Patterns of selection: stress resistance and energy storage in density-dependent populations of Drosophila melanogaster

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Abstract

Populations of Drosophila melanogaster subjected to extreme larval (CU) or adult (UC) densities for multiple generations were assayed for a variety of life history characters. When reared under either crowded or uncrowded larval conditions, populations which had been selected to tolerate the limitation of resources imposed by extreme larval (CU) crowding, exhibited greater starvation resistance and lipid content than did populations which do not routinely undergo larval density-dependent regulation. Previous studies have shown that the CU populations do not show a correlated increase in longevity; as has been generally observed for these characteristics in age-structured populations of D. melanogaster. This suggests that density-dependent natural selection may not always shape life histories of the same characteristic in the same direction that age-specific selection does. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Evolutionary biology seeks to explain the mechanisms by which the environment may shape a population’s life history characteristics. The attempt to determine whether there are patterns through which populations may evolve has been the focus of many studies (Anderson, 1971; Charlesworth, 1971; Rose et al., 1996; Roughgarden, 1979; Travis and Mueller, 1989). The interaction between age-specific natural selection and density-dependent natural selection has only recently begun to be investigated, with only some characteristics showing common patterns of response to laboratory selection (Borash et al., 2000; Mueller et al., 1993; Shiotsugu et al., 1997).

In age-specific populations of Drosophila melanogaster, it has been repeatedly observed that selection for increased late-life fertility imparts a subsequent increase in starvation resistance at the expense of early fecundity (Rose, 1984; Service, 1987; Service et al., 1985). Consequently, the selection for increased starvation tolerance imparts an increase in late life fertility but a decrease in early fertility (Chippindale et al., 1996; Rose et al., 1992). These characteristics appear to respond to selection in a predictable fashion (although see Rose et al., 1996).

Stemming from work investigating the effect of the environment in shaping life histories is the role of trade-offs between life history characters (Chippindale et al., 1993; Partridge and Harvey, 1988; Reznick 1985, 1992; Tucic et al., 1997). Assays of demographically and stress selected D. melanogaster have yielded much evidence that energetic allocation may affect the trade-off of survival and reproduction (Chippindale et al., 1993; Graves et al., 1992; Service 1987, 1989). It is known that experimentally reducing the caloric intake of rodents increases their longevity through transforming energy for reproduction into energy used for survival (Holehan and Merry, 1985; Masoro, 1988; Rose, 1991).

Dietary manipulation of D. melanogaster has been performed before, in the attempt to understand the phenotypic trade-offs associated with longevity and reproduction (Chippindale et al., 1993; Service et al., 1985). Results from age-specific populations of D. mel-
D. melanogaster show that a marked increase in longevity is associated with correlated increases in both absolute lipid content and starvation resistance (Chippindale et al., 1993; Service, 1987; Service et al., 1985). Populations that have been selected for starvation resistance for many generations show increased absolute lipid content and greater longevity than their controls (Chippindale et al., 1993).

The density at which a population is reared may have important life history consequences for a population (Bakker, 1959; Chiang and Hodson, 1950; Joshi, 1997; Joshi and Mueller, 1996; Mueller, 1988; Sewell et al., 1975; Sokolowski et al., 1997). Many of these adaptations involve changes in larval behavior or physiology and are a direct consequence of selection caused by crowded larval conditions (Mueller et al., 1993). However, it is clear that the environment experienced by larvae may have an impact on adult fitness including reproduction (Prout and McChesney, 1985). In part, these outcomes are mediated by the effects of larval crowding on adult size (Chiang and Hodson, 1950). In general, adult females that emerge from crowded larval cultures are smaller and thus, produce fewer eggs.

Previous experiments (Mueller et al., 1993), have shown that populations which have undergone multiple generations of selection for tolerance to crowded larval conditions show no increase in longevity relative to their uncrowded controls. However, it appears that populations subjected to crowded larval conditions appear to show slightly greater adult fecundity (unpublished data), relative to their control populations. Fecundity, as well as starvation tolerance are correlated with lipid content (Djawdan et al., 1998).

