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Density-dependent natural selection in *Drosophila*: Adaptation to adult crowding

AMITABH JOSHI,‡ WAN-PIN WU and LAURENCE D. MUELLER*

Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525, USA

Summary

The effects of adult crowding on two components of fitness were studied in three sets of *Drosophila melanogaster* populations, subjected to life-stage-specific, density-dependent natural selection in the laboratory for over 50 generations. Three days of crowding, early in adult life, were observed to increase mortality significantly during the episode of crowding, as well as decrease subsequent fecundity. Populations selected for adaptation to high adult densities suffered significantly lower mortality during episodes of adult crowding, as compared to populations selected specifically for adaptation to larval crowding, as well as control populations typically maintained at low larval and adult densities. Moreover, populations adapted to larval crowding seemed to be adversely affected by adult crowding to a greater extent than the controls, raising the possibility of trade-offs between adaptations to larval and adult crowding, respectively. Preliminary evidence suggests that the populations adapted to adult crowding may have evolved a propensity to stay away from the food medium, which is where most deaths occur when adults are crowded in culture vials.

Keywords: adult crowding; density-dependent selection; *Drosophila melanogaster*; life-history

Introduction

The significant role of density-dependent natural selection in moulding the evolution of life histories and adaptive strategies is now widely recognized (MacArthur and Wilson, 1967; Pianka, 1970; Boyce, 1984; Elgar and Catterall, 1989; Travis and Mueller, 1989). Moreover, explicit mathematical models of density-dependent and age-specific selection have underscored the importance of the timing and precise mechanisms of density-dependent regulation of fitness components for the evolution of particular life-history strategies in populations (Charlesworth, 1971, 1980; King and Anderson, 1971; Clarke, 1972; Roughgarden, 1979; Iwasa and Teramoto, 1980; Asmussen, 1983; Nunney, 1983; Mueller, 1988a). Nevertheless, there have been very few systematic investigations of patterns and processes in the evolution of specific adaptations to crowding in carefully controlled, and relatively well characterized, laboratory populations (reviewed in Roff, 1992; Joshi and Mueller, 1996; Joshi *et al.*, 1996; Joshi, 1997). Most of these studies have used laboratory populations of *Drosophila* (e.g. Taylor and Condra, 1980; Mueller, 1988b, 1990; Joshi and Mueller, 1988, 1993, 1996; Bierbaum *et al.*, 1989; Mueller *et al.*, 1993), and have typically focused on the impact of larval crowding on the evolution of larval and adult fitness components.

*Author to whom all correspondence should be addressed.
‡*Present address*: Evolution and Behaviour Laboratory, Animal Behaviour Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bangalore 560 064, India.

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Interestingly, despite numerous laboratory and field studies documenting the impact of adult density on a variety of fitness correlates in many species (Pearl *et al*., 1927; Park, 1932; Utida, 1941; Davis, 1945; Chiang and Hodson, 1950; Frank *et al*., 1957; Tanner, 1966; Mueller and Ayala, 1981; Graves and Mueller, 1993; Condit *et al*., 1994; Tonn *et al*., 1994; Ostfeld and Canham, 1995), there has never been, to our knowledge, any empirical study of the evolution of specific adaptations to high levels of adult crowding. There have been attempts to document differences in components of fitness among extant wild populations, and ascribe them to inferred density differences in the past (Pianka, 1970; Gadgil and Solbrig, 1972; Abrahamson and Gadgil, 1973; McNaughton, 1975). In such studies, it is not possible to assert unambiguously that any observed differences are due to density-dependent selection. Moreover, it is also impossible to link crowding experienced during a specific life stage to any observed differences among populations.

The inability to tease apart the evolutionary effects of larval versus adult crowding has also been a problem in interpreting results from the very few studies comparing adult fitness correlates in *Drosophila* populations subjected to varying density-dependent selection regimes (Taylor and Condra, 1980; Mueller and Ayala, 1981; Mueller *et al*., 1993). Taylor and Condra (1980) measured body size, longevity and age-specific fecundity on two pairs of *r*- and *K*-selected populations of *Drosophila pseudoobscura*. They observed significantly greater longevity in the *K*-selected females, and also found some evidence suggesting that *K*-selected females may have greater late-life fecundity than *r*-selected females. Their results are, however, difficult to interpret in terms of adaptations to larval or adult crowding because their *K*-selected lines were subjected to crowding both as larvae and as adults, and their *r*-selected lines were under direct selection for decreased egg–adult development time, which could potentially affect the evolution of fitness components quite independently of density.

