Evolution, 55(7), 2001, pp. 1363–1372

CORRELATED RESPONSES TO SELECTION FOR FASTER DEVELOPMENT AND EARLY REPRODUCTION IN *DROSOPHILA*: THE EVOLUTION OF LARVAL TRAITS

N. G. PRASAD,[1] MALLIKARJUN SHAKARAD,[1,2] D. ANITHA,[1] M. RAJAMANI,[1] AND AMITABH JOSHI[1,3,4]
[1]*Evolutionary Biology Laboratory, Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, PO Box 6436, Jakkur, Bangalore 560 064, India*
[3]*E-mail: ajoshi@jncasr.ac.in*

Abstract.—Studies on selection for faster development in *Drosophila* have typically focused on the trade-offs among development time, adult weight, and adult life span. Relatively less attention has been paid to the evolution of preadult life stages and behaviors in response to such selection. We have earlier reported that four laboratory populations of *D. melanogaster* selected for faster development and early reproduction, relative to control populations, showed considerably reduced preadult development time and survivorship, dry weight at eclosion, and larval growth rates. Here we study the larval phase of these populations in greater detail. We show here that the reduction in development time after about 50 generations of selection is due to reduced duration of the first and third larval instars and the pupal stage, whereas the duration of the second larval instar has not changed. About 90% of the preadult mortality in the selected populations is due to larval mortality. The third instar larvae, pupae, and freshly eclosed adults of the selected populations weigh significantly less than controls, and this difference appears during the third larval instar. Thereafter, percentage weight loss during the pupal stage does not differ between selected and control populations. The minimum amount of time a larva must feed to subsequently complete development is lower in the selected populations, which also exhibit a syndrome of reduced energy expenditure through reduction in larval feeding rate, larval digging and foraging activity, and pupation height. Comparison of these results with those observed earlier in populations selected for adaptation to larval crowding and faster development under a different protocol from ours reveal differences in the evolved traits that suggest that the responses to selection for faster development are greatly affected by the larval density at which selection acts and on details of the selection pressures acting on the timing of reproduction.

Key words.—Development time, *Drosophila melanogaster,* foraging behavior, larval feeding rate, life history, preadult mortality, pupation height.

Received June 3, 2000. Accepted March 8, 2001.

Two of the important selection pressures operating on insects whose larvae inhabit ephemeral habitats in the wild are overcrowding and the necessity to complete preadult development relatively fast. Larval growth rates in *Drosophila* are thought to be partly shaped by a trade-off between faster development and adult size (Santos et al. 1988; Partridge and Fowler 1993), and this trade-off has been extensively studied. The evolution of *Drosophila* life-history traits in response to larval and adult crowding has also been studied extensively (reviewed by Joshi 1997; Mueller 1997). When populations of *Drosophila* are kept at very high larval density for many generations, larval feeding rates and the minimum food required for larvae to complete development increase relative to controls (Joshi and Mueller 1988, 1996; Mueller 1990). Yet, populations maintained at high larval density do not exhibit faster development, increased larval growth rate, or increased adult weight at eclosion when assayed at moderate densities (Santos et al. 1997). Selection for faster development, however, results in decreased adult weight at eclosion (Nunney 1996; Chippindale et al. 1997) and reduced larval growth rates (Prasad et al. 2000). Unlike in the case of adaptation to larval crowding, not much is known about larval behaviors related to food acquisition in populations subjected to selection for faster development.

In an ongoing study in our laboratory we have successfully selected four populations of *D. melanogaster* for faster development and early reproduction, relative to control populations that had been maintained on a three-week discrete generation cycle with no conscious selection on development time for over 200 generations. Over 70 generations of selection, we have observed a reduction of about 20% in egg-to-eclosion development time and survivorship, about 42% in adult dry weight at eclosion, and about 28% in larval growth rate (Prasad et al. 2000). Because selection for faster development directly impinges on the preadult life stages, one may expect that the genetic correlations among the various traits expressed in the juvenile stages may play a major role in molding the outcome of selection. The genetic control of larval instar duration in *Drosophila* is not well understood, although it is known that the durations of the first and the second instar and the early part of the third instar can be environmentally manipulated, whereas the duration of the third instar after attainment of the minimum critical size for pupation does not respond to environmental manipulation (Bakker 1961).

Reduction in the duration of the larval stage in *Drosophila* is also constrained by the necessity of early third instar larvae attaining a critical size required to successfully pupate and eclose. The critical size is known to be environmentally (De Moed et al. 1999) and genetically (Robertson 1963) alterable, and flies with a larger adult body size are known to often take longer to attain the minimum critical size (Robertson 1963). Therefore, in a population where shorter development time, smaller adult body size, and lower growth rates have evolved, one may expect the evolution of shorter time to the attainment of minimum critical size and/or smaller minimum critical size itself.

[2] Present address: Poornaprajna Institute of Scientific Research, 4, Sadashivanagar, Bangalore 560 080, India.
[4] Corresponding author.

Many energy costly larval behaviors connected with resource acquisition are known to have an impact on larval growth, minimum food requirement for pupation, and preadult fitness (Joshi and Mueller 1996; Joshi 1997). Consequently, the optimal levels of expression of such behaviors may be expected to depend upon the selection regime, with the relative importance to fitness of adult size (and therefore fecundity) and development time shifting the optimum in one or the other direction. Larval feeding rates (Sewell et al. 1975; Joshi and Mueller 1988, 1996), the height above the medium that larvae pupate (Mueller and Sweet 1986; Joshi and Mueller 1993), and foraging path length (Sokolowski et al. 1997) are known to increase as an adaptation to larval crowding. Similarly, the propensity of larvae to dig deep into the medium is a trait that has been shown to possess ample additive genetic variance (Godoy-Herrera 1994) and is, therefore, likely to respond to selection in the laboratory.

At this time, little is known about the correlated responses of larval behaviors or of the preadult life-stage-specific mortality rates to selection for faster development. After about 40 generations of selection, larvae from faster-developing populations were observed to pay a mortality cost for their rapid development (Chippindale et al. 1997; Prasad et al. 2000). In the only study to examine larval and pupal development time and mortality separately, Chippindale et al. (1997) found that preadult mortality in the accelerated development time populations was evenly distributed over the larval and pupal life stages, whereas the bulk of the reduction in egg-to-eclosion development time was due to a shortening of the larval life stage. Larval feeding rates in the accelerated development time populations did not differ significantly from the controls after 50 generations of selection (Chippindale et al. 1997), although Borash et al. (2000) reported increased feeding rates and preadult viability of these selected populations after 100 generations of moderate relaxation of selection. Pupation height in the selected populations were significantly lower than in control populations after about 50 generations of selection (Chippindale et al. 1997).