In principle, adaptations to crowded larval conditions may be constrained or affected by the effects of these adaptations on adult fitness. One physiological aspect of adult reproduction is female fecundity, which is correlated to the amount of available lipid in D. melanogaster (Djawdan et al., 1998). In this study, we investigated the physiological effects of density-dependent natural selection on energy storage and stress resistance in adults, in order to determine if adaptation to either high adult or high larval densities affected the expression of these adult traits. Additionally, we determined, whether the relationship between genetically correlated characteristics in populations under density regulation is similar to results seen for populations subject to age-specific natural selection.

2. Materials and methods

2.1. Populations employed

All populations used in these assays were derived from the five B-lines of Rose (1984). The CU populations were created in January 1990; and the UC and UU populations were derived in September 1991. The CU, UC and UU populations were kept in five-fold replicate with discrete generations in continuous light at 25°C. The five CU populations were crowded as larvae (1000–1500 eggs per 6-dram vial, 40 replicate vials per replicate) with eclosing adults being kept in uncrowded conditions (60–80 adult flies per 8-dram vial) on fresh banana–molasses medium transferred every other day for approximately one week. The five UC populations were uncrowded as larvae (60–80 eggs per 8-dram vial, 60 replicate vials per replicate population), with eclosing adults being crowded (~200 adults per 8-dram vial) at two weeks from egg collection. Adults were transferred to fresh food every other day for one week. The five UU populations were reared in uncrowded conditions as larvae for two weeks, with eclosing adults being kept in uncrowded conditions. Adults were transferred to fresh food every other day for one week. Each generation, the adult population size of each replicate, for each of the two selection regimes and the UU controls was in excess of 1200 flies, which would minimize the effects of inbreeding depression. A more detailed explanation of the rearing protocol for these populations can be found in Joshi and Mueller (1996).

Before any experiments were performed, all populations were separately reared under uncrowded larval and adult conditions, in order to remove any environmental and maternal effects, which might confound the observation of subtle genetic differentiation between the populations. At the time of the following experiments, the CU populations had undergone >90 generations of selection for larval crowding tolerance, and the UU and UC populations had undergone >75 generations in their particular regime.

2.2. Rearing conditions of flies

Eggs laid over 8 h on banana–molasses medium, supplemented with live yeast paste, were placed in vials at two separate densities. A low rearing density of exactly 60 eggs per 8-dram vial (10 vials per population), and a high rearing density of exactly 500 eggs per 6-dram vial (5 vials per population) were used. Eggs were collected on small cubes of non-nutritive agar, so as not to affect the food levels within the vials. While 500 eggs per vial was considerably less than the density of the stock CU populations, we chose this density to represent a crowded culture as we required a large set of adults for the assays. All vials contained exactly 5 ml of banana–molasses food medium. Checks for eclosing flies were performed every 6 h, at which time the adults were assayed for starvation tolerance or frozen for lipid content analysis (Fig. 1).
Fig. 1. The experimental design. CU, UC, and UU populations were reared in a common, uncrowded environment for two generations in order to remove non-genetic effects. Then eggs from each population were placed into vials that were either uncrowded (60 eggs/8-dram vial) or crowded (500 eggs/6-dram vial). Developmental time and egg-to-adult viability/survivorship were assayed. Adults eclosed from these vials were used to measure dry weight, lipid content, and starvation tolerance.

2.3. Developmental time/viability assay

Developmental time checks for eclosing flies were performed every 6 h, at which time the number and sex of newly emerged adults were recorded. Dead adults, stuck in the food at the bottom of the vial, were counted as eclosed adults, and were carefully removed from the food medium. Checks were halted, for a replicate series of vials, after 72 h during which no new adults had emerged from that set of vials.

2.4. Starvation time assay

Flies emerging from the development time experiment were frozen, dried (1 h at 60°C), and weighed (in batches of 10 flies of each sex per population per density). At least three separate replicate batches were weighed for each sex from each replicate population. After weighing, the flies were placed into a petroleum ether extractor for 24 h, after which, they were dried for 30 min at 60°C, and re-weighed. The absolute lipid content was calculated as the initial dry weight minus the dry weight after lipid extraction.

2.5. Dry weight/lipid content assay

Flies emerging from the development time experiment were refrigerated, dried (1 h at 60°C), and weighed (in batches of 10 flies of each sex per population per density). At least three separate replicate batches were weighed for each sex from each replicate population. After weighing, the flies were placed into a petroleum ether extractor for 24 h, after which, they were dried for 30 min at 60°C, and re-weighed. The absolute lipid content was calculated as the initial dry weight minus the dry weight after lipid extraction.

2.6. Statistical analysis

The evaluation of significant effects was made with the aid of analysis of variance (ANOVA) implemented on SAS for Windows version 6.08. Population (CU versus UC versus UU), sex (male and female), and density (uncrowded versus crowded) were treated as fixed effects. Population replicate was treated as a block effect because of the common origin of CUi, UCi, and UUi populations. Survivorship and development time data were transformed using the arcsin square-root transformation. Multiple comparisons were performed using the Tukey–Kramer or Scheffe method. All results shown are that of non-transformed data.

3. Results

3.1. Developmental time assays

Results show that for development time (Fig. 2), larval crowding significantly prolonged mean eclosion time. At low rearing density (60 eggs per 8-dram vial), there were no significant differences in development time among any of the three populations tested for either sex. At low rearing density, female flies eclosed faster than male flies, on an average of 4.5 h sooner. At high larval density (500 eggs per 6-dram vial), both males and females from the CU populations developed faster than males or females from the UU and UC populations. Additionally, the UU populations developed faster than the UC populations. When reared at high larval density, there were no developmental time differences between males and females.
Fig. 2. Developmental time. The effects on developmental time of larval rearing density and selection history of the CU, UC, and UU populations, all having undergone development from egg to adult in either uncrowded or crowded larval conditions. The error bars are standard errors around the mean of the five replicates.

3.2. Viability

The ANOVA showed that population was not a significant factor affecting egg-to-adult survivorship ($F_{1, 4}=0.17; P=0.66$). At low larval rearing density, there were no differences in viability among any of the populations assayed. At a density of 500 eggs per vial, the viabilities declined significantly from the low density level, but, again, there were no significant differences between any of the populations assayed (Fig. 3).

3.3. Starvation time

The ANOVA showed only population ($F_{2, 8}=11.407; P<0.005$), density ($F_{1, 4}=16.287; P<0.01$), and sex ($F_{1, 4}=18.408; P<0.01$) as significant factors affecting starvation resistance. Both males and females from the CU populations, reared at low larval density, demonstrated longer times to death by starvation than did males and females from either the UC populations or the UU controls ($P<0.01$). Additionally, males and females from the UU control populations, reared at low larval density, demonstrated longer time to death by starvation than did males and females from either the UC populations or the UU controls. However, when reared at high larval densities, both males and females from the UC populations and the UU controls survived for the same period of time in the absence of food.

High larval rearing density caused both males and females to decline in their ability to tolerate starvation, regardless of population. For populations reared at the same larval densities, females always displayed equal, if not greater starvation tolerance than did males.

3.4. Dry weight/lipid content assays

The ANOVA for dry weight revealed that selection regime ($F_{1, 4}=3.29; P>0.05$) was not an overall significant factor influencing dry weight at eclosion. CU females and UU females from uncrowded larval rearing density were significantly heavier than uncrowded larval rearing density UC females. CU and UU males from uncrowded larval rearing density weighed the same, but both were heavier than UC males. Females eclosing from crowded larval culture showed no differences in dry weight, while CU males from high larval rearing density.
Table 1
Summary of results for dry weight, lipid content, and percent lipid assays. Lipid content was determined as the dry weight minus the after extraction weight. Percent lipid content was calculated as \([\text{Dry Weight (mg)} - \text{Lipid (mg)}]/\text{Dry Weight (mg)}\). SE: standard error around the mean of the five replicates.