So far, the clearest evidence for adaptation to adult crowding has come from studies on three pairs of *Drosophila melanogaster* populations subjected to selection at low and high densities, respectively (the *r*- and *K*-populations of Mueller and Ayala, 1981). The *K*-populations were observed to have greater rates of population growth at high adult densities (Mueller and Ayala, 1981), as well as greater tolerance to the detrimental effects of adult crowding on longevity (Mueller *et al*., 1993). Although these results were relatively unambiguous, the *K*-populations, nevertheless, differed from the *r*-populations in several features of their maintenance regime other than adult density (Mueller *et al*., 1993). Larval density in the *K*-populations was typically much higher than in the *r*-populations. More importantly, the *K*-populations were maintained with overlapping generations, permitting reproduction throughout life, whereas the *r*-populations were maintained on a discrete generation cycle that restricted reproduction to the first few days of adult life. These features of the *r*- and *K*-selection regimes, therefore, confounded the potential effects of age-specific selection and density-dependent selection on the larval and adult stages, making it difficult to ascribe unequivocally any observed changes in these populations to specific causes (Mueller *et al*., 1993).

This drawback with the *r*- and *K*-system motivated the creation of a set of 15 populations subjected to crowding specifically during either the larval or the adult stage: the CU, UC and UU populations (described in Mueller *et al*., 1993, Joshi and Mueller, 1997). The first letter in these population designations refers to larval density and the second letter to adult density (e.g. CU: crowded as larvae, uncrowded as adults). These populations constitute a powerful system for studying the evolutionary effects of extreme crowding at different life stages, because they allow observed differences among populations subjected to varying selection regimes to be ascribed unambiguously to density-dependent selection acting specifically on the larval or adult stage of the life cycle.

In this paper, we report results from two experiments in which we investigated the effects of adult crowding on key fitness components in the CU, UC and UU populations, to ascertain whether populations reared at high adult densities exhibit increased tolerance to adult crowding. We also examined the issue of whether populations selected for adaptations to high larval densities had undergone any correlated evolutionary changes in their ability to withstand adult crowding.

Materials and methods

Experimental populations

This study used three sets of five replicate populations of *D. melanogaster* that had each been subjected to differing levels of larval or adult density for over 50 generations (Mueller *et al.*, 1993). All populations were maintained on banana-molasses food at 25°C and continuous light, and had a generation time of about 3 weeks. Population sizes each generation were about 2000–4000 breeding adults. The five populations crowded as larvae ($CU_1 \ldots CU_5$) were reared at densities of 1000 or more larvae per 6 dram vial. Eclosing adults were collected daily from these vials, and kept at a low density of about 60–80 adults per 8 dram vial. 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 five populations crowded as adults ($UC_1 \ldots UC_5$) were reared at low larval densities of 60–80 larvae per 8 dram vial; eclosed adults were collected from these vials on the 13th day after egg-lay and kept in 8 dram vials at densities of about 160–200 adults per vial. Thus, the three sets of populations differed in the degree of larval or adult crowding to which they were exposed, with the UU populations acting as controls to both the UC and CU populations. 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. All three sets of populations were derived from the five B populations of Rose (1984), each B population being used as the progenitor of one CU, one UC and one UU population. Consequently, CU, UC 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.

First experiment: 3 days of adult conditioning

Collection of adult flies for assays. Prior to initiating the assays described below, all test populations were passed through one complete generation of identical rearing conditions, so as to eliminate any differences among selected lines due to environmental or maternal effects. Eggs were collected from the adults of each population and placed in 8 dram vials at low densities of 60–80 eggs per vial. Eclosing adults from these vials were then collected into cages; eggs laid by these adults were collected into 8 dram vials at low densities of 60–80 eggs per vial. Adult flies eclosing in these vials were collected one day after eclosion, and put into one of two conditioning treatments: crowded (75 males and 75 females per vial) or uncrowded (25 males and 25 females per vial). All conditioning vials contained exactly 5 ml food medium. The two conditioning densities were chosen to approximate the adult densities experienced by the UC (crowded) and the CU/UU (uncrowded) populations in their respective selection regimes. For each population, seven vials were set up at each conditioning density, resulting in a total of 210 vials (3 selection regimes $\times$ 5 replicate populations $\times$ 2 conditioning densities $\times$ 7 vials). The flies remained in these vials for 3 days, after which they were used for setting up the fecundity and longevity assays described below. Any flies that died during the 3 days of conditioning were sexed, and the number of male and female dead in each vial was recorded.

