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Interactions between density-dependent and age-specific selection in *Drosophila melanogaster*

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Summary

1. Density-dependent natural selection and age-specific natural selection are important determinants of life-history evolution. A variety of laboratory populations of *Drosophila melanogaster* have been created to study the effects of these selection mechanisms.
2. Two types of populations have been selected for reproduction early (B) and late (O) in life. These have exhibited changes in life span and resistance to stresses, such as desiccation, starvation, ethanol vapours and flying to exhaustion.
3. Similarly, two types of populations have been selected at high adult and larval densities (K) and low adult and larval densities (r). These have exhibited changes in characters like larval feeding rates, pupation height and minimum food required for successful pupation.
4. To study whether age-specific and density-dependent selection act on the same traits either directly or through indirect effects, such as pleiotropy or linkage, we have examined the B and O populations for the traits that have become differentiated in the r and K populations and vice versa.
5. In general, there is a lack of similar response, except for starvation resistance which is greater in the K populations than the r populations.
6. We have tested, for the first time, longevity in all four types of populations as a function of adult density. The O populations show greater longevity than the B populations at all densities and this difference does not depend on density. In contrast, the K populations are able to resist the decline in longevity caused by increasing density much more effectively than are the r populations.
7. Lastly, a new set of populations, called CU, has been derived from the B populations and is maintained by crowding the larval life stage but raising adults under low densities. The CU populations have evolved increased feeding rates, pupation height and larval viability at high density relative to the B populations. These changes parallel the changes seen in the r and K populations and demonstrate the importance of crowding in the larval stages for much of the evolution seen in the r and K populations.

Key-words: Ageing, evolutionary physiology, fruit flies, life-history evolution, r and K selection
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Introduction

A number of *Drosophila* laboratory evolution studies have now been conducted focusing on age-specific or density-dependent selection (e.g. Mueller & Ayala 1981; Rose 1984; Luckinbill & Clare 1985). A major concern in the studies of age-specific selection has been the dependence of the response to selection on density (Clare & Luckinbill 1985; Service, Hutchinson & Rose 1988); it is now apparent that larval rearing densities can be critical in such experiments. On the other hand, 'r-*vs*-K' selection also gives rise to varying patterns of selection on different adult life-cycle stages, and thus age-specific selection (see Mueller 1987). Inadvertently, it seems as if research on these two different types of selection has been probing at their interactions, albeit unsystematically. A further problem in these experiments is that there is little in the way of theoretical expectations to guide the interpretation of those experimental results that indicate a possible interaction between age-specific and density-dependent selection.

Age-specific and density-dependent mechanisms of selection infuse discussions of such specific problems as the cost of reproduction, r and K selection, the evolution of ageing, etc. Implicitly, these modes of selection are also related to questions concerning patterns of pleiotropy between fitness components, the maintenance of genetic variation for fitness characters, and response to selection on fitness characters.

One of the features that has characterized the literature on life-history evolution from the start is ambiguity about patterns of results. Many of the original ideas about r and K selection also seem to entail different conceptions about the age structure of selection associated with r and K selection (MacArthur & Wilson 1967; Pianka 1970; discussed further in Mueller 1988a). This way of thinking is in fact quite natural; different ecological settings are expected to give rise to characteristic associations between age-dependent and density-dependent selection. Unfortunately, our theoretical understanding of the combined effects of the two forms of selection is limited to some local results in age-structured population genetics theory (Charlesworth 1980). Indeed, there are no general, theoretically motivated, expectations as to the effects of interactions between age-specific and density-dependent selection. However, recent experimental work on selection for different ages at reproduction has suggested that the outcome of evolution subject to this age-specific treatment may interact with the density under which larvae are raised (Luckinbill & Clare 1985; Service *et al.* 1988; Partridge & Fowler 1992).

Before the 1980s, most of the available evidence bearing on life-history evolution was circumstantial, chiefly consisting of comparative or phenotypic evidence, of little direct significance for the evolutionary genetics of life history. There were a few relevant experiments, but these either gave inconsistent results (e.g. Mertz 1975) or were interpreted in odd ways (e.g. Wattiaux 1968; Lints & Hoste 1974, 1977; these authors being neo-Lamarckians).

At the end of the 1970s, there was a burgeoning of evidence bearing on age specificity in selection. Much of this work has employed *Drosophila* (e.g. Rose & Charlesworth 1980; Rose 1984; Luckinbill & Clare 1985; Service *et al.* 1988). Other organisms have also been used: meadow grass (Law 1979), petunia (Roach 1986), and frogs (Travis, Emerson & Blouin 1987) being examples. There is now abundant evidence for antagonistic pleiotropy between life-history characters (e.g. Rose & Charlesworth 1981a,b; Luckinbill *et al.* 1984; Roach 1986; Service *et al.* 1988). There is also evidence for mutation accumulation affecting later life-history characters (Kosuda 1985; Mueller 1987; Service *et al.* 1988). The relationship between theory and experiment in this area has been one of useful interaction, in that theory has generated clear expectations that have been tested experimentally.

