Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10905–10906, December 1991
Evolution

Evolution of behavior by density-dependent natural selection

(low-density selection/high-density selection/*Drosophila melanogaster*)

PINGZHONG GUO*, LAURENCE D. MUELLER, AND FRANCISCO J. AYALA†

Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717

Contributed by Francisco J. Ayala, September 3, 1991

ABSTRACT **Theories of density-dependent natural selection predict that evolution should favor those genotypes with the highest per capita rates of population growth under the current density conditions. These theories are silent about the mechanisms that may give rise to these increases in density-dependent growth rates. We have observed the evolution of six populations of *Drosophila melanogaster* recently placed in crowded environments after nearly 200 generations at low-population density in the laboratory. After 25 generations in these crowded cultures all six populations showed the predicted increase in population growth rates at high-population density with the concomitant decrease in their growth rates at low densities. These changes in rates of population growth are accompanied by changes in the feeding and pupation behavior of the larvae: those populations that have evolved at high-population densities have higher feeding rates and are less likely to pupate on or near the food surface than populations maintained at low densities. These changes in behavior serve to increase the competitive ability of larvae for limited food and reduce mortality under crowded conditions during the pupal stage of development. A detailed understanding of the mechanisms by which populations evolve under density-dependent natural selection will provide a framework for understanding the nature of trade-offs in life history evolution.**

One of the most useful fusions of theory from ecology and evolution is the theory of density-dependent natural selection (1–4)—often called *r*- and *K*-selection, where *r* and *K* refer to low- and high-density conditions, respectively. These theories have been used to support the contention that adaptation to high or low density will involve different suites of characters and that it is unlikely that one set of traits will perform optimally in either extreme environment. To study this problem we have undertaken a series of laboratory experiments with *Drosophila melanogaster* (5, 6). The original design of this experiment was to take three replicate samples from a genetically variable population and maintain them at low larval and adult density (*r* populations) and from the same source population take three samples maintained at high larval and adult densities (*K* populations). Over time the *r* and *K* populations have become genetically differentiated with respect to a variety of traits: at low density the *r* populations have higher per capita growth rates than the *K* populations, whereas at high densities the opposite is observed (6); the *K* populations have increased larval competitive ability relative to the *r* larvae (7), and this increase seems to be due to increased feeding rate of the *K* larvae (8); and, finally, *K* larvae have an increased tendency to pupate off the surface of the medium compared to *r* larvae (9).

The interpretation and weight that may be given these previous observations is constrained for the following reason. The original *r* and *K* populations were started from stocks that had been neither at very low nor very high densities and, hence, the low and the high density environment were both novel to the flies. Consequently, it is not possible to assess whether the differences that have developed between the *r* and *K* populations are due to both populations changing or just one changing and the other remaining unchanged. One further source of concern is that the results have not been replicated, and recent studies of mosquitoes (10) have failed to observe changes in rates of population growth. These problems are addressed in the current study.

MATERIALS AND METHODS

Three new low-density populations were created at generation 198 of the experiments just mentioned (6) by taking three samples of F_1 offspring from all possible pairwise crosses of the three *r* populations; these new populations are called $r \times r$. This process creates three low-density populations, each with the combined genetic variation of the three independent *r* populations (which might have individually lost some genetic variation due to random genetic drift). Also during generation 198 samples from each of the six (three *r* and three $r \times r$) low-density populations were then moved to new cultures and maintained in the *K* environment. The three populations derived from the *r* populations and placed at high density are called *rK*, and the three populations derived from the $r \times r$ populations are called $r \times rK$.

Each *r* population is maintained by allowing 50 adults to lay eggs for 24 hr in a half-pint culture with 40 ml of cornmeal/sugar/flour food. The total population consists of 10 such cultures. Fourteen days after egg laying, newly emerged adults are collected, and the process is repeated. The *K* populations are maintained by a serial-transfer system. Each week, newly emerged adults are added to those surviving from previous weeks and become with them the adult egg-laying population.

