

Maternal investment affects offspring phenotypic plasticity in a viviparous cockroach

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Maternal effects, crossgenerational influences of the mother's phenotype on phenotypic variation in offspring, can profoundly influence the fitness of offspring. In insects especially, social interactions during larval development also can alter life-history traits. To date, however, no experimental design, to our knowledge, has manipulated the prenatal and postnatal environments independently to investigate their interaction. We report here that the degree of maternal nutrient investment in developing embryos of the viviparous cockroach *Diploptera punctata* influences how quickly neonate males become adults and how large they are at adulthood. An offspring's probability of reaching adulthood in fewer than four molts increased with birth weight: the heavier neonates were, consequently, more likely to become smaller adults. Social interaction also affected nymphal development and adult size. Nymphs reared in pairs molted fewer times than solitary nymphs and, thus, became smaller adults. The social effect on developmental trajectory was, however, eliminated by experimentally increasing the level of maternal nutrient investment per offspring, which was accomplished by removing one of the female's paired ovaries (allometric engineering). We conclude that a particular prenatal environment can result in different offspring phenotypes under different postnatal social conditions. By investing more in each offspring, however, *D. punctata* mothers, because they are viviparous, are able to produce broods with environmentally (socially) independent phenotypes.

A central tenet of life-history theory, and a direct consequence of anisogamy, is that the mother can elevate provisions per individual offspring by reducing clutch size, and conversely, she can reduce this investment and increase offspring number (1, 2). Such maternal provisions can result in crossgenerational phenotypic plasticity, profoundly affecting suites of life-history traits and offspring fitness. With greater physiological dependency of offspring on the mother, as in viviparous animals, females that invest more in fewer embryos are expected to produce larger offspring (as in fish) (3). However, because postnatal growth and development are then shaped by environmental effects, negative instead of positive correlations between maternal investment and offspring phenotype can emerge.

The social environment of an insect can greatly influence its development. For example, a desert locust (*Schistocerca gregaria*) nymph reared at high density grows significantly faster than one reared alone (4) and such "group effects" (5) have now been characterized in dozens of insect species. In some insect species, the nymphs of several cockroach species (e.g., *Diploptera punctata*) for instance, interaction between just two individuals is sufficient for expression of a group effect (6, 7). Beyond affecting its immediate physiology and development, the social condition of a nymph can influence its adult traits. Cricket larvae, for instance, reared in groups of 10 grow faster and become larger adults than those reared alone (8, 9), whereas grouped cockroach larvae also grow more quickly than isolated ones but become smaller adults (6, 10). Because adult size is often related to fecundity (11) and reproductive success (12), it is expected that group effects would influence individual fitness and, there-

fore, play a major role in population dynamics, as in locusts, for example (13, 14).

Parental influence on expression of group effects in offspring remains a largely uninvestigated issue. Maternal age was found to be a determinant of whether a cricket larva grew faster under grouped conditions (15, 16), but how a mother affected its offspring's sensitivity to social conditions was not ascertained. We speculated that the viviparous beetle cockroach, *D. punctata*, would be an ideal species with which to address this issue because maternal investment into progeny is substantial but variable. Embryos increase 50-fold in dry mass during gestation (17), as the embryos ingest the nutritive secretion of the uterine lining (18), and at parturition, a brood can exceed its mother's weight by 50% (19). Paired male nymphs of *D. punctata* usually reach adulthood after just three molts, but they reach adulthood in four molts when isolated (6, 7). Therefore, the potential seemed great for a beetle cockroach mother to contribute substantially to phenotypic variation in neonates and possibly influence postnatal development of offspring.

Materials and Methods

Insect Maintenance. Cockroaches were reared at $27 \pm 0.3^\circ\text{C}$ under a 12:12 h light/dark photoperiodic regime in which they were provided a continuous supply of rat chow and water. Adult females were mated shortly after eclosion and maintained thereafter in groups of 10–20 in 150×25 -mm Petri dishes under conditions similar to the colony.

Experimental Insects. Newborn male nymphs from the same brood were weighed within 8 h of parturition and either isolated or paired with newborn female larvae in 100×20 -mm Petri dishes, with each dish containing rat chow and water. Males were subsequently monitored daily for molting and adult eclosion.

Allometric Engineering. An allometric engineering approach (20) was used to investigate the effect of birth weight on development. Unilaterally ovariectomized females oviposit one-half of the eggs of normal females, and their offspring are significantly larger than offspring of normal females (21). Females were mated on the first day of adulthood and unilaterally ovariectomized (hemiovariectomized). They were anesthetized with CO_2 and immobilized with plasticine on a paraffin block, and a longitudinal slit was made with fine scissors in the seventh abdominal tergite. One ovary was removed through this slit (22), and when the operation was completed, the females were placed in groups of 10–20 in 150×25 -mm Petri dishes containing water, rat chow, and shelters constructed with filter paper. Sham-operated females were treated similarly, but their ovaries were left intact.

