Density-dependent natural selection in *Drosophila*: correlations between feeding rate, development time and viability

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Abstract

We have previously hypothesized that density-dependent natural selection is responsible for a genetic polymorphism in crowded cultures of *Drosophila*. This genetic polymorphism entails two alternative phenotypes for dealing with crowded *Drosophila* larval cultures. The first phenotype is associated with rapid development, fast larval feeding rates but reduced absolute viability, especially in the presence of nitrogenous wastes like ammonia. The second phenotype has associated with it the opposite set of traits, slow development, slow feeding rates and higher viability. We suggested that these traits are associated due to genetic correlations and that an important selective agent in crowded larval cultures was high levels of ammonia. To test this hypothesis we have examined viability and larval feeding rates in populations kept at low larval densities but selected directly for (i) rapid egg-to-adult development, (ii) tolerance of ammonia in the larval environment and (iii) tolerance of urea in the larval environment. Consistent with our hypothesis we found that (i) larvae selected for rapid development exhibited increased feeding rates, and decreased viability in food laced with ammonia or urea relative to controls, and (ii) larvae selected to tolerate either ammonia or urea in their larval environment show reduced feeding rates but elevated survival in toxin-laced food relative to controls. It would appear that development time and larval feeding rate are important characters for larvae adapting to crowded cultures. The correlated fitness effects of these characters provide important insights into the nature of density-dependent natural selection.

Introduction

An important feature of early research on life history evolution, especially in relation to density-dependence, was the idea of trade-offs (MacArthur & Wilson, 1967). The early arguments were not genetic in nature but rather focused on the seemingly obvious point that individual organisms will ultimately have finite energy resources to divide among many competing needs (Cody, 1966). In our own work on density-dependent natural selection in *Drosophila* we have identified genetically based trade-offs that affect density-dependent rates of population growth (Mueller & Ayala, 1981; Mueller *et al*., 1991) and efficiency of food utilization (Mueller, 1990; Joshi & Mueller, 1996).

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More recently we found that there was a genetic polymorphism within populations that had adapted to crowded larval cultures (Borash *et al*., 1998). Two phenotypic classes were identified that differed in the time they emerged from a crowded culture. The early emerging phenotype had a high larval feeding rate but low absolute viability especially in cultures in which the larval nitrogen waste product ammonia had been added. The later emerging phenotype had a slower feeding rate but higher absolute viability under several different conditions. These results were coupled with the observation that in crowded larval cultures the levels of ammonia increased exponentially during the course of the 20 days of larval growth.

These observations lead to the following hypothesis. The selection pressures in crowded *Drosophila* cultures change over the time it takes a larva to develop since the levels of food and levels of waste products are changing continuously and predictably over this period. Early in the life of the culture rapid development and high competitive ability are at a premium whereas late in the life of the culture the ability to tolerate waste products and low food levels are more important. The early phenotypic class has a high competitive ability due to their high feeding rate (Joshi & Mueller, 1988), but reduced efficiency of food utilization and tolerance of ammonia, presumably as a result of the genetic coupling of these traits with feeding rates. The late developing phenotype has the opposite set of traits that are most useful in the older, crowded *Drosophila* cultures.

If this hypothesis is true then the following predictions can be made concerning the evolution of these correlated sets of traits. Selection for rapid development should result in the appearance of the traits associated with the early phenotypes: high feeding rates and low viability especially in the presence of ammonia. Selection for the ability to tolerate ammonia in the larval environment should result in traits associated with the late phenotype: slow feeding rates but elevated viability in the presence of waste products. We test these predictions in this paper by studying *Drosophila* populations selected for rapid development and another set cultured for many generations in larval food with ammonia added. In addition we have tested a third set of populations that have been selected to tolerate urea in their larval food. These populations are interesting to address the question of whether the evolved response to larval ammonia is highly specific or whether it confers general resistance to other toxic nitrogenous compounds.