In this paper, we used populations of *D. melanogaster* selected for faster development and early reproduction, relative to controls, to address the following questions: What is the relative contribution of the three larval instars and the pupal stage to the observed reduction in preadult development time and survivorship? Has the minimum critical size necessary for completing development evolved in the selected populations? Have larval feeding rates, larval digging behavior, larval foraging path lengths, and pupation height evolved in the selected populations? Repetition of studies of correlated responses to selection is desirable because observed patterns of correlations among life-history traits are often affected by seemingly small differences in either the genetic composition of the strains used or in laboratory protocols (Joshi and Mueller 1996; Rose et al. 1996; Reznick and Ghalambor 1999; Harshman and Hoffmann 2000). The populations used in this study share common ancestry with the populations used by Chippindale et al. (1997) in their studies on evolution of faster development, as well as with those used for many of the studies on adaptation to larval crowding (Mueller 1990; Joshi and Mueller 1996). Thus, our results are amenable to comparison with these previous studies and can be used to investigate parallels between the evolutionary consequences of larval overcrowding and selection for faster development.

Materials and Methods

Experimental Populations

This study was done on eight populations of *D. melanogaster,* of which four served as controls and four were subjected to selection for faster development and early reproduction relative to the controls. The control populations employed here were the four populations ($JB_{1–4}$) described in detail by Sheeba et al. (1998). The JB populations are maintained in incubators on a 21-day discrete generation cycle at 25°C, about 90% relative humidity, and constant light on banana-jaggery food. The larval density is regulated at about 60–80 larvae per 8-dram vial (9-cm height × 2.4-cm diameter) with 6 ml of food. The number of breeding adults is about 1800 per population, and the adults are maintained in Plexiglas cages (25 cm × 20 cm × 15 cm) with abundant food. Eggs are collected from these flies by placing petri dishes with food into these cages for 18 h. The eggs collected off these food plates are then dispensed into 40 vials at a density of 60–80 eggs per vial. On the 12th, 14th, and the 16th day after egg collection, the eclosed flies are transferred into fresh food vials; on the 18th day after egg collection, all the eclosed flies are collected into Plexiglas cages containing a petri dish of food on which a generous smear of yeast-acetic acid paste has been applied. Three days later, eggs are collected for the next generation. The four JB populations are ultimately descended from a single population of *D. melanogaster* (about 450 generations ago), the IV population described by Ives (1970). The immediate ancestors of JBs are the UU populations described by Joshi and Mueller (1996), which had been maintained for over 100 generations on a 21-day discrete generation cycle at 25°C and constant light. The four JB populations, therefore, had been independent evolutionary entities for more than 450 generations and had been on a three-week cycle for more than 100 generations at the time the present study was initiated.

The four populations selected for faster development and early reproduction were derived from the four JB populations and are designated as $FEJ_{1–4}$ (F, faster development; E, early reproduction; J, JB derived). Each FEJ population was derived from one JB population; thus, selected and control populations bearing identical numerical subscripts are more closely related to each other than to other populations with which they share a selection regime (JB_i and FEJ_i are more closely related than JB_i and JB_j or FEJ_i and FEJ_j; $i,j = 1–4$). Consequently, control and selected populations with identical subscripts were treated as blocks in the statistical analysis.

The selected populations are maintained on a regime similar to the JB populations except that 80 vials of 60–80 larvae are collected per population and monitored closely for eclosions once the pupae begin to darken. The first 15 or so flies that eclose in each vial are collected into Plexiglas cages with abundant food and a generous smear of live yeast-acetic acid paste. Typically the breeding adult number is about 1000–1200 per population. Eggs are collected from these flies on the third day after eclosion by placing fresh food plates into these cages for 1 h. The eggs are then dispensed into 80 vials

at a density of 60–80 eggs per vial. Thus, selection is essentially on the total egg to eclosion development time and on fecundity at an adult age of three days posteclosion. The major differences between the FEJ and JB populations, thus, are that: (1) eggs are collected from the FEJs three days posteclosion to initiate the next generation, whereas in JBs the eggs are collected nine to 10 days posteclosion; (2) the egg-lay window for FEJ is 1 h, whereas for JB it is 18 h; (3) in FEJs only the first 25% of the flies to eclose have an opportunity of contributing to the next generation, whereas in JBs all the flies that eclose by the 12th day after egg collection can contribute to the next generation; and (4) the number of breeding adults in FEJs is about 1000–1200, whereas in JBs it is about 1800.

Collection of Flies for Assays

Imposition of different maintenance regimes can induce nongenetic parental effects. Consequently, all selected and control populations were maintained under common rearing conditions for one complete generation prior to assaying to eliminate all such nongenetic effects. Eggs were collected from the running cultures and dispensed into vials with about 6 ml of food at a density of 60–80 eggs per vial. On the 12th day after egg collection, by which time all normally developing individuals would have eclosed, the flies were collected into Plexiglas cages with abundant food. The adult numbers were usually 1200–1800 per population. They were supplied with live yeast-acetic acid paste for two days prior to egg collection for assays. The progeny of these flies, hereafter referred to as standardized flies, were used for the various assays.

Life-Stage-Specific Development Time and Survivorship Assays

After 56 generations of selection had elapsed, the contribution of different preadult life stages to the overall egg-to-eclosion development time and survivorship was assessed. Eggs of approximately identical age were collected from the standardized flies by placing a fresh food plate in the cage for 1 h. The plate was then replaced by another food plate. After 1 h this plate, too, was discarded and a third food plate was kept in the cage for 30 min. Eggs for the assay were collected from the last food plate and dispensed into vials with 5 ml of food at a density of 30 eggs per vial and incubated. Eighty-five such vials were set up per population. Thirty-six hours after the midpoint of the 30-min egg collection window, five vials from each population were removed from the incubator and the larvae killed by immersion in hot water. The number of the first, second, and third instar larvae in each vial was determined by looking at their mouth hooks. This procedure was repeated at 2-h intervals, until 66 h had elapsed from the midpoint of the 30-min egg collection window. From these data, median times of each molt were obtained by extrapolation. The difference between the median hatching time and the median time of first molt was taken as the duration of first instar, and so on. The five vials that were left over were used to determine pupation and development times. After the first pupa (P1 pupa, as described by Ashburner 1989) was observed, the vials were checked regularly at 2-h intervals. Any new pupae that had formed were scored and marked using a color pen. The observations were continued until no new pupae were formed in any of the vials for two consecutive days. The vials were then monitored for the first eclosion. Thereafter, the vials were checked regularly at 2-h intervals and the number of eclosing males and females recorded. These observations were continued until no flies eclosed for three consecutive days in any of the vials. From these data, we obtained mean egg-to-pupation development time, mean egg-to-eclosion development time, and larval and pupal survivorship for each vial.

