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DENSITY-DEPENDENT NATURAL SELECTION IN *DROSOPHILA*: EVOLUTION OF GROWTH RATE AND BODY SIZE

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Abstract.—*Drosophila melanogaster* populations subjected to extreme larval crowding (CU lines) in our laboratory have evolved higher larval feeding rates than their corresponding controls (UU lines). It has been suggested that this genetically based behavior may involve an energetic cost, which precludes natural selection in a density-regulated population to simultaneously maximize food acquisition and food conversion into biomass. If true, this stands against some basic predictions of the general theory of density-dependent natural selection. Here we investigate the evolutionary consequences of density-dependent natural selection on growth rate and body size in *D. melanogaster*. The CU populations showed a higher growth rate during the postcritical period of larval life than UU populations, but the sustained differences in weight did not translate into the adult stage. The simplest explanation for these findings (that natural selection in a crowded larval environment favors a faster food acquisition for the individual to attain the same final body size in a shorter period of time) was tested and rejected by looking at the larva-to-adult development times. Larvae of CU populations starved for different periods of time develop into comparatively smaller adults, suggesting that food seeking behavior in a food depleted environment carries a higher cost to these larvae than to their UU counterparts. The results have important implications for understanding the evolution of body size in natural populations of *Drosophila*, and stand against some widespread beliefs that body size may represent a compromise between the conflicting effects of genetic variation in larval and adult performance.

Key words.—Body size, critical size, density-dependent selection, development time, *Drosophila melanogaster*, efficiency, feeding rate, growth rate, trade-offs.

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An important contribution to the theory of life-history evolution are the consequences of natural selection in a density-regulated population (Roughgarden 1979; Boyce 1984; Travis and Mueller 1989; Charlesworth 1994). Empirical testing of the theory of density-dependent selection, often called r- and K-selection (MacArthur and Wilson 1967; Boyce 1984), have rendered some contradictory results (cf. Luckinbill 1978; Mueller and Ayala 1981). This is probably not surprising since organisms with quite different life histories were used in the experiments. The studies with *Drosophila melanogaster* (Mueller and Ayala 1981; Mueller et al. 1991) yielded consistent results with the predictions: populations kept at low densities (r-populations) had higher rates of population growth when tested at low densities than populations kept at high density (K-populations), whereas the opposite was true when all populations were tested at high densities. However, Mueller (1990) and Joshi and Mueller (1996) have recently suggested a trade-off between larval competitive ability and efficiency at converting food into biomass when *Drosophila* populations have been maintained under crowded larval conditions. This suggestion is at odds with some basic predictions of theoretical models of density-dependent natural selection that take into account species-specific details (Mueller 1988a). This is an uncomfortable situation. Perhaps the relevant parameters are not properly incorporated into the models, or important additional phenomena have to be taken into account.

In holometabolous insects like *Drosophila*, the larval life stage is quite important. Many of the adaptations to different levels of larval crowding (Joshi and Mueller 1988, 1993; Guo et al. 1991; Mueller et al. 1993) involve changes in larval behavior and physiology that may impinge on other phases of the life cycle. For instance, successful selection for postponed senescence critically depends on larval densities, and high densities are generally required to produce a selection response (Clare and Luckinbill 1985; Luckinbill and Clare 1985, 1986; Service et al. 1988; Zwaan et al. 1991). Additionally, larval foraging and growth rate establish strong connections between adult and larval stages. Thus, in stocks artificially selected for body size (thorax length) a correlated response in larval development time has been demonstrated, with large lines taking longer to develop than unselected or small lines (Reeve 1954; Robertson 1960, 1963; Partridge and Fowler 1993; Santos et al. 1994). An analogous correlated response has been observed in populations artificially selected for late life reproduction (Partridge and Fowler 1992; Chippindale et al. 1994). Recently, Chippindale et al. (1996) have shown that larval lipid acquisition seems to play a major role in the evolution of adult starvation resistance. This large body of evidence clearly indicates that characters expressed at one life stage for which evolutionary responses have been achieved may nevertheless be genetically correlated with characters expressed at another life stage. To the extent that genetic variance for a trait limited to one life stage is dependent on the environmental conditions experienced by the other life stage(s), genotype-environment interaction can be a serious problem when inferring evolutionary links between characters from correlated responses to selection.

When food is limited, as in the case of a crowded larval environment, the competitive ability of *D. melanogaster* lar-

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vae is largely determined by their feeding rates, and therefore growth rates (Bakker 1961, 1969; Burnet et al. 1977; Joshi and Mueller 1988). Models of larval competition in *Drosophila* suggest that superior competitors, i.e., those individuals that have a higher growth rate, will enjoy increases in both viability and adult size in food-limited environments (Mueller 1988a). On the other hand, some verbal arguments sustained by direct empirical tests, and by quantitative genetic experiments, suggest that evolution in a crowded larval environment should favor individuals with shorter development time and, hence, smaller adult size (Wilkinson 1987; Santos et al. 1992a, 1994; Partridge and Fowler 1993). There is no logical contradiction here: the first suggestion concerns phenotypic size and the second genetic size. If both suggestions are correct, however, it would imply that genetically smaller but otherwise phenotypically larger flies are favored in crowded environments. A negative regression between body size of flies from the wild and their laboratory offspring, as found by Prout (1958), is clearly not an indication that this is true. It is most likely explained by genotype-environment interaction (Riska et al. 1989).

Here we are concerned with the evolutionary outcome of density-dependent natural selection on growth rate and body size in *Drosophila.* Clearly, growth rate, and its correlative development rate (assuming adult size is constant), are important characters in *Drosophila* life history, since small changes in their values may have very large effects on fitness (Lewontin 1965). Larval survival, and all the evolutionary relevant traits in the adult stage (female fecundity, male mating success, longevity, starvation resistance, body size, etc.), are closely connected to growth rate. Earlier selection experiments for rate of development in *Drosophila* proved it difficult to increase development rate (Hollingsworth and Maynard Smith 1955; Clarke et al. 1961), clearly suggesting a long history of strong directional selection in the base population. Some recent experiments, however, report a substantial increase in the rate of development of *D. melanogaster* together with "predicted" correlated responses in body size, namely slow-developing larvae ended with a larger adult size than fast-developing larvae (Zwaan et al. 1995; Nunney 1996). The base population in this experiment had a past history of uncrowded larval conditions, and the authors conclude that this environment favors large body size and slow development time. The situation, however, may not be so simple. It is well known that larval growing period in *D. melanogaster* can be divided in two: a flexible period of growth from egg-hatch to the attainment of a critical weight early in the third instar, and a fixed growth period from the critical weight to pupariation. At the critical stage larvae have grown to less than half their final achievable body weight (Bakker 1959; Church and Robertson 1966). There is no reason a priori to think that the developmental profiles of genotypes should be exactly the same in these two periods of larval growth. In fact, there are some detailed results suggesting that they are not (Ruiz-Dubreuil et al. 1996). This clearly complicates the "straightforward" links between growth rate, development rate, and body size.

There are plenty of examples in the *Drosophila* literature showing that body size may, or may not, be correlated with development rate (see, e.g., the different results in Robertson [1963, 1964] on the correlation of body size and development time in different diets, and the discussion in Chippindale et al. [1994] on the possible reasons for some divergent results in the correlations involving development time, longevity, starvation resistance, and body size). Genotype-environment interaction can obviously enhance or reverse some genetic correlations (Service and Rose 1985). However, some confusion could probably be avoided by distinguishing between evolutionary mechanisms that, to some extent, impinge on one or both periods of larval growth. We will show here that density-dependent natural selection increases growth rate during the postcritical period of larval growth without modifying the development time or the adult body size. By doing so, we provide additional evidence that supports the previous suggestions by Mueller (1990) and Joshi and Mueller (1996) that there is a trade-off between larval food acquisition and food conversion in populations of *Drosophila* kept at high larval densities.

