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# Density-dependent natural selection in *Drosophila*: trade-offs between larval food acquisition and utilization

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## Summary

Natural selection at high densities has often been postulated to favour the evolution of greater efficiency of food use. Contrary to this expectation, a previous study suggested the existence of a trade-off between larval feeding rate and efficiency at using food to complete larval development in populations of *Drosophila melanogaster* subjected to crowding for many generations. In this paper, we confirm the generality of such a density-dependent trade-off between food acquisition and utilization by demonstrating its occurrence in a new set of *Drosophila* populations subjected to extreme larval crowding. We suggest that such trade-offs between food acquisition and food use may represent a general phenomenon in organisms exhibiting scramble competition. We test and reject the possible mechanistic explanation that decreased efficiency of food use in faster-feeding larvae may merely be a consequence of a faster passage of food through the gut, leading to incomplete assimilation of nutrients and energy.

*Keywords*: *Drosophila melanogaster*; food acquisition; food utilization; efficiency; trade-offs; density-dependent selection

## Introduction

Ever since the discussion of the effects of natural selection in crowded environments by MacArthur and Wilson (1967), density-dependent selection has been recognized as an important force affecting the evolution of life histories and has become one of the major components of the theory of evolutionary ecology (Pianka, 1970; Anderson, 1971; Charlesworth, 1971; Clarke, 1972; Roughgarden, 1979; Boyce, 1984; Travis and Mueller, 1989). However, systematic empirical testing of predictions of the theory of density-dependent natural selection has been relatively rare (Luckinbill, 1978) and largely confined to one set of six laboratory populations of *Drosophila melanogaster* subjected to different levels of crowding (Mueller, 1991; Mueller *et al.*, 1993).

Most evolutionary ecology theory is motivated by observations of diversity in nature and seeks to explain that diversity within the neo-Darwinian framework. However, in order to test the specific predictions of the theory critically, it is desirable, if not crucial, to use carefully controlled laboratory systems in which specific ecological factors can be manipulated one or a few at a time, and their genetic effects over many generations unambiguously ascertained (Travis and Mueller, 1989). In any such study, the traits of interest are likely to be metric components of fitness. Such traits are often extremely sensitive to even fairly subtle variation in assay conditions (Leroi *et al.*, 1994).

Consequently, a primary concern about the interpretation of such laboratory studies is whether the observed evolutionary effects of specific environmental factors are, in fact, of sufficiently general occurrence as to be legitimately considered to constitute a definite pattern of responses to a particular form of selection, as opposed to being an artefact of the specific materials and methods employed in one series of experiments. It is only when observed patterns of responses to specific

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selective forces pass this test of robustness, that they can unambiguously be used in refining and expanding our theoretical understanding of evolutionary ecology.

One of the most detailed and systematic investigations of the evolutionary consequences of extreme crowding was carried out over several years by Mueller and his co-workers, using laboratory populations of *D. melanogaster* maintained at extreme densities for over 200 generations (reviewed in Mueller, 1991; Mueller *et al.*, 1993). This series of experiments identified several traits that had diverged between uncrowded and crowded populations (designated as *r* and *K* populations, respectively) due to density-dependent natural selection. Relative to the *r* populations, the *K* populations showed the following characteristics.

(1) Elevated population growth rates at high densities, but depressed growth rates at low densities (Mueller and Ayala, 1981; Mueller *et al.*, 1991).
(2) Increased size and viability under crowded larval conditions (Bierbaum *et al.*, 1989).
(3) Increased larval competitive ability (Mueller, 1988b).
(4) Increased larval feeding rates (Joshi and Mueller, 1988).
(5) Increased pupation height (Mueller and Sweet, 1986; Guo *et al.*, 1991; Joshi and Mueller, 1993).
(6) Increased minimum larval food requirement for pupation (Mueller, 1990).

The observation that *K* populations actually needed more food than *r* populations in order to pupate successfully was somewhat counter-intuitive and suggested a fundamental trade-off between larval food acquisition and utilization. The greater minimum food requirement in the *K* populations was not accompanied by an increase in the size of eclosing adults (Mueller, 1991), a trait positively correlated with female fecundity and, therefore, fitness (Mueller, 1987). It appeared that the *K* populations, though faster feeders than the *r* populations, were less efficient when it came to utilizing the food they ate (Mueller, 1990, 1991). This observation was contradictory to expectations from both verbal (MacArthur and Wilson, 1967) and mathematical (Mueller, 1988a) models. It should be noted that 'greater efficiency of food utilization' (*sensu* Mueller, 1990) implies the ability to complete development successfully, and to attain a given adult size, on relatively smaller amounts of food.

In this paper we report results from several experiments done on a set of populations subjected to low or high densities in order to test the robustness of the observations made on the *r* and *K* populations. These populations were derived from a different source than the *r* and *K* populations and have a different maintenance regime. If the same trade-off between larval food acquisition and utilization was exhibited by these new populations, it would be a strong indication that this trade-off represents a fundamental constraint affecting density-dependent evolution in *Drosophila*. We also test the hypothesis that this trade-off may merely be the result of a faster passage of food through the gut of individuals with high feeding rates, resulting in potentially lower levels of assimilation of nutrients and energy.

## Materials and methods

### *Experimental populations*

This study used two sets of five replicate populations that had each been subjected to differing levels of larval density for over 50 generations. All populations were maintained on banana-molasses food at 25°C and continuous light and had a generation time of approximately 3 weeks; the number of breeding adults per population was typically well over 1000 flies. The five crowded populations ($CU_1 \ldots CU_5$) were reared at densities of 1000 or more larvae per 6 dram vial (2.2 cm diameter × 8.4 cm height). Eclosing adults were collected daily from these vials and kept at a low

density of approximately 60–80 adults per 8 dram vial (2.4 cm diameter × 9.5 cm height). The five uncrowded populations ($UU_1 \ldots UU_5$) were reared at low larval densities of 60–80 larvae per 8 dram vial; eclosing adults were subjected to the same density as the CU populations. The two sets of populations thus differed only in the degree of larval crowding and were kept at low density as adults. Prior to initiating a new generation, all the eclosed adults from a population were dumped into a Plexiglass cage ($25.5 \times 20 \times 14.4$ cm$^3$) and supplied with liberal amounts of live yeast paste for 2 days before egg collection.

Both sets of populations were derived from the five *B* populations of Rose (1984), each *B* population being used as the progenitor of one CU and one UU population. Consequently, CU and UU populations bearing the same numerical subscript are more closely related to each other, as compared to other populations subjected to the same density regime.

*Collection of larvae for assays*

Prior to initiating the assays described below, all test populations were passed through two generations of identical rearing conditions in order to eliminate any differences between selected lines due to environmental or maternal effects. Eggs were collected from the adults of each population and placed in vials at low densities of 60–80 eggs per 8 dram vial. Eclosing adults from these vials were then collected into cages and allowed to lay eggs for 2–4 h on Petri dishes containing non-nutritive agar with a generous smear of live yeast paste. Immediately upon conclusion of the egg-laying period, pieces of agar with eggs on them were cut out and placed on fresh agar Petri dishes to ensure that hatching larvae would not have access to any food. These Petri dishes were then incubated at 25°C for 18–20 h, whereupon newly-hatched first instar larvae were collected for the various assays.

*Measurement of pupation height*

Pupation heights were measured in 8 dram vials containing 5 ml of banana-molasses food. Five such vials were set up for each population and 50 newly-hatched first instar larvae were placed into each vial. At the first sign of pupation, the vials were checked every 8 h and any eclosed adults found were discarded. This was done to prevent eclosing adults from laying eggs in the vials, because pupation height is strongly affected by larval density (Mueller and Sweet, 1986; Joshi and Mueller, 1993). At low densities, such as those used in this assay, eclosion occurs relatively synchronously over a short period of time. Therefore, the experiment was terminated 7 days after the first eclosion. At that point, pupation height was measured as the distance, to the nearest millimetre, from the surface of the medium to the point between the spiracles of the pupa (Mueller and Sweet, 1986).