Dry Weight Assay

At generation 40 of FEJ selection, the dry weight of third instar larvae, pupae, and adults from selected and control lines was assayed. Eggs were collected at a density of 50 eggs per vial and 12 such vials were set up per population, of which four vials each were used to weigh third instar larvae, pupae, and adults at eclosion. The third instar larvae were picked up with a moist paintbrush and were immediately frozen. They were later grouped into batches of five, without sexing, and were placed into previously weighed aluminium foils, which in turn were placed in clean dry vials. Twelve such replicate vials were set up for each population. After drying at 70°C for 36 h, these were cooled and immediately weighed along with the aluminium foil. The difference in the initial and final weights of the foil yielded the cumulative dry weight of five larvae. The procedure for determining the weights of pupae was essentially the same, except that the P1 pupae were picked off the walls of the vial. Dry weights of freshly eclosed adults were measured by collecting adults within 2 h of eclosion and freezing them. The adults were later sexed and weighed in batches of five flies each, after drying for 36 h at 70°C. Eight such batches were weighed per sex per population.

Critical Minimum Feeding Time

We assayed the minimum time of larval feeding required for individuals to successfully complete development (henceforth, critical minimum feeding time) after 48 generations of FEJ selection. Eggs from standardized flies were collected off agar plates and transferred on to a petri dish containing nonnutritive agar. Twenty-five freshly hatched larvae from the plates were transferred to a petri dish containing a thin layer of nonnutritive agar overlaid with 3 ml of 42.5% yeast suspension. Thirty such petri dishes were set up per population and were randomly distributed within the incubator. Later, at intervals of 4 h, 10 plates of each population were pulled out at random, and a total of 150 larvae from these plates were removed from the food, gently washed in water, and transferred into 10 vials containing 5 ml of nonnutritive agar at a density of 15 larvae per vial. These transfers were done at 46, 50, and 54 h after hatching. Each vial was then monitored for pupation and eclosion.

Larval Feeding Rate

After 65 generations of selection, the feeding rates of FEJ and JB larvae were measured at physiologically equalized

ages. This was done by collecting eggs from the standardized FEJ flies 6 h later than the JB flies. Thus, at the time of assay, FEJ larvae were 42 h old, whereas JB larvae were 48 h old and consequently were in the same relative stage of their larval development. Twenty-five newly hatched larvae were transferred into petri dishes containing a thin layer of nonnutritive agar overlaid with 3 ml of 42.5% yeast suspension. Four such petri dishes were set up per population. The larvae were allowed to feed for 42 (FEJ) or 48 (JB) h, by which time they were in the early third instar. At this point, 25 larvae from each population were assayed for feeding rates, following the procedure of Joshi and Mueller (1996), by placing them individually in a small petri dish (5-cm diameter) containing a thin layer of agar overlaid with a thin layer of 10% yeast suspension. After allowing for a 15-sec acclimation period, feeding rates were measured as the number of cephalopharyngeal sclerite retractions in two consecutive 1-min intervals. Selected and control populations, matched by the subscripted indices, were assayed together, with one larva from the selected population and one from the control population being assayed alternately.

Pupation Height

Pupation heights were measured after 65 generations of selection. Thirty eggs were placed in vials (20-cm height × 2.5-cm diameter) containing 5 ml food. Once all the individuals had pupated, the pupation heights were measured following Mueller and Sweet (1986), as the distance from the surface of the medium to the point between the anterior spiracles of the pupae. Any pupae on the surface of the food were given a pupation height of zero.

Foraging Path Length

After 65 generations of selection had elapsed, eggs were collected from the standardized flies on banana-jaggery food with a 1-h window. Eggs from FEJs were collected 6 h later than the JBs to equalize their physiological ages at the time of assay. Twenty-five newly hatched larvae were transferred into petri dishes containing a thin layer of nonnutritive agar overlaid with 3 ml of 42.5% yeast suspension; six such plates were set up per population. Forty-eight hours after transfer of JB larvae and 42 h after transfer of FEJ larvae, the foraging path lengths were measured. A single larva was placed in the center of a petri dish containing a thin layer of agar overlaid with a very thin layer of 50% yeast suspension. A 15-sec duration was allowed for acclimation. Five minutes later, the larva was removed from the petri dish and the path made by it on the yeast suspension was traced onto a transparent plastic sheet. The lengths of these paths were later measured. Thirty larvae were assayed per population.

Larval Digging Behavior

Larval digging behavior was measured after 65 generations of selection, following Godoy-Herrera (1994) with some modifications. Eggs were collected from the standardized flies on banana-jaggery food with a 1-h egg-laying window. Thirty eggs were then collected and placed into a vial containing 5 ml of charcoal-banana-jaggery medium overlaid with 3 ml of regular banana-jaggery medium. Ten such vials were set up per population. After 90% of the larval duration had elapsed for FEJ and JB larvae, the larvae were fixed by pouring hot water into the vials and were then taken out of the food and observed under the microscope. Larvae with charcoal stained guts were scored as diggers, and the fraction of diggers was calculated for each vial.

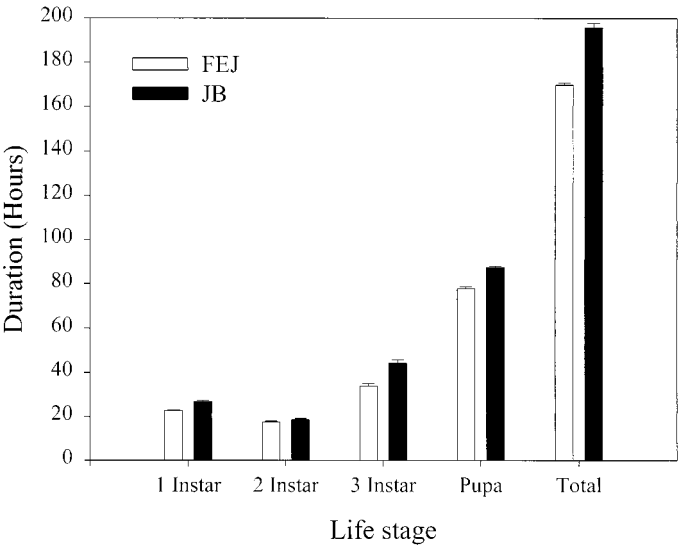

FIG. 1. Mean duration of different preadult life stages in FEJ and JB populations. The error bars represent standard errors around the mean, constructed using the variation among replicate population means within selection regimes. Total, egg-to-eclosion development time.

Statistical Analysis

Data from all the assays were subjected to separate mixed-model analyses of variance (ANOVA), treating block as a random factor and selection as a fixed factor crossed with block. For the critical minimum feeding time assay, the duration for which the larvae were allowed to feed before being transferred to agar vials, was treated as a fixed factor crossed with selection regime and block. All the fractional data (survivorship and digging behavior) were arcsine–square root transformed (Freeman and Tukey 1950) before analysis. In all cases, the population means were used as the units of analysis and, therefore, only fixed-factor effects and interactions can be tested for significance. All analyses were implemented using Statistica for Windows (rel. 5.0B, StatSoft 1995).