Materials and Methods

Experimental Populations

All 10 populations used here were derived from the five B-populations of Rose (1984). These B populations had been maintained under standard laboratory conditions used in this study, for several hundred generations at effective populations of about 800–1200 (Mueller, unpubl. data). The new populations created from the Bs were named according to the larval and adult densities they were maintained at. The first letter of their names (either U or C) indicates the larval density (either uncrowded or crowded) and the second letter indicates the adult density. The five CU populations ($CU_i \ldots CU_5$) were started in January of 1990, and the five UU populations ($UU_1 \ldots UU_5$) were started in September 1991 (CU_i and UU_i were derived from B_i and hence are more closely related than either of the pairs CU_i and CU_j, or UUi and UU_j, $i \neq j$). All populations are maintained on banana-molasses food at 25°C (24:0 light:dark period) and uncontrolled humidity, and have a generation time of approximately three weeks. The CU populations are crowded as larvae (~1000–1500 eggs per 6-dram vial), whereas the UU populations are uncrowded as larvae (~60–80 eggs per 8-dram vial). The two sets of populations differ only in the degree of larval crowding they experience: adults of all populations are kept at a low density of approximately 50–60 flies per 8-dram vial on fresh food and transferred every other day for approximately one week. Prior to initiating a new generation, all the eclosed adults from a population are dumped into a Plexiglas cage ($25.5 \times 20 \times 14.4$ cm^3) and supplied with liberal amounts of live yeast paste for two days before egg collection. The number of breeding adults per population is typically well over 1000 flies. Complete details of the derivation and maintenance of these populations have previously been described by Joshi and Mueller (1996).

Collection of Larvae for Assays

Before the experimental assays described below were performed, all test populations were passed through two generations of identical rearing conditions to eliminate any en-

vironmental and residual nongenetic parental effects. These common rearing conditions consist of raising eggs at low density (50–60 per 8-dram vial) and collecting progeny at two-week intervals. Two days prior to egg collections the flies had been given liberal amounts of live yeast paste to avoid females retaining their eggs. Eggs for the experiment were collected over a two-hour period on petri dishes containing nonnutritive agar with a generous smear of live yeast. Immediately upon conclusion of the egg-laying period, the yeast was removed by gently washing the petri dishes with distilled water. These petri dishes were then incubated at 25°C for 20 h, whereupon newly hatched first instar larvae were collected for the various assays. There was no indication of differences in embryonic development between CU and UU populations.

Larval Feeding Rate

For each population, 20 larvae were assayed for feeding rate at 0, 12, 24, 36, 48, 60, and 72 h after egg-hatch, respectively. For all assays subsequent to hour 0, larvae were maintained at 25°C on petri dishes containing nonnutritive agar that had about 6 mL of yeast paste (37.6 g of dry yeast in 100 mL water) on the surface. To equalize rearing densities, six petri dishes were set up for each population, each of which contained 30 larvae. At every 12-h interval, 20 larvae from a single petri dish were assayed for feeding rate, and the petri dish was then discarded. Larval feeding rate was measured as the number of cephalopharyngeal retractions per minute, following techniques described by Joshi and Mueller (1988, 1996). Larvae were placed one at a time in a petri dish (9-cm diameter) containing agar coated with a thin layer of 10% yeast suspension (10 g of dry yeast in 100 mL water). After a 15-sec acclimation period, the number of cephalopharyngeal sclerite retractions was recorded for one minute under a dissecting microscope. Pairs of CU and UU populations, matched by subscripted indices, were assayed together, with one larva from each population being measured alternately. Due to a mishap in handling, the data for hour 12 included larvae from only four blocks (CU_2–CU_5, UU_2–UU_5).

It is obvious that the conditions under which feeding rates are measured are quite different than the actual vial environment in which evolution has proceeded. This opens up the possibility that the relative differences in feeding rates observed by these assay techniques will bear little resemblance to the relative feeding rates in the "natural" environment due to genotype × environment interactions. Feeding rates are of interest in the first place because of their correlation with fitness-related traits such as competitive ability. The relationship between larval feeding rates, as measured by the techniques described above, and competitive ability, measured by several independent techniques, has been confirmed several times by independent studies, in different laboratories with different *Drosophila* stocks (Burnet et al. 1977; Joshi and Mueller 1988). These results suggest that feeding rates collected here permit us to reliably rank fast and slow feeders (good and bad competitors) independently of the specific environment. There also have not been, to our knowledge, any detailed measurements of food consumed by larvae as a function of their feeding rate. While it is generally assumed that feeding rate and food consumed are positively correlated the exact relationship is not know, nor is it critical for this study.

Measurements of Larval Growth, Critical Size, and Development Time

Pairs of CU and UU populations, matched by subscripted indices, were assayed together in a randomized block experimental design. For each population, 45 newly hatched first instar larvae were placed onto petri dishes with nonnutritive agar that had 6 mL of yeast paste (37.6 g of dry yeast in 100 mL water; i.e., approximately 33.42 mg yeast/larva) on the surface. We found it more convenient to set up two series of petri dishes for each population: one series was used to record larval growth, and the other series to determine critical sizes; i.e., the ability of a larva to pupate and differentiate even though it is no longer allowed to feed. The total number of petri dishes was 30 (15 + 15) per population, placed at 25°C and randomly distributed on the same incubator shelf. At successive intervals of time, two petri dishes per population containing individuals of the same age were taken out of the incubator. Sixty larvae were removed from the yeast paste and gently rinsed with distilled water to clean any food adhering to the surface. Thirty larvae were transferred to an 8-dram vial with 5 mL of nonnutritive agar to determine whether they could pupate and eclose. These larvae were handled with care to avoid any injury, and were checked for activity before introducing the vial in the incubator at 25°C. The other 30 larvae were rolled on a paper towel to dry and were weighed together alive to the nearest 0.01 mg on a Mettler microbalance (model AT261). Afterward, they were dried for 36 h at 80°C, and weighed again. The emerging adults from the vials were counted, sexed, and weighed twice: alive and dried at 80°C. When considering the results of weight loss as a function of the starvation period between larval removal and puparium formation (see below), it should be kept in mind that the larvae were randomly distributed in the two sets of 30 larvae each. There is, therefore, no reason to believe that these two sets are inherently different.

After approximately 90 h in the petri dish, the larvae are close to pupation. Most of them go out from the yeast paste and pupate in the top cover or the lateral side of the petri dish. Two batches of 30 pupae each (~105 h) per population were placed in vials with nonnutritive agar and the eclosed adults were sexed and weighed twice (alive and dried at 80°C). The recorded weights were taken as the figures at the adult stage, and are the averages of 20–25 flies per sex weighed together. The pupal cases of these adults were also weighed.

Emerging adults from the agar vials were counted every six hours. Larva-to-adult development time was estimated as the average number of hours from egg-hatch (the time when first-instar larvae were placed in the petri dishes with the yeast paste) to adult emergence, where all females and males counted at a particular scoring were taken as having emerged at the midpoint in time between that scoring and the previous one.

