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A Genetic Polymorphism Maintained by Natural Selection in a Temporally Varying Environment

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ABSTRACT: Environments that are crowded with larvae of the fruit fly, *Drosophila melanogaster,* exhibit a temporal deterioration in quality as waste products accumulate and food is depleted. We show that natural selection in these environments can maintain a genetic polymorphism with one group of genotypes specializing on the early part of the environment and a second group specializing on the late part. These specializations involve trade-offs in fitness components. The early types emerge first from crowded cultures and have high larval feeding rates, which are positively correlated with competitive ability but exhibit lower absolute viability than the late phenotype, especially in food contaminated with the nitrogenous waste product, ammonia. The late emerging types have reduced feeding rates but higher absolute survival under conditions of severe crowding and high levels of ammonia. Organisms that experience temporal variation within a single generation are not uncommon, and this model system provides some of the first insights into the evolutionary forces at work in these environments.

Keywords: Drosophila melanogaster, density-dependent selection, nitrogen wastes, ammonia, urea.

An important goal of evolutionary biology is to develop an understanding of the role the natural environment has in molding adaptations and affecting allele frequency change (Partridge and Harvey 1988; Roff 1992; Stearns 1992). In the classical models of natural selection, the fitness of a genotype was assumed to be constant and thus unresponsive to changes in the environment. The theory of density-dependent natural selection was one of the first attempts to alter this view of evolution by developing a theory that permitted fitness to be a function of local population size (Anderson 1971; Roughgarden 1971; Smouse 1976; Felsenstein 1979; Asmussen 1983; Mueller 1988*a;* Tanaka 1996).

Another important innovation in the theory of evolution was the examination of the outcome of natural selection when fitness varied over generations. This theory has included models in which the environment passed through fixed cycles, varied at random, or possessed some autocorrelated variation (Wright 1948; Kimura 1954; Dempster 1955; Haldane and Jayakar 1963; Gillespie 1973, 1991; Hartl and Cook 1973; Jensen 1973; Felsenstein 1976). However, all of these theoretical models are similar in their assumption that within a generation the environment assumed a fixed state and that genotypes could be characterized by a set of constant fitness values.

An unexplored but feasible extension of this theory would examine environments that go through a temporal sequence of deterioration within a generation, for instance, habitats that are ephemeral and change rapidly over time. This deterioration of the environment could be a function of ecological conditions like population density. One example may be excrement from large mammals that serves as a habitat for many insects and microorganisms but dries out and decays over time. For *Drosophila,* similar conditions may occur in fresh fruit that falls to the ground and starts to decay. *Drosophila* larvae in these habitats may often be at suboptimal densities (Grimaldi and Jaenike 1984). The concentration of organic compounds also changes over time in *Drosophila* cultures. In particular, *Drosophila* food that initially has high levels of ethanol shows a marked decline in ethanol levels and an increase in acetic acid levels as the cultures age (Hageman et al. 1990).

Many plant species may find themselves in environments in which the quality declines over time. For instance, goldenrod usually occupies recently cleared, early successional habitats. As additional species settle nearby, the shading and competitive environment are altered significantly (Abrahamson and Weiss 1997). A similar

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sort of successional series can be created by fires; selection pressures may change over time in these environments (Scheiner 1989). Any character, like dispersal ability or development time, that exposes individuals to different slices of these sorts of temporal decays makes it possible for selection to act on traits that differentially adapt organisms to these changing aspects of the environment.

This study develops a model system in which to study adaptation to these types of heterogeneous environments. Here we examine populations of *Drosophila melanogaster* used to study density-dependent natural selection, specifically through crowding in the larval stage (Mueller et al. 1993). While the populations are cultured on a fully discrete regime of reproduction, the larval environment shows a gradual deterioration over time as food is depleted, nitrogen wastes accumulate, and dead larvae decay. This temporal variation permits some genotypes to specialize on the early part of the environmental sequence and others to specialize on the late part of the sequence.