Fecundity assay. After the 3 day conditioning period, the fecundity of females was assayed at two different densities: high (25 males and 25 females per vial) and low (1 male and 1 female per vial). This was done to determine whether an episode of adult crowding affects the subsequent sensitivity of female fecundity to adult density at the time of egg laying. For each population × conditioning combination, 15 vials were set up at the low assay density; the number of vials set up at the high assay density varied from 4 to 8, based on the availability of flies that survived the conditioning treatments. Flies to be assayed were placed in 8 dram vials containing about 3 ml of charcoal-sucrose medium (Rose and Charlesworth, 1981), and a dab of live yeast paste on the side of the vial to ensure an abundant supply of food (Mueller and Huynh, 1994). The flies were given exactly 24 h to lay eggs in these vials, after which the adults were discarded and the number of eggs in each vial counted.

Second experiment: 5 days of adult conditioning

The second experiment was conducted after the results of the first experiment had strongly suggested that the UC flies were better adapted to tolerate adult crowding. It also appeared that UC flies, in both crowded and uncrowded vials, tended to spend more time away from the food surface, close to the sponge plug at the top of the vial (A. Joshi, personal observation). In the second experiment, we increased both the degree and duration of the adult crowding, in an attempt to differentiate more clearly among the response to adult crowding exhibited by the different selection lines. We also measured the gain in weight of flies during the first 5 days of adult life; if UC flies indeed tend to avoid the food in the vials, they might be expected to gain less weight during this time, as compared to their UU and CU counterparts.

As in the first experiment, test populations were passed through one complete generation of identical rearing conditions prior to being assayed. Virgin females were collected within 3 h of eclosion and frozen for subsequent weighing. Flies were collected in batches of eight females; six or seven such batches were collected for each replicate population. Prior to weighing, the flies were dried in an oven at 80°C for 24 h. In addition, two adult conditioning treatments were set up: crowded (100 males and 100 females per vial) and uncrowded (20 males and 20 females per vial). All conditioning vials contained exactly 5 ml food medium. For each population, 10 vials were set up at each conditioning density, and the flies remained in these vials for 5 days. Any flies that died during the 5 days of conditioning were sexed, and the number of male and female dead in each vial was recorded. For each population, six to seven batches of eight females each were collected on the sixth day after eclosion, from among the survivors of the uncrowded conditioning treatment. These flies were frozen, dried and weighed as described earlier for the virgin flies. Due to the extremely high mortality in the crowded conditioning vials (see Results), we were unable to collect a reasonable sample of flies from the crowded treatment for weighing.

Statistical analysis

All analyses of variance (ANOVA) were performed using the procedure GLM of SAS for Windows version 6.08. Due to the pattern of relatedness among the CU, UC and UU populations (CU_i, UC_i and UU_i are more closely related to each other than any of them is to other populations with which they share the same selection regime, $i = 1 \ldots 5$), sets of CU, UC and UU populations, matched by subscripted indices, were treated as random blocks in the analyses. Selection regime and conditioning density were treated as fixed effects crossed within each block. Mortality data from both experiments were subjected to the arcsin square-root transformation prior to analysis (Freeman and Tukey, 1950). For these data, sex was treated as a crossed, fixed factor, along with selection and conditioning, and the units of analysis were individual estimates of mortality from each vial.

For the data from the fecundity assay, selection, conditioning and density of measurement were treated as crossed, fixed factors. The units of analysis were estimates of mean fecundity across all females in a vial. Consequently, individual data points from the high assay density were mean fecundities (averaged across 25 females in a vial), whereas those from the low assay density were fecundities of individual females in a vial. For the dry weights of female flies at days 0 and 6 after eclosion, the units of analysis were the weights, in grams, of individual batches of eight flies; the ANOVA model included selection regime and day of measurement as fixed main effects crossed with the random blocks.