Methods and materials

Accelerated development populations

The derivation and maintenance of the accelerated development populations used in the present study are described by Chippindale *et al*. (1997). All of the populations are descended from a common ancestral population, the Ives population (Ives, 1970; Fig. 1). In 1980, replicate populations were derived from this ancestral population and subjected to two different demographic laboratory selection regimes as described in Rose (1984; Fig. 1). The B treatment (baseline) was maintained on the same discrete 2-week life cycle as the ancestral population. The O treatment (Old) was progressively selected for increased late fertility and longevity. Normally, the O populations reproduce at 8 weeks post-eclosion. Both B and O treatments are five-fold replicated. The B populations undergo their entire life-cycle in vials, including mating and egg laying, while the O populations are dumped into plexi-glass cages 2 weeks after egg collection and are maintained there until egg collection at 10 weeks. In 1989, intermediate selection generation treatments were derived from the B and O populations: the CB and CO populations. The CB populations were the direct descendents of the

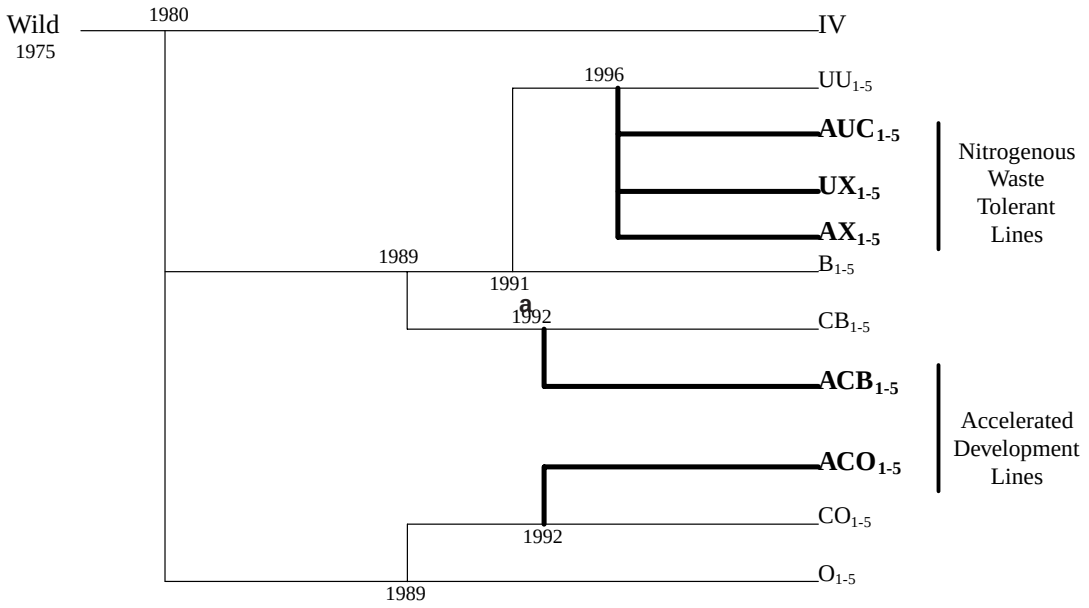

Fig. 1 The derivation of the accelerated development selection regime lines (ACB and ACO), their controls (CB and CO), and the nitrogenous waste tolerance selection regime lines (AUX, AX and UX). The graph is not chronologically to scale.

B populations, while the CO populations were derived from the O populations (see Rose *et al.*, 1992; Fig. 1). These 10 'C' populations spend the first 2 weeks in vials, reared in an identical manner to the B populations, after which the flies are dumped into plexi-glass cages and maintained until the eggs for the next generation are collected 2–3 weeks later.

In 1992, the accelerated development populations, ACB and ACO, were derived from the CB and CO populations, respectively (Fig. 1). Accelerated development selection was imposed on each of the derived populations by selecting for approximately the first 20% of the flies emerging from each of the uncrowded populations. The earliest emerging adults were dumped into plexi-glass cages and allowed to lay eggs for 24 h, after which eggs were collected for the next generation (for details see Chippindale *et al.*, 1997). By generation 100 of selection both ACB and ACO had generation times of 8 days. After approximately 110 generations, additional selection was suspended and all the populations were maintained on a fixed 9-day generation life-cycle. At the time of the present experiments approximately 210 generations had elapsed since the start of selection.