Methods

Populations

The two selected populations are both derived from a long-standing laboratory-adapted population called the B's (Rose 1984). One population called the *UU* population has evolved in the laboratory under uncrowded larval (50–80 larvae/8-dram vial) and adult (50 adults/8-dram vial) densities (Joshi and Mueller 1996). Each *UU* population consists of 40 vials. The second population, called *CU,* is maintained the same as the *UU*'s except that larvae are crowded (>1,000 larvae/6-dram vial). Each *CU* population consists of 20 vials. The *UU* and *CU* populations had evolved for approximately 117 and 145 generations, respectively, before the onset of the experiments. Each of these populations is replicated fivefold so that differences between the *CU* and *UU* populations due to natural selection (a deterministic process) can be separated from differences arising due to genetic drift (a stochastic process; Rose et al. 1996). All populations have breeding adult numbers of more than 1,500 adults each generation. Eggs from each of these populations (the five *CU* and the five *UU*) were collected and raised under the same high larval densities (fig. 1). Early adults are those that emerge during the first 72 h of adult eclosion. Previous work suggested that this sample will include about 15%–20% of all eclosed adults. During the next 9–10 d, eclosing adults were removed daily from their crowded cultures and not used. Flies emerging after this period were collected for about 48–72 h and classified as late adults. Eggs were then collected from each of the four population types (*CU*-early, *CU*-late, *UU*-early, and *UU*-late) and passed through two generations of common conditions, which consisted of low larval and adult densities. This type of standardization insures that any observed phenotypic differences between the four population types cannot be attributed to different environments the individuals were raised in (acclimation) or the different environments the mothers of the tested individuals were raised in (maternal effects). Consequently, these phenotypic differences ought to be due to genetic differences between the populations (Clausen et al. 1941). Since each population was replicated fivefold there were a total of 20 experimental populations on which the experimental assays were done.

Feeding Rates

Eggs were collected from adults that had been through the two-generation standardization procedure described in figure 1. Newly hatched larvae from these eggs were raised on petri dishes with agar and live yeast paste. At 48 h of larval development, feeding rates of 20 larvae per population were measured by methods described elsewhere (Joshi and Mueller 1988), with the following modifications. At least 1 min of feeding behavior was videotaped with a camera attached to the dissecting microscope. Feeding rates were then counted from videotape records by two different people. If any feeding rates for a single larva differed by more than 10 retractions per minute the results were rechecked by each investigator. All feeding rates were completed during 1 wk. Since all populations could not be finished on a single day, populations were broken into blocks. On a single day, all the populations with the same subscript were tested (e.g., CU_1-early, CU_1-late, UU_1-early, UU_1-late). Thus, the possibility exists that for tests conducted on different days uncontrolled experimental variables could produce differences in feeding rates. This experimental design is handled by the block design ANOVA discussed later. A second feeding rate assay was conduced without the *UU*-derived populations. This experiment involved an independent derivation of the early and late populations as described previously. We present these results only to illustrate that the large differences between the *CU*-early and -late population feeding rates are repeatable phenomena.

Viability

Adults that had undergone the standardization procedure were used to collect 60 eggs on nonnutritive agar. These eggs were placed in 8-dram vials with 5 mL of food,