RESULTS

Life-Stage-Specific Development Time and Survivorship

By 50 generations of selection, overall mean egg-to-eclosion development time in FEJ populations was 26 h less than that in JB populations (Prasad et al. 2000). In the present study, the duration of the first and third larval instars and of the pupal stage were significantly shorter in the FEJ populations, relative to the JB controls (Fig. 1, Table 1). The duration of the second instar, however, did not differ significantly between FEJ and JB populations (Fig. 1, Table 1).

TABLE 1. Summary of results of separate ANOVAs on mean life-stage-specific development time.

Stage	Effect	df	MS	*F*	*P*
First instar	selection	1	32.0	96.0	0.002
	block	3	1.5	—	—
	selection × block	3	0.3	—	—
Second instar	selection	1	2.0	2.0	0.252
	block	3	0.3	—	—
	selection × block	3	1.0	—	—
Third instar	selection	1	218.1	81.2	0.003
	block	3	11.0	—	—
	selection × block	3	2.7	—	—
Pupa	selection	1	184.6	141.2	0.001
	block	3	3.7	—	—
	selection × block	3	1.3	—	—
Total	selection	1	1361.1	290.7	<0.001
	block	3	19.7	—	—
	selection × block	3	4.7	—	—

TABLE 2. Summary of results of separate ANOVAs on mean preadult life-stage-specific survivorship.

Stage	Effect	df	MS	*F*	*P*
Larva	selection	1	0.051	23.730	0.017
	block	3	0.003	—	—
	selection × block	3	0.002	—	—
Pupa	selection	1	0.015	5.571	0.099
	block	3	0.001	—	—
	selection × block	3	0.003	—	—

The overall larval and pupal durations in the FEJ populations were shorter than in the JB populations by about 16 h (a reduction of ~15%) and 10 h (a reduction of ~11%), respectively (Fig. 1).

We earlier reported a 13% reduction in preadult survivorship of FEJ populations relative to controls (Prasad et al. 2000). In the present study, almost 90% of the difference between FEJ and JB populations in egg-to-eclosion survivorship was accounted for by reduced larval survivorship in the FEJ populations (Fig. 2). Separate ANOVAs done on the larval and pupal survivorship data revealed a significant main effect of selection on larval, but not on pupal, survivorship (Table 2).

Life-Stage-Specific Dry Weight

Third instar larvae, pupae, and freshly eclosed adults (averaged across sexes) of the FEJ populations were significantly lighter than their JB counterparts (Fig. 3). The difference in dry weight was apparent in the third instar larvae and remained relatively unchanged through the pupal duration, even though absolute dry weights of both FEJ and JB populations changed with life stage assayed (Fig. 3, Table 3).

Critical Minimum Feeding Time

After feeding for only 46 h, very few larvae survived in either FEJ or JB populations, whereas mean survivorship rose to about 0.4 when larvae were allowed to feed for 54 h (Fig. 4). The ANOVA revealed significant effects of selection, feeding time, and selection × feeding time interaction (Table 4). Multiple comparisons revealed no significant difference in mean survivorship of FEJ and JB larvae after 46 ($t = 0.58$, df = 6, $P > 0.05$) and 54 h ($t = 1.3$, df = 6, $P > 0.05$) of feeding, whereas after 50 h of feeding, FEJ larvae had sig-

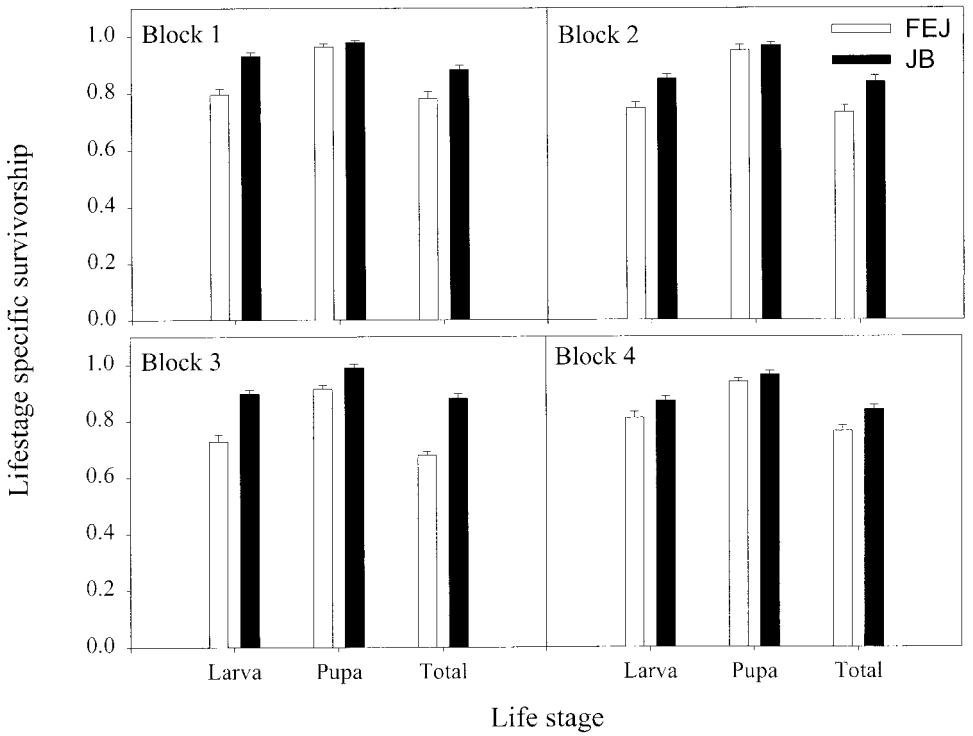

FIG. 2. Mean life-stage-specific preadult survivorship in FEJ and JB populations. The error bars represent standard errors around the mean, constructed using the variation among replicate vials within populations. Total, egg-to-eclosion survivorship.