Nitrogenous waste-tolerant populations

Fifteen nitrogenous waste-tolerant populations were derived from the five UU populations (Joshi & Mueller, 1996). The UU populations originally derived from the B populations had been maintained at low larval and adult densities, on a 3-week generation cycle, for over 100 generations, when these populations were created. The new populations created from the UU populations were named in accordance with the particular nitrogenous waste product they encounter as larvae. The AX selection regime line (Fig. 1) experiences ammonium chloride (pH about 5.5, equivalent to the food pH) as larvae. Each generation, for each of the five AX populations, 60 8-dram vials with an equal concentration of ammonium chloride (NH_4Cl) added to the plain banana-molasses food medium (Rose, 1984) were inoculated with approximately 60–80 eggs laid on non-nutritive agar. Plastic sleeves were inserted in the vials, so that pupae may be removed before eclosion, and hence only the larvae are exposed to ammonia. After >90% of the larvae had pupated, the sleeves were removed and placed into plexi-glass cages, with the plain banana-molasses food plate.

After a majority of adults had eclosed, yeasted food plates were inserted into the plexi-glass cages, to stimulate female oviposition, so that eggs may be collected and the next generation started. Initial levels of ammonia and urea were chosen to reduce viability but permit the collection of about 1000 breeding adults. It was expected that in the absence of ammonia or urea there would be around 3500 adults produced from all vials. While we did not precisely count eggs or adults we aimed to keep urea and ammonia levels high enough to result in only one-third of the eggs surviving. When it appeared that survival was significantly above this level the urea or ammonia levels were increased (Fig. 2). The UX selection regime was maintained in a similar fashion, with the exception that they received urea-supplemented food as larvae. The AUC regime served as a control, and did not receive any nitrogenous waste exposure as larvae. During the larval feeding phase, all populations were maintained in incubators under constant conditions (25 °C, 24 h light, and constant humidity). In natural environments *Drosophila* larvae would not be expected to encounter urea since ammonia is the natural nitrogen waste product (Borash *et al.*, 1998). In these experiments the final levels of ammonia in the AX populations were roughly 10 times greater than the levels reached in crowded *Drosophila* cultures (Borash *et al.*, 1998).

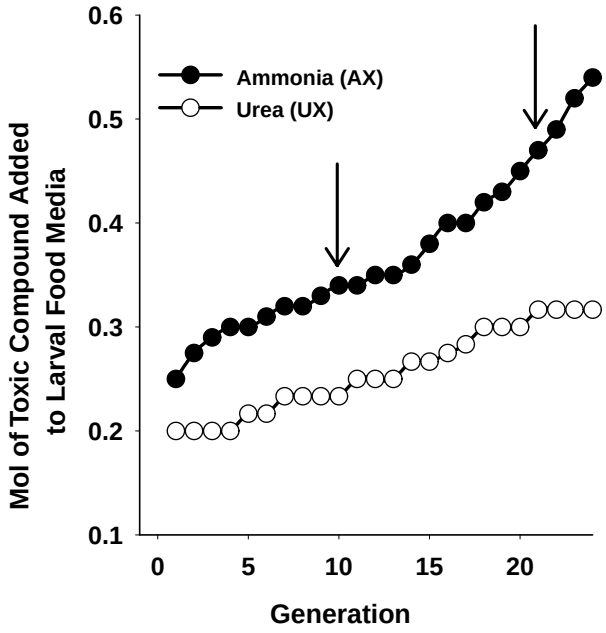

Fig. 2 The increase of supplemented levels of ammonium chloride (NH_4Cl) and urea added to the larval food medium of the AX and UX selection regimes, respectively. The arrows indicate the generations when assays of viability and feeding rates were performed.

Pre-experimental conditioning

Before any experiments were performed, all populations were removed from selection for two full, 2-week generations and reared under identical larval and adult conditions, to remove environmental or maternal effects that may confound the inference of genetic differences between the populations. Assays were performed after 10 and 21 generations of selection, for the nitrogenous waste tolerance populations.

Experiments with the accelerated populations were performed separately.

Experimental assays

Viability

Eggs laid over a 4–6-h period from the CO/CB/ACO/ACB populations were placed in 8-dram vials at exact densities of 60 per vial. The following food treatments were used: plain food, plain food supplemented with 0.266 mol urea, or 0.350 mol NH_4Cl. Eight vials per food treatment were set up for each population. At the onset of eclosion, newly emerged adults were removed, using CO_2 anaesthesia, every 8 h, sexed and counted. Checks were stopped after 2 days had passed with no new adults emerging. The same process was repeated for the nitrogenous waste tolerance stocks.