Statistics. To estimate the probability of a nymph reaching adulthood in three or four stadia as a function of its birth weight

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Table 1. Social effects on larval development and weight at adulthood in males isolated or paired from birth to adulthood

Social condition	No. of progeny*	Length of development, days	Adult weight, mg
All isolated	61	67.2 ± 0.9	155.5 ± 1.9
Isolated, three stadia	17 (27.9%)	57.2 ± 0.6	146.3 ± 3.1
Isolated, more than three stadia	44 (72.1%)	71.1 ± 0.5	159.1 ± 2.2
All paired	67	57.3 ± 0.9	147.5 ± 1.8
Paired, three stadia	42 (62.7%)	52.3 ± 0.3	141.0 ± 1.6
Paired, more than three stadia	25 (37.3%)	65.8 ± 0.6	158.6 ± 2.7

Means ± SEM for length of larval development and adult weight are shown for all isolated and paired males and for those that became adults in either three or more than three stadia.

*Percentage of isolated and paired larvae becoming adults in three or more than three stadia is given in parentheses.

and social condition, logistic regression analysis was implemented in SAS 6.12 (SAS Institute, Cary, NC). Other statistical analyses are described below.

Results and Discussion

Paired males completed the larval stage in 85.3% of the time taken by isolated nymphs, representing a highly significant social effect on development ($Z = 6.88$, $P < 0.001$, Mann–Whitney test) (Table 1). The shorter duration of the immature stage in paired males was reflected in a significantly greater proportion ($\chi^2 = 14.208$, $df = 1$, $P < 0.001$) of paired males than isolated males becoming adults in three stadia (i.e., after three molts). The remaining males in both groups passed through four stadia almost exclusively. An increase in the number of stadia was significant in two ways. Males passing through more than three stadia took $\approx 25\%$ more time to become adults than three-stadia males, and they weighed on average $\approx 10\%$ more at adulthood (Table 1).

To examine whether neonate size, a reflection of maternal investment, affected nymphal development, we weighed newborn males and recorded whether they passed through three or more stadia before adulthood under both isolated and paired conditions (Fig. 1). Logistic regression analysis showed that the probability (π) of a male reaching adulthood in three stadia depended on both its birth weight (w) and, as expected, social condition (s , dummy-coded “0” for isolated and “1” for paired males). The coefficients of the two variables were highly significant ($Z = 8.20$ for w and $Z = 8.37$ for s , $P < 0.001$), justifying the inclusion of both variables in a main-effects model [$\text{logit}(\pi) = -7.085 + 0.350w + 2.177s$]. The model was not improved by incorporation of an interaction term ($\chi^2 = 0.162$, $df = 1$, $P = 0.67$, likelihood ratio statistic), so the effect of birth weight on development was examined with the main-effects model. Exponentiating the coefficient of weight in this model gave an odds ratio of 1.42 (95% confidence interval, 1.31–1.54) indicating that the odds of becoming an adult in three stadia increased by $\approx 42\%$ for any 1-mg increase in birth weight, regardless of social condition.

To confirm that birth weight affected development, we adopted an allometric engineering approach (20): neonate size was increased experimentally by unilaterally ovariectomizing mated females on the first day of adulthood. Both sham-operated and hemiovariectomized females were kept under conditions similar to normal mated females. Unilaterally ovariectomized females oviposit one-half of the eggs of normal females and provision their reduced brood internally throughout gestation. It is important to note that under normal lab rearing conditions, *D. punctata* females sometimes incubate as few as six embryos (23). Male hatchlings from hemiovariectomized females were heavier at birth than those from sham-operated females ($Z = 6.12$, $P < 0.001$, Mann–Whitney test) (Table 2), and a far greater proportion of males from hemiovariectomized females reached adult-

hood in only three stadia when isolated ($\chi^2 = 25.568$, $df = 1$, $P < 0.001$). Notably, a higher proportion of isolated males from hemiovariectomized females than paired males from normal females became adults in three stadia (Table 1). Greater prenatal maternal provisioning, therefore, offsets the retarding effect of social isolation on their offspring’s development. These results, coupled with those of Woodhead (23), show that the maternal environment plays a major role in determining male fitness in this species. Males that pass through four stadia become larger adults (Table 1), which are, under some circumstances, more successful in fertilizing females (23). On the other hand, three-stadia males become adults more quickly, and early reproduction may impart a fitness advantage.