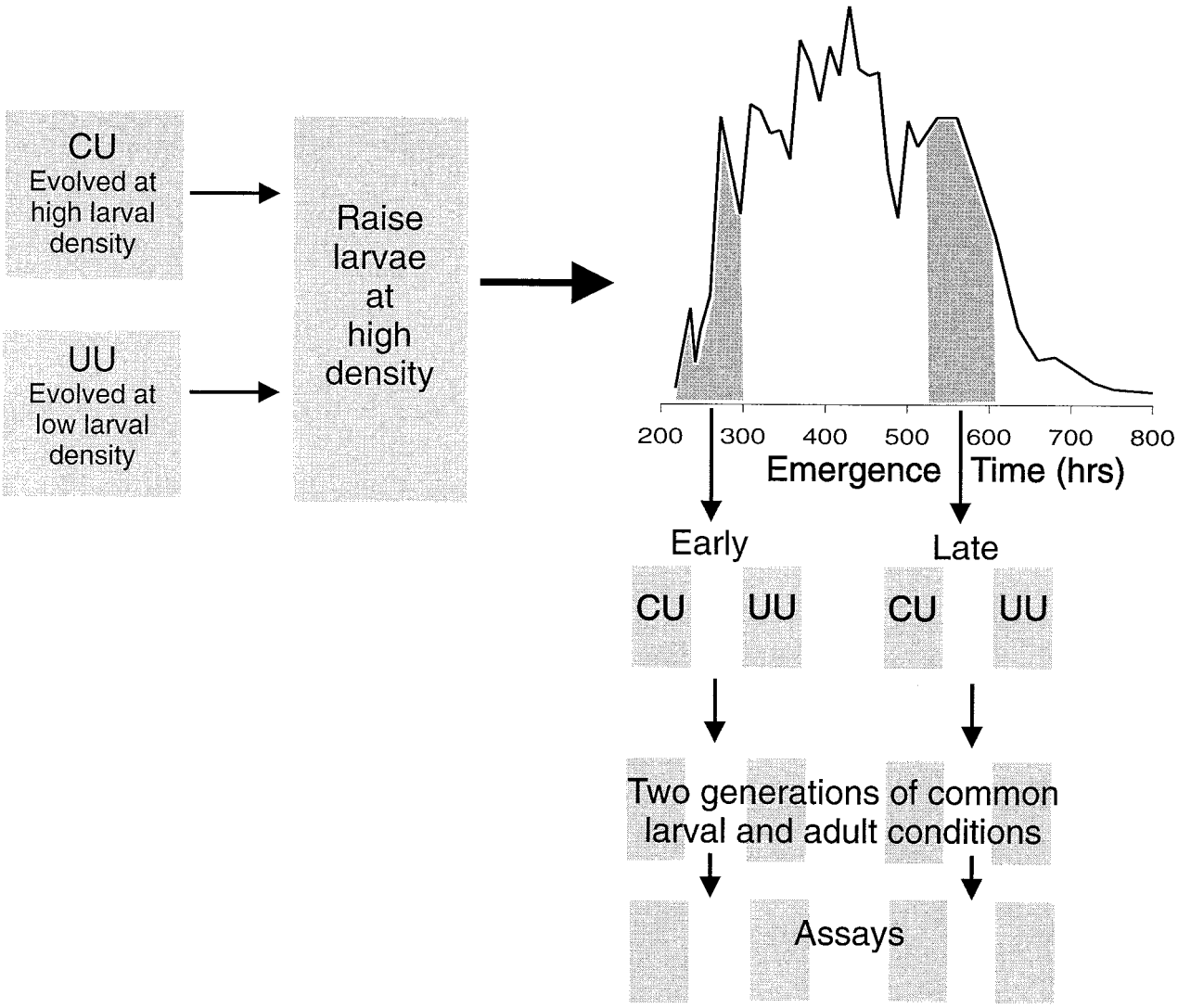

Figure 1: The experimental procedures for isolating and testing the early and late phenotypes. The source material came from two types of populations. The *CU* have evolved under crowded larval conditions and the *UU* have evolved under uncrowded larval conditions. Each type of population is replicated fivefold.

which contained the following: standard banana-molasses food, standard food with 0.25 M ammonium chloride, or standard food with 0.3 M urea added. Each treatment was replicated eight times for each of the 20 populations. The high density experiment was conducted on standard food with 1,000 eggs per vial and replicated five times for each population. These experiments were performed on all populations simultaneously. Prior to performing an ANOVA the viability data were subject to an arcsin square-root transformation.

Ammonia, Urea, and Ethanol

From each of the five *CU* and *UU* populations, 1,000 eggs were placed in 6-dram vials containing 5 mL of standard banana-molasses food. Each of these vials was replicated 20 times. On each experimental day, four vials from a given population were removed, and ~1 mL of food was stored at −20°C. Larvae were excluded from the samples as much as possible. Food homogenates were prepared by grinding 100 mg of food in 4 mL of water. Homogenates were stored at −70°C until assayed. Ammonia concentrations were determined using a nicotinamide adenine dinucleotide–linked assay (Mondzac et al. 1965). The reaction mixture (3 mL final volume) contained 100 mM potassium phosphate (pH 8), 1 mM ethylenediaminetetraacetic acid (EDTA), 30 mM α-ketoglutarate, 0.15 mM NADH (reduced), and 0.1 mL food homogenate. After measuring the initial absorbance at 340 nm, 10 μL of glutamate dehydrogenase

(G-2626; Sigma Chemical Co., St. Louis) was added. The final absorbance was measured after 60 min at room temperature and compared with a blank to which water was added instead of food homogenate. Ammonia concentrations were calculated using a molar absorbance coefficient for NADH of 6,220. Urea assays were performed under conditions identical to the ammonia assays, except that 10 μL urease (U-1875; Sigma Chemical Co.) was also added to the reaction mixture. Ethanol levels were determined using Sigma kit BBB-3. Assays were performed over several days, but all samples from a given *CU* and *UU* population pair were assayed simultaneously on a given day.