Much less work has been done on density-dependent selection. Luckinbill (1978, 1984) performed an early study on the covariation of r and K parameters in populations of bacteria and protozoa. He did not find the trade-off between r and K that had been suggested by proponents of the verbal theory (e.g. Pianka 1970). Mueller & Ayala (1981), Mueller & Sweet (1986), Mueller (1988b), Joshi & Mueller (1988), Bierbaum, Mueller & Ayala (1989) and Mueller, Guo & Ayala (1991) report a series of experiments involving sustained culture of populations at high and low densities. More in keeping with density-dependent selection theory, populations had relatively higher fitness at those densities at which they had been cultured for some time. Mueller (1988a) provides a discussion of the relationship between theory and experiment in this area.

One of the striking features of experimental work on life-history evolution is how difficult it is to separate age-specific and density-dependent factors in evolutionary–genetic experiments. In the experiments on *Drosophila* life-history genetics, a critical factor has been larval rearing densities (Clare & Luckinbill 1985; Luckinbill & Clare 1985; Service *et al.* 1988). At lower larval rearing densities, there is relatively less additive genetic variance for age-specific life-history characters. On the other hand, the density-dependent selection experiments of Mueller and colleagues have involved alterations in age structure in their experimental populations, giving rise to changes in age-specific selection patterns (see Mueller 1987). Thus, it seems evident that age-specific and density-dependent patterns of selection are difficult to separate, even under controlled laboratory conditions.

In the present study, we compare populations of *Drosophila melanogaster* Meigen that have been subjected to age-specific selection and density-dependent selection. In particular, we evaluate age-specific selected lines for their differentiation for characters that have been found to be involved in the response to density-dependent selection and vice versa. In addition, we report the creation and initial analysis of *D. melanogaster* lines selected specifically for adaptation to conditions of larval crowding.

Materials and methods

STOCKS

Age-selected stocks

Five *D. melanogaster* stocks, called 'O', have been selected for postponed ageing since February 1980 (Rose 1984). These have also been paired with five control stocks, 'B', which have been maintained from young females only, also since Feburary 1980. Larvae in the B and O cultures have always been maintained at low larval densities (60–100 vial^{-1}). This selection has continued for a total of 12 years. A number of observations have been made about the biology of the B–O difference. Firstly, life span is increased in the O lines by reductions in rates of mortality at every adult age (Rose 1984). Secondly, later fertility is increased somewhat, but earlier fecundity is considerably depressed (Rose 1984), a depression which is not relieved by crossing (Hutchinson & Rose 1991). These findings had been obtained earlier in a comparable experiment with less replication (Rose & Charlesworth 1981b). Perhaps more importantly, they were presaged by quantitative genetic analysis of the base population from which B and O stocks were derived. The base population exhibited pronounced negative genetic correlations between early fecundity and later survival and egg laying (Rose & Charlesworth 1981a).

Thirdly, a variety of functional characters are altered.

1. Overall body size is not affected by postponed senescence, but early ovary weight is reduced (Rose *et al.* 1984), at the same time as early fecundity is reduced.
2. O adult survival times under stress are enhanced for starvation, desiccation, and 15% ambient ethanol, but not for high temperatures or high levels of ambient ethanol (Service *et al.* 1985).
3. Early O metabolic rate and locomotion are depressed, but later locomotion is enhanced (Service 1987).
4. O lipid levels are increased in a strain-, age- and gender-dependent fashion that mimics that of starvation time (Service 1987). This suggests that the starvation resistance of O stocks is a result of increased lipid levels.
5. Flight duration is increased in the O stocks at every adult age (Graves & Rose 1990).

Density-selected stocks

The basis of this experimental programme was the creation of populations subjected to different density regimes. Six replicate population cultures of *D. melanogaster* were initiated in November 1978. Three were kept at high population densities in a serial transfer system (Mueller & Ayala 1981), and therefore were designated 'K' populations, while three were kept at low larval and adult densities, and so were designated 'r' populations. The r and K populations also differed in age of reproduction (Mueller 1987). The r populations reproduced during the first 6 days of adult life, while the K populations reproduced indefinitely, although the bulk of reproduction probably occurred during the first 2 weeks of adult life.

Relative to the r populations, the K populations showed:

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

ASSAY METHODS

Prior to initiating the assays described below, all test populations were passed through two generations of common conditions. Specifically, for two generations adults from the selected populations lay eggs that are then placed at low larval densities and raised under identical conditions to those of their controls. The flies that emerge from these cultures were then used to produce larvae or adults that were used in the assay procedure. These protocols ensure that differences observed between control and selected populations were due to genetic differences and not to environmental or maternal differences.