Results

First experiment: 3 days of adult conditioning

Mortality during conditioning. The ANOVA results for mortality during the 3 days of adult conditioning showed significant effects of sex and conditioning density, as well as a significant selection × sex × conditioning interaction (Table 1). In general, males suffered greater mortality than females, and flies in the crowded conditioning treatment suffered greater mortality than those subjected to uncrowded conditioning (Fig. 1). In the uncrowded conditioning treatment, CU males suffered significantly greater mortality ($P < 0.01$) than UC males; all other differences were non-significant (Fig. 1a). In contrast, in the crowded conditioning treatment, CU flies suffered the highest mortality, followed by flies from the UU populations. The UC flies suffered the lowest levels of mortality. This pattern was consistent across both sexes, and all differences among populations were significant at the 0.01 level (Fig. 1b). The overall levels of mortality over 3 days of crowding, however, were fairly low (Fig. 1).

Table 1. ANOVA for arcsin square-root transformed mortality in the UU, UC and CU populations during the 3 days of crowded (150 flies per vial) or uncrowded (50 flies per vial) conditioning in the first experiment

Source	d.f.	MS	*F*	*P*
Block (Blk)	4	0.1460	25.85	< 0.0005
Selection (Sel)	2	0.2469	1.93	> 0.1
Conditioning (Cond)	**1**	**0.4249**	**19.23**	**< 0.025**
Sex	**1**	**0.1204**	**16.49**	**< 0.025**
Blk × Sel	8	0.1282	22.68	< 0.0005
Blk × Cond	4	0.0221	3.91	< 0.005
Blk × Sex	4	0.0073	1.30	> 0.25
Sel × Cond	2	0.1325	2.07	> 0.10
Sel × Sex	2	0.0138	0.63	> 0.25
Cond × Sex	1	0.0002	0.06	> 0.25
Blk × Sel × Cond	8	0.0639	11.32	< 0.0005
Blk × Sel × Sex	8	0.0218	3.85	< 0.0005
Blk × Cond × Sex	4	0.0027	0.48	> 0.25
Sel × Cond × Sex	**2**	**0.0116**	**5.04**	**< 0.05**
Blk × Sel × Cond × Sex	8	0.0023	0.41	> 0.25
Error	360	0.0056		

Note: Significant fixed main effects and interactions are indicated in **bold** type (terms enclosed in parentheses denote abbreviations, not nested effects).

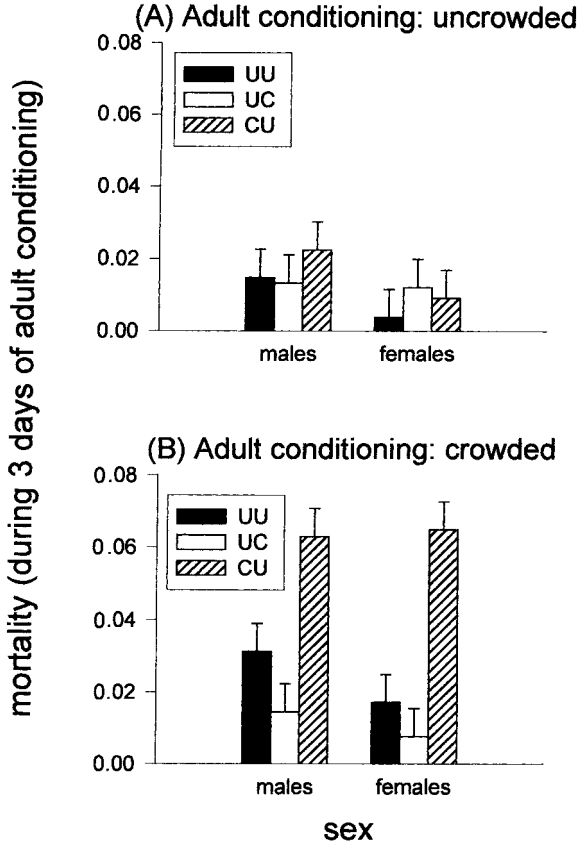

Figure 1. Mean mortality of flies during the 3 days of uncrowded (A) or crowded (B) conditioning as adults in the first experiment. The error bars depict 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least-squares estimates of the standard errors of cell means in a randomized block ANOVA on untransformed mortality data.

Effect of adult crowding on fecundity. The ANOVA results for fecundity after 3 days of adult conditioning showed significant effects of conditioning density and the density at which fecundity was measured (Table 2). In general, fecundity decreased with increasing density of conditioning and/or measurement, and these effects were more or less additive (Fig. 2). The pattern of mean fecundity after crowded and uncrowded conditioning also suggested that the detrimental effect of adult crowding on fecundity may be greatest on the CU females and least on the UC females. After the uncrowded conditioning treatment (Fig. 2a), the mean fecundity of UU females, averaged across the two measuring densities, was significantly lower ($P < 0.02$) than that of females from both the UC and CU populations (UU $<$ CU $=$ UC). However, after the crowded conditioning treatment (Fig. 2b), UU and CU females did not differ from each other in mean fecundity, although both populations showed significantly lower fecundity ($P < 0.005$) compared to the UC populations (UU $=$ CU $<$ UC). Strictly speaking, if the appropriate interaction effect is not significant, then significant differences seen in multiple comparisons should, at best, be considered suggestive of, rather than evidence for, the existence of meaningful differences among cell means in an ANOVA. We have included the results from these multiple comparisons for two reasons. The

Table 2. ANOVA for fecundity of UU, UC and CU females after 3 days of crowded (150 flies per vial) or uncrowded (50 flies per vial) conditioning

Source	d.f.	MS	*F*	*P*
Block (Blk)	4	523.89	3.62	< 0.01
Selection (Sel)	2	980.65	0.81	> 0.25
Conditioning (Cond)	**1**	**3 892.40**	**18.63**	**< 0.025**
Density (Den)	**1**	**11 014.33**	**20.74**	**< 0.025**
Blk × Sel	8	1 215.16	8.39	< 0.0005
Blk × Cond	4	208.98	1.44	> 0.20
Blk × Den	4	531.05	3.67	< 0.01
Sel × Cond	2	145.59	1.99	> 0.10
Sel × Den	2	49.17	0.11	> 0.25
Cond × Den	1	54.32	0.45	> 0.25
Blk × Sel × Cond	8	73.29	0.51	> 0.25
Blk × Sel × Den	8	452.36	3.12	< 0.005
Blk × Cond × Den	4	121.39	0.84	> 0.25
Sel × Cond × Den	2	29.44	0.72	> 0.25
Blk × Sel × Cond × Den	8	40.72	0.28	> 0.25
Error	582	144.87		

Note: Fecundities were measured at low (1 male and 1 female per vial) and high (25 males and 25 females per vial) densities for each combination of population and conditioning. Significant fixed main effects and interactions are indicated in **bold** type (terms enclosed in parentheses denote abbreviations, not nested effects).

pattern of differences in fecundity among the UU, UC and CU populations is the same as that seen for the effect of adult crowding on mortality in this study, and on longevity (Joshi and Mueller, 1997) in these populations; the likelihood of the same pattern arising by chance in three separate assays of different fitness components is rather remote. Moreover, the lack of a significant selection × conditioning interaction in the ANOVA is entirely due to the anomalous response of one population (CU_5) to adult crowding. In our laboratory, we have previously noted that this population differs dramatically from the other four CU populations for several fitness traits related to fecundity in *Drosophila*; for example, dry weight and lipid content (D.J. Borash and L.D. Mueller, unpublished data). Indeed, removing block 5 (UU_5, UC_5, CU_5) from the dataset has the effect of rendering the selection × conditioning interaction highly significant ($F = 10.02$, $P < 0.02$); the magnitude of the other ANOVA effects is not significantly altered.

Second experiment: 5 days of adult conditioning

Mortality during conditioning. Increasing the duration of adult conditioning from 3 days, in the first experiment, to 5 days, in the second experiment, resulted in higher mortality overall (Fig. 3), especially in the crowded treatment (compare Fig. 3b to Fig. 1b). In the more severely crowded conditioning treatment of the second experiment, females, in general, suffered higher mortality than males ($P < 0.01$), although the difference between the sexes was significant only in the crowded conditioning treatment ($P = 0.001$). Overall, the effect of selection regime was significant, as were the selection × sex, selection × conditioning and conditioning × sex interactions (Table 3). The rank order of mortality suffered by the selection lines during the 5 days of crowded conditioning (CU > UU > UC) was the same as in the first experiment. Under the more severe crowding of the second experiment, however, only the differences between the UC and CU