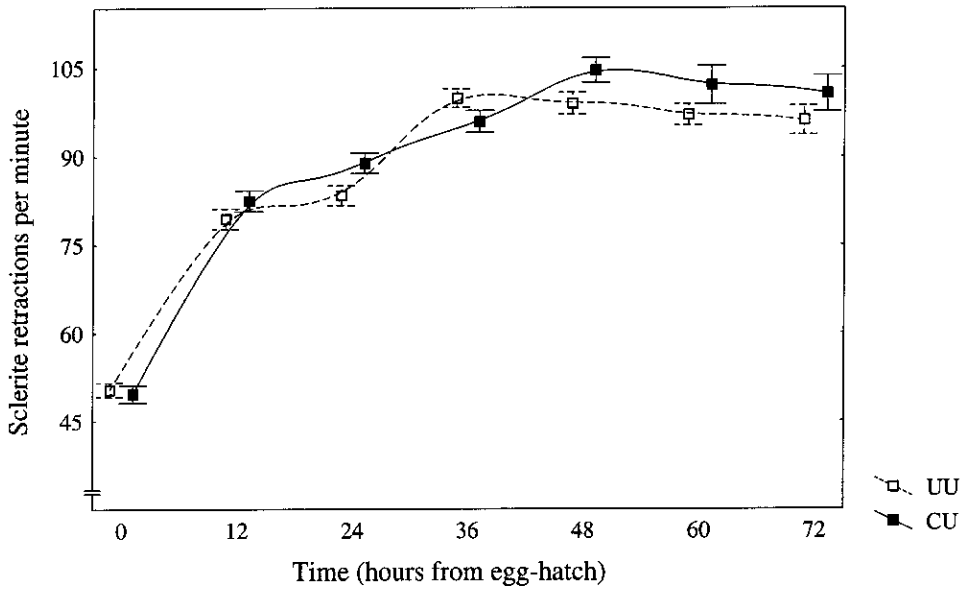

FIG. 1. The feeding rate, measured as sclerite retractions per minute, of larvae from the CU and UU populations at various times after egg-hatch. The error bars are 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least squares estimates of the standard errors of cell means in the randomized block ANOVA. The points have been connected by cubic spline interpolation to enhance visibility.

Statistical Analyses

STATISTICA® (1993) and SAS for Windows version 6.08 (1991) were used for the statistical analyses. The unit of analysis here is the population, and the pairs of populations assayed together were treated as random blocks in the analyses of variance. Each data point of wet weight or dry weight is obtained from statistically independent groups of individuals, and a completely randomized block design rather than a repeated measured design is more appropriate in the present case.

RESULTS

Larval Feeding Rates

In general, feeding rates of larvae increased with time for both CU and UU populations, especially during the first 36 hours from egg-hatch (Fig. 1). Overall, the feeding rate of CU larvae was greater than their UU counterparts, although many of the differences in the earlier phases of larval growth were not statistically significant. At 60 and 72 h from egg-hatch, the CU larvae were feeding significantly faster than the UU larvae, and the difference seemed to be increasing with time (Fig. 1). This pattern is reflected in the significant effects of time, selection (CU vs. UU), and the selection-by-time interaction in the ANOVA (Table 1). The block effects confound two possible sources of variation: experimental conditions that vary over time and ancestry effects since both CU_i and UU_i populations are derived from a common ancestor. Our own feeling is that most of this variation is due to the differences in experimental conditions rather than ancestry. There are two reasons for this belief. First, we have seen the absolute feeding rates of larvae occasionally vary noticeably in different experiments for no apparent reason other than environmental factors we have been unable to control. Second, in other experiments in our laboratory (Joshi and Mueller, unpubl. data) we have seen that the effects of ancestry on feeding rates is detecable for a few generations, but is then quickly overwhelmed by the effects of the environment the populations evolve in. Since the CU and UU populations are many dozens of generations removed from their common ancestor, we think it unlikely that the block effects reflect such ancestry.

TABLE 1. Analysis of variance for larval feeding rate. The analysis was done on the measurements of sclerite retraction rate on 20 individual larvae from the different populations (data plotted in Fig. 1). Selection: selection regime, CU or UU.

Source	df	MS	*F*	*P*
Block	4	12950.928	52.71	< 0.001
Selection	1	8674.298	13.89	0.020
Time	6	67604.585	11.07	< 0.001
Block × selection	4	624.645	2.54	0.038
Block × time	23	6105.989	24.85	< 0.001
Selection × time	6	1269.794	3.75	0.010
Block × selection × time	23	338.395	1.38	0.110
Error	1306	245.683		

Growth Curves: Wet Weight

Figure 2 shows the wet weight increase for the 10 populations. All the populations show the same basic pattern: an approximately exponentially shaped growth curve during the first 72 hours of larval life, when they are about to reach the maximum wet weight, followed by a decline in wet weight to pupariation. The patterns agree quite well with those previously described by Bakker (1959) and Church and Robertson (1966) for different populations of *D. melanogaster* growth at 25°C.

Even though qualitatively similar, the ANOVA revealed

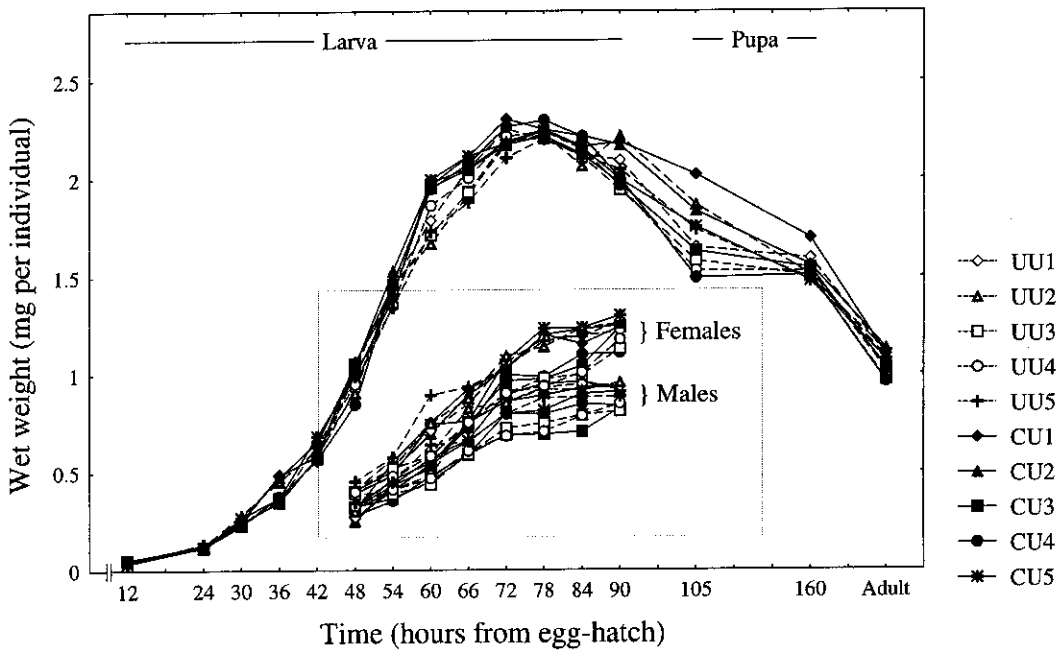

FIG. 2. Average wet weight per individual during growth in each of the 10 populations subjected to different regimes of larval crowding. "Adult" refers to the average of females and males that emerged from 105-hour pupae placed into agar vials. Embodied in the figure are the average weights of females and males, separately, which had emerged from the puparia formed by larvae that were allowed to feed for different periods of time.

significant quantitative effects of the selection regime by time interaction on wet weight (Table 2). To graphically illustrate the differences, Figure 3 shows the average wet weights for each selection regime, together with deviation histograms indicating the number of blocks where $CU_i > UU_i$, or $CU_i < UU_i$, at each period of time. After 54–60 h of larval growth, CU larvae tend to be consistently heavier than UU larvae. This trend remains until the larvae are close to pupation, when there are no longer differences between the two selection regimes. Comparisons of average adult wet weights indicated no statistically significant differences between selection regimes ($F_{1,4} = 0.003$; $P = 0.960$) when larvae are allowed to feed until pupariation.