Starvation resistance

This was assayed by measuring the amount of time it took a fly to become completely unresponsive to mechanical stimulation under conditions of high humidity and no nutrition. The starvation environment was created by placing flies in groups of four into standard shell vials, and then inserting half a sponge stopper to seal in the flies, such that the lower edge of the sponge was 4–5 cm above the bottom of the vial. A cotton-ball was inserted into the part of the vial away from the flies on the other side of the sponge. Three millilitres of water was squirted into the cotton wool and the open end of the vial was sealed with Parafilm. The flies were then observed at regular intervals, every 4–8 h, the vial being agitated if a fly was motionless. Death due to starvation was inferred when a fly did not respond to agitation on three consecutive checks. The time of first inanition was then used as the time of death (see also Service *et al.* 1985).

Desiccation resistance

This was assayed in a fashion analogous to that used to assay starvation resistance. The main difference was that 3 g of Dri-Rite desiccant replaced the water-soaked cotton wool in the vial. Flies were checked more often, every 1–2 h, because they die much faster due to desiccation than starvation (Service *et al.* 1985).

Ethanol resistance

This was assayed like starvation resistance, except that the cotton wool was soaked with a 15% ethanol solution. Flies were checked more often, as for desiccation resistance, because they die much faster (see Service *et al.* 1985).

Glycogen

This was assayed by microseparation of glycogen after the technique of van Handel (1965a,b) and was performed on the five replicate B and O populations both before and after desiccation. Three females were used for each assay; the females were chosen to be of equal size distributions between the two lines. A total of five assays, each consisting of 15 females, was performed for each population, in the two lines.

Longevity

This was assayed using groups of mated flies, where the groups varied in size, as discussed below. All longevity assays were conducted on banana–molasses medium, at 25°C, in shell vials, with transfer to new vials every 2–3 days without anaesthesia.

Tethered flight duration

Flies were lightly etherized and then individually tethered to a light test piece of fishing line by use of Duco cement. The flies were stimulated to fly by passing light air current along the head–tail axis. Flies were flown to exhaustion by immediate stimulation upon cessation of flight.

Larval feeding rate

Newly hatched first-instar larvae were placed in a watch glass containing medium. Larvae were collected for assay 48 h later. Larvae were placed individually on a Petri dish containing 3% agar and a thin solution of 2% live yeast. The larva was given 1·5 min to acclimate and then cephalopharyngeal retractions were recorded for 1 min using a dissecting microscope and a hand counter.

Pupation height

Fifty newly hatched first-instar larvae were collected and placed in 6-dram vials with 10 ml of standard medium. After pupation, the height of each pupa was recorded as the distance from the top of the food medium to the spiracles of the pupal case. Any pupa touching the surface was given a height of 0. Experiments were run in 12:12 L:D.

Minimum food requirements

This was determined by placing larvae individually in vials with measured amounts of food (Mueller 1990). For each population, five food levels were used and 50 larvae per food level were tested. The three pairs of populations B_2–B_4 and O_2–O_4 were tested at 2, 3, 4, 5 and 6 mg of yeast per larva. The fraction of larvae surviving on 2 mg of yeast was not uniformly low (range 0·24–0·94). Subsequently, for B_1 and O_1 and B_5 and O_5, the food levels were 0·1, 0·5, 1, 2 and 5 mg of yeast. While testing the O_2 and B_2 populations, a number of vials at all food levels inadvertently had no larvae placed in them. Thus, the total sample size for these experiments ranged from 39 to 49.

SURVEY OF EXTANT STOCKS

B and O populations

These stocks were compared with each other for the following larval characters: minimum food requirements, pupation height and feeding rate, as discussed above.

r and K populations

These stocks were compared with each other for the following adult characters: starvation, ethanol and desiccation resistance; tethered flight duration; glycogen levels; and longevity with one mated pair per vial.

Comparison of adult density effects in r, K, B and O populations

All 16 of the extant stocks were compared for the effect of varying adult density on longevity. The density treatments used with the r and K stocks were 2, 4, 20, 64, 80, 128 and 200, with 50:50 sex ratios initially. For B and O stocks, the densities were 4, 16, 20, 32, 50, 64, 80, 128, 160 and 200, with sex ratios as before. Vials were changed every 2 days after the first 5 days. Dead flies were not replaced.

CREATION OF CU LINES

One of the major problems facing the comparison of B–O with r–K stocks is that they were founded from different wild populations of *D. melanogaster*, making background effects potentially obscure. In addition, the r and K stocks were difficult to interpret in terms of selection mechanisms, because there were several important differences between the selection regimes imposed on these stocks: larval density, adult density and time of reproduction (Mueller & Ayala 1981). Accordingly, we derived five replicate populations from the B populations of Rose (1984), and selected these replicates under conditions of larval crowding (500 larvae/8-dram vial for 12 gener-

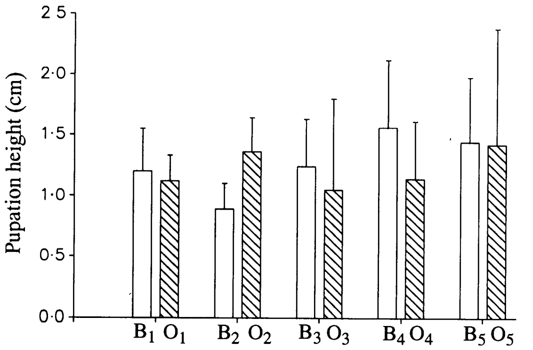

Fig. 1. Pupation heights with 95% confidence intervals in the five B (denoted by subscripts 1–5) and five O populations.

ations; 1000+ larvae/6-dram vial since) for 30 generations. These stocks were thus selected specifically for fitness under high larval densities, with no adult crowding.

Similarly, a series of populations has been derived from B called UC which are uncrowded as larvae (50–80 larvae per 8-dram vial) and crowded as adults (~150 adults per vial). These populations are cultured from young adults (1 week old) and a generation is competed in 3 weeks.

These stocks have been compared with B stocks for pupation height, feeding rate, longevity and resistance to larval crowding. The last assay involved rearing larvae at densities of 50 and 500 larvae per 8-dram vial, with the number of flies emerging at 12-h periods being recorded. These data allowed estimation of both average development for a stock, as well as its viability.

Results

COMPARISON OF LARVAE FROM AGE-SELECTED STOCKS

Four B populations have greater pupation heights than their matched O population but none of those differences is significant (Fig. 1). The one B population that has a lower pupation height than its matched O population (B_2 *vs* O_2) does show a significant difference (Fig. 1). There is clearly no consistent difference in pupation height between the B and O populations.

The results from the feeding rate study show that four B populations have lower feeding rates than their matched O populations (Fig. 2). One of those differences is statistically significant (B_5 *vs* O_5). If all five pairs of populations are compared by the non-parametric Wilcoxon signed-ranks test, the differences are not significant either (P = 0·0625). The average O population has a feeding rate which is 5·9 counts greater than the average B population. While it is not possible to conclude that this difference is statistically significant, it is clearly much less than the difference between the average r and K populations (21 c.p.m.; Mueller & Sweet 1986).

The minimum food requirement results were analysed with a log-linear model. For the pairs of populations with exactly 50 samples at each food level, there are two dimensions in the table of counts of surviving larvae: food level (five values) and population (B *vs* O). Differential ability to survive on these various food levels would be indicated by a significant two-way effect in the model. This has been tested by comparing the goodness of fit to a model without the two-way interaction (food by population) to the fully saturated model (no degrees of freedom). The increase in the likelihood ratio χ^2 statistic for the reduced model and its significance is shown in Table 1. The B_2 and O_2 experiment had numerous food levels which had different numbers of vials. For these data there are three factors: food level, population and status (alive or dead). As a result, there are three different two-way interaction terms. The significance of the food by population interaction is tested by computing the difference in likelihood ratio χ^2 between the model with all two-way interactions to the one without the food by population interaction. It is clear from these results (Table 1) that there are no significant differences between the B and O populations in minimum food requirements.

COMPARISON OF ADULTS FROM r AND K STOCKS

The low-density assays are shown in Figs. 3 and 4. There was no significant difference in longevity in either sex between the r and K lines, and there was no

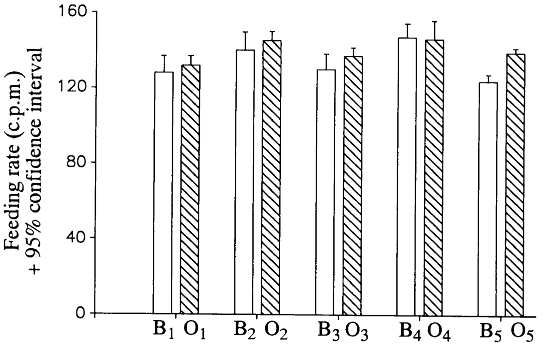

Fig. 2. Feeding rates and 95% confidence intervals in the five B and O populations.

Table 1. Results of log-linear analysis on the minimum food requirement tests

Populations	df	Test statistic	Probability
B_1 and O_1	4	2·686*	0·61
B_2 and O_2	4	2·805†	0·59
B_3 and O_3	4	2·202*	0·70
B_4 and O_4	4	0·883*	0·93
B_5 and O_5	4	0·496*	0·97

* Likelihood ratio χ^2.
† Partial χ^2.

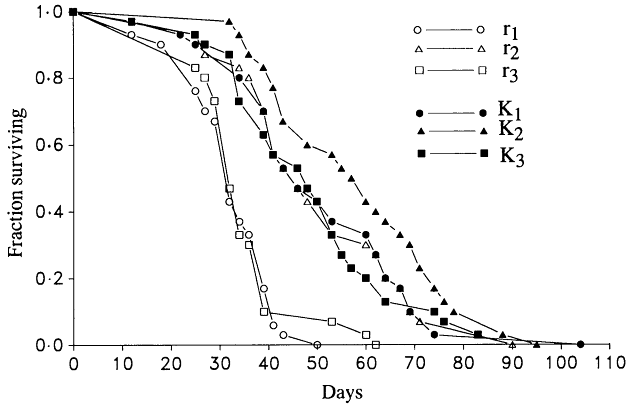

Fig. 3. The fraction of adult females that survive to various ages in the three r and three K populations.

sex difference within lines. As discussed below, this absence of a difference is not characteristic of these lines when adults are crowded.

Figure 5 shows the stress resistance and flight duration results for r and K females. Only the starvation resistance character is significantly differentiated. The males are not differentiated for starvation or desiccation resistance. Likewise the glycogen content of the r and K adults are not significantly different (Table 2).

EFFECT OF ADULT CROWDING ON LONGEVITY

Figure 6 shows the effect of adult density on longevity in the B and O stocks, while Fig. 7 shows the corresponding results for the r and K stocks. The B and O results involve a consistent differentiation of treatments that does not depend on density. By contrast, there is a substantial density dependence of differentiation of r and K stocks. Using a Newman–Kuels multiple comparison test, we find that longevities in the r populations are not significantly different from each other at 2, 4, and 20 adults, but this group has significantly greater longevity than those at 50. This non-parametric test is not sensitive to the distribution of longevity, which may be skewed. The longevity at 50 is also greater than the longevity at a density of 80 adults in the r populations. By comparison the longevities at 2, 4, 20, 50 and 80 adults are not significantly different from each other in the K populations. These results strongly suggest that K stocks have evolved resistance to the deleterious effects of adult crowding. It is noteworthy that when B and r populations are compared over varying densities, they exhibit the same sensitivity to adult density. This suggests that the K populations differ because of their selection history, not some feature specific to their ancestry relative to the other stocks.

Table 2. The glycogen content (μg ml^{-1}, three flies per 5 ml) of adult r and K males and females

	Sex			
	Male		Female	
	Population			
	K	r	K	r
Mean	8·5	6·6	17·2	14·9
95% confidence interval	±2·8	±7·5	±6·3	±3·3

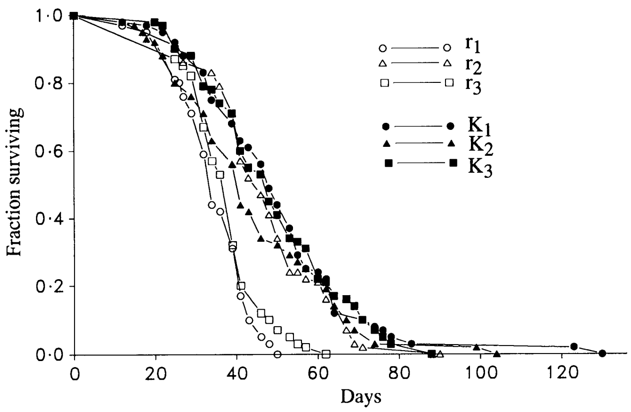

Fig. 4. The fraction of adult males that survive to various ages in the three r and three K populations.

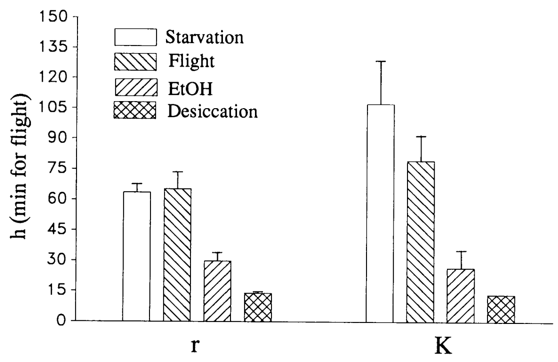

Fig. 5. The average (±1 SE) of females from the three r and three K populations for resistance to various stresses as described in the text.

SELECTION WITH LARVAL CROWDING: CU STOCKS

No differences in the pupation heights and feeding rates of the B and CU populations after 12 generations of selection in the CU environment were found (Figs. 8 and 9). At this time there were no consistent differences between the two groups of populations. Since one possible explanation for this observation is that the intensity of selection was too weak, the level of larval crowding was increased at that time (see Materials and methods). The differences in pupation height and feeding rate in the ensuing generations show a rapid differentiation of the two groups of lines (Figs. 8 and 9). The pupation height and feeding rates have increased in the CU populations relative to the B populations.

Larval development times indicate that the B populations develop more rapidly than CU populations at both densities (Table 3). The larval viabilities show a density by population interaction (Table 3). At the low larval density the populations have similar viabilities, but at high larval density the CU larvae are 33% more likely to survive than B larvae.

To test for significant differences between UC and B or CU and B we have used the Wilcoxon signed-rank test for paired observations, these tests do not require longevities to be normally distributed. In these cases $\mathrm{CU_l}$ or $\mathrm{UC_l}$ is paired with $\mathrm{B_l}$ since $\mathrm{B_l}$ is the parental population. The longevities of the CU, UC and B populations show no significant differences (Figs. 10 and 11). However, the median longevities of four of the UC populations are greater than their controls while the longevities of the fifth pair was equal. Nevertheless the differences between the mean UC and B longevities was small — 3 days.