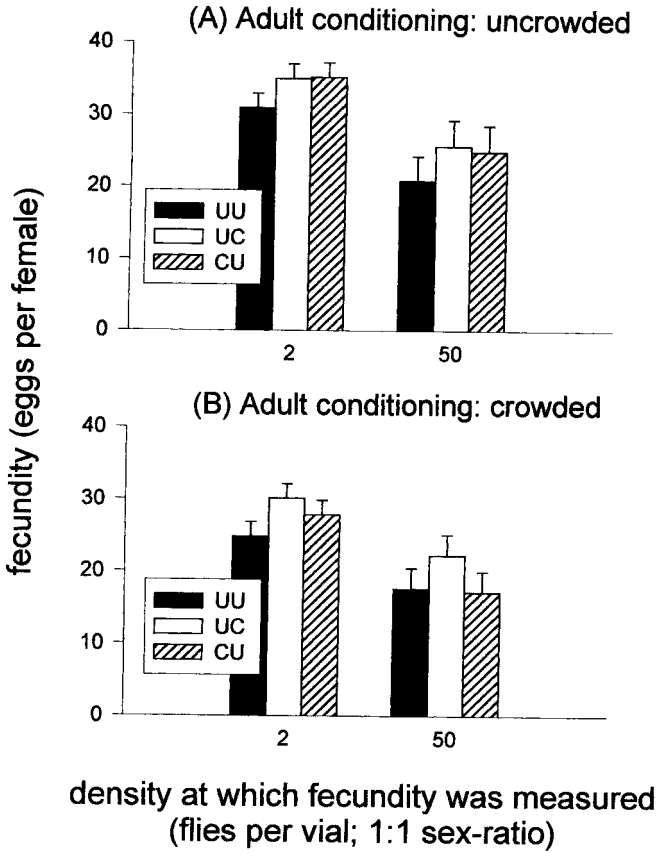

Figure 2. Mean 24 h female fecundity after 3 days of uncrowded (A) or crowded (B) conditioning as adults. The error bars depict 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least-squares estimates of the standard errors of cell means in the randomized block ANOVA.

($P = 0.0001$) and UC and UU ($P = 0.0001$) populations were significant; the populations did not differ significantly in mortality during the 5 days of uncrowded conditioning.

Dry weight at days 0 and 6 of adult life. Overall, the effect of selection regime on dry weight of females was only marginally significant ($P = 0.045$); there were, however, significant effects due to day (virgins at day 0 post-eclosion *vs* mated flies after 5 days of uncrowded conditioning) and the selection × day interaction (Table 4). Both the UU and CU populations showed a significant ($P < 0.001$) increase in weight over the 5 days of adult conditioning at low density; the increase in weight exhibited by the UC populations, on the other hand, was negligible ($P = 0.16$) (Fig. 4).

Discussion

The pattern of mortality experienced by the UU, UC and CU populations during adult conditioning, in both the first and second experiments, clearly shows that adult flies from the UC