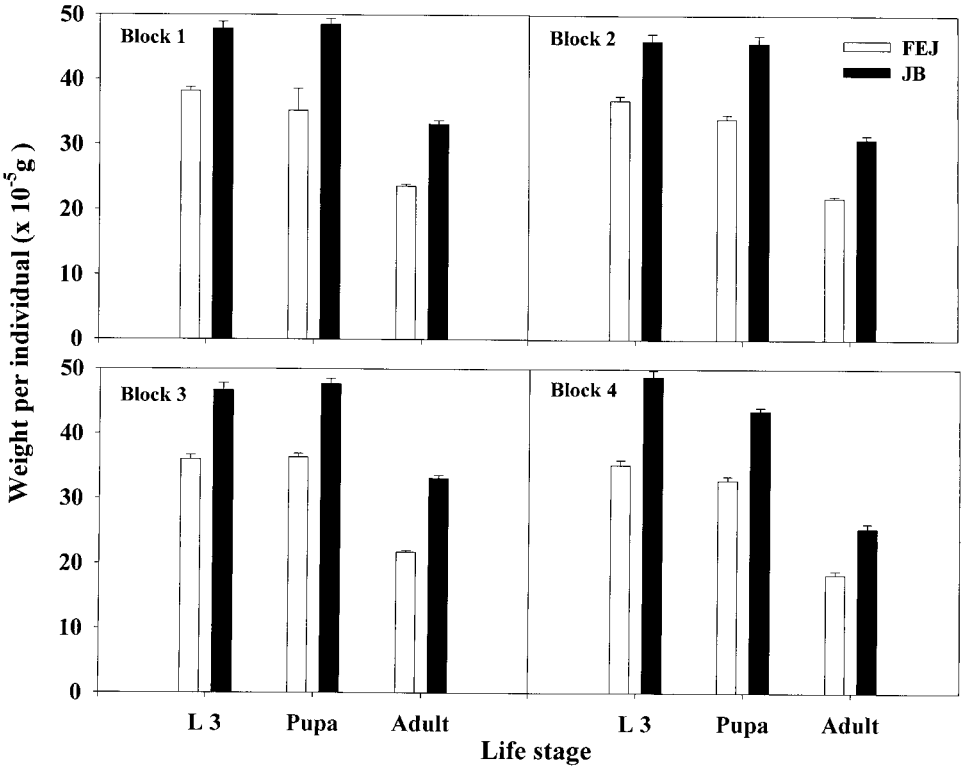

FIG. 3. Mean dry weight of third instar larvae, pupae, and freshly eclosed adults of FEJ and JB populations. The data for adults have been averaged over sexes. The error bars represent standard errors around the mean, constructed using the variation among replicate batches of five individuals within populations. L3, third instar.

nificantly greater mean survivorship than JB larvae ($t = 4.79$, df = 6, $P < 0.005$; Fig. 4).

Larval Behaviors

FEJ larvae had a significantly lower mean feeding rate, pupation height, and foraging path length than the JB larvae (Table 5). The fraction of diggers (larvae digging > 5 mm into the medium during feeding) in the FEJ populations was also significantly less than in the JB controls (Table 5).

DISCUSSION

Although faster development has been successfully selected for in *Drosophila* (Zwaan et al. 1995; Nunney 1996; Chippindale et al. 1997), the relative contribution of different preadult stages to the response to selection has not been examined in detail. Because *Drosophila* larvae inhabit ephemeral habitats (such as rotting fruits) in the wild, faster development has been thought to have been under strong natural selection in wild populations (Clarke et al. 1961; Robertson 1963; Partridge and Fowler 1992). However, adaptation to ephemeral habitats will typically involve a need to deal with high larval density in addition to the need to complete development fast (Nunney 1990). Perhaps due to this confounding of selection pressures in ephemeral habitats, adaptation to larval crowding and selection for faster development in *Drosophila* have often implicitly been assumed to have similar evolutionary outcomes (Tantawy and El-Helw 1970; Wilkinson 1987; Santos et al. 1988; Prout and Barker 1989; Partridge and Fowler 1993; Borash et al. 2000). Of course, one of the major consequences of larval crowding in *Drosophila* cultures is that food runs out well before most individuals have attained the critical minimum size, thereby placing a heavy fitness premium on rapid development. Yet, our results suggest that the suites of traits that evolve in response to these seemingly similar selection pressures are actually strikingly different.

TABLE 3. ANOVA results for mean life-stage-specific dry weights.

Effect	df	MS	*F*	*P*
Selection	1	645.9	1278.3	<0.001
Block	3	9.2	—	—
Life stage	2	543.5	235.3	<0.001
Selection × block	3	0.5	—	—
Selection × life stage	2	0.9	0.7	0.523
Block × life stage	6	2.3	—	—
Selection × block × life stage	6	1.4	—	—

Life-Stage-Specific Development Time and Mortality

Selection for faster development in the FEJ populations has resulted in large changes in the temporal organization of preadult development. After 56 generations of selection, the pupal duration was substantially reduced and accounted for almost 33% of the total reduction in egg-to-eclosion development time (Fig. 1). This novel finding is in contrast to a previous observation that pupal duration did not change over

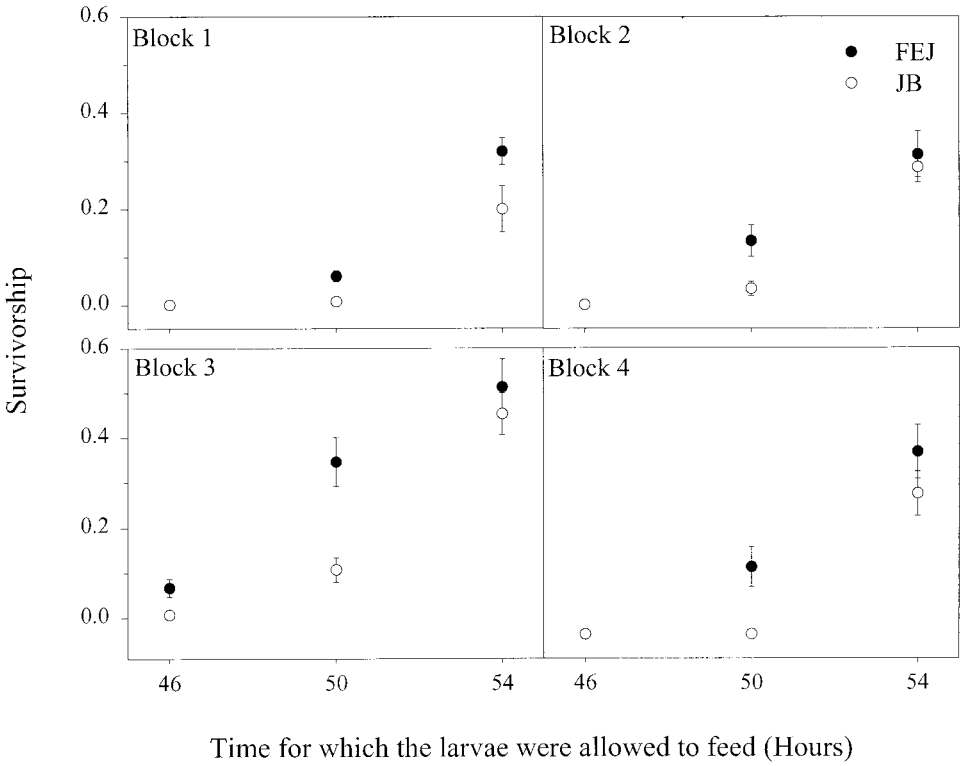

FIG. 4. Mean survivorship of the larvae of FEJ and JB populations after feeding for different periods of time. The error bars represent standard errors around the mean, constructed using the variation among replicate vials within populations.