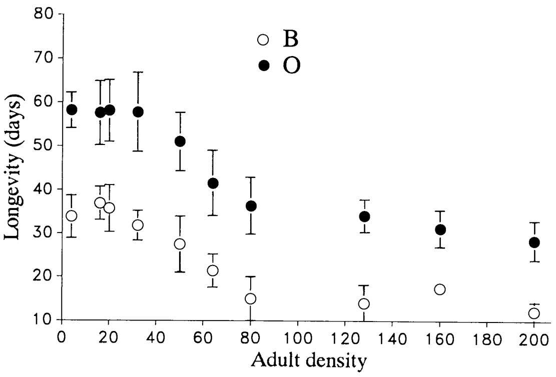

Fig. 6. The average longevity (±1 SE) of the B and O populations at 10 different adult densities.

Discussion

AGE-SPECIFIC AND DENSITY-DEPENDENT SELECTION REGIMES GIVE RISE TO DIFFERENT RESPONSES FOR MOST CHARACTERS

None of the characters that responded to density-dependent selection was found to respond in a statistically significant manner in stocks subjected to different age-specific selection regimes only. This conclusion might be qualified to the extent that the feeding rate data suggest that there has been a small change in the feeding rates of the O populations, a change that might be statistically detectable with massive replication. However, even if the effect does prove to be significant, its magnitude (a difference of about six cephalopharyngeal contractions between the B and O populations, compared with a difference of 21 contractions between r and K) is small. Therefore, the general pattern seems to be one in which the response to age-specific selection is distinct from that to density-dependent selection.

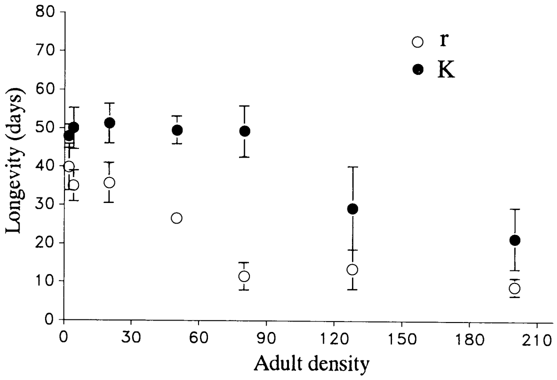

Fig. 7. The average longevity (±1 SE) of the r and K populations at seven different adult densities.

It remains true that K selection does not affect some of the characters involved in the response to age-specific selection: desiccation resistance, ethanol resistance and flight duration. However, starvation resistance is enhanced in the K lines, relative to the r lines. Interestingly, longevity at increased adult densities is also enhanced in the K populations (Fig. 7). Perhaps the lipid resources that presumably

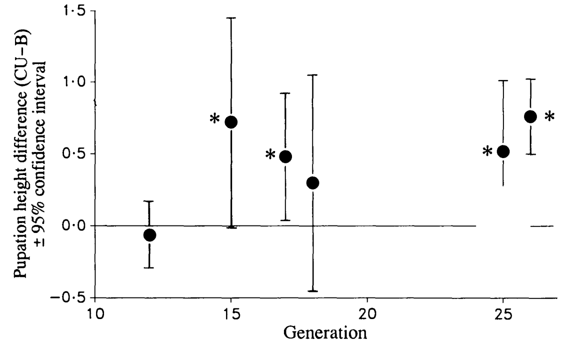

Fig. 8. The difference in mean pupation height (±1 SE) of the five CU and five B populations over 14 generations of selection.

* Significant differences between the populations were assessed by a random blocks design ANOVA.

underlie enhanced starvation resistance (cf. Service 1987) are used by K flies to enhance their adult survival under conditions of crowding. A problem with this interpretation is that the O flies have enhanced starvation resistance, but no specific enhancement in their resistance to adult crowding, compared with the B or r flies. Therefore, it might be more reasonable to regard the enhanced starvation resistance of K flies as a response to selection distinct from their increased resistance to adult crowding.

Another possible interpretation of the differences between the r and K populations is that the r populations have been inbred and hence show reduced values of the phenotypes in question. There are several reasons why we think this alternative is unlikely for the characters examined here. The B populations have been kept at very large population sizes and show no evidence of inbreeding depression (Hutchinson & Rose 1991). The value of longevity at various densities (Figs. 6 and 7) and starvation resistance in the B populations are quite similar to the r populations. The only character in the r populations that has shown signs of inbreeding depression are traits which act late in life (Mueller 1987). Thus, starvation resistance in early life would not be expected to be depressed. Although longevity differences are seen between the r and K populations, they appear most dramatically at higher population densities, not at low population densities, where flies live the longest.

There is a fundamental confusion in the comparison of the r–K differentiation with the B–O differentiation, that arises from the age-specific selection imposed on the K flies, which can lay eggs at later ages than the r flies. Thus the r–K stocks have both density-dependent and age-specific selection differences. The B and O cultures have been handled so as to minimize the role of crowding effects upon both larvae and adults. The results presented here suggest that the B and O populations indeed have not differentiated from each other with respect to adaptations arising from density-dependent selection.