Statistics

The evaluation of significant effects was made with the aid of ANOVA implemented on SAS for Windows (SAS Institute 1991). Population (*CU* vs. *UU*), period (early vs. late), and food type (standard vs. ammonia, etc.) were treated as fixed effects. Population replicate was treated as a block effect because of the common origin of CU_i and UU_i populations, and in the case of the feeding rate experiments these populations shared a common day of analysis (Joshi and Mueller 1996). Survivorship data were transformed using the arcsin square-root transformation. Multiple comparisons were done using the Tukey-Kramer method.

Results

Evolution in Theory

We start by developing a simple population genetic model that illustrates how evolution might work in a temporally variable environment. Assume a single locus with two alleles, A_1 and A_2. For genotype A_iA_j, the fraction that emerges early in the environmental profile is v_{ij}. The viability of this early emerging group is e_{ij}. The remaining portion of the genotypes $(1 - v_{ij})$ emerges during the late portion and their viability is l_{ij}. If we assume there is complete assortative mating, for example, early emerging types only mate with other early types, then we must keep track of genotype frequencies since eggs will not be in Hardy-Weinberg proportions. Let the frequency of genotype A_iA_j among zygotes be x_{ij}. Then the frequency of the A_1 allele in the early emerging adult population is

$$p_1' = \frac{(x_{11}v_{11}e_{11} + 1/2\, x_{12}v_{12}e_{12})}{\overline{w}'},$$

where the mean fitness is $\overline{w}' = x_{11}v_{11}e_{11} + x_{12}v_{12}e_{12} + x_{22}v_{22}e_{22}$. The frequency of the A_1 allele in the late portion of the adult populations is

$$p_1'' = \frac{[x_{11}(1 - v_{11})l_{11} + 1/2\, x_{12}(1 - v_{12})l_{12}]}{\overline{w}''},$$

where the mean fitness is $\overline{w}'' = x_{11}(1 - v_{11})l_{11} + x_{12}(1 - v_{12})l_{12} + x_{22}(1 - v\overline{w}_{22})l_{22}$. Then the genotype frequencies in the next generation are given by

$$\tilde{x}_{11} = (p_1')^2E + (p_1'')^2L,$$

$$\tilde{x}_{12} = 2p_1'(1 - p_1')E + 2p_1''(1 - p_1'')L,$$

and

$$\tilde{x}_{22} = (p_2')^2E + (p_2'')^2L,$$

where $E = \overline{w}'/(\overline{w}' + \overline{w}'')$ and $L = \overline{w}''/(\overline{w}' + \overline{w}'')$.

If one allele is fixed (A_i, $i = 1, 2$) the condition for the increase of the rare alternative allele is $v_{11}e_{12} + (1 - v_{12})l_{12} > v_{ii}e_{ii} + (1 - v_{ii})l_{ii}$. If mating is completely random, then the initial increase condition is the same, although the internal equilibrium frequencies have not been determined. With the aid of this relationship, we can predict the conditions necessary for a protected polymorphism (Hartl and Clark 1989) or the point at which natural selection will not fix either the A_1 or the A_2 allele. For example, consider the point illustrated in figure 2. Here all three genotypes have the same early viability: the "early genotype," A_1A_1, has a high fraction of adults emerging early (0.5) but low viability in the late environment (0.1). Conversely, the "late genotype," A_2A_2, has fewer adults emerging early (0.1) but higher viability in the late environment (0.5). The heterozygotes have an intermediate viability and emergence fractions, demonstrating that a polymorphism is possible without overdominance in each component of fitness. The important point is that there are broad conditions under which both alleles may be stably maintained by evolution due to genotypes with differing abilities to do well in either the early or late portion of the environment.