Figure 2 shows the average wet weights of adults that had emerged from the puparia formed by larvae that were allowed to feed in the yeast paste for different periods of time. The first thing to notice is that no single adult emerged before 48 hours of larval life. This was expected because it has long been known (Beadle et al. 1938; Bakker 1959, 1961; Robertson 1963) that larvae must be allowed to grow to a minimum size, which corresponds to a critical stage in development early in the third instar, before they acquire the capacity to pupate and differentiate. In *D. melanogaster*, the second molt occurs between 42 and 48 hours at 25°C (Demerec 1950; Bakker 1959). This result was confirmed here by removing from the yeast paste 10–12 larvae per population after 48 hours of growth and looking at their mouthparts. All but one larva (in population CU_4) had a row of several teeth in their mandibular hooks, characteristic of larvae in the third instar (Demerec 1950).

TABLE 2. Analysis of variance for larval and pupa wet weight measured at different times after egg-hatch (data plotted in Fig. 2). Selection: selection regime, CU or UU.

Source	df	MS	*F*	*P*
Block	4	0.0220		
Selection	1	0.0782	25.97	0.007
Time	14	6.7911	1144.52	< 0.001
Block × selection	4	0.0030		
Block × time	56	0.0059		
Selection × time	14	0.0084	3.44	< 0.001
Block × selection × time	56	0.0024		

Interestingly, we observed a statistically significant selection regime by time interaction when the wet weights of the adults emerged from the larvae starved for different periods of time were compared ($F_{7,28} = 2.43$; $P = 0.044$, results not shown). CU larvae tend to metamorphose into smaller adults if starved before 66 hours (and obviously not before 48 h) of larval growth when food is abundant. This pattern is more dramatically illustrated in Figure 4, where we plot the wet weight loss during the starvation periods (i.e., the difference between the wet weight midpoint of the larvae and the wet weight midpoint of the adults produced by the larvae placed into the agar vials). Even though CU larvae are able to sustain a greater growth rate than UU larvae (Fig. 3), they lose proportionally more weight during the starvation period and/or the puparium stage. This is true for both females and males, and no statistically significant selection regime by sex interaction is detected in the ANOVA (Table 3).

In all data presented here, both sexes were grouped until the adult stage. Each data point in Figure 3 is the average of 30 × 5 individuals, and no indication was found of an influence of feeding period on the sex ratio of emerging adults (see below). It seems, therefore, reasonable to conclude that

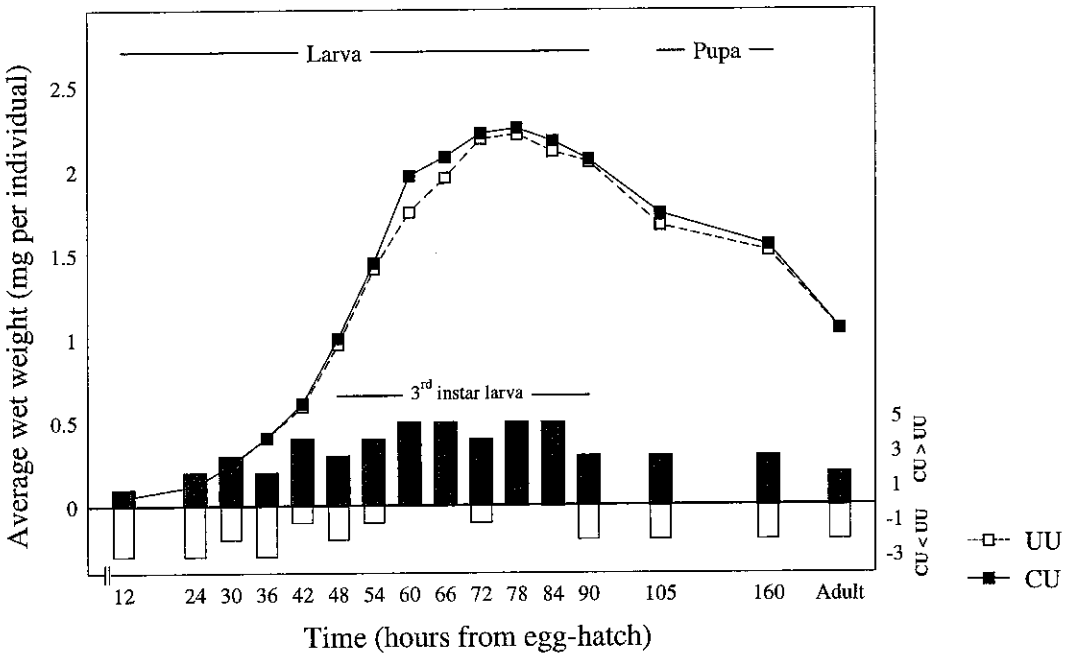

FIG. 3. Average wet weight over the five replicate UU or CU populations. The deviation histograms indicate the number of times when $CU_i > UU_i$ or $CU_i < UU_i$ ($i = 1, 2, \ldots, 5$).

the differences we have detected between the CU and UU populations are indeed real and not due to any consistent bias in the sex ratio between selection regimes. In fact, we found consistent differences in weight loss between CUs and UUs regardless the sex of the flies (Fig. 4). The statistically significant effect of sex in Table 3 simply reflects the fact that females were always heavier than males, although the sexual dimorphism was reduced in adults emerging from larvae that had fed only 48–78 h approximately.

Growth Curves: Dry Weight

All data concerning the dry weights are shown in Figures 5, 6, and 7. Dry weight increases up to 90 hours, and there is a slight decrease in dry weight of the puparium, which may be attributable to the energetically costly process of metamorphosis (Keister and Buck 1974).

The dry weight differences between the CU and UU populations are even clearer than the differences in wet weights. Throughout most of the third-instar period CU larvae were consistently heavier than UU larvae, but this difference disappeared during the puparium stage (Fig. 6 and Table 4). As before, no statistically significant differences between selection regimes were found in the weight of adults raised from larvae allowed to feed until pupariation ($F_{1,4} = 0.387$; $P = 0.568$). We did not detect any difference in the weight of pupal cases ($F_{1,4} = 0.178$; $P = 0.695$, results not shown), and the higher decrease in dry weight of the CU puparia might reflect a higher metabolic cost during metamorphosis.

Figure 7 plots the dry weight loss during the starvation periods, and Table 5 gives the corresponding ANOVA results. The patterns are similar to those shown in Figure 4 for wet weight, but now there seems to be a sustained dry weight loss all through starvation periods because the selection regime by time interaction was only marginally significant ($P = 0.063$).

Critical Sizes

Only larvae that had stayed on the food for 48 hours or longer were able to pupate and differentiate into adults. It is interesting to note that a few tiny puparia were observed in some 42-h vials, but they did not further develop to the adult stage. Figure 8 shows the proportion of flies raised from the larvae transferred to vials with nonnutritive agar at different periods of time. The survivorship data were transformed using the arcsine square-root transformation (Sokal and Rohlf 1981) and subjected to a three-way ANOVA with time and selection regime (CU vs. UU) as fixed effects, and block as random. Time was the only statistically significant effect ($F_{8,32} = 23.90$; $P < 0.001$).