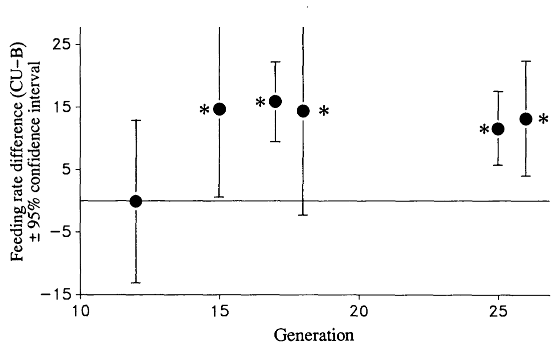

Fig. 9. The difference in mean feeding rate (±1 SE) of the five CU and five B populations over 14 generations of selection. * Significant differences between the populations were assessed by a random blocks design ANOVA.

Table 3. Viability (measured as the fraction surviving), male and female development times (DT; measured in days) ±95% confidence intervals, for the CU and B populations and two different larval densities

	Density 50			Density 500		
Population	Viability	Male-DT	Female-DT	Viability	Male-DT	Female-DT
B_1	0·78 ± 0·09	8·25 ± 0·30	8·07 ± 0·27	0·45 ± 0·11	15·3 ± 0·7	14·4 ± 0·9
B_2	0·75 ± 0·13	8·34 ± 0·39	8·14 ± 0·27	0·19 ± 0·35	11·1 ± 10·2	10·8 ± 8·8
B_3	0·73 ± 0·16	8·37 ± 0·26	8·14 ± 0·27	0·32 ± 0·04	12·9 ± 3·5	12·7 ± 3·2
B_4	0·81 ± 0·12	8·29 ± 0·27	8·20 ± 0·29	0·36 ± 0·25	11·7 ± 3·3	11·8 ± 3·1
B_5	0·87 ± 0·05	8·32 ± 0·33	8·21 ± 0·33	0·46 ± 0·09	14·9 ± 1·8	14·2 ± 1·2
B_{ave}	0·79 ± 0·07	8·31 ± 0·06	8·15 ± 0·07	0·36 ± 0·13	13·2 ± 2·3	12·8 ± 2·0
CU_1	0·76 ± 0·16	8·67 ± 0·08	8·59 ± 0·08	0·42 ± 0·12	13·5 ± 2·2	13·3 ± 1·5
CU_2	0·75 ± 0·07	8·59 ± 0·12	8·53 ± 0·19	0·50 ± 0·07	14·9 ± 0·4	13·9 ± 0·6
CU_3	0·74 ± 0·18	8·65 ± 0·14	8·61 ± 0·14	0·41 ± 0·07	14·7 ± 0·3	14·0 ± 0·9
CU_4	0·77 ± 0·12	8·66 ± 0·20	8·50 ± 0·22	0·51 ± 0·10	13·8 ± 1·0	13·7 ± 1·5
CU_5	0·74 ± 0·13	9·02 ± 0·47	8·81 ± 0·25	0·50 ± 0·11	13·3 ± 1·7	13·3 ± 1·7
CU_{ave}	0·75 ± 0·02	8·72 ± 0·21	8·61 ± 0·15	0·47 ± 0·06	14·0 ± 0·9	13·7 ± 0·4