Pupation height was measured for each population in five replicate vials that were initially seeded with 50 larvae following standard methods (9). The larvae used in these analyses are two generations removed from the selection regimes, so that environmental effects have been controlled for by making them identical for all populations. The pupation height is defined as the distance (cm) between the surface of the food and the spiracles of the pupa. Any pupa that is touching the surface of the food is given a pupation height of 0.

Feeding rates are measured in 20 larvae per population as described (8). Sixty-eight-hour-old larvae are removed from their cultures and placed on a Petri dish with agar and a thin layer of yeast solution. After 1 min to adjust to the new conditions the number of cephalopharyngeal contractions made by the larva in 1 min are recorded.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Department of Biology, Beijing Teachers College, Beijing 100037, People's Republic of China.
†To whom reprint requests should be addressed.

RESULTS AND DISCUSSION

Because the *r* populations have had nearly 200 generations to adapt to the low-density environment, we expect little further change in the low-density populations over short periods of time. However, the *rK* and $r \times rK$ populations have been placed in a new environment and will be expected to adapt if there is selection and the appropriate genetic variation exists in these populations. Our previous work (11) suggests that adaptation in high-density environments may be accomplished by increasing larval competitive ability (measured by larval feeding rates) and reducing pupal mortality (by increasing pupation height). We note that our experimental design provides a control for every experimental *K* population: e.g., the control for experimental population rK_1 will be its parental population r_1. The null hypothesis is that density has no effect on behavior and, hence, the feeding rate and pupation height of the rK_1 and r_1 populations are the same (and likewise for the other control and experimental populations). However, if natural selection at high density favors different phenotypes than at low density, we expect to see the feeding rates and pupation height of the experimental populations elevated relative to their controls.

The results are shown in Figs. 1 and 2. After 25 generations of selection significant changes in the behavior of the *rK* and $r \times rK$ populations have been observed. In every case the pupation height of the high-density population is greater than its control (Fig. 1) and in five out of six cases these differences are statistically significant. Similar consistent results are seen in the feeding rates of the experimental and control populations (Fig. 2). In this instance all six comparisons yield statistically significant differences, so that the *rK* and $r \times rK$ larvae have higher feeding rates than their controls. These results clearly show that populations of *Drosophila* adapt to crowded environments by increasing larval feeding rates and pupation height.

It is known from several studies that feeding rate is highly correlated with larval competitive ability and perhaps the major determinant of competitive ability (8, 12, 13). It is also thought that increased competitive ability will contribute to increased viability and increased female fecundity in crowded cultures (14). Recently, evidence has shown that *r* populations placed in crowded environments are expected to undergo strong directional selection for increased pupation height because nearly

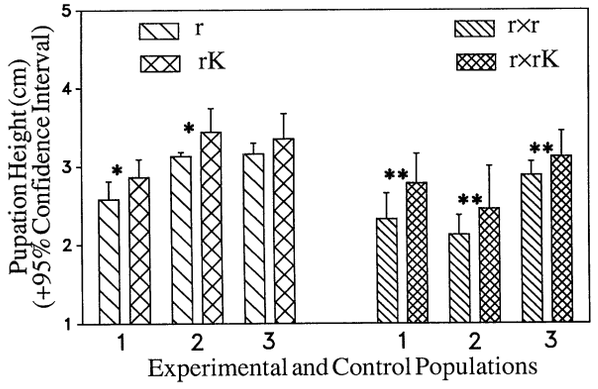

FIG. 1. Mean pupation height of each population. Histograms are arranged in pairs: an experimental and its control population. For instance, the two histograms on the far left just above 1 are for the r_1 and rK_1 populations. A nested analysis of variance shows significant effects due to *r* vs. *rK* treatments ($P < 0.01$) and $r \times r$ vs. $r \times rK$ treatments ($P < 0.01$). Planned contrasts are made comparing the mean pupation height of each control r_i or $r \times r_i$ to its experimental population rK_i or $r \times rK_i$, respectively. Statistically significant differences from the contrasts are indicated (*, $P < 0.05$; **, $P < 0.01$).