36 generations of selection in populations successfully selected for faster development (Chippindale et al. 1997), even though substantial additive genetic variation for pupal duration in *Drosophila* has previously been demonstrated (Tantawy and El-Helw 1970). Because our flies share a common ancestry with those used by Chippindale et al. (1997), the lack of reduction in pupal duration in their study is somewhat surprising. However, one major difference between our selection protocol and that followed by Chippindale et al. (1997) may explain this apparent discrepancy. Chippindale et al. (1997) collected eggs from the flies as soon as enough eggs were available (within 24 h after eclosion), whereas our flies were held in cages for two full days posteclosion and supplied with yeasted food before eggs were collected, on the third day after eclosion, to start the next generation. Therefore, it is possible that our FEJ flies postpone or compensate for some aspect of development related to reproduction (e.g., ovary and ovariole maturation, sperm maturation) until after eclosion, thereby making a reduction of pupal duration evolutionarily possible. Indeed, the time taken from eclosion to first copulation is significantly greater in FEJ than in JB populations (M. Shakarad, N. G. Prasad, M. Rajamani, A. Joshi, unpubl. data), which is opposite of what was seen by Chippindale et al. (1997).

TABLE 4. ANOVA results for mean survivorship in the critical minimum feeding time assay.

Effect	df	MS	*F*	*P*
Selection	1	0.098	27.045	0.014
Block	3	0.070	—	—
Time	2	0.685	199.998	<0.001
Selection × block	3	0.004	—	—
Selection × time	2	0.026	8.084	0.020
Block × time	6	0.003	—	—
Selection × block × time	6	0.003	—	—

The reduction in the duration of the different instars in the FEJ populations was not symmetrical, with only the first and third instar duration being reduced after 56 generations of selection (Fig. 1). We have also observed that there are no significant differences between the egg hatching time and egg hatchability in the FEJ and JB populations (N. G. Prasad, unpubl. data). This finding is consistent with the observations of Chippindale et al. (1997) and indicates that the difference between the larval duration in the FEJ and JB populations is almost entirely due to reduced duration of the first and third larval instars in the FEJ populations. It is not clear at this time why the duration of the second larval instar did not respond to selection. Possibly, the first and third larval instars are predominantly feeding stages and a reduction in their duration, therefore, does not impose a strong mortality cost, at least early in the selection response. We have earlier speculated that preferential reduction of predominantly feeding phases in preference to preadult phases where major developmental changes are occurring may be the explanation for the reduced larval growth rate in FEJ populations (Prasad et al. 2000). Yet, studies on populations related to ours but selected for adaptations to larval crowding indicate that second instar larvae put on weight at a higher rate and have

TABLE 5. Population means (± SE) of larval behavioral traits in the selected (FEJ) and control (JB) populations. All mean differences between selection regimes were significant in one-way ANOVAs (*P*-values for the *F*-tests are shown in parentheses below each trait).

Selection	Population	Feeding rate (bits/min) ($P = 0.002$)	Pupation height (cm) ($P < 0.001$)	Path length (cm) ($P = 0.01$)	Fraction of diggers ($P = 0.011$)
FEJ	1	157.48 (5.72)	1.81 (0.21)	6.90 (0.74)	0.01 (0.01)
	2	142.60 (6.41)	1.29 (0.39)	3.60 (0.79)	0.00 (0.00)
	3	172.80 (6.27)	1.91 (0.25)	3.70 (0.60)	0.08 (0.03)
	4	125.48 (9.10)	0.96 (0.17)	3.90 (0.64)	0.03 (0.01)
JB	1	188.84 (5.41)	4.96 (0.25)	8.85 (1.14)	0.48 (0.04)
	2	186.92 (6.78)	4.55 (0.29)	7.55 (1.02)	0.30 (0.03)
	3	205.96 (5.84)	5.45 (0.49)	6.58 (0.82)	0.28 (0.05)
	4	153.68 (11.55)	4.18 (0.09)	5.93 (0.83)	0.31 (0.06)

higher feeding rates than first instar larvae (Santos et al. 1997). Of course, it is also possible that the duration of the second instar has already been optimized by selection in the wild. Studies on the larval weight gain over time and on instar specific mortality rates may help clarify this issue.

The preadult mortality cost to faster development that we observed in the FEJ populations was almost entirely due to larval mortality (Fig. 2), whereas the difference in preadult mortality between the selected and control populations of Chippindale et al. (1997) was evenly distributed over the larval and pupal stages, although there was no significant reduction in pupal duration. Chippindale et al. (1997) speculated that increased pupal mortality in their selected populations was due to decreased larval resource provisioning that affected some aspect(s) of pupal metabolism. Our results suggest an alternative possibility that the increased pupal mortality seen by Chippindale et al. (1997) was, in fact, due to some aspect(s) of selection in the adult stage. Possibly, selection for reduced duration from eclosion to egg laying in the protocol of Chippindale et al. (1997) exacted a cost in pupal mortality, whereas in our FEJ populations the two-day holding period in cages before egg collection is buffering pupal survivorship. We suspect that this is a more likely explanation of the increased pupal mortality seen by Chippindale et al. (1997), because our FEJ populations have greatly reduced larval feeding rates (Table 5) and third instar FEJ larvae weigh substantially less than their JB counter parts (Fig. 3). If pupal mortality were causally related to reduced larval provisioning, the FEJ populations would also be expected to show higher pupal mortality than the JB controls. The exact reasons for increased larval and pupal mortality in populations that have evolved rapid development under selection are, however, not known at this time.

Larval Behavior and Minimum Critical Size

The observation of reduced larval feeding rate, foraging path length, pupation height, and the fraction of diggers in the FEJ populations (Table 5) is consistent with a scenario of the evolution of reduced energy expenditure and with the observation by Chippindale et al. (1997) of reduced pupation height in their accelerated development populations. This suite of evolved behaviors in the FEJ populations is also consistent with earlier observations that rover phenotypes have significantly higher pupation heights than sitter phenotypes (Sokolowski and Hansell 1983) and that populations that have evolved higher larval feeding rates under density-dependent selection show a greater frequency of rovers (Sokolowski et al. 1997).

However, Borash et al. (2000) have reported increased larval feeeding rates in the ACO and ACB populations of Chippindale et al. (1997), relative to their controls. Borash et al. (2000) interpret this result in terms of an earlier reported (Borash et al. 1998) polymorphism in populations of *Drosophila* adapted to very high larval density. In that study, the CU populations described by Joshi and Mueller (1996) were seen to consist of individuals falling into at least two categories. Individuals eclosing early from crowded larval cultures had high feeding rates and relatively poor egg-to-adult viability and tolerance to nitrogenous metabolic wastes, whereas individuals eclosing later had lower feeding rates, but higher viability and tolerance to metabolic wastes (Borash et al. 1998). Consequently, Borash et al. (2000) interpret the faster feeding rate of ACO and ACB populations of Chippindale et al. (1997) as reflecting a direct relationship between faster feeding and faster development.