Evolution in the Laboratory

Several aspects of the larval environment change over the time that larvae develop in crowded cultures. Of course food is depleted, and in our own laboratory the volume of food is often reduced by 50%–80%. Since the food contains a growing population of yeast there is also an accumulation of acetic acid (Hageman et al. 1990). We have sampled the food from crowded cultures and measured levels of urea and ammonia (fig. 3). These data show that there are almost no detectable levels of urea (contrary to previous reports; Botella et al. 1985) but a significant and steadily increasing amount of ammonia. Thus, larvae that are more slowly developing in crowded cultures are more likely to be exposed to high levels of ammonia through ingestion of polluted food. Over this

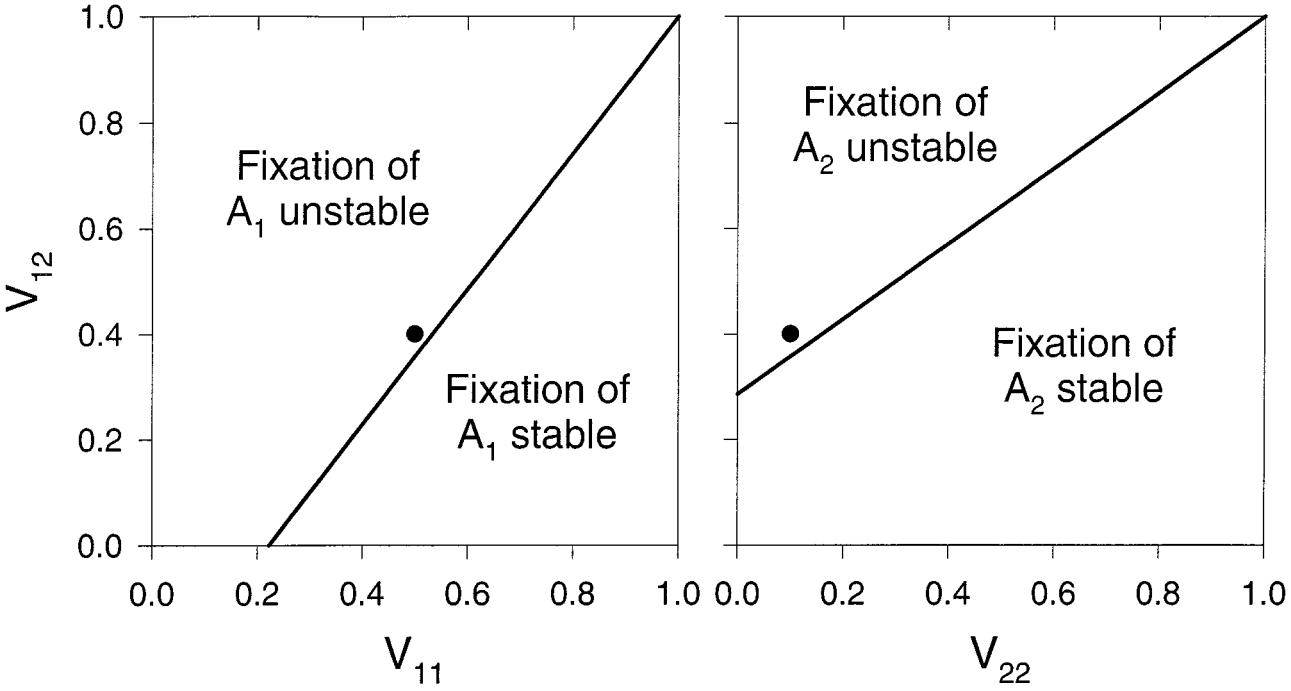

Figure 2: The boundary behavior of the model of selection in a temporally variable environment. In this example, combinations of the genotype-specific propensity to emerge early (v_{ij}) outline stable from unstable regions. The point represents one particular combination of values that results in the fixation of the A_1 allele and the fixation of the A_2 allele being unstable equilibria. Consequently, both alleles must be stably maintained in the population by natural selection. The other values for the model parameters were: $l_{11} = 0.1$, $l_{12} = 0.3$, $l_{22} = 0.5$; $e_{11} = e_{12} = e_{22} = 1$.

same period of time the levels of ethanol in crowded cultures drops dramatically in both *CU* and *UU* cultures (fig. 4).

To test whether these environments could harbor a polymorphism similar to the one described by the previous model, we studied two types of laboratory populations of *Drosophila melanogaster*. We have isolated two subpopulations from the *UU* and *CU* populations that we call early and late, as illustrated in figure 1. There are two important features of the protocol outlined in figure 1. The use of two generations of common environmental conditions just prior to the assays insures that any differences observed between the four population groups (*CU*-early, *CU*-late, *UU*-early, *UU*-late) will be due to genetic differences among the populations, not environmentally induced differences. Also, if there are differences between the *CU*-early and the *CU*-late but not between the *UU*-early and the *UU*-late, then we can reasonably infer that natural selection due to larval crowding is the cause of the observed genetic differences.

