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EVOLUTION OF HIGHER FEEDING RATE IN *DROSOPHILA* DUE TO DENSITY-DEPENDENT NATURAL SELECTION

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Population density plays an extremely important role in determining the evolution of certain attributes of populations. The earliest investigations in this direction were conducted by MacArthur and Wilson (1967), who analyzed the nature of selection pressures acting on populations at extreme densities. They designated the characteristic types of selection operating at low and high densities as *r*-selection and *K*-selection, respectively. The terms *r*-selection and *K*-selection may be taken to encompass, respectively, the selection pressures occurring in environments with density-independent and density-dependent population regulation (Mueller and Sweet, 1986). The theory dealing with the evolutionary consequences of extreme population density implies that *K*-selection should result, among other things, in an enhanced ability to compete successfully for limiting resources (MacArthur and Wilson, 1967; Southwood, 1976), and this phenomenon has been modeled by a number of workers (Charlesworth, 1971; Anderson, 1971; Anderson and Arnold, 1983; Asmussen, 1983).

The evolution of higher competitive ability in populations of *Drosophila melanogaster* subjected to *K*-selection has been demonstrated (Mueller, 1988). These experiments used competition for food among larvae as one of the indicators of competitive ability, and the results correlated well with the outcome of actual competition experiments in terms of differential survival of larvae from *r*- and *K*-selected lines when reared with larvae of a third strain (Mueller, 1988). Larval competition for food in *Drosophila* is of the "scramble" type; the ablest competitor is one that can effectively consume food at the fastest rate. Thus, when food is limiting, different individuals will obtain different quantities in accordance with their competitive ability (Bakker, 1961). Effective consumption of food implies consumption of food in an amount sufficient to permit completion of development.

Larval competitive ability thus depends on many factors, such as feeding rate, relative time spent on molting, minimum food requirement for pupation, initial weight, and resistance to crowding. Most of these factors themselves depend on a number of factors. Experimental studies indicate that, in laboratory populations of *D. melanogaster*, the outcome of larval competition for food depends primarily on the feeding rate (Bakker, 1961).

An important component of the feeding rate, and one which is amenable to measurement, is the number of bites that the larva takes from the food medium per unit time (Bakker, 1961). This can be measured as the number of sclerite retractions per minute. Sewell et al. (1975) demonstrated the existence of a fair amount of additive genetic variation for retraction rates in populations of *D. melanogaster* which respond readily to bidirectional selection, giving nonoverlapping populations with fast and slow feeding individuals, respectively. This implies that sclerite-retraction rate is a character that would respond to natural selection. In this paper we report the differences in rates of sclerite retraction in larvae of the *r*- and *K*-selected *D. melanogaster* populations used by Mueller (1988); these differences could be a significant component of the earlier recorded (Mueller, 1988) