvertent selection during experiments can bias estimates of variance components or, in our experiment, possibly explain the difference between the high- and low-density lines. Sex-biased selection on body size could explain why the treatment difference was maternally inherited. However, we believe that sex-biased

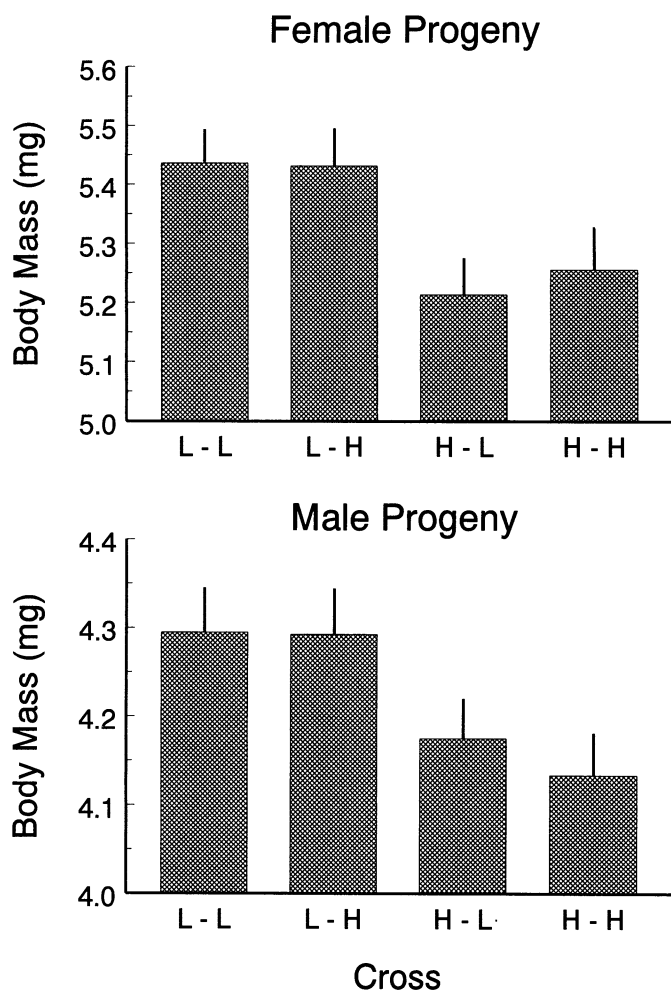


FIG. 4. The influence of maternal and paternal rearing density on the body size of their progeny in the seed beetle, *Callosobruchus maculatus* (mean \pm SEM). L-L = low-density line, H-H = high-density line, L-H = low-density mother crossed with a high-density father, and H-L = a high-density mother crossed with a low-density father. Note that progeny body size was influenced by the density that their mother was reared at but not the density that their father was reared at, as expected if variation in body size was inherited via an environmentally based maternal effect. Means and standard errors were calculated across family means (i.e., each family within each line was treated as a single data point).

selection does not explain the maternal inheritance of the body size difference for three reasons. First, the high and low density lines converged on a common body size by generation 3 in both replicates (Fig. 3). If the difference between the lines represented a response to selection, and was thus genetic, it should have persisted beyond generation 2. Second, our experiment was designed to minimize inadvertent selection on body size; every family contributed the same number of individuals to be parents of the subsequent generation. Although some of these females failed to lay eggs, there was no difference in the average size of females that laid eggs and those that did not. Third, that the sex-ratio of emerging progeny did not differ from 1:1 and did not vary with density indicates that sex-biased selection within families is unlikely. Thus, we conclude that selection does not

TABLE 5. The influence of maternal and paternal rearing density on progeny body size in the seed beetle, *Callosobruchus maculatus*. The analyses (SAS Proc GLM, Type III sums-of-squares) demonstrate that environmentally based variation in body size is maternally inherited. Family, nested within the maternal density*paternal density interaction, was included in each analysis because siblings are not independent of each other. The *F*-ratios for the maternal density, paternal density, and interaction effects were calculated with the MS[Family(maternal density*paternal density)] in the denominator.

	Female progeny		Male progeny	
	df	<i>F</i>	df	<i>F</i>
Maternal density	1	8.60**	1	6.80**
Paternal density	1	0.07 ns	1	0.17 ns
Maternal*paternal density	1	0.12 ns	1	0.13 ns
Family (maternal*paternal density)	239	2.67***	240	2.71***
<i>R</i> ² =		0.47		0.46

** *P* < 0.01; *** *P* < 0.001; ns = not significant.

account for our observation that the body size difference between treatments is maternally inherited.