Feeding rate

Eggs laid over a 3–4-h period were collected and placed onto a Petri-dish of non-nutritive agar, and allowed to hatch. After 24 h, larvae were collected and placed onto a new Petri-dish with a liberal amount of 37 g/100 mL live yeast-distilled water solution spread over the top of an agar base, so that all larvae would begin feeding at the same time. The larvae were allowed to feed on this medium for 48 h before measurements of sclerite retraction rates, a measure of larval feeding (Joshi & Mueller, 1988), were made. A single larvae was placed onto an agar plate with a thin layer of 10 g/100 mL live yeast-distilled water solution on top, and allowed to adjust to the environment for at least 30 s, which was a suitable length of time for acclimatization (Joshi & Mueller, 1996). Then the larvae were recorded for at least 1 min of feeding using a video camera (Hitachi CCD colour camera, model KP-C553) mounted on a dissecting microscope. The recording has a date and time camera titler connected to the VCR, so that the elapsed time of feeding can be followed.

Feeding rates were measured during a 1-week period, with the selection regimes broken up into blocks of populations bearing the same subscript being measured at the same time (i.e. AUCi, AXi, UXi or ACBi, ACOi, CBi, COi). See Borash *et al*. (1998) for more detailed descriptions of this process.

Our feeding rate protocol differs from previous techniques employed to measure this character (Joshi & Mueller, 1996; Chippindale *et al*., 1997; Santos *et al*., 1997). These earlier techniques involved a single person measuring one live feeding larva viewed through a microscope. This method does not allow individual feeding rate measures to be checked. Obtaining accurate feeding measures is particularly difficult for rapidly feeding larvae. Videotaping permits individual measurements to be recounted and slowed down if necessary. Videotape of each larva feeding was viewed by at least two people, and if separate measurements were off by more than 10% that larva was re-checked.

We would like to point out that the work of Chippindale *et al*. (1997) found no difference in feeding rates between the accelerated developmental populations (ACB and ACO) and their controls (CB and CO). Chippindale *et al*. (1997) used a large number of students to measure feeding rates of different larvae. Individuals with less experience are prone to giving biased results, where feeding rates of fast feeding larvae are underestimated. Because we videotaped the larvae, several students could repeatedly survey each larva and at speeds slower than real time if necessary. The increased accuracy our method affords may be sufficient to account for the different findings.

Statistical analysis

All statistical analysis used SAS for Windows 98. In the analysis of variance (ANOVA), selection regime was a fixed effect, and replicate was a random effect. For the viability assays, data were arcsin transformed before the ANOVA in order to normalize the data. Additionally, for the viability assays, food type was a fixed effect in our analysis. Differences between specific selection treatments were tested by Scheffe's test.

Results

Larval feeding rates in *Drosophila melanogaster* are highly correlated with competitive ability (Sewell *et al*., 1975; Burnet *et al*., 1977; Joshi & Mueller, 1988). The ANOVA indicated that populations which had undergone selection for accelerated development (ACB and ACO) had faster feeding rates relative to their controls (CB and CO). There were no differences between the accelerated development populations (ACB vs. ACO), nor were the control populations significantly different (CB vs. CO) in sclerite retractions measured per minute (Fig. 3).

Populations which had evolved with nitrogenous waste added to their larval food medium (AX and UX) displayed slower feeding rates than their controls (AUC) in both the generation 10 (not shown) and the generation 21 assays (Fig. 3). Scheffe's test to correct for multiple comparisons revealed that the mean feeding rates for the AX and UX were significantly lower than the AUC feeding rate. However, there was no detectable difference between the UX and AX populations (Fig. 3).

