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DENSITY-DEPENDENT NATURAL SELECTION IN *DROSOPHILA*: EVOLUTION OF PUPATION HEIGHT

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MacArthur and Wilson (1967) were the first to explore systematically the evolutionary consequences of extreme population densities. There are, however, few controlled experiments aimed at determining the outcome of evolution at extreme densities (except see Luckinbill, 1978, 1979; Taylor and Condra, 1980; Mueller and Ayala, 1981*b*; Barclay and Gregory, 1981). Consequently, we have continued the examination of *r*- and *K*-selection in laboratory populations of *Drosophila* in order to document and understand phenotypic differences which appear to be both extensive and substantial. We note here that by "*r*- and *K*-selection" we mean the selection that takes place in environments with density-independent and density-dependent population regulation respectively. The connection to the parameters *r* and *K* of the logistic equation is merely a historic artifact.

A number of phenotypic differences have been documented for the *r*- and *K*-selected populations used in this study. In all cases these phenotypic differences can be ascribed to genetic differences between the populations. After eight generations of selection a trade-off in rates of population growth was observed (Mueller and Ayala, 1981*b*). After 36 generations differences were observed in density-dependent larval viability, and the size of the adult flies emerging from crowded cultures (Bierbaum et al., unpubl.). After nearly 130 generations of selection a large decline in the late fecundity of *r*-selected flies has been observed (Mueller, unpubl.). We have now initiated a number of studies aimed at understanding, in more detail, the biological phenomena responsible for the larval viability differences at high densities. In this paper we document differences in pupation height between the *r*- and *K*-selected populations which may account for some of the differences in egg to adult survival.

There appears to be substantial genetic variation for pupation height in *D. melanogaster* (Markow, 1979; Sokolowski and Hansell, 1983; Bauer, 1984; Bauer and Sokolowski, 1985) with both the second and third chromosome contributing to the trait (Bauer and Sokolowski, 1985). Markow (1979) has been successful in selecting for both high and low pupation height. There is also a substantial environmental component to pupation height. A number of studies have shown that pupation height increases markedly with increasing larval densities in *D. melanogaster* (Sokolowski and Hansell, 1983) and *D. simulans* (Ringo and Wood, 1983). Chiang and Hodson (1950) note that in crowded cultures larvae pupating on the sides of vials, close to the surface of the medium, are more likely to be dislodged and drowned by larvae crawling up the sides. Likewise, any larva pupating on the surface of the medium is likely to be buried if there is still an actively feeding larval population. These observations clearly suggest that the fitness of genotypes which vary in pupation height may be a function of larval density.

MATERIALS AND METHODS

Selection Regime

Three *r*-selected and three *K*-selected populations have been maintained by methods described previously (Mueller and Ayala, 1981*b*) for nearly 130 generations prior to the start of this study. Briefly, the *r*-selected populations are generated by putting 50 adults that are three to six days old in a standard half-pint culture for 24 hours. At the end of this period the adults are discarded and 14 days later another 50 adults are chosen at random from the 200 to 400 progeny, aged three days and the whole process repeated. The *K*-selected populations are maintained by the serial transfer system (Mueller and Ayala, 1981*a*) at carrying capacity which is about 800–1200 adult flies per half-pint culture. All cultures consist of 40 ml of cornmeal-flour-sugar-agar medium with one facial tissue inserted to control moisture and are kept at 23°C with a 12L:12D cycle.

Casual observation of high and low density populations shows that there is extreme crowding of both adults and larvae in the *K*-selected populations and little evidence of crowding in the *r*-selected populations. The medium becomes very loose and fluid in the high density populations while it remains relatively dry and solid in the low density treatments. In the *K*-selected populations the tissue is invariably buried in the medium, hence almost all larvae pupate on the side of the bottle while many crawl all the way to the cotton plug and pupate on it. In the *r*-selected populations most larvae seem to pupate in the tissue (which remains intact) or on the surface of the medium.