One phenotype that evolves in response to larval crowding is competitive ability (Mueller 1988*a*, 1988*b*). In environments with limited food, increased competitive

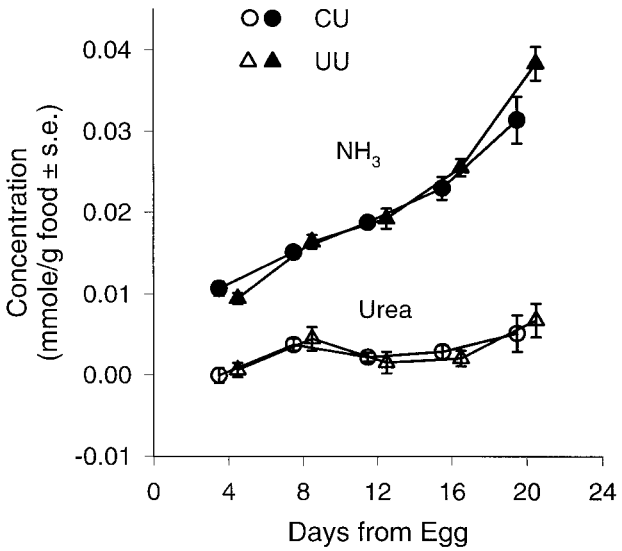

Figure 3: Levels of environmental urea and ammonia in crowded cultures of the *CU* (adapted to crowded larval conditions) and *UU* (adapted to uncrowded larval conditions) populations.

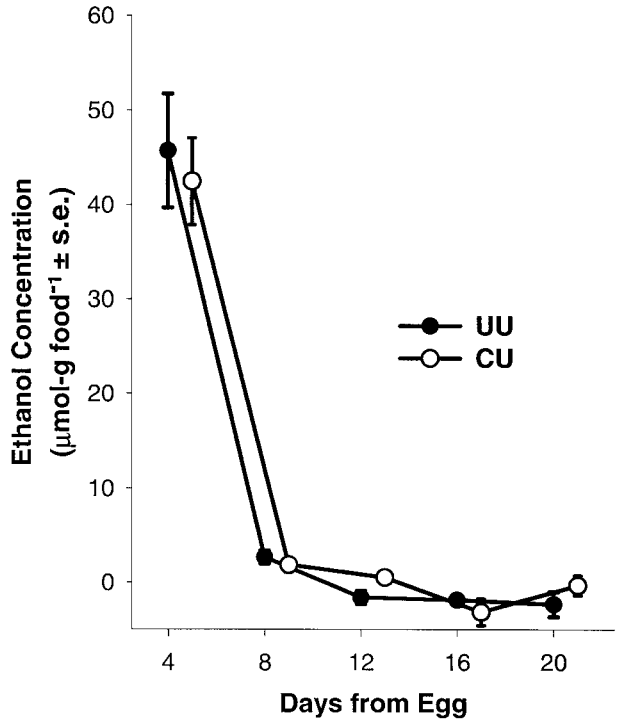

Figure 4: Levels of environmental ethanol in crowded cultures of the *CU* (adapted to crowded larval conditions) and *UU* (adapted to uncrowded larval conditions) populations. Freshly made food contains 3% ethanol, equal to 650 μmoles per gram of food.