Figure 9 shows the proportion of males in the emerging adults. The points are scattered around 50%, and no indication was found for a departure from the 1:1 sex ratio due to selection regime (CU vs. UU) or feeding period.

Development Times

Larva-to-adult development times are plotted in Figure 10. Some retardation was observed in the larvae that had fed only 48 hours. This was mainly due to a few flies in each of those vials that took a relatively long time before emerging. However, no retardation in successful development to adult stage was detected when the larvae fed 54 hours or longer. Our observations were in general agreement with those of Bakker (1959).

There were no consistent differences in the development times between selection regimes (CU vs. UU) or sexes, and time was the only statistically significant effect (Table 6).

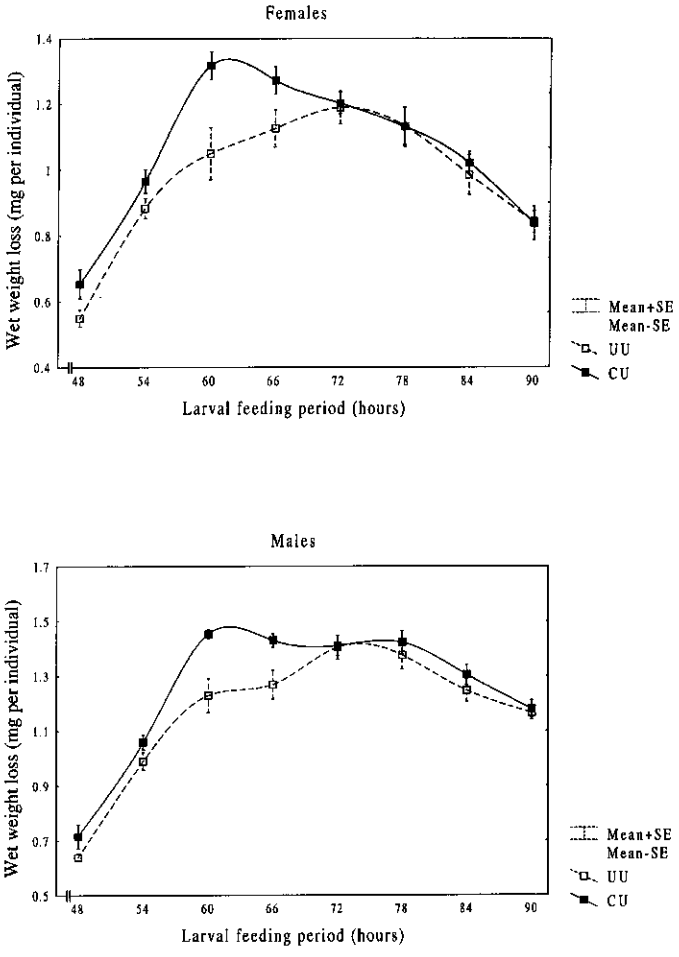

FIG. 4. Wet weight loss during the different starvation periods. The points have been connected by cubic spline interpolation to enhance visibility. The weight loss equals the difference between the wet weight of larvae at a particular age and the wet weight of adults raised without food from that age (see text).

TABLE 3. Analysis of variance for wet weight loss during the different starvation periods (data plotted in Fig. 4). Selection: selection regime, CU or UU.

Source	df	MS	*F*	*P*
Block	4	0.0987		
Selection	1	0.2637	31.45	0.005
Sex	1	1.5419	246.23	< 0.001
Time	7	1.0355	61.54	< 0.001
Block × selection	4	0.0084		
Block × sex	4	0.0063		
Block × time	28	0.0168		
Selection × sex	1	0.0002	0.03	0.869
Selection × time	7	0.0344	4.80	0.001
Sex × time	7	0.0407	31.77	< 0.001
Block × selection × sex	4	0.0070		
Block × selection × time	28	0.0072		
Block × sex × time	28	0.0013		
Selection × sex × time	7	0.0011	0.83	0.571
Block × selection × sex × time	28	0.0013		

DISCUSSION

The suggested trade-off between larval food acquisition and utilization when *Drosophila* populations are subjected to extreme larval crowding (Mueller 1990; Joshi and Mueller 1996) was the point of departure for the present experiments. Of the characteristics determining food acquisition, the rate of feeding, which is largely genetically determined (Sewell et al. 1975; Burnet et al. 1977), is obviously the most important. Previous work on the CU populations, selected for larval crowding and related populations that had a crowded larval phase, had also documented increases in larval feeding rate (Joshi and Mueller 1988, 1996; Guo et al. 1991; Mueller et al. 1993). This trait is also very important in relation to larval competitive ability in crowded cultures, as has been repeatedly stressed by Bakker (1961, 1969) and other authors (Sewell et al. 1975; Burnet et al. 1977; Joshi and Mueller 1988; Mueller 1988b; Ruiz-Dubreuil et al. 1996). The possible mechanistic explanation for the trade-off, namely that decreased efficiency of food use in populations subjected to density-dependent natural selection is due to a faster passage of food through the gut, was recently tested and rejected by Joshi and Mueller (1996). These authors hypothesized that the elevated levels of larval activity expressed by the CU populations could translate into a higher energetic cost (Berrigan and Lighton 1993) and, in turn, into a lower efficiency of CU larva at utilizing food to complete development as compared to their UU counterparts. This scenario contradicts the verbal argument in MacArthur and Wilson (1967), and the more formal theoretical predictions in Mueller (1988a), that evolution in a high density environment should favor individuals more efficient at turning food into biomass.

The results in the present paper clearly show that faster feeding rates of CU larvae do translate into a greater larval weight after the second molt, when larvae have grown less than half their final achievable body weight. The greater weight of third instar CU larvae did not, however, carry over into the adult phase. A possible interpretation of these findings is simply that natural selection in a crowded larval environment favors a faster food acquisition for the individual to attain the same final body size in a shorter period of time. (Note that this suggestion is apparently supported by the data in Fig. 2, when CU and UU larvae have approximately the same wet weight after 90 h of larval growth. This could be indicative that CU larvae were closer to the puparium stage than UU larvae.) If true, this would conciliate somehow the theoretical predictions of density-dependent natural selection and the previously suggested trade-off between larval food acquisition and utilization in *Drosophila*. Body size is known to be positively correlated with adult fitness components (Santos et al. 1992b; Partridge and Fowler 1993 and references therein), and that strategy would allow the individual to gain a demographic advantage in increasing populations (e.g., Cole 1954; Lewontin 1965) without the costs associated at evolving a smaller adult soma. In many areas this could be very important, because *Drosophila* larvae may often be at suboptimal local densities when ephemeral resources are abundant (Atkinson 1979; Grimaldi and Jaenike 1984; Sevenster 1992). The available evidence, however, does not support the simple explanation, at least when CU and UU larvae develop under optimum growing conditions. We did not detect differences in larva-to-adult development time between