<table>
<thead>
<tr>
<th>Rearing lipid Density</th>
<th>Weight (mg/fly) se</th>
<th>Proportion Lipid se</th>
</tr>
</thead>
<tbody>
<tr>
<td>UU females Low</td>
<td>0.0708 0.0021</td>
<td>0.338 0.0043</td>
</tr>
<tr>
<td>UC females Low</td>
<td>0.0655 0.0025</td>
<td>0.312 0.00407</td>
</tr>
<tr>
<td>CU females Low</td>
<td>0.0774 0.0025</td>
<td>0.346 0.00418</td>
</tr>
<tr>
<td>UU females High</td>
<td>0.0543 0.0025</td>
<td>0.211 0.00418</td>
</tr>
<tr>
<td>UC females High</td>
<td>0.0543 0.0025</td>
<td>0.210 0.00418</td>
</tr>
<tr>
<td>CU females High</td>
<td>0.0637 0.0025</td>
<td>0.216 0.00418</td>
</tr>
<tr>
<td>UU males Low</td>
<td>0.0629 0.0025</td>
<td>0.259 0.00429</td>
</tr>
<tr>
<td>UC males Low</td>
<td>0.0488 0.0025</td>
<td>0.204 0.00418</td>
</tr>
<tr>
<td>CU males Low</td>
<td>0.0643 0.0026</td>
<td>0.263 0.00429</td>
</tr>
<tr>
<td>UU males High</td>
<td>0.0419 0.0025</td>
<td>0.154 0.00429</td>
</tr>
<tr>
<td>UC males High</td>
<td>0.0375 0.0025</td>
<td>0.148 0.00411</td>
</tr>
<tr>
<td>CU males High</td>
<td>0.0513 0.0026</td>
<td>0.163 0.00407</td>
</tr>
</tbody>
</table>

Crowded larval conditions limited the amount of lipid that both males and females could eclose with, across all populations \((F_{1,5}=30.74; P<0.01)\). CU females, from both high and low larval density conditions eclosed with a greater amount of lipid reserves, than did the UU or UC females. In the low larval density treatment, the UU females had a higher lipid content than the UC populations, whereas, the UU and UC females contained the same amount of lipid when reared in uncrowded larval conditions. CU and UU males emerging from uncrowded larval conditions contained equal amounts of lipid, but both contained more lipid than the UC males. For males eclosing from crowded larval conditions, the CU populations (Scheffe Test: \(P<0.01\)) contained more lipid than the UU populations, which, in turn, eclosed with more lipid than males from the UC populations.

Percent lipid content was calculated by subtracting the absolute lipid content from the dry weight and then dividing by the dry weight. There were no differences in male percent lipid among populations when they were reared at low larval density. Males from the CU populations demonstrated a greater percent lipid content than males from UU or UC populations only when reared at high larval density, (sex×density effect \(F_{1,5}=25.48; P<0.01\)). At low rearing density, females from all three populations have similar percent lipid content, but CU females from high density have a greater fraction of their body weight as lipid than either the UU or UC populations (see Table 1 for summary of results).

4. Discussion

Populations of *D. melanogaster* selected for either increased late fertility or starvation resistance (Chippindale et al. 1996, 1998; Rose, 1984; Service,
1987; Service et al., 1985) show correlated increases in metabolic components; such as lipid content (Djawdan et al., 1998). Additionally, they show a marked increase in developmental time and a corresponding increase in egg-to-adult survivorship (Chippindale et al., 1996). It was interesting to see whether these patterns through which life history characteristics have repeatedly evolved in age-specific populations of *D. melanogaster* are similar in density-dependent populations of *D. melanogaster*.

While the CU populations do develop faster when reared in crowded larval culture, there does not exist a detectable difference in egg-to-adult survivorship, as would be expected in populations selected for extreme larval crowding tolerance. One explanation for the lack of differentiated viabilities may be due to the larval rearing density employed for these experiments. The CU population is routinely maintained at a larval density of >1000 larva per 6-dram vial. Our experimental density was less than half of the normal rearing density. Our experimental crowded density may not have been sufficiently stressful to observe viability differences between the populations, even though it did cause a dramatic reduction in viability within populations. Previous results with *r*- and *K*-selected populations, revealed that the *K*-selected populations, which had been reared in both crowded larval and adult conditions, had greater egg-to-adult survivorship, when reared in crowded conditions (Bierbaum et al., 1989). Additionally, late-emerging sub-populations derived from the CU populations demonstrated greater egg-to-adult survivorship relative to the UU populations, when reared at densities of exactly 1000 eggs per vial (Borash et al., 1998).