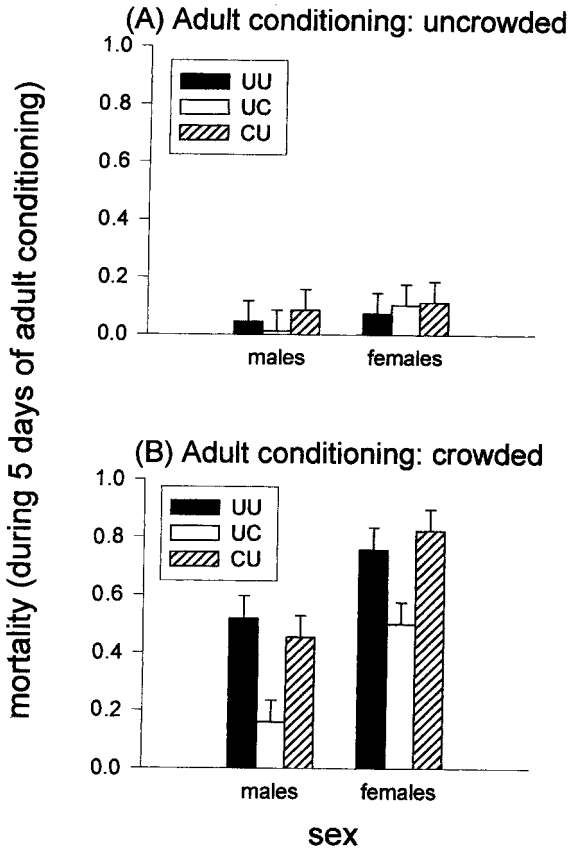

Figure 3. Mean mortality of flies during the 5 days of uncrowded (A) or crowded (B) conditioning as adults in the second experiment. The error bars depict 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least-squares estimates of the standard errors of cell means in a randomized block ANOVA on untransformed mortality data.

populations were the least affected by crowding, followed by those from the UU and CU populations, in that order (Figs 1 and 3). The same pattern of sensitivity to adult crowding was also seen for fecundity in the first experiment, as well as longevity (Joshi and Mueller, 1997), clearly indicating that the UC populations have evolved increased tolerance to the detrimental effects of adult crowding in response to being maintained at high adult densities. Although not previously documented in an unambiguous manner, this result is exactly what would be intuitively expected, given that adult crowding is known to have detrimental effects on fitness in *Drosophila*.

The decline of female fecundity with increasing adult density in *Drosophila* is a well-documented phenomenon, and one that is known to be at least partly independent of the decrease in *per capita* food availability caused by increasing density (Mueller, 1985). Typically, studies of the effect of density on fecundity have involved measuring fecundity on female flies at different densities, as opposed to examining the effect of a discrete period of crowding on subsequent egg production (reviewed in Mueller, 1985). Our results clearly demonstrate that brief episodes of crowding can have a detrimental effect on subsequent fecundity, regardless of the density at the time of measurement (Fig. 2). The lack of a significant conditioning density × measuring density interaction in

Table 3. ANOVA for arcsin square-root transformed mortality in the UU, UC and CU populations during the 5 days of crowded (200 flies per vial) or uncrowded (40 flies per vial) conditioning in the second experiment

Source	d.f.	MS	*F*	*P*
Block (Blk)	4	0.7776	27.01	< 0.0005
Selection (Sel)	**2**	**2.9396**	**7.91**	**< 0.025**
Conditioning (Cond)	**1**	**61.3488**	**379.81**	**< 0.0001**
Sex	**1**	**8.1810**	**24.42**	**< 0.01**
Blk × Sel	8	0.3714	12.90	< 0.0005
Blk × Cond	4	0.1615	5.61	< 0.005
Blk × Sex	4	0.3350	11.64	< 0.0005
Sel × Cond	**2**	**1.4645**	**12.43**	**< 0.01**
Sel × Sex	**2**	**0.1852**	**4.56**	**< 0.05**
Cond × Sex	**1**	**2.5096**	**18.71**	**< 0.025**
Blk × Sel × Cond	8	0.1178	4.09	< 0.0005
Blk × Sel × Sex	8	0.0406	1.41	> 0.15
Blk × Cond × Sex	4	0.1341	4.66	< 0.0025
Sel × Cond × Sex	2	0.1155	2.07	> 0.15
Blk × Sel × Cond × Sex	8	0.0557	1.93	> 0.05
Error	527	0.0288		

Note: Significant fixed main effects and interactions are indicated in **bold** type (terms enclosed in parentheses denote abbreviations, not nested effects).

Table 4. ANOVA for dry weight of virgin females (day 0) and females that were kept at a density of 20 males and 20 females per vial for 5 days (day 6) of adult conditioning in the second experiment

Source	d.f.	MS ($\times 10^{-6}$)	*F*	*P*
Block	4	1.45	92.24	< 0.0005
Selection	**2**	**0.53**	**4.68**	**< 0.05**
Day	**1**	**4.18**	**163.59**	**< 0.0001**
Block × selection	8	0.11	7.25	< 0.0005
Block × day	4	0.03	1.62	> 0.15
Selection × day	**2**	**0.54**	**8.98**	**< 0.01**
Block × selection × day	8	0.06	3.85	< 0.0005
Error	177	0.02		

Note: Significant fixed main effects and interactions are indicated in **bold** type.

the ANOVA (Table 2) indicates that previous exposure to high density does not markedly affect the sensitivity of female fecundity to the density of adults at the time of egg-laying. Since we assayed fecundity only once, at the end of the conditioning period, our data do not, however, permit an assessment of how episodes of crowding may affect the long-term fecundity profile of females.