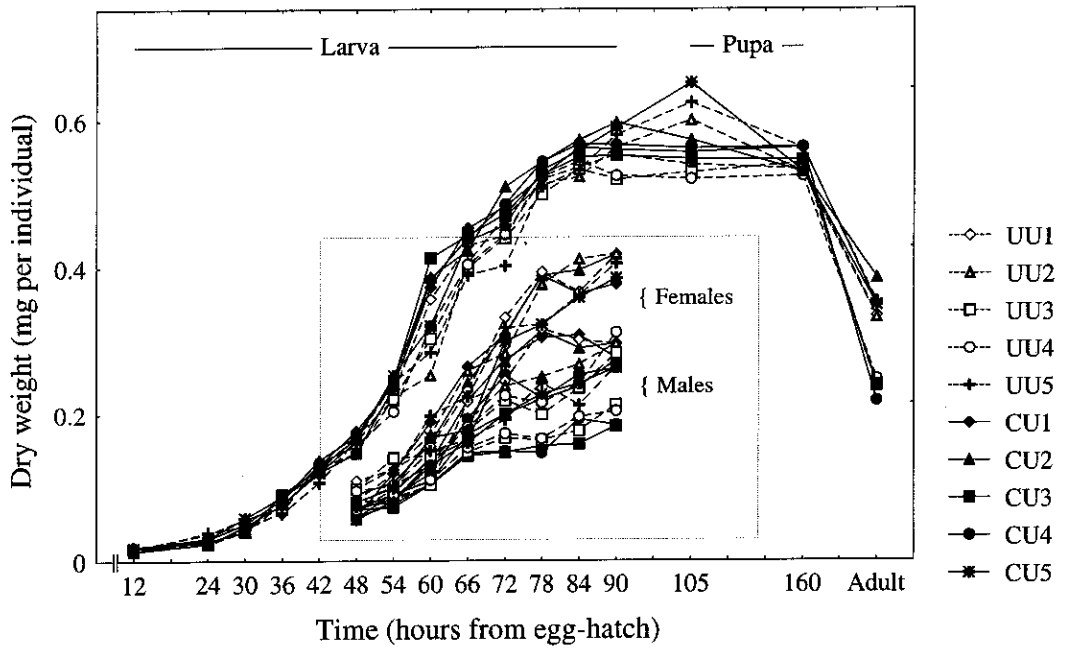

FIG. 5. Average dry weight per individual during growth in each of the 10 populations subjected to different regimes of larval crowding. "Adult" refers to the average of females and males that emerged from 105-hour pupae placed into agar vials. Embodied in the figure are the average weights of females and males, separately, which had emerged from the puparia formed by larvae that were allowed to feed for different periods of time.

selection regimes, and previous work with the same populations also failed to observe statistically significant differences in development time between the CUs and UUs (D. J. Borash, G. T. Ho, and L. D. Mueller, unpubl. data).

The lack of correlation between larval growth rate and the rate of development to arrive at the adult stage is a reasonable expectation when all the evidence from this and previous work is taken into account. The differences between CU and UU larvae in feeding rate are manifested in a time-dependent manner, becoming prominent after the final 30 hours or so of larval development, when the weight gain assays (Figs. 3, 6) show a consistent difference between selection regimes. The larvae of *D. melanogaster* must reach a critical body weight early in the third stage before they can pupate and

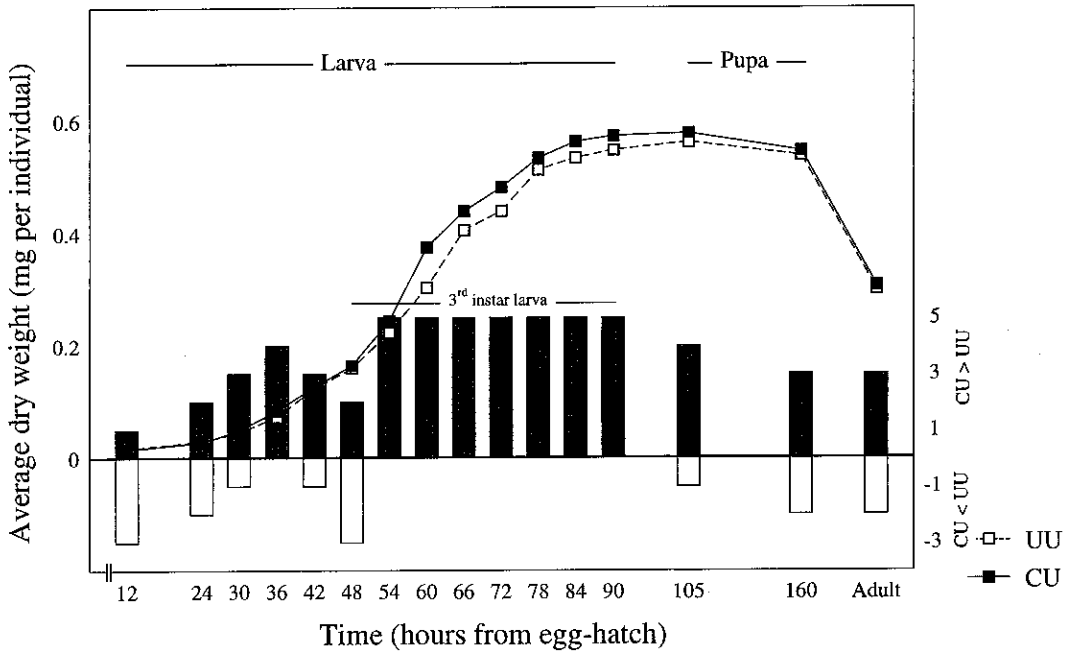

FIG. 6. Average dry weight of larvae and pupae over the five replicate UU (dashed lines) or CU (solid lines) populations. The deviation histograms indicate the number of times when $CU_i > UU_i$ (solid histograms) or $CU_i < UU_i$ (open histograms) ($i = 1, 2, \ldots, 5$).

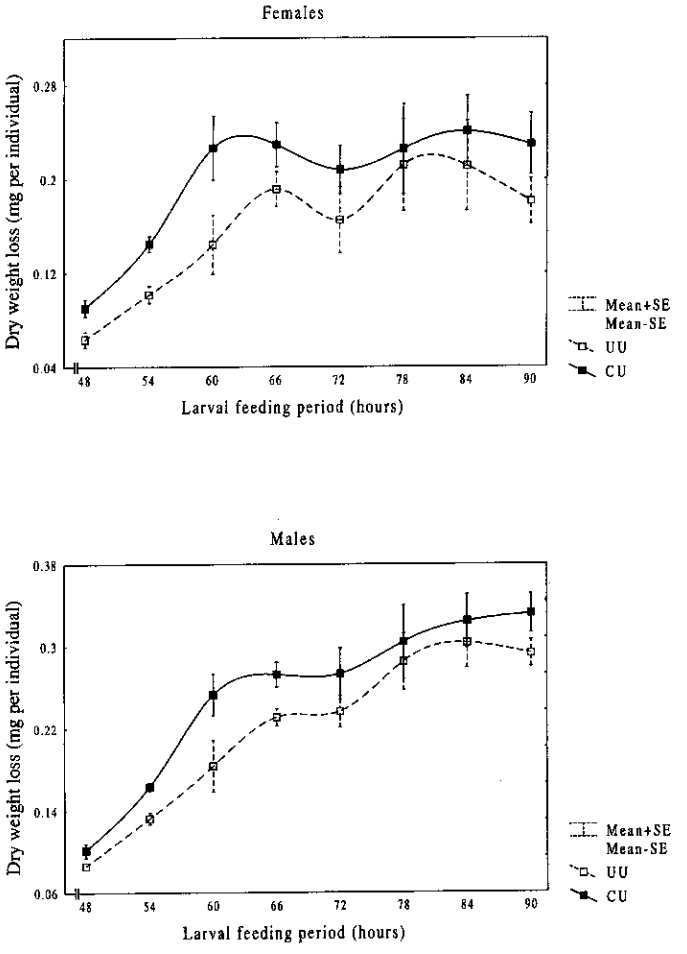

FIG. 7. Dry weight loss during the different starvation periods. The points have been connected by cubic spline interpolation to enhance visibility. The weight loss equals the difference between the dry weight of larvae at a particular age and the dry weight of adults raised without food from that age (see text).