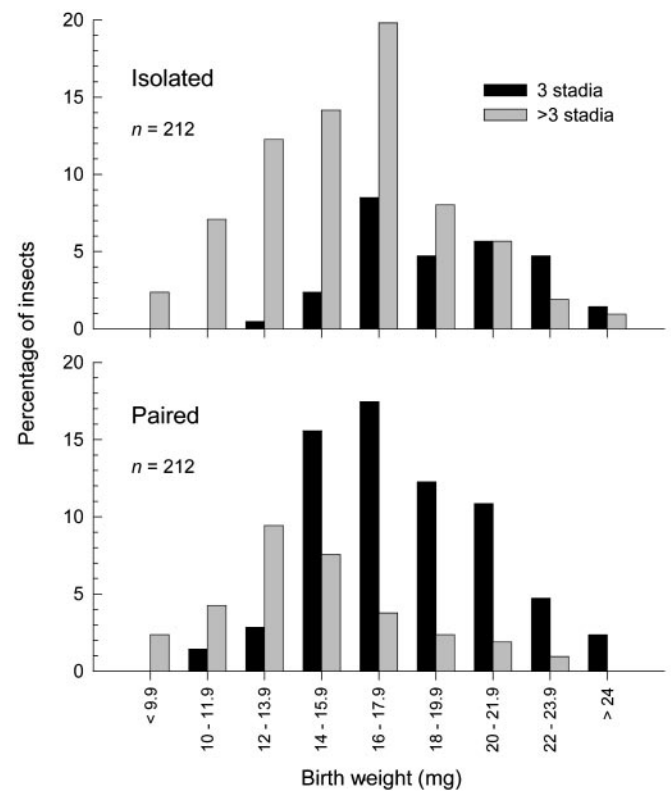


Fig. 1. Number of stadia (molts) in relation to birth weight in isolated and paired males. Neonates were weighed to the nearest 0.1 mg and isolated (Upper) or paired (Lower) until adult eclosion. The number of stadia that each insect passed through before adulthood was recorded. Cockroaches have been placed in weight categories for graphical purposes only, and the two bars in a category show percentages of insects developing by either three or more than three stadia. All bars in a graph total 100%. The mean weights of isolated and paired males at birth were both 16.8 ± 0.2 mg.

Table 2. Effect of maternal unilateral ovariectomy (hemiovariectomy) on progeny development

Mother	No. of progeny	Birth weight (mg)	No. of stadia		Development time (days)
			Three	More than three	
Sham-operated*	36	17.2 ± 0.8	7 (19.4%)	29 (80.4%)	66.2 ± 1.4
Hemiovariectomized	43	25.6 ± 0.7	34 (79.1%)	9 (20.9%)	58.5 ± 1.0

*The newborn male offspring of 10 sham-operated and 20 unilaterally ovariectomized females were weighed and reared in isolation until adult eclosion. The total number of larval molts was recorded for each insect. Values represent the number and percentage (in parentheses) of insects becoming adults in either three or more than three stadia.

The interplay between social and maternal effects on development has received little attention in insects. Our results show, for the first time, that interaction of the prenatal maternal environment with the postnatal social environment determines the course of development. The level of maternal contribution in *D. punctata* influences the number of stadia through which males pass before adulthood and determines the degree to which a nymph's development is affected by its social environment. Whereas newborn male larvae of intermediate birth weight showed great phenotypic plasticity in development varying primarily between three and four stadia under paired and isolated conditions, respectively, the developmental trajectory of low- and high-birth-weight neonates was essentially predetermined and independent of the social environment. This result is interesting because the immatures of many insect species retain the ability to alter their development in response to changing environmental conditions, even when parental effects have prompted them initially to enter a particular developmental pathway. For instance, adult female locusts of the gregarious phase give birth to nymphs that are gregarious in both behavior and physiology, but these nymphs, when isolated, almost immediately lose gregarious traits and become solitary in behavior (13).

A female's investment in offspring represents a balance between clutch size and the mass of each offspring at parturition. These results suggest that females might modify their investments in offspring in response to environmental conditions. Production of small offspring may be advantageous under conditions in which postnatal development is socially facilitated,

i.e., crowding. In sparse population densities, females should invest more in each offspring to offset the subsequent retarding effect of social isolation on nymphal development. Interestingly, however, the adults that result from larger than average embryos, reach adulthood in only three stadia in *D. punctata* and are small. They, in turn, produce smaller than average embryos that develop more slowly as nymphs, especially if isolated; undergo four stadia before adulthood; and, thus, become large adults. This striking inversion of offspring phenotype across generations (an apparent negative maternal effect) results from opposing directional selection during successive generations. The net effect is to stabilize the size of adults but provide extensive size variation, which makes possible a range of maternal investments in offspring and, thus, variation in life-history tactics and traits.

It will be of continuing interest to determine whether social factors outweigh maternal influences on postnatal development in the ≈4,000 oviparous and ovoviviparous cockroach species and whether pseudoplacental viviparity in the beetle cockroach has brought about maternal modulation of offspring development and size at adulthood. The means by which social effects on development are offset by maternal factors in other animal species is also of great interest.

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