The ANOVA of the arcsin-transformed viability data revealed that both selection regime and the selection × food-type interaction were significant factors determining viability in the accelerated development populations and their controls. When reared on plain food, the CB, CO and ACO populations were significantly more viable than the ACB populations (Fig. 4A). When reared on food containing 0.350 mol ammonium chloride, all populations suffered a dramatic decline in viability. The CB and CO populations were significantly better at tolerating the damaging effects of ammonia in

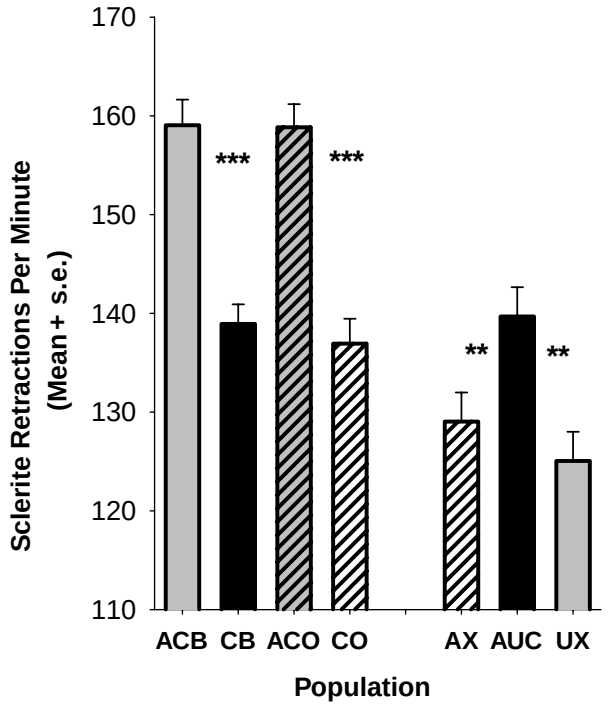

Fig. 3 Mean feeding rates. The mean feeding rate, measured as the number of sclerite retractions per minute, for the populations selected for accelerated development and the populations selected for ammonia and urea tolerance at generation 21. The error bars are standard errors around the mean of the five replicate populations of each selection regime. $^{**}P < 0.01$, $^{***}P < 0.005$.

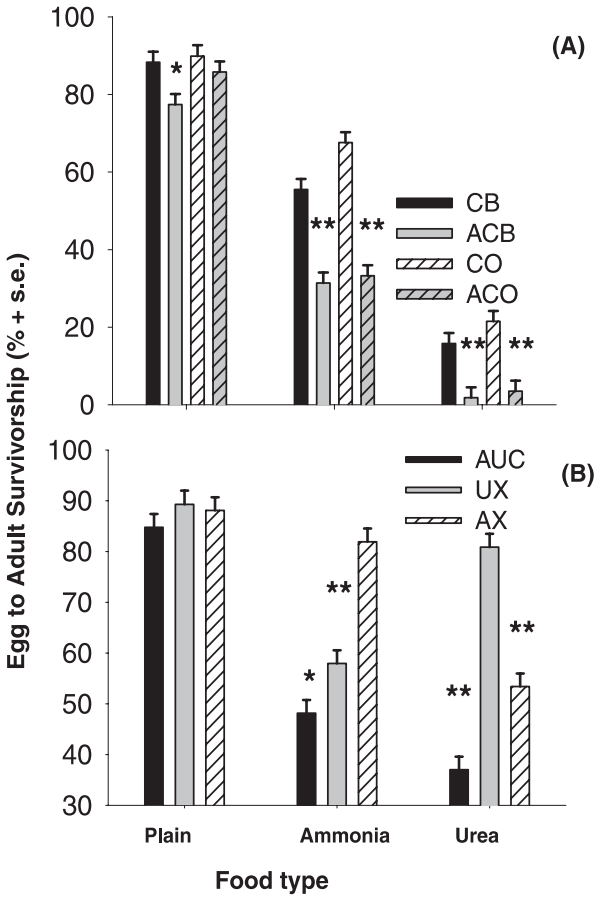

Fig. 4 Mean viability. (A) The mean viability of the populations selected for accelerated development and their controls, measured in three different food types: plain food, food supplemented with ammonium chloride, or food supplemented with urea. The error bars are standard errors around the mean of the five replicate populations of each selection regime. (B) The mean viability of the populations selected for ammonia and urea and their controls. The error bars are standard errors around the mean of the five replicate populations of each selection regime. $^{*}P < 0.05$, $^{**}P < 0.01$.

their larval food medium, compared to ACB and ACO populations. When reared on food containing 0.266 mol urea, all populations suffered an even more dramatic decline in viability, due to the toxic effects of urea. The CB and CO populations were significantly better at tolerating the damaging effects of urea in their larval food medium than were the ACB and ACO populations.