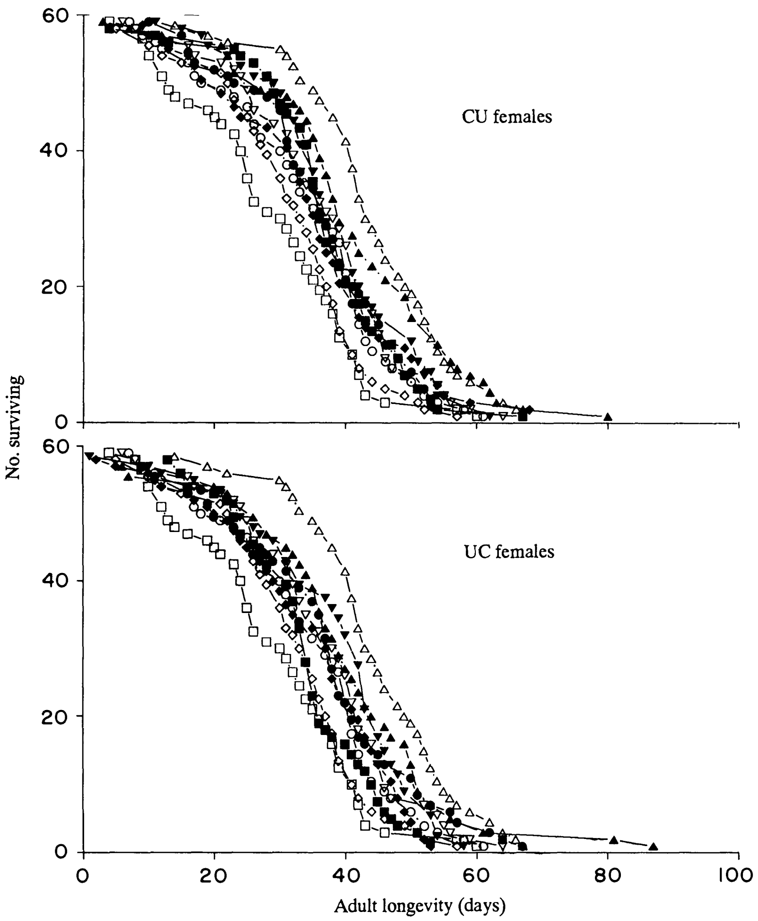

Fig. 10. The number (from 60 initially) of adult females that survive to various ages in the five B (open symbols) and five CU or UC (closed symbols).

Thus the B–O contrast appears to involve age-specific selection primarily, free of any significant admixture of density-dependent selection.

However, the comparison of the two selection regime differences, B–O *vs* r–K, is nonetheless deficient with respect to the clear articulation of the effects of density-dependent selection *vs* age-specific selection, given the confusion of selection mechanisms acting to differentiate K from r stocks. This was the motivation for the creation of the CU stocks, which have been selected specifically for adaptations to larval crowding. Further research devoted to the clarification of the multiple selection mechanisms in life-history evolution requires stocks of this kind rather than the r–K stocks.

SOME OF THE RESPONSES TO THE K SELECTION REGIME ARISE WITH SELECTION UNDER LARVAL CROWDING ALONE

Notwithstanding these strictures, it is important to note that the CU stocks give similar differentiation of larval characters to those observed in the comparison of r and K stocks. Pupation height and larval feeding rate were both increased in CU stocks relative to B stocks, as they were in K stocks relative to r stocks (Mueller & Sweet 1986; Joshi & Mueller 1988). Another interesting result is the test of the dependence of larval viability on larval density in CU *vs* B stocks. CU stocks have greater resistance to viability depression resulting from crowding, suggesting that they are indeed adapted to more crowded larval conditions. This result was obtained for r and K stocks also (Bierbaum *et al.* 1989), which again strengthens the connection between some of the K differentiation and selection under conditions of larval crowding.

Conclusion

The responses of *Drosophila* populations to age-specific selection and density-dependent selection do not appear to be generally similar. It is not the case that K and O populations, both of which have been selected for adaptations to ecologically demanding regimes, compared to their matched r and B controls, normally respond by equivalent mechanisms. This is not to preclude the possibility that some of the characters associated with adaptation to age-specific selection or density-dependent selection are related,

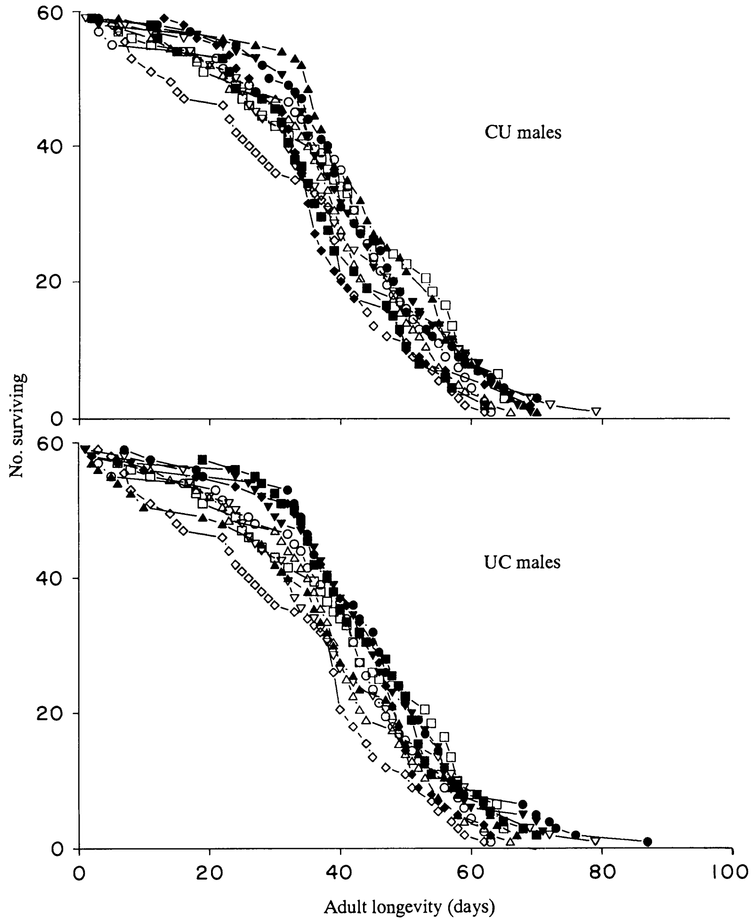

Fig. 11. The number (from 60 initially) of adult males that survive to various ages in the five B (open symbols) and five CU or UC (closed symbols).

only to assert that the overall adaptive syndromes are not similar.

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