In addition to adult and larval density there are several other factors which differ between these populations which may be important in their evolution. The populations differ in the magnitude of genetic drift they experience. Genetic drift will clearly have the greater impact on the *r*-selected populations. The life of adult flies is never artificially terminated in the *K*-selected populations, hence they may live to be many weeks old, while all flies are less than a week old in the reproducing population of *r*-selected flies. The differing age structure of the populations should not be an important factor during the evolution of pupation heights. However, experiments need to be designed to sort out

TABLE 1. Mean (standard error) of pupation heights (in mm) for *r*- and *K*-selected populations and their F_1 progeny.

Line	Yeast (g)/number of larvae			
	0.0473/100	0.0946/200	0.0518/100	0.0563/100
K-1	10.7 (0.76)	13.7 (0.64)	9.7 (0.77)	12.0 (0.98)
r-1	5.2 (0.55)	5.4 (0.45)	5.1 (0.68)	6.9 (0.71)
K-2	7.9 (0.55)	11.9 (0.73)	8.0 (0.61)	10.4 (0.69)
r-2	5.0 (0.49)	†	6.7 (0.53)	4.8 (0.56)
K-3	6.3 (0.75)	13.8 (0.58)	11.4 (0.88)	12.3 (1.02)
r-3	2.4 (0.35)	8.2 (0.53)	4.7 (0.51)	5.8 (0.69)
K-F_1	12.0 (0.97)	—	14.0 (0.89)	16.1 (1.10)
r-F_1	6.1 (0.70)	—	6.2 (0.65)	9.1 (0.77)

† Only 100 larvae added.

the possible effects of genetic drift from natural selection.

Pupation Height Measurements

Pupation heights were determined for three *r*-selected populations (*r*-1, *r*-2, *r*-3), three *K*-selected populations (*K*-1, *K*-2, *K*-3) and F_1 hybrids of the *r*- and *K*-selected lines (*r*-F_1, *K*-F_1). Since pupation heights may vary over time for the same population (Bauer and Sokolowski, 1985) pairs of lines were run at the same time and will be compared in the statistical analysis. *K*- and *r*-selected populations with the same number and the two F_1 populations were assayed simultaneously.

Any consistent differences between the *r*- and *K*-selected populations in pupation height are most likely due to differential selection for this quantitative trait in the two environments. However, another possible interpretation is that genetic drift has fixed deleterious recessive alleles in the *r*-selected populations which have pleiotropic effects on pupation height. If this has occurred we expect that the F_1 larvae of the *r*-selected populations will have quite different pupation heights than their parents.

The F_1 populations were derived as follows. For each possible heterotypic cross, *r-i* male × *r-j* female (i,j = 1,2,3; $i \neq j$), the reciprocal cross was also carried out. Females from like crosses (e.g., *r*-1 ♂ × *r*-2 ♀ and *r*-1 ♀ × *r*-2 ♂) were paired together after mating and allowed to lay eggs. Equal numbers of first instar larvae were taken from the three different crosses to start each experiment. The same procedure was also used to create the *K*-F_1 population.

In 8-dram vials (22 × 95 mm) 10 ml of non-nutritive Kalmus medium was added and after solidifying, measured amounts of dry yeast were placed on the surface and moistened with 2.67 ml water per gram yeast. For all selected lines and the hybrids three yeast levels were used: 0.0473, 0.0518, and 0.0563 g. For the selected populations a fourth experiment was conducted with a total of 0.0946 g yeast but double the number of larvae. Thus, the amount of yeast per larva was the same as in the 0.0473 treatment.

One hundred first-instar larvae were collected using techniques similar to Nunney (1983) and added to each vial except the vial with 0.0946 g yeast to which 200 larvae were added. The position of these vials was randomized in the incubator and they were subject to conditions identical to those experienced by the selected populations. The larvae used to start these experiments were all progeny of adults which had been raised under common conditions of density, food, light, etc. Thus, environmental effects of the selection regimes should have been eliminated. After all larvae had either died or pupated, pupation height was measured to the nearest millimeter as the distance from the surface of the medium to the point between the spiracles of the pupa. Any pupa touching the surface of the medium was given a pupation height of zero.