Yet, other studies indicate that faster feeding does not result in faster development at low densities, such as those at which the ACO and ACB populations were reared. Neither the CU populations (Santos et al. 1997) nor the progeny of early eclosing flies from crowded CU populations (D. J. Borash, pers. comm.) exhibit faster development than controls, when assayed at low density. In fact, we suspect the reason for the faster feeding rate of the Chippindale et al. (1997) accelerated-development populations observed by Borash et al. (2000) is because they did not assay larvae of physiologically matched ages (the first authors of these papers also agree that this is a likely explanation: D. J. Borash, pers. comm.; A. K. Chippindale, pers. comm.). After 48 h from egg hatching, larvae from their accelerated populations would have been in mid-to-late third instar, whereas the control larvae were probably caught in very early third instar, right after molting, at which point feeding rates are low.

Our results show that the FEJ populations have evolved a smaller critical minimum feeding time, thereby attaining the critical size earlier than the JB controls (Fig. 4). The reduction in minimum feeding time, however, is only about 2 h, and our data do not allow any direct inference about the evolution of minimum critical size in the FEJ populations to be drawn. The results from the larval behavioral assays, however, do tend to rule out a simplistic explanation that the FEJ popu-

lations achieve the same critical size as the JB populations, but earlier, by simply feeding faster. The evolution of lower larval feeding rates (Table 5), lower larval growth rates (Prasad et al. 2000) in the FEJ populations, and the lower weight of FEJ third instar larvae (Fig. 3) suggest that the reduction in critical minimum feeding time is a reflection of reduced critical size in these populations.

Density-Dependent Selection and Selection for Faster Development

Selection for faster development and for adapting to larval crowding share some superficial similarity in that individuals failing to eclose before a certain point in time die, either because food runs out or because the experimenter does not include them in the pool of breeding adults. A comparison of results from density-dependent selection experiments and experiments in which shorter development time was selected for, however, makes it clear that the evolutionary outcomes of these two types of selection regime are very different. *Drosophila* populations maintained at very high larval densities evolve increased population growth rates at high density (Mueller and Ayala 1981), competitive ability (Mueller 1988), larval feeding rate (Joshi and Mueller 1988, 1996), pupation height (Mueller and Sweet 1986; Joshi and Mueller 1993, 1996), larval tolerance to metabolic waste (Shiostsugu et al. 1997; Borash et al. 1998), foraging path length (Sokolowski et al. 1997), and minimum food required for pupation (Mueller 1990; Joshi and Mueller 1996). When assayed at low larval densities, populations adapted to larval crowding do not differ from controls in egg to eclosion development time and survivorship or in adult dry weight at eclosion (Santos et al. 1997), although they exhibit greater fecundity, lipid content, and starvation resistance (D. J. Borash, pers. comm.).

Thus, *Drosophila* populations evolve enhanced competitive ability when evolving at high larval density, primarily by becoming better at acquiring food fast, even though this ability comes at the cost of decreased efficiency at converting food to biomass (Mueller 1990; Joshi and Mueller 1996), perhaps partly offset by greater efficiency at assimilating lipids. Larvae in such populations are also better able to withstand relatively high levels of metabolic waste, another aspect of life in crowded *Drosophila* cultures (Shiostsugu et al. 1997; Borash et al. 1998). In contrast, the evolution of reduced preadult development time in our FEJ populations is accompanied by increased preadult mortality (Fig. 2) and reduced feeding rate, pupation height, foraging path length, digging propensity (Table 5), and minimum food required for completion of development (Fig. 4).

The differences in the suite of traits that evolve under high larval density and under selection for fast development can be understood in terms of one fundamental aspect in which these selection regimes differ. At high larval densities there is a clear environmental signal, in the form of food running out, available to the larvae such that they can make the switch from feeding to pupation. Therefore, it is not necessary, in principle, for larvae to speed up the developmental process in terms of real time. What is probably more important in this context is for the larvae to acquire food faster than others, such that they attain the critical size for pupation before food runs out. Under truncation selection for faster development, however, there is no external signal available to larvae indicating that they need to switch from feeding to pupation. In this context, a speeding up of the developmental processes, such that an internal signal for pupation is triggered earlier in real time, is of crucial importance. We speculate that this is a likely explanation for why the overall intrinsic timing of developmental events is unchanged in populations adapted to larval crowding, whereas the FEJ populations exhibit large changes in the temporal organization of preadult development.

Overall, our results clearly illustrate that the density at which selection occurs can greatly affect the evolution of life-history traits. Selection for faster development imposed through food limitation at high density and direct selection for faster development in moderate-density food-rich conditions lead to the evolution of entirely different suites of traits. Thus, larval and adult densities need to be controlled when performing selection experiments, and some knowledge of density is required when speculating about possible selection pressures in wild populations. It is also apparent that a relaxation of selection pressures on adult life-history traits can greatly affect the response of preadult traits to selection acting on the preadult life stages. Thus, relatively relaxed selection on reproduction very early in adult life in the FEJ populations appears to have permitted the evolution of a substantially reduced pupal duration in contrast to the results of Chippindale et al. (1997). Thus, even when selection pressures acting on development time are similar, differences in early adult life expectancy could yield different responses to selection. In organisms undergoing complete metamorphosis, where larval provisioning is a major determinant of adult resource reserves, it is intuitively obvious that selection acting on preadult life stages can profoundly affect the responses of adult life-history traits to selection acting upon them. There is substantial empirical evidence for such genetic constraints on life-history evolution that exert their effects in the direction of the unfolding of the ontogeny (Partridge and Fowler 1992; Roper et al. 1993; Chippindale et al. 1994, 1996, 1997; Zwaan et al. 1995). Our results suggest that such constraints on life-history evolution can also exert their influence against the direction of the unfolding of the ontogeny, often leading to unexpected and counter intuitive correlated responses to selection.

Acknowledgments

We thank N. B. Jyothi, D. N. Belavadi, M. Das, V. Sheeba, K. Shubha, K. Shankar Murthy, and N. Rajanna for help in the laboratory; D. J. Borash, L. D. Mueller, and A. K. Chippindale for much insightful discussion; and C. Lopez-Fanjul and two anonymous reviewers for helpful comments on the manuscript. L. D. Mueller also helped us find some of the relevant literature, and D. J. Borash kindly shared unpublished data with us. NGP thanks the Council for Scientific and Industrial Research, Government of India, for financial assistance in the form of a Junior Research Fellowship. This work was supported by funds from the Department of Science and Technology, Government of India.

Literature Cited

Ashburner, M. 1989. *Drosophila:* a laboratory handbook. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bakker, K. 1961. An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. Archs. Neerl. Zool. 14:200–281.

Borash, D. J., A. G. Gibbs, A. Joshi, and L. D. Mueller. 1998. A genetic polymorphism maintained by natural selection in a temporally varying environment. Am. Nat. 151:148–156.

Borash, D. J., H. Teotonio, M. R. Rose, and L. D. Mueller. 2000. Density dependent natural selection in *Drosophila*: correlations between feeding rate, development time and viability. J. Evol. Biol. 13:181–187.