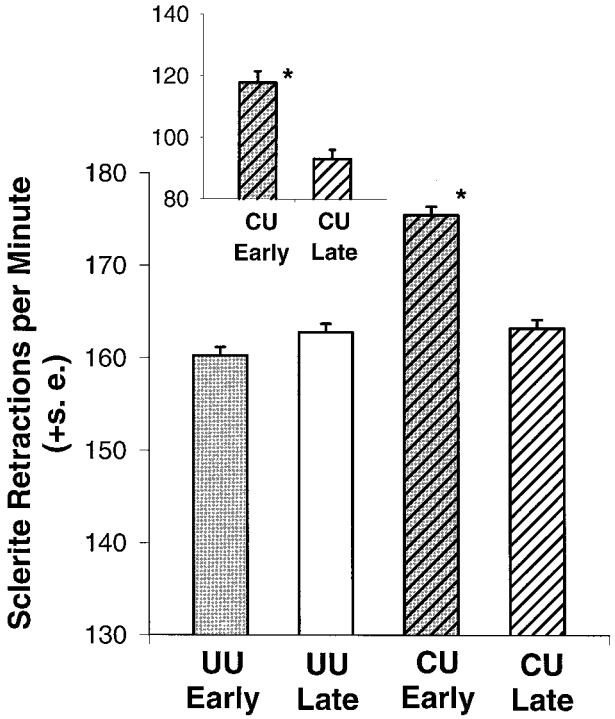

Figure 5: Larval feeding rates for four different populations, with * indicating the population with the significantly increased feeding rate. An ANOVA of these data indicates that there is a significant population (*CU* vs. *UU*) by period (early vs. late) interaction ($P < .005$). This result is due almost entirely to the significantly greater feeding rates of the *CU*-early larvae compared with the *CU*-late larvae (one-tailed test, $P < .0004$). The *UU*-late versus the *UU*-early, in contrast, show no significant difference. The small insert shows the additional test of feeding rates on just the *CU* populations, which also reveal large and significant differences ($P < .001$).

ability affects viability and male mating success and female fecundity—through changes in adult size—in a frequency dependent manner; for example, the fitness benefits enjoyed by good competitors are greatest when they are rare (Mueller 1988*a,* 1988*b*). It has been shown several times that competitive ability in *Drosophila* larvae is highly correlated with larval feeding rate (Burnet et al. 1977; Joshi and Mueller 1988). The larval feeding rates of the four populations derived in figure 1 were measured and compared (fig. 5). These results show that the *CU*-early larvae feed at a significantly higher rate than the *CU*-late larvae but that there is no difference between the *UU*-early and *UU*-late larvae.

Egg-to-adult viability was also examined under four different conditions. Larvae from the four populations were raised at low densities but in three different food environments: standard food, standard food with ammonia (0.25 M), and standard food with urea (0.3 M). In all three cases the *CU*-late larvae show a significantly higher viability than the *CU*-early larvae, while there are no significant differences between *UU*-early and *UU*-late (fig. 6). On ammonia the *CU*-early larvae also have a significantly lower viability than the *UU*-early larvae (one-tailed test, $P < .03$). This suggests that the differentiation of the *CU*-early and -late subpopulations is due to both the *CU*-late larvae becoming more tolerant of ammonia and the *CU*-early larvae becoming less tolerant. Larvae were also raised in standard food at high larval densities. Again the *CU*-late larvae had a significantly higher rate of survival than the *CU*-early population and no difference was observed between *UU*-early and late (fig. 6). If these absolute viabilities are expressed as fractions relative to the *CU*-late viability, then the relative fitness of the *CU*-early larvae at high density (0.84) and in ammonia (0.84) is much less than their relative fitness at low density (0.96), even though there are significant differences between *CU*-early and -late populations in all three cases. Nevertheless, the fitness advantage of the *CU*-late types is most pronounced under conditions of crowding and high ammonia concentrations.