After 21 generations of selection for either urea or ammonia tolerance, there were no detectable viability differences among all populations when reared as larvae on plain food (Fig. 4B). When reared as larvae on food supplemented with 0.350 mol ammonium chloride, the AX populations displayed the greater viability, relative to the UX and AUC populations. The UX populations had a significantly greater viability than that of the AUC controls. When reared as larvae on food supplemented with 0.266 mol urea, the UX populations displayed the greatest viability relative to the AX and AUC populations. The AX populations had a significantly greater viability than that of the AUC controls. The generation 10 results were identical in terms of the direction of the differences shown above. The magnitudes of the differences were larger in the generation 21 assay.

Discussion

Borash *et al*. (1998) studied the conditions under which genetic polymorphisms may be maintained in a temporally varying environment in laboratory-adapted populations of *Drosophila melanogaster*. The theoretical work and experimental work of Borash *et al*. (1998) suggested that trade-offs in fitness components were a key to the maintenance of genetic polymorphisms. It was suggested that these trade-offs were reflected in a suite of genetic correlations between development time, feeding rate and tolerance of ammonia.

This study offers further evidence of causal genetic links between feeding rate, viability and sensitivity to nitrogenous waste. Populations which have been subjected to extreme demographic selection for early

eclosion (ACB and ACO) demonstrate a reduced tolerance to both ammonia and urea. It had been previously found that the evolution of decreased developmental time is coupled with a reduction in viability on plain food (Chippindale *et al*., 1997). In our experiments the differences in viability on plain food have disappeared in the ACO and CO populations and a small difference remains between the ACB and CB populations. When assayed in environments supplemented with nitrogenous compounds, however, the populations selected for rapid development show pronounced viability reductions relative to their controls. In ammonia-laced larval food the viability of the ACB populations is reduced to 57% of the CB level while the ACO viability is reduced to 49% of the CO.

There may be several explanations for the different observations of ACO and CO viability in this study and in Chippindale *et al*. (1997). (1) It might be that mutations with compensating effects on viability have arisen in the 85 generations since the Chippindale study (Lenski, 1988). However, in these relatively small populations (approximately 1000 adults) it is unlikely to have a repeated beneficial mutation event in five replicate populations in just 85 generations. (2) An alteration in the selection regime may have relaxed selection slightly, permitting viability to increase. Chppindale's study occurred during the active phase of selection in which the earliest developing adults were chosen for the next generation. That procedure was relaxed around generation 110 and adults were simple collected after 9 days compared with the peak of selection at 8 days. This sight relaxation of selection may have resulted in improved viability of the selected stocks since the relative fitness values of development time to viability had now been altered.

Populations that have adapted to high levels of nitrogenous waste show higher viabilities in the presence of these toxic compounds, while displaying lower feeding rates relative to that of their controls. Moreover, it appears that tolerance to nitrogenous substances was not specific, as populations which have evolved tolerance to one nitrogenous waste show some tolerance to a chemically distinct nitrogenous waste. There appears to be no cost associated with this response in environments not supplemented with nitrogenous substances, as the viabilities were not significantly different when the UX, AX and AUC populations were reared as larvae on plain food. This further indicates that genes conferring tolerance to one unique nitrogenous compound also provide a measurable degree of tolerance to a related, but chemically distinct, nitrogenous compound.

It appears that the ability to feed faster involves a reduction in the ability to tolerate nitrogenous waste. The mechanistic basis of this association is not completely understood. Since there is an increased influx of toxic food into a faster-feeding organism, it must have the ability to dispose of the nitrogenous substances or suffer the adverse effects created by their presence. In urea-selected populations, an increase in the excretion of urea may be the major mechanism responsible for tolerance (V. Pierce, personal communication). We have not ruled out that these populations have reduced the uptake of ingested nitrogen compounds through a decrease in gut permeability to that waste. These mechanisms, coupled or not, could have produced the observed patterns.

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