TABLE 2. Frequency of larvae pupating on the medium surface for *r*- and *K*-selected populations and their F_1 progeny.

Line	Yeast (g)/number of larvae			
	0.0473/100	0.0946/200	0.0518/100	0.0563/100
K-1	0.10	0.13	0.10	0.08
r-1	0.39	0.41	0.43	0.33
K-2	0.18	0.22	0.13	0.10
r-2	0.37	†	0.29	0.42
K-3	0.22	0.09	0.18	0.18
r-3	0.58	0.31	0.53	0.47
K-F_1	0.17	—	0.12	0.13
r-F_1	0.47	—	0.45	0.29

† Only 100 larvae added.

RESULTS

In Table 1 the means and standard errors of the pupation heights for each line at each yeast level are given. For one population (*r*-2) and one yeast level (0.0946 g/200 larvae) only half the number of larvae were added accidentally. These results have been omitted since a valid comparison to the *K*-2 population is not possible. Every *K*-selected population at all yeast levels shows higher average pupation heights relative to the appropriate *r*-selected population. The differences are statistically significant in all but one case (*K*-2 versus *r*-2 at 0.0518 g yeast/100 larvae). It is also clear (Table 1) that increasing the density of larvae while keeping the amount of food per larva constant results in increased pupation height (compare the second column in Table 1 to the first column).

The low average pupation height of the *r*-selected

populations is in large part due to the large number of larvae pupating on the surface of the medium (Table 2). In *r*-selected populations the fraction of pupae on the surface of the medium is two to four times as great as in the corresponding *K*-selected population (Table 2).

The large differences in pupation height and fraction of pupae on the surface seen in the selected populations are also present in the F_1 progeny (Tables 1, 2). Thus, pleiotropic effects of recessive alleles which seem to have caused a reduction in the late fecundity of the *r*-selected flies (Mueller, unpubl.) do not seem to be responsible for the differences in pupation heights.

Discussion

After demonstrating the existence of additive genetic variation for pupation height Markow (1979) concludes that "the importance of this intraspecific variation is still unclear . . . ." The extreme environments that we have created here clearly result in differential selection pressures for pupation height. Although it is unclear if there is an advantage to low pupation height in the "*r*" environments it seems clear that any genetic predisposition to pupate away from the medium surface will be adaptive in the high density environments. In the *K*-selected populations the medium is soft and moist in addition to being crowded. As discussed previously, there are a number of hazards in such environments to pupae on the surface or close to it.

The importance of genetically determined pupation site choice has been demonstrated for *D. willistoni* under high density conditions (De Souza et al., 1970). In this study it was found that allelic variation at a single locus determined whether larvae pupated in food cups or on the bottom of the population cage. Under crowded conditions, when pupation sites in the food cups were scarce, the genotypes that pupated on the bottom of the cage appeared to have an advantage and rapidly increased in frequency. This was accompanied by a concomitant increase in the carrying capacity of the population. The simple genetic control for this behavior in *D. willistoni* clearly implicates a different type of behavior than the pupation height measured for *D. melanogaster*. However, it is clear that larval behavior influencing pupation site choice can be important under varying density regimes. In the present study the increased pupation height of the *K*-selected populations may account, in part, for their increased viability and higher rates of population growth at high densities (Mueller and Ayala, 1981*b*; Bierbaum et al., unpubl.). Other possible explanations include increased efficiency of food utilization or increased digging behavior of the *K*-selected populations. This latter explanation assumes larvae which dig deeper may have access to food at the bottom of the vial which is normally unused even in crowded cultures. These possibilities are currently under investigation.

The results from the F_1 progeny clearly allow us to eliminate the explanation that genetic drift in the *r*-selected populations has fixed (different) recessive alleles with pleiotropic effects on pupation height. The consistency of the response between all replicate *r*- and *K*-selected populations and the known genetic basis of pupation height clearly implicates selection as the causative agent for the observed differences.

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