*Measurement of larval feeding rate*

For each population, 50 newly-hatched first instar larvae were moved onto Petri dishes with non-nutritive agar that had a generous amount of yeast paste (37.6 g yeast in 100 ml water) on the surface. The larvae were allowed to feed on this yeast for 48 h. At that point, 25 larvae from each population were assayed for feeding rate, which was measured as the number of cephalopharyngeal retractions per minute (Joshi and Mueller, 1988). Larvae were placed one at a time onto a small Petri dish (3.5 cm diameter) containing agar coated with a thin layer of 10% yeast suspension (10 g yeast in 100 ml water). After a 15 s acclimation period, the number of cephalopharyngeal sclerite retractions was recorded for 1 min under a dissecting microscope. Previous studies of larval feeding rates typically had acclimation periods of 90 s (Sewell *et al.*, 1975; Joshi and Mueller, 1988; Mueller *et al.*, 1993). Reducing the acclimation period to 15 s, however, has no significant effect on the feeding rate (A. Joshi, unpublished data) and considerably reduces the time taken for the

assay. Pairs of CU and UU populations, matched by subscripted indices, were assayed together, with one larva from each population being measured alternately.

*Measurement of larval food passage time*

For each population, 50 newly-hatched first instar larvae were moved onto Petri dishes with non-nutritive agar that had a generous amount of yeast paste (37.6 g yeast in 100 ml water) on the surface. The larvae were allowed to feed on this yeast for 48 h. At that point, 50 larvae from each population were moved onto small Petri dishes (3.5 cm diameter) containing agar covered by 1 ml of a green indicator solution. The indicator solution was a 4 : 1 mixture of 10% yeast solution and Schilling Green Food Color. Preliminary studies had determined that feeding on this solution for 2–3 h has no significant effect on either feeding rate or survival to eclosion. The larvae were then monitored under a dissecting microscope for indications that their guts were fully loaded with the indicator solution. When most larvae in a population had their entire gut filled with the green indicator solution, 40 larvae were removed from the Petri dish, gently rinsed in water and rolled on a paper towel to dry. This was done to remove any indicator solution from their body surface. The larvae were then moved to a small Petri dish containing agar coated with 1 ml of 10% yeast solution and observed continuously under a dissecting microscope. Any larva that entirely eliminated the green indicator solution from its gut was removed from the Petri dish, and the time of gut clearing noted. The food passage time was measured as the time taken, in minutes, to clear the gut entirely of green indicator solution while feeding on 10% yeast solution. Pairs of CU and UU populations, matched by subscripted indices, were assayed together.

*Measurement of minimum food requirements*

For each population, 150 newly-hatched first instar larvae were placed singly in 8 dram vials containing 5 ml of non-nutritive agar with varying amounts of yeast added as a solution (0.37 g yeast in 40 ml water) to the agar surface. Populations were tested at three separate food levels, corresponding to 40, 60 and 80 μl, respectively, of the yeast solution. Fifty larvae from each population were subjected to each food treatment. Eclosing adults were collected twice daily over the next 4 weeks and refrigerated for weighing at the end of the experiment. After 4 weeks, vials were scored into categories depending on what level of development (adult, pupa or larva) the individual in that vial attained. In vials with no pupae, the presence of an active larva was determined by looking for tracks of larval movement on the surface of the yeast. Vials with no sign of larval activity were presumed to have no larvae placed in them and were not used for the analysis. Eclosed adults from each population × food level combination were segregated by sex and dried in an oven at 80°C for 24 h. They were then allowed to cool for 20 min and weighed.

*Statistical analysis*

All statistical analyses were performed using SAS for Windows version 6.08. Due to the pattern of relatedness among CU and UU populations ($CU_i$ and $UU_i$ are more closely related than either of the pairs $CU_i$ and $CU_j$ or $UU_i$ and $UU_j$, $i \neq j$), pairs of CU and UU populations, matched by subscripted indices, were treated as blocks in the analyses of variance for feeding rate, food passage time and pupation heights. In the minimum food requirement experiment, each population yielded only one observation each for larval survivorship and the mean adult weight of eclosed males and females. Therefore, in the analyses of variance for these three traits, the five CU and five UU populations were treated as replicates rather than blocks. Survivorship data for each population were transformed using the arcsin square-root transformation (Freeman and Tukey, 1950); all other analyses were done on untransformed data. The ANOVA models used in these analyses are summarized in Table 1. Multiple comparisons for feeding rate and larval survivorship were done using Fisher's least significant differences.

Table 1. Summary of ANOVA models used in the analyses

| Trait | Factors used in ANOVA | Units of analysis |
|---|---|---|
| Pupation height | Block (1 ... 5), selection regime (CU/UU) | Vials |
| Larval feeding rate | Block (1 ... 5), selection regime (CU/UU) | Individual larvae |
| Larval food passage time | Block (1 ... 5), selection regime (CU/UU) | Individual larvae |
| Larval survivorship | Food level, selection regime (CU/UU) | Populations |
| Adult dry weight | Food level, selection regime (CU/UU), sex | Populations |

For each analysis, the model included main effects of the factors listed, as well as all possible interaction effects.

## Results

The mean pupation heights of CU and UU populations did not differ in any consistent manner, the only significant effect in the ANOVA being that of the block × selection regime interaction (Table 2). The feeding rates of CU larvae were significantly greater than those of UU larvae in each pair of populations tested (Fig. 1). The ANOVA revealed significant effects of both block ($F = 7.0, p < 0.01$) and selection regime ($F = 64.13, p < 0.01$). The block × selection regime interaction was not significant ($F = 0.61, p = 0.66$). The larval food passage time, on the other hand, did not differ consistently between the CU and UU populations (Table 3).

In the minimum food requirement experiment, the dry weight of eclosing adults was unaffected by either selection regime or sex (Table 4). At each food level, the weights of males and females from both the CU and UU populations were similar; weights of flies from all populations increased consistently with the amount of food provided (Fig. 2). Overall survivorship from larva to eclosion was higher in the UU populations at all three food levels (Fig. 3). However, none of the differences was significant at the 0.05 level; the only significant ANOVA effect was that due to the food level ($F = 55.64, p = 0.0001$). The survival to pupation of CU larvae, on the other hand, was significantly lower than that of UU larvae at the two lower food levels, corresponding to 40 and 60 μl of yeast suspension, respectively (Fig. 4). At the third food level (80 μl of yeast suspension), the survival to pupation of CU and UU larvae was not significantly different. This pattern of differences in larval survivorship to pupation across the different food levels is reflected in the significant food × selection regime interaction in the ANOVA (Table 5). Larval survival to pupation was positively correlated to both overall survival to eclosion ($r = 0.658, p = 0.0001$), as well as pupal survivorship ($r = 0.548, p = 0.0017$).

Table 2. Analysis of variance for pupation height

| Source | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Block | 4 | 17.414 | 1.01 | 0.42 |
| Selection | 1 | 5.264 | 0.06 | > 0.5 |
| Block × selection | 4 | 85.198 | 4.93 | 0.002 |
| Error | 40 | 17.283 | | |

The analysis was done on mean pupation height of all individuals in a vial. Five such vials were set up per population. Selection: selection regime, CU or UU. MS, mean squares.

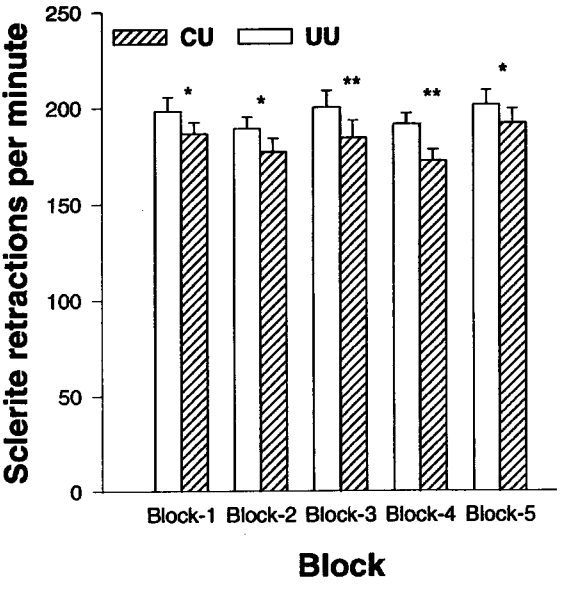


Figure 1. Mean number of sclerite retractions per minute for larvae from the CU and UU populations. The error bars denote 95% confidence intervals. In every block, the mean sclerite retraction rate of CU larvae was significantly greater than their UU counterparts. The asterisks denote the results of multiple comparisons ($*p < 0.05$, $**p < 0.01$).

Table 3. Analysis of variance for larval food passage time

| Source | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Block | 4 | 3428.26 | 9.81 | 0.0001 |
| Selection | 1 | 1651.06 | 0.86 | >0.25 |
| Block × selection | 4 | 1911.15 | 5.47 | 0.0003 |
| Error | 202 | 349.38 | | |

Selection: selection regime, CU or UU. MS, mean squares.

## Discussion

### *Larval food acquisition and utilization*

MacArthur and Wilson (1967) first hypothesized that natural selection at high densities would tend to favour the evolution of greater efficiency in the utilization of food for successful completion of development. Explicit mathematical models incorporating many specific features of the laboratory ecology of *Drosophila* suggested that either increased competitive ability, or a decreased minimum food requirement for pupation, may be favoured by selection if the two traits are affected by common loci exhibiting antagonistic pleiotropy (Mueller, 1988a).

In the study reported here, CU larvae had higher feeding rates than UU larvae (Fig. 1). Feeding rates of *Drosophila* larvae are known to be good indicators of larval competitive ability (Sewell *et al.*, 1975; Joshi and Mueller, 1988). The results of the minimum food requirement experiment

Table 4. Analysis of variance for dry weight of adults eclosing at different food levels in the minimum food requirement experiment

| Source | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Food | 2 | 0.00691 | 80.98 | 0.0001 |
| Selection | 1 | 0.00002 | 0.27 | 0.6087 |
| Sex | 1 | 0.00001 | 0.12 | 0.7293 |
| Food × selection | 2 | 0.00009 | 1.14 | 0.3277 |
| Food × sex | 2 | 0.00003 | 0.36 | 0.6988 |
| Selection × sex | 1 | 0.000007 | 0.08 | 0.7768 |
| Food × selection × sex | 2 | 0.00004 | 0.48 | 0.6207 |
| Error | 48 | 0.000085 | | |

The analysis was done on the mean weight of all eclosed adults, segregated by sex, for each population $CU_i$, $UU_i$. Selection: selection regime, CU or UU. Food: amount of food provided to individual larvae, 40, 60 or 80 µl of yeast suspension. MS, mean squares.

strongly suggest that CU populations are less efficient at utilizing food to complete development, as compared to their UU counterparts. The significantly lower survival to pupation of CU larvae at the lower food levels (Fig. 4) clearly indicates a greater minimum food requirement for pupation in the CU populations. Given the positive correlations between larval survival to pupation and both pupal and overall survivorship, the lack of statistically significant differences between the overall survival of the CU and UU populations (Fig. 3) is most likely a consequence of the small sample sizes involved, because relatively few individuals survived to eclose as adults. The sample sizes for

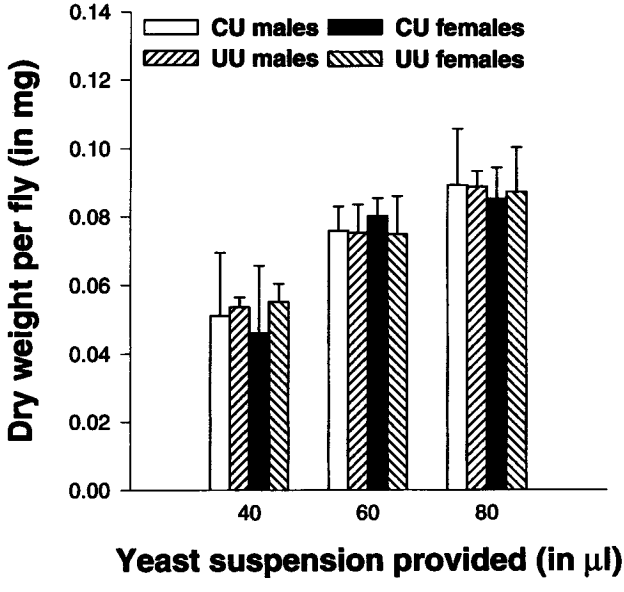


Figure 2. Mean dry weight of male and female flies from CU and UU populations that survived to eclosion at different levels of food. The error bars denote 95% confidence intervals about the mean weight across the five replicate populations of each kind (CU and UU).

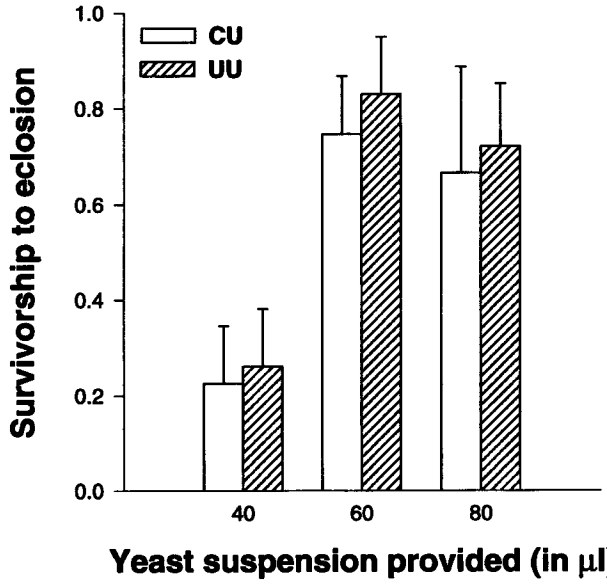


Figure 3. The fraction of larvae surviving to eclosion at different levels of food. The error bars denote 95% confidence intervals about the mean across the five replicate populations of each kind (CU and UU). Noneof the differences are statistically significant.

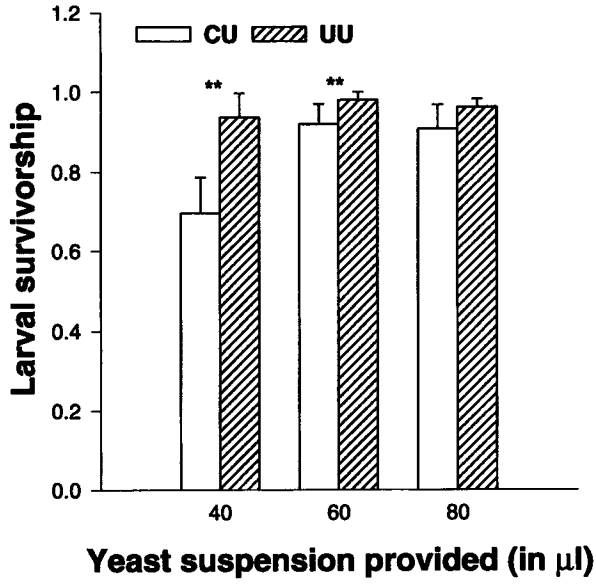


Figure 4. The fraction of larvae surviving to pupation at different levels of food. The error bars denote 95% confidence intervals about the mean across the five replicate populations of each kind (CU and UU). The asterisks denote the results of multiple comparisons (**$p < 0.01$).

Table 5. Analysis of variance for arcsin square-root transformed data on survivorship to pupation of CU and UU larvae in the minimum food requirement experiment

| Source | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Food | 2 | 0.12049 | 14.05 | 0.0001 |
| Selection | 1 | 0.33708 | 39.31 | 0.0001 |
| Food × selection | 2 | 0.04207 | 4.91 | 0.0164 |
| Error | 24 | 0.00858 | | |

Selection: selection regime, CU or UU. Food: amount of food provided to individual larvae, 40, 60 or 80 µl of yeast suspension. MS, mean squares.

the estimates of larval survival to pupation were considerably larger. Moreover, survival at different food levels was assayed under conditions enabling a distinction to be made between populations if differences in minimum food requirements for pupation existed. Consequently, these assay conditions differ substantially from the conditions to which the populations are subjected under their respective maintenance regimes. In the actual cultures, increased food requirements for successful pupation may well be an accurate reflection of increased food requirements for the completion of development to eclosion.

In principle, greater efficiency of food utilization may be attained either by completing development on less food or by eclosing as a larger adult. In our study, however, the eclosing CU adults were no heavier than their UU counterparts (Fig. 2 and Table 4), thereby ruling out the possibility that efficiency of food use may be reflected in size (and, therefore, future fecundity), rather than numbers.

Thus, our results, together with the results of Mueller (1990) on minimum food requirements in the *K* populations, suggest that competitive ability and efficiency of food use cannot, in fact, be simultaneously maximized in *Drosophila* populations kept at high densities. Larval competition in *Drosophila* is of the scramble type; the ablest competitor is one that can consume food at the fastest rate. Increased larval feeding rates, as characterized by cephalopharyngeal retraction rates, are highly correlated to increased competitive ability (Sewell *et al.*, 1975; Burnet *et al.*, 1977; Joshi and Mueller, 1988). This is likely to be the case, under crowded conditions, with other organisms with predominantly exploitative forms of competition.