Our interpretation that these experiments demonstrate environmental inheritance of body size variation could be flawed if seeds are small enough that food for larvae is exhausted during larval development. In this case, smaller eggs may produce larvae that emerge as smaller adults because on average they require more food to attain a specific size than larvae hatching from a larger egg. Our interpretation assumes that food is not limiting for larvae when they are reared at densities of one beetle per seed. There is substantial evidence that this assumption is appropriate. The *V. unguiculata* seeds are large relative to beetle body sizes (\approx 50 times larger by mass). There is only a small effect of larval density on emergence body size at the lowest larval densities (between one and seven larvae per seed; Fig. 2), and the total biomass of all adults emerging from seeds actually increases over this range of densities indicating that there is enough food within a single seed to rear more than one normal size beetle. Within larval densities of one, two, or three larvae per seed (experiment 1) there was no evidence that seed size affected the size of beetles that emerged from the seed. Females are larger than males (see Figs. 2, 3, 4) and eat more of the seed than males (unpubl. data), but the effect of maternal density on progeny body size is similar for progeny of both sexes.

The persistence of environmental variation across generations in *C. maculatus* was an unexpected result. In a similar experiment with another seed beetle, *Stator limbatus*, progeny developing from eggs laid by high-density mothers compensated for their small egg size by developing longer to eventually emerge at the same average body size as progeny developing from eggs laid by low-density mothers (Fox 1997b). We expected the same result for *C. maculatus* because previous experiments (including one using this same population) suggested that body size is targeted in this species—progeny compensated for small eggs by developing longer to attain what appeared to be a genetically targeted body size. First, as mothers age they lay increasingly smaller eggs, and their progeny compensate for this decrease in egg size by devel-

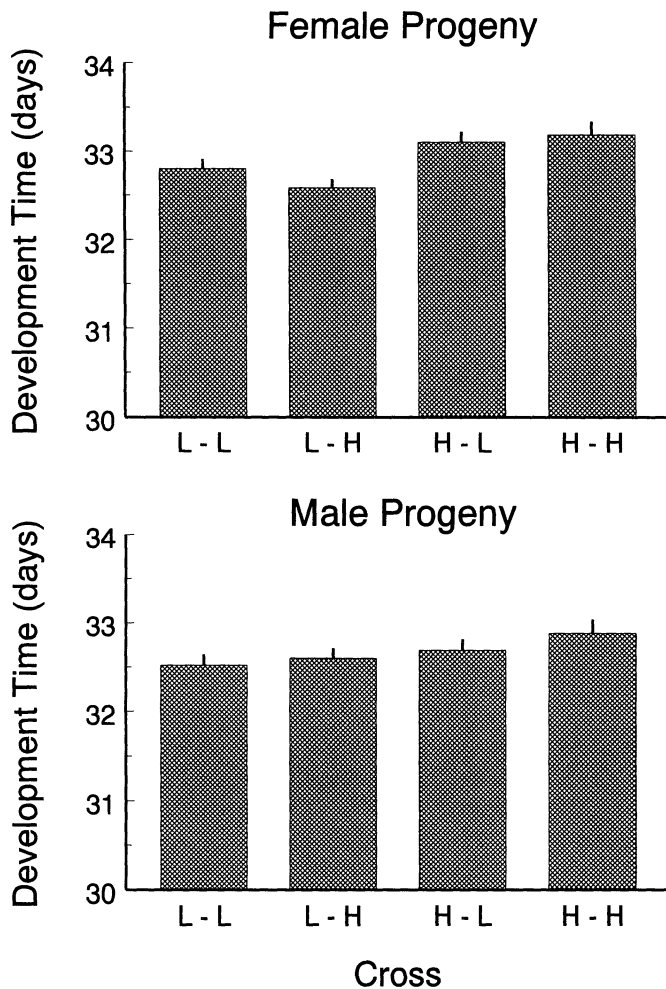


FIG. 5. The influence of maternal and paternal rearing density on the body size of their progeny in the seed beetle, *Callosobruchus maculatus* (mean \pm SEM). L-L = low-density line, H-H = high-density line, L-H = low-density mother crossed with a high-density father, and H-L = a high-density mother crossed with a low-density father. Note that there is evidence that H-H and H-L progeny partially compensated for the small size of their eggs by developing longer, although this compensation was insufficient to entirely alleviate the influence of maternal density on their final body size when they emerged as adults.