differentiate (Bakker 1961; Robertson 1963; Fig. 2). The duration of the precritical period can be highly variable depending on the available resources but, after the critical weight is reached, hormonal changes lead to a fixed period of postcritical growth to pupariation (Bakker 1959; Robertson 1963; Burnet et al. 1977). Genetic differences in larval development time can be obtained by either: (1) increasing the critical weight to successful pupariation without altering the growth rate, as has been shown to happen when artificial selection for large body size is practiced (Robertson 1963; L. Partridge, R. E. Langelan, K. Fowler, and V. French, unpubl. data); or (2) by increasing the larval feeding rate at the flexible period of growth that precedes attainment of critical mass for pupation (Ruiz-Dubreuil et al. 1996). Neither the critical size or the precritical growth period seem to be modified under density-dependent natural selection. The critical sizes of CU and UU populations were not statistically different, although our results do not provide direct empirical evidence concerning the minimum amount of food necessary for successful development. More direct evidence was obtained by Joshi and Mueller (1996), who followed the methods originally described by Chiang and Hodson (1950) and

TABLE 4. Analysis of variance for larval and pupa dry weight measured at different times after egg-hatch (data plotted in Fig. 5). Selection: selection regime, CU or UU.

Source	df	MS	*F*	*P*
Block	4	0.0003		
Selection	1	0.0134	105.67	0.001
Time	14	0.4811	889.73	< 0.001
Block × selection	4	0.0001		
Block × time	56	0.0005		
Selection × time	14	0.0010	5.50	< 0.001
Block × selection × time	56	0.0002		

supplied a number of individual larvae with different amounts of food and recorded their survivorship. Their results, however, were not very clear-cut. CU larvae, selected for larval crowding, appeared to have a lower survival to pupation than UU larvae, but there were no differences in the fraction of larvae surviving to eclosion at any level of food used. Overall, CU and UU populations do not appear to be substantially different during the precritical growth period (up to 48 h of larval life; Figs. 2, 3) and reach the critical size at approximately the same time. This finding is in agreement with Robertson's (1963) conclusion that larval growth rate in the precritical period is maximized in any environment to which the population is adapted, and would be difficult or impossible to increase by selection (see also Burnet et al. 1977). However, in the fixed postcritical growth period, superior feeding rate in the CU populations leads to greater larval growth.

The results on weight loss during the starvation periods suggest that food seeking behavior in a nonnutritive or food depleted environment carries a higher cost to the CU larvae. Increased locomotory activity of larvae on agar has been observed by Green et al. (1983) and Ruiz-Dubreuil et al. (1996). The CU larvae exhibit higher levels of locomotory activity during foraging as compared to the UU (Sokolowski et al., unpubl.). Graf and Sokolowski (1989) compared the foraging behavior of rover (i.e., larvae with long foraging path lengths; see also Sokolowski 1980) and sitter (short path lengths) larvae on agar and yeast

TABLE 5. Analysis of variance for dry weight loss during the different starvation periods (data plotted in Fig. 7). Selection: selection regime, CU or UU.

Source	df	MS	*F*	*P*
Block	4	0.0387		
Selection	1	0.0557	65.54	0.001
Sex	1	0.1306	107.96	< 0.001
Time	7	0.0853	20.54	< 0.001
Block × selection	4	0.0009		
Block × sex	4	0.0012		
Block × time	28	0.0042		
Selection × sex	1	0.0004	2.74	0.173
Selection × time	7	0.0017	2.22	0.063
Sex × time	7	0.0053	13.92	< 0.001
Block × selection × sex	4	0.0002		
Block × selection × time	28	0.0008		
Block × sex × time	28	0.0004		
Selection × sex × time	7	0.00006	0.38	0.905
Block × selection × sex × time	28	0.0002		

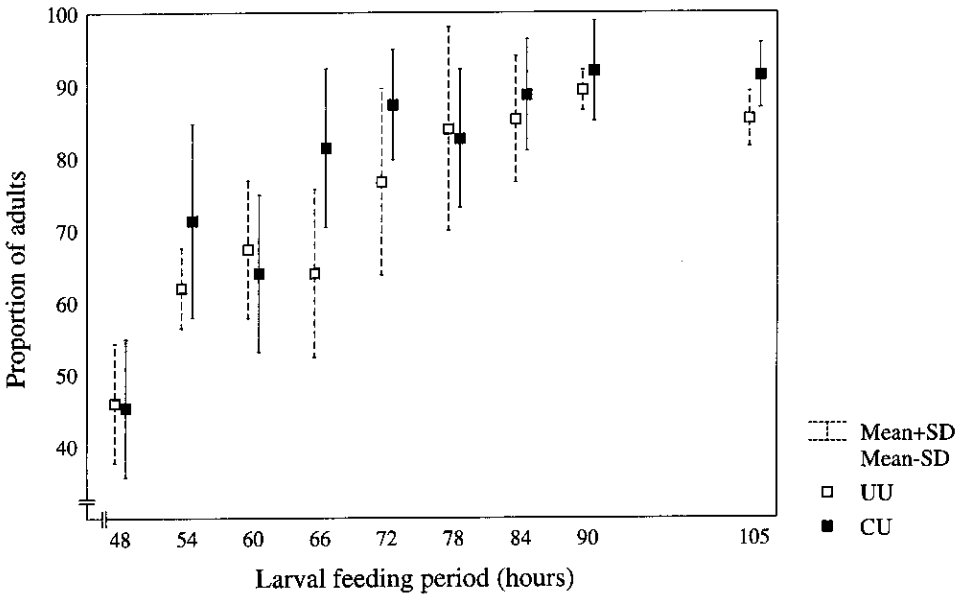

FIG. 8. Percentage of adults emerged from pupae formed by larvae with different feeding periods in the CU and UU populations.

at different stages of development. Greatest expression of the rover phenotype was observed in the postcritical period (72 and 96 h), but absence of food reduced its expression. We do not have data on levels of locomotory activity of CU and UU larvae in the absence of food. However, the highly significant selection regime by time interaction in Table 3 (see also Fig. 4) suggests that differences in the energetically costly locomotory behavior between CU and UU larvae remain or even increase when removed from food onto agar. In any case, larval behavior in the agar vials does not seem to be the only explanation for our findings. When larvae are allowed to remain in the food until the pupa stage, CU larvae are consistently heavier than their corresponding controls (Fig. 6). During the energetically costly process of metamorphosis, a higher decrease in dry weight of CU puparia results in no differences in initial adult size between selection regimes (CU vs. UU). Additional evidence that faster feeding rates, and hence higher growth rates, appear to have a cost comes from the observations that lowering larval density in CU populations results in an evolutionary decline of feeding rate over time (A. Joshi and L. D. Mueller, unpubl. data). In conclusion, the previous suggestion that CU larvae are less efficient at converting food into biomass because of the higher energetic costs associated with larval activity and growth (Joshi and Mueller 1996), seems sound.