The experiments in figure 6 measured absolute viabil-

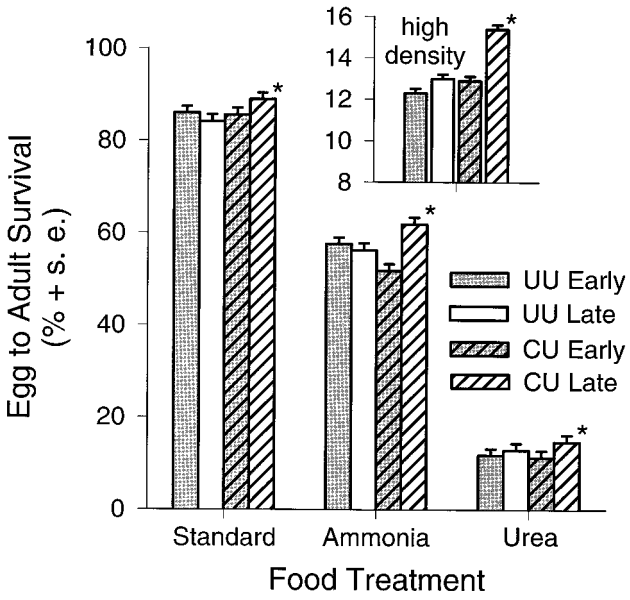

Figure 6: The egg-to-adult survival at low density on three different food types and at high density on standard food, with * indicating the population with the significantly increased survival rate. An ANOVA of the low density data indicates that there is a significant population (*CU* vs. *UU*) by period (early vs. late) interaction ($P < .025$). For each food treatment, the viability of the *CU*-late subpopulation was significantly greater that the *CU*-early subpopulation, and there were no significant differences between the two *UU* subpopulations. At high density there was also a significant population by period interaction ($P < .025$) with the *CU*-late subpopulation having a significantly greater viability than the *CU*-early subpopulation (one-tailed test, $P < .0008$).

ity differences of early or late phenotypes only. Although competitive ability affects fitness through changes in viability, these viability effects can only be seen when good competitors are placed in competition with poor competitors. The results in figure 6 show that the *CU*-early phenotype suffers a reduction in absolute viability relative to the *CU*-late phenotypes.

Discussion

It appears that natural selection in crowded *Drosophila* cultures has led to a polymorphism that can be dissected along an axis of developmental times. Fast developing larvae have high feeding rates and reduced exposure to the late part of the larval environment that is characterized by low levels of food and high levels of ammonia. Conversely, the more slowly developing larvae have higher absolute viability, especially under conditions of high levels of waste products and also under very crowded conditions. A crucial component of this polymorphism is the trade-off between feeding rates and absolute viability. Additional support for this trade-off comes from recent experiments in which the *CU* populations were cultured at reduced larval densities and experienced a significant decline in feeding rates relative to similar populations kept at high larval densities (Joshi and Mueller 1996). These observations further justify the important role that trade-offs play in the theory of life-history evolution (Stearns 1992).

While we have emphasized the temporal aspects of the environmental variation, there are similarities between the environmental decay in the *CU* populations and temporal variation. In the *CU* environments, only a portion of the total population experiences the early environment (by this we mean the completion of development in this time interval) and only a portion experiences the late environment. In standard models of temporal variation, the entire population would be assumed to experience each new environmental state.

Although numerous studies have investigated the chemical environment of larvae under natural conditions (e.g., Fogelman and Abril 1990), relatively few have done so in laboratory culture. Botella et al. (1985) found that urea and uric acid levels changed over time in relatively uncrowded cultures. Urea is an uncommon nitrogenous waste product in insects, and uric acid production is usually associated with terrestriality (Cochran 1985). We could not detect uric acid, and urea levels were much lower than ammonia (fig. 3). The levels of urea and uric acid measured by Botella et al. (1985) were no higher than a few mmol/kg food. These values are similar in magnitude to the urea concentrations we measured under much more crowded conditions but were probably not high enough to be toxic. In our populations, ammonia appears to be the primary nitrogenous waste product, although we cannot exclude the possibility that it is generated microbially.

Ethanol in *Drosophila* cultures may evaporate or be converted to acetic acid by microbes (Hageman et al. 1990). In our crowded conditions, over 90% of the ethanol initially present in fresh foods disappears within 4 d, and insignificant quantities are present after 8 d (fig. 4). The rate of disappearance is much slower in food held without larvae or with uncrowded larvae (A. G. Gibbs, unpublished observations). The larvae may use ethanol as a major energy source during early development (Geer et al. 1993), although microbial degradation may also occur.

Even under uncrowded conditions, other chemical changes are likely. For example, changes in ammonia and acetic acid levels will affect the pH of the medium. The

osmotic strength increases as water evaporates and the larvae consume the food (V. A. Pierce, personal communication), and microbial activity can affect the chemical composition of the medium. It is clear that the environmental conditions of *Drosophila* larvae can be quite variable, even under relatively well-defined laboratory conditions.

A standard complaint concerning studies of lab-evolved populations is that the laboratory environment is unrealistically simple and thus cannot provide useful insights about evolution in nature. The *Drosophila* model system described here has revealed one mechanism in which crowding produces environmental heterogeneity. In addition, this model system provides a means for understanding the manner in which this environmental heterogeneity affects evolution. As noted in the introduction, the type of environmental heterogeneity discussed here is likely to be found in a wide variety of organisms and environments, not just crowded ones.

Acknowledgments

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