The data on the dry weight of females from the UU, UC and CU populations, as freshly eclosed virgins and after 5 days of adult life at low density (Fig. 4, Table 4), lend some credence to the speculation that the decreased mortality of UC flies in crowded cultures may in part be due to their having evolved a tendency to stay away from the food medium, which is where most deaths occur in crowded *Drosophila* cultures. In stark contrast to the UU and CU populations, females from the UC populations underwent almost no increase in weight during the first 5 days of adult life at a

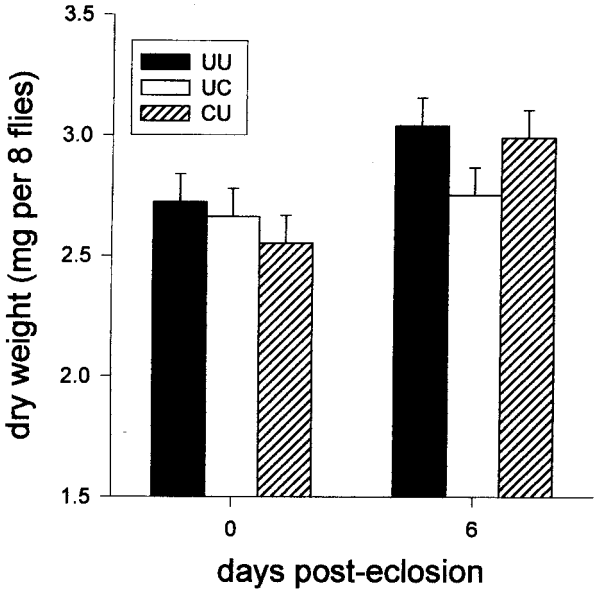

Figure 4. Dry weight of virgin females and females that were kept at a density of 20 males and 20 females per vial for 5 days of adult conditioning in the second experiment. The error bars depict 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least-squares estimates of the standard errors of cell means in the randomized block ANOVA.

very moderate density of 20 males and 20 females per vial (Fig. 4). The UC flies, especially in crowded vials, tend to congregate in the upper part of the vial, near the sponge plug; UU and CU flies, on the other hand, tend to spend a lot of time on the food surface (A. Joshi, personal observation). Taken together, these two observations suggest that crowded adult conditions may possibly impose a selective advantage to flies with a tendency, perhaps due to negative geotaxis, to spend more time in the upper part of the vial, away from the food surface. Though by no means conclusive, the current results suggest that further studies on this behavioural aspect of adult life may help us to understand the mechanisms underlying adaptations to adult crowding in laboratory populations of *Drosophila*.

The consistently greater susceptibility to adult crowding of the CU populations, compared to the UU controls, is very interesting, inasmuch as it suggests the possibility of a hitherto unsuspected trade-off between adaptations to larval and adult crowding, indicating that studies of the performance of UC larvae under crowded larval conditions may be worth pursuing to determine whether the trade-off between adaptation to larval and adult crowding is symmetrical. This finding also underscores the importance of being able to separate the effects of crowding during different life stages in any attempt to study how density-dependent selection may shape the evolution of life histories.

In other experiments in our laboratory, we have documented the adaptation of the CU populations to their high-density larval rearing conditions. The CU populations have evolved higher larval feeding rates (Joshi and Mueller, 1996) and greater tolerance to metabolic waste, relative to the UU populations (Shiotsugu *et al.*, 1997), as a consequence of being reared at high larval densities. It is, therefore, clear that both the UC and CU populations have diverged from the UU control lines as a result of adapting to their respective density-dependent selection regimes.

The mechanisms of adaptation to larval crowding in these populations, and indeed in *Drosophila* in general, are much better studied and understood than are the mechanisms conferring enhanced tolerance to adult crowding. The possibility of a trade-off between adaptation to larval and adult crowding suggested by this study highlights the importance of elucidating exactly how the UC populations are able to withstand the detrimental effects of adult crowding, and why the CU populations are so sensitive to high adult densities. Such an understanding will be important for a clearer appreciation of the physiological and genetic factors that may shape the evolutionary response of populations to extreme density experienced at various stages of the life cycle.

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