Populations routinely subjected to extreme larval crowding exhibited an increase in the ability to tolerate starvation stress, when reared in either crowded or uncrowded larval conditions. As expected, death by starvation was faster for populations that had been reared under crowded larval conditions. This occurs, as flies emerging from crowded conditions do so at a smaller size (Chiang and Hodson, 1950), presumably, with less available metabolic resources with which to tolerate a stress such as starvation (Miller and Thomas, 1958).

Previous results with *r*- and *K*-selected populations, demonstrated that the *K*-populations, which had been reared in both crowded larval and adult conditions, had superior starvation resistance (Mueller et al., 1993), relative to that of the *r*-selected populations, which were reared in uncrowded larval and adult conditions. As the UC populations do not experience an increase in starvation tolerance relative to the UU controls, we can tease apart the *r*- and *K*- populations’ starvation result, as being largely influenced by selection for tolerance to larval crowding.

A subsequent increase in lipid content was detected in the CU populations. In many cases, the UC populations showed the lowest starvation tolerance and the lowest level of lipid. As lipid content and starvation tolerance have been shown to be genetically correlated traits (Chippindale et al., 1993; Service, 1987; Service et al., 1985), our findings serve to indicate that this genetic trade-off between energetic allocation and stress tolerance also exists in populations that have undergone density-dependent natural selection.

The UC populations often show the lowest lipid levels and starvation resistance. Previous studies of these populations have shown that they have evolved significantly greater ability to tolerate a crowded adult environment (Joshi et al., 1997), perhaps by limiting the duration which flies remain near the food, at the bottom of the vial. It appears that the ability to survive in a crowded adult culture, however, has not increased the UC population’s ability to tolerate starvation stress, either behaviorally or physiologically.

In Zwaan et al. (1991), populations of *D. melanogaster* were found to exhibit greater starvation resistance and percent lipid when they were reared in crowded larval culture. These results may be due to the fact that Zwaan’s starvation and lipid assays were conducted on flies that were already 28 days post-eclosion, whereas our assays began to measure the characteristics within 6 h after emergence. It is well known that an organism’s phenotypic characters may change with age (Chippindale et al., 1993; Rose, 1984; Service, 1989). Zwaan’s reasoning for using day 28 as the initial assay time was based on the work by Service (1987), which showed that flies derived from the IV population (Rose, 1984), reached a maximal lipid concentration at day 21. The life history of the flies that Service used is more than likely distinct from that experienced by Zwaan’s flies. As a result, without assaying whether Zwaan’s flies behave similarly at day 21, there is no direct evidence to suggest what their peak lipid concentration will be at day 21.

Additionally, the techniques employed by Zwaan et al. (1991) may not be adequate to measure starvation resistance. Their protocol to measure starvation tolerance used an agar plug at the bottom of the vial, serving as a water source. While agar is non-nutritive to *D. melanogaster*, other organisms (particularly bacteria and yeast) may use agar as a rich growth medium. It is possible that the flies were able to acquire food from microorganisms growing on the surface of the agar plug. As further evidence of this possibility, they report that their female flies survived starvation stress in excess of 180 h. Zwaan et al. (1991) employed flies with no history of starvation stress selection, however, many of their populations tolerated starvation stress longer than *D. melanogaster* that had been directly selected for starvation tolerance for multiple generations (Chippindale et al., 1996; Rose et al., 1992).

It has been shown that density-dependent and age-spe-
cific populations of *D. melanogaster* can demonstrate similar evolutionary outcomes for larval feeding rate and viability (Borash et al., 2000). However, Mueller et al. (1993) revealed that not all life history characteristics will evolve along the same pathways for populations under density-dependent natural selection and populations under age-specific natural selection. Previous studies with the CU populations show that they do not always follow the patterns of correlated characters seen in age-specific populations of *D. melanogaster*. While the CU populations do exhibit superiority for starvation tolerance relative to the UU and UC populations, there is no indication of CU superiority for the genetically correlated characteristic longevity (Mueller et al., 1993). The genetic correlation between starvation tolerance and longevity has been observed repeatedly in starvation selected and postponed senescent populations of *D. melanogaster* (Chippendale et al. 1993, 1996; Service, 1987; Service et al., 1985). While we do see that adaptation to tolerate extreme larval crowding does alter several important life history characteristics, it may not affect the same characteristics to the same degree, as seen in age-specific populations of *D. melanogaster*.

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