One possible mechanism underlying such a trade-off between larval food acquisition and utilization could be the faster passage of food through the gut of faster feeding individuals, leading to lowered rates of food assimilation (Burnet *et al.*, 1977; Scribner and Slansky, 1981). This does not seem to be the case in the CU and UU populations studied by us, as they do not differ significantly in larval food passage time (Table 3). There are various other possible causes for an increased minimum food requirement in the CU populations. In addition to being faster feeders, the CU populations are also more tolerant to toxic metabolic waste in the food medium (J. Shiotsugu, A.M. Leroi, H. Yashiro, M.R. Rose and L.D. Mueller, unpublished manuscript), and exhibit elevated levels of larval locomotory behaviour (M.B. Sokolowski, personal communication). All of these traits can be reasonably expected to exact an energetic cost, thereby contributing to an increased minimum food requirement for pupation. As it appears that trade-offs between larval food acquisition and utilization may be of general occurrence, studies on larval energy metabolism

in crowded and uncrowded populations would be very useful in attempting a deeper understanding of the physiological basis of such trade-offs.

*Generality of results*

The results of this study confirm the existence of a trade-off between larval food acquisition and utilization in *Drosophila* populations subjected to extreme crowding. The populations used in this study were completely unrelated to the *r* and *K* populations in which evidence for such a trade-off was first seen (Mueller, 1990). Moreover, significant aspects of maintenance, such as food, temperature and the containers in which the flies were reared, also differed between the two sets of populations (CU, UU and *r*, *K*). The experiments reported in this paper also avoided the potential confounding effects of factors other than larval density, because, unlike the *r* and *K* populations, the CU and UU populations differed only in larval density and not in population size, age structure or adult density (Mueller *et al.*, 1993). The parallel increase of the larval feeding rate (Fig. 1) and minimum food required for pupation (Fig. 2) in both sets of crowded populations, therefore, indicates that this trade-off represents a general and fundamental constraint on the manner in which laboratory populations of *Drosophila* can adapt to extremely high densities.

On the other hand, the increased pupation height of the *K* populations, relative to the *r* populations, appears to have been mediated by specific aspects of their maintenance regimes. The lack of a similar pattern of pupation height change in the CU and UU populations provides a good example of a genotype × environment interaction affecting a response to selection. In an earlier study of pupal mortality at varying heights above the food surface in vials, we found evidence for strong selection for increased pupation height even at low densities (Joshi and Mueller, 1993). Getting buried in the food medium is a major cause of mortality for individuals pupating on or near the food surface in *Drosophila* cultures and this effect is exacerbated if the medium is soft and moist, as in a crowded culture (Chiang and Hodson, 1950; Sameoto and Miller, 1968). The *r* and *K* populations, though assayed for pupation height in vials (Mueller and Sweet, 1986), were maintained in half-pint milk bottles and it appeared that the *r* populations escaped selection for increased pupation height by pupating on the paper tissue inserted into the culture bottles to control moisture, rather than pupating on the food surface or on the sides of the bottles close to the food surface (Joshi and Mueller, 1993). The CU and UU populations used in the current study were maintained in vials as larvae. Moreover, the banana-molasses food that these populations were reared on is softer and more moist than the cornmeal food on which the *r* and *K* populations were raised. Consequently, it is likely that the UU populations have been experiencing selection for increased pupation height and this is reflected in the lack of differentiation between the UU and CU populations for this trait (Table 2).

When the CU populations were established, they were maintained at larval densities of approximately 500 larvae per 8 dram vial for 12 generations. During this period, the CU populations did not diverge significantly from their controls in either pupation height or larval feeding rate (Mueller *et al.*, 1993). From that point on, the level of larval crowding was increased to over 1000 larvae per 6 dram vial. Over the first 14 generations of selection at this higher level of crowding, the pupation height and larval feeding rate in the CU populations became significantly higher than in their controls (Mueller *et al.*, 1993). This initial divergence in pupation height would seem to indicate varying intensities of selection for increased pupation height in the CU and UU populations. In the approximately 40 generations between the study of Mueller *et al.* (1993) and the experiments reported here, the difference in pupation height between the CU and UU populations has diminished to non-significance, suggesting that UU populations have undergone selection for increased pupation height, albeit at lower intensities than those experienced by their CU counterparts in their much more crowded cultures.

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