Bakker (1961, 1969) suggested that genetic differences in feeding rates, and therefore growth rates, could entirely ex-

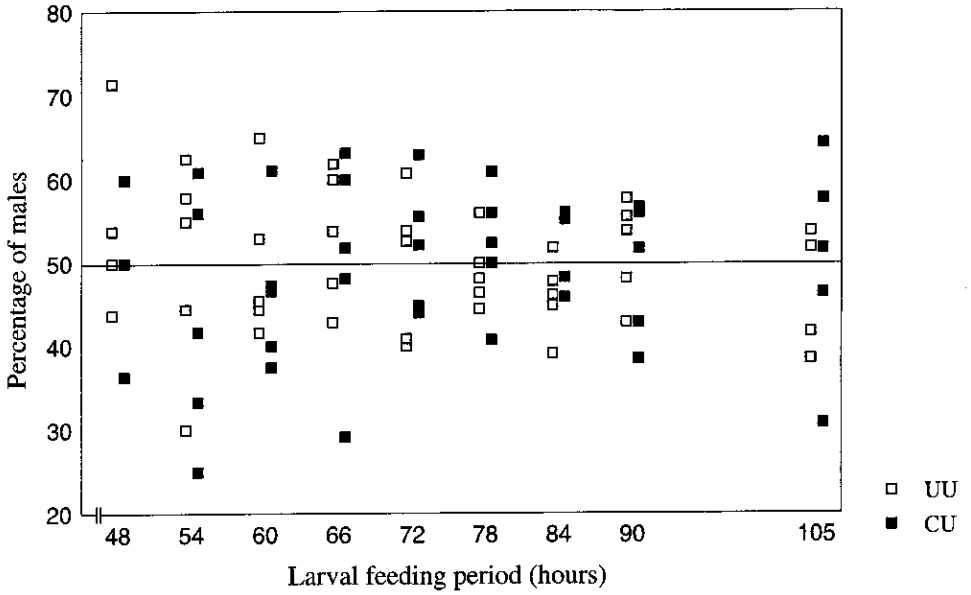

FIG. 9. Sex ratio of adults emerged from pupae formed by larvae with different feeding periods in the CU and UU populations.

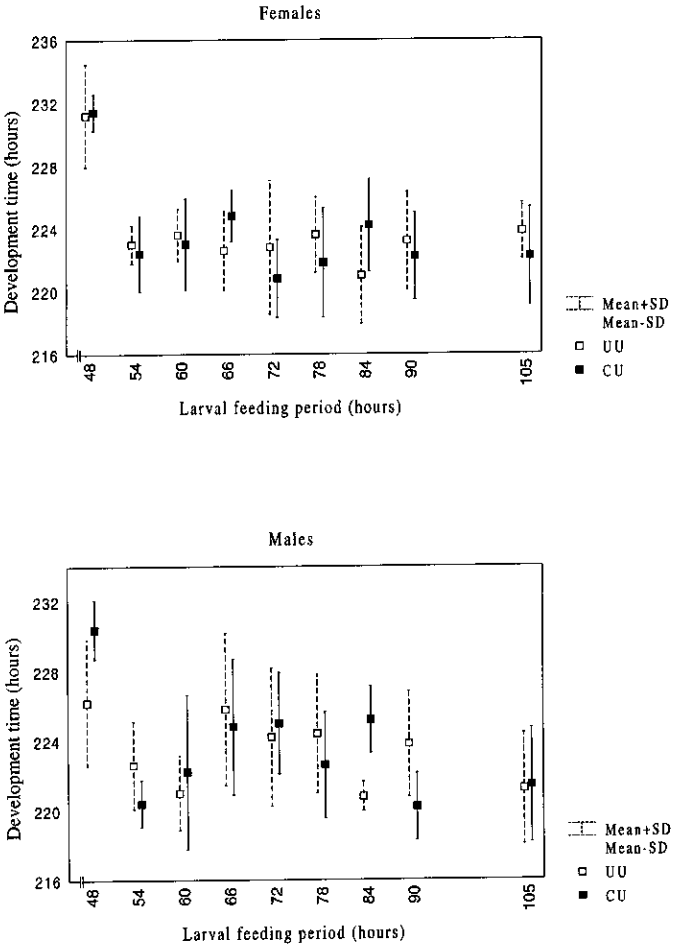

FIG. 10. Larva-to-adult development times of larvae allowed to feed for different periods for both sexes in the CU and UU populations.

TABLE 6. Analysis of variance for larva-to-adult development time in the different starvation periods (data plotted in Fig. 10). Selection: selection regime, CU or UU.

Source	df	MS	*F*	*P*
Block	4	3.9639		
Selection	1	0.0056	0.001	0.983
Sex	1	4.0500	0.32	0.602
Time	8	118.8431	18.14	< 0.001
Block × selection	4	11.9083		
Block × sex	4	12.6750		
Block × time	32	6.5514		
Selection × sex	1	2.4500	0.33	0.596
Selection × time	8	19.4181	2.16	0.059
Sex × time	8	16.9375	1.71	0.134
Block × selection × sex	4	7.4083		
Block × selection × time	32	9.0083		
Block × sex × time	32	9.9063		
Selection × sex × time	8	7.7125	1.08	0.401
Block × selection × sex × time	32	7.1396		

plain differences in competitive ability between *Drosophila* strains. Models of viability in food-limited environments consistent with Bakker's results have been developed by de Jong (1976) and Nunney (1983). In short, the basic parameters that determine competitive interactions in these models are the minimum amount of food necessary for survival and the probability of acquiring food per unit of time. Thus, it is possible to make exact predictions about the outcome of competition between CU and UU populations from the results in Joshi and Mueller (1996), and the information contained in the present work. Growth rates during the precritical period, and critical sizes to successful development, are approximately the same for CU and UU larvae, but adult size would be chiefly determined by the genetically determined growth rates and the available food supply during the postcritical period of larval life. The prediction is, therefore, that competition between CU and UU larvae for limited food would, other things being equal, mostly reduce adult size of UUs relative to adult size of CUs, with little effect on viability or development time as far as some food still remains for postcritical larval growth. However, a serious problem with this scenario is that it overlooks an important ingredient in crowded cultures: the increased concentrations of toxic metabolic wastes that have significant effects on viability and development time (Weisbrot 1966; Dawood and Strickberger 1969; Ménsua and Moya 1983; Botella et al. 1985; Moya and Botella 1985). An evolutionary increase in resistance to the effects of larval crowding has been reported in populations cultured at high larval density (Bierbaum et al. 1989; Mueller 1995). This adds an important source of variation that might preclude any straightforward interpretation on larval competitive ability. A possible experimental protocol to overcome this problem is to raise UU larvae in an environment that mimics the accumulation of metabolic wastes but that is otherwise uncrowded. If there is an evolutionary increase in resistance to metabolic wastes without undesired correlated responses in growth rate and critical size, these populations should probably be used as the appropriate controls in any competition experiment that looks at the effects of density-dependent natural selection in *Drosophila*.

Finally, our results also have important implications to the understanding of the evolution of body size in natural populations of *Drosophila*. There is a general belief that body size in *Drosophila* may reflect an evolutionary compromise between the conflicting effects of genetic variation in larval and adult performance, because large body size increases the fitness of adults, but also increases the larval critical size and, hence, development time, which results in a decrease in larval survival in crowded environments (Wilkinson 1987; Santos et al. 1992a, 1994; Partridge and Fowler 1993; but see Santos 1996). This prediction is clearly not met here. Evolution in a crowded larval environment does not result in genetically smaller adults, but in an increase of growth rate and a lower efficiency of larvae at utilizing food to complete development.

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