Chippindale, A. K., D. T. Hoang, P. M. Service, and M. R. Rose. 1994. The evolution of development in *Drosophila melanogaster* selected for postponed senescence. Evolution 48:1880–1899.

Chippindale, A. K., T. J. F. Chu, and M. R. Rose. 1996. Complex trade-offs and the evolution of starvation resistance in *Drosophila.* Evolution 50:753–766.

Chippindale, A. K., J. A. Alipaz, H.-W. Chen, and M. R. Rose. 1997. Experimental evolution of accelerated development in *Drosophila.* 1. Developmental speed and larval survival. Evolution 51:1536–1551.

Clarke, J. M., J. Maynard Smith, and K. C. Sondhi. 1961. Asymmetrical response to selection for rate of development in *Drosophila subobscura.* Genet. Res. 2:70–81.

De Moed, G. H., C. L. J. J. Kruitwagen, G. de Jong, and W. Scharloo. 1999. Critical weight for the induction of pupariation in *Drosophila melanogaster:* genetic and environmental variation. J. Evol. Biol. 12:852–858.

Freeman, M. F., and J. W. Tukey. 1950. Transformations related to angular and the square root. Ann. Math. Stat. 21:607–611.

Godoy-Herrera, R. 1994. Biometrical analysis of larval digging behaviour in *Drosophila melanogaster*. Behav. Genet. 24:427–432.

Harshman, L. G., and A. A. Hoffmann. 2000. Laboratory selection experiments using *Drosophila:* what do they really tell us. Trend. Ecol. Evol. 15:32–36.

Ives, P. T. 1970. Further studies of the South Amherst population of *Drosophila melanogaster*. Evolution 38:507–518.

Joshi, A. 1997. Laboratory studies of density-dependent selection: adaptations to crowding in *Drosophila melanogaster.* Curr. Sci. 72:555–561.

Joshi, A., and L. D. Mueller. 1988. Evolution of higher feeding rate in *Drosophila* due to density-dependent natural selection. Evolution 42:1090–1093.

———. 1993. Directional and stabilizing density-dependent natural selection for pupation height in *Drosophila melanogaster*. Evolution 47:176–184.

———. 1996. Density-dependent natural selection in *Drosophila:* trade-offs between larval food acquisition and utilization. Evol. Ecol. 10:463–474.

Mueller, L. D. 1988. Evolution of competitive ability in *Drosophila* by density-dependent natural selection. Proc. Natl. Acad. Sci. USA 85:4383–4386.

———. 1990. Density-dependent natural selection does not increase efficiency. Evol. Ecol. 4:290–297.

———. 1997. Theoretical and empirical examination of density dependent selection. Annu. Rev. Ecol. Syst. 28:269–288.

Mueller, L. D., and F. J. Ayala. 1981. Trade-off between *r*-selection and *K*-selection in *Drosophila* populations. Proc. Natl. Acad. Sci. USA 78:1303–1305.

Mueller, L. D., and V. F. Sweet. 1986. Density-dependent natural selection in *Drosophila:* evolution of pupation height. Evolution 40:1354–1356.

Nunney, L. 1990. *Drosophila* on oranges: colonization, competition, and coexistence. Ecology 71:1904–1915.

———. 1996. The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: an example of a fitness trade-off. Evolution 50:1193–1204.

Partridge, L., and K. Fowler. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46:76–91.

———. 1993. Responses and correlated responses to artificial selection on thorax length in *Drosophila melanogaster.* Evolution 47:213–226.

Prasad, N. G., M. Shakarad, V. M. Gohil, V. Sheeba, M. Rajamani, and A. Joshi. 2000. Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time. Genet. Res. 76:249–259.

Prout, T., and J. S. F. Barker. 1989. Ecological aspects of the heritabiltiy of body size in *Drosophila buzzatii.* Genetics 123: 803–813.

Reznick, D. N., and C. Ghalambor. 1999. Sex and death. Science 286:2458–2459.

Robertson, F. W. 1963. The ecological genetics of growth in *Drosophila.* 6. The genetic correlation between the duration of the larval period and body size in relation to larval diet. Genet. Res. 4:74–92.

Roper, C., P. Pignatelli, and L. Partridge. 1993. Evolutionary effects of selection on age at reproduction in larval and adult *Drosophila melanogaster.* Evolution 47:445–455.

Rose, M. R., T. J. Nusbaum, and A. K. Chippindale. 1996. Laboratory evolution: experimental wonderland and the cheshire cat syndrome. Pp. 221–241 *in* M. R. Rose and G. V. Lauder, eds. Adaptation. Academic Press, San Diego, CA.

Santos, M., A. Ruiz, A. Barbadilla, J. E. Quezada-Diaz, E. Hasson, and A. Fontdevila. 1988. The evolutionary history of *Drosophila buzzatii.* XIV. Larger flies mate more often in nature. Heredity 61:255–262.

Santos, M., D. J. Borash, A. Joshi, N. Bounlutay, and L. D. Mueller. 1997. Density-dependent natural selection in *Drosophila:* evolution of growth rate and body size. Evolution 51:420–432.

Sewell, D., B. Burnet, and K. Connolly. 1975. Genetic analysis of larval feeding behavior in *Drosophila melanogaster*. Genet. Res. 24:163–173.

Sheeba, V., N. A. A. Madhyastha, and A. Joshi. 1998. Oviposition preference for novel *versus* normal food resources in laboratory populations of *Drosophila melanogaster*. J. Biosci. 23:93–100.

Shiotsugu, J., A. M. Leroi, H. Yashiro, M. R. Rose, and L. D. Mueller. 1997. The symmetry of correlated responses in adaptive evolution: an experimental study using *Drosophila.* Evolution 51:163–172.

Sokolowski, M. B., and R. I. C. Hansell. 1983. Elucidating the behavioral phenotype of *Drosophila melanogaster* larvae: correlations between larval foraging strategies and pupation height. Behav. Genet. 13:267–280.

Sokolowski, M. B., H. S. Pereira, and K. Hughes. 1997. Evolution of foraging behavior in *Drosophila* by density-dependent selection. Proc. Natl. Acad. Sci. USA 94:7373–7377.

Statsoft. 1995. Statistica. Vol. I. General conventions and statistics I. Statsoft, Inc., Tulsa, OK.

Tantawy, A. O., and M. R. El-Helw. 1970. Studies on natural populations of *Drosophila.* IX. Some fitness components and their heritabilities in natural and mutant populations of *Drosophila melanogaster.* Genetics 64:79–91.

Wilkinson, G. S. 1987. Equilibrium analysis of sexual selection in *Drosophila melanogaster.* Evolution 41:11–21.

Zwaan, B. J., R. Bijlsma, and R. F. Hoekstra. 1995. Artificial selection for developmental time in *Drosophila melanogaster* in relation to the evolution of aging: direct and correlated responses. Evolution 49:635–648.

Corresponding Editor: C. López-Fanjul