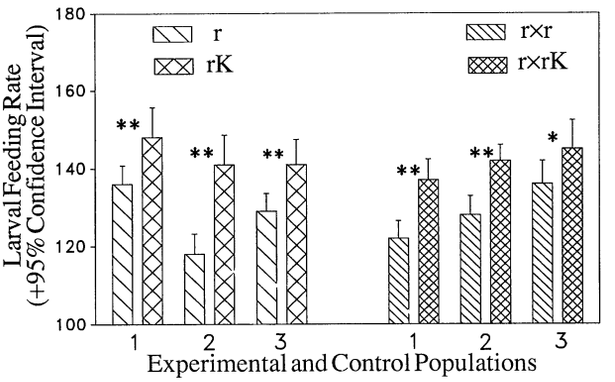

FIG. 2. Mean feeding rate of each experimental and control population measured as number of cephalopharyngeal contractions per min. Data are organized as in Fig. 1. Average numbers of these contractions are shown. The analysis of variance shows significant effects of the treatments *r* vs. *rK* ($P < 0.01$) and $r \times r$ vs. $r \times rK$ ($P < 0.01$). Significance of the planned comparisons are indicated using the same notation as in Fig. 1.

80% of pupae on the food surface die in crowded cultures (A. Joshi and L.D.M., unpublished data). Thus, these behavioral traits have close ties to components of fitness that are directly affected by population density.

However, these increases in pupation height and feeding rates may incur costs to the organism. For instance, recent evidence suggests that the evolution of increased feeding rates may be accompanied by decreased efficiency of food use (15). Although pupating off the surface of the food in crowded cultures is favored over pupating on the food, those larvae that pupate too high also suffer increased mortality compared with those at intermediate heights (A. Joshi and L.D.M., unpublished data). The evolution of animal behavior as a consequence of natural selection is a subject of considerable interest—and much debate because so little is definitively known about the subject. Our study demonstrates how two important behaviors (feeding rate and pupation height) readily evolve as a means of adaptation to particular environmental conditions. Moreover, our study provides a behavioral basis for understanding the adaptation of populations to variable density conditions. A more thorough understanding of the trade-offs involved in evolution by density-dependent natural selection may be accomplished by additional study of these behavioral traits.

Support for this work was provided, in part, by grants from the Department of Energy and the National Institutes of Health.

1. Anderson, W. W. (1971) *Am. Nat.* **105,** 489–498.
2. Charlesworth, B. (1971) *Ecology* **52,** 469–474.
3. Clarke, B. (1972) *Am. Nat.* **106,** 1–13.
4. Roughgarden, J. (1971) *Ecology* **52,** 453–468.
5. Mueller, L. D., Guo, P. Z. & Ayala, F. J. (1991) *Science* **253,** 433–435.
6. Mueller, L. D. & Ayala, F. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1303–1305.
7. Mueller, L. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4383–4386.
8. Joshi, A. & Mueller, L. D. (1988) *Evolution* **42,** 1090–1093.
9. Mueller, L. D. & Sweet, V. (1986) *Evolution* **40,** 1354–1356.
10. Bradshaw, W. E. & Holzapfel, C. M. (1989) *Am. Nat.* **133,** 869–887.
11. Mueller, L. D. (1991) *Philos. Trans. R. Soc. London Ser. B* **332,** 25–30.
12. Sewell, D., Burnet, B. & Connolly, K. (1975) *Genet. Res.* **24,** 163–173.
13. Burnet, B., Sewell, D. & Bos, M. (1977) *Genet. Res.* **30,** 149–161.
14. Mueller, L. D. (1988) *Am. Nat.* **132,** 786–809.
15. Mueller, L. D. (1990) *Evol. Ecol.* **4,** 290–297.