TABLE 6. The influence of maternal and paternal rearing density on progeny development time in the seed beetle, *Callosobruchus maculatus*. Analyses as in Table 5.

	Female progeny		Male progeny	
	df	F	df	F
Maternal density	1	13.61***	1	3.02 ns
Paternal density	1	0.33 ns	1	1.01 ns
Maternal*paternal density	1	1.47 ns	1	0.17 ns
Family (maternal*paternal density)	239	1.73***	240	2.07***
$R^2 =$		0.47		0.46

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

oping longer to emerge at the same body size as their siblings that developed from larger eggs (laid when their mother was younger; Fox 1993; Fox and Dingle 1994). Second, using a half-sib quantitative genetic analysis, Fox (1994a) detected large maternal effects on progeny development time, but found no evidence that maternal effects persisted into the adult stage of their progeny. In the larval density experiments described here, however, *C. maculatus* larvae did not compensate for reduced egg size by developing longer to attain a targeted adult body size. Instead, they developed a similar length of time in each treatment (experiment 2), or only slightly longer if they hatched from a small egg (experiment 3), and emerged smaller if their mother had been reared at high density.

This difference between previously published results (Fox 1993, 1994a; Fox and Dingle 1994) and the results of this current study suggests that there are either different ways to make small eggs in response to different environmental stresses (i.e., eggs may vary in composition), or that egg size is not the mechanism by which environmental variation in adult body size is inherited (or both). Maternal environmental effects on egg composition have been demonstrated in other animals, including insects, and may provide an explanation for our observations. For example, many studies have found that egg size does not necessarily correlate well with egg content (review in Bernardo 1996b). In the gypsy moth, *Lymantria dispar*, 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). In insects, yolk proteins serve as the primary source of energy for offspring prior to the initiation of feeding (Kunkel and Nordin 1985), and yolk protein content may vary according to maternal experiences. In the gypsy moth, 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 may also influence the concentration of other 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.

We suspect that nongenetic inheritance of body size variation may be common in some animals, and particularly in parasitic insects. Environmental conditions are well documented to affect the body size of egg-laying females in insects that complete development inside discrete resource patches such as a host insect (e.g., parasitoid), seed (e.g., seed beetles) or leaf (leafminers; Hespenheide 1991). These discrete resource patches can be limiting in nature (e.g., Fox and Mousseau 1995), such that natural selection often favors mothers that either lay clutches of eggs (Godfray 1987, 1994; Ives 1989) or readily superparasitize hosts (Hubbard et al. 1987; Ives 1989; Mangel 1992; Visser et al. 1992; Wilson and Lesells 1994). Thus, variation in larval competition among hosts is one of the primary factors structuring the behavior and development of larvae (e.g., Messina 1991, in press; Godfray 1994; Fox et al. 1996). In response to this increasing competition, developmental plasticity has frequently evolved in

which individuals can mature at a substantially smaller than normal size, providing the opportunity for the inheritance of environmental variation in body size—maternal size generally affects egg size and/or composition, which in turn can affect progeny growth and development. Thus, food stress (due to increasing clutch size or superparasitism) in one generation can affect not only growth of the stressed individuals, but also, in at least some parasitic insects, growth of individuals in and population dynamics of subsequent generations via environmentally based maternal effects.

Like larval density, variation in diet quality (e.g., seed quality or seed species) in one generation can also influence the growth of individuals in subsequent generations, such that maternal host preferences affect the growth not only of their progeny, but also of their grandprogeny. For example, in *S. limbatus*, females ovipositing on *Cercidium floridum*, a relatively poor-quality host plant, produce larger daughters that in turn produce larger and faster developing granddaughters, as compared to females ovipositing on *Acacia greggii* (a relatively high-quality host plant) (Fox et al. 1995). Controlled experiments have demonstrated that environmentally based maternal effects alone can explain these results. Similar diet-mediated maternal effects have been reported for other insects (Morris 1967; Haukioja and Neuvonen 1987; Rossiter 1991, 1994, 1996; Rossiter et al. 1993). For example, in the gypsy moth, *L. dispar*, the plant species that a mother develops on can affect the composition of her eggs (Rossiter et al. 1993, Rossiter 1994), which subsequently affects the growth and development of her progeny. In the greenbug, *Schizaphis graminum*, offspring of corn-reared parents attain larger adult size than offspring of sorghum-reared parents (McCaughey et al. 1990). However, diet-mediated maternal effects have so far been identified for only a few organisms, probably because few studies have looked for them. We suspect that they are very common in nature, and likely both ecologically and evolutionarily important, and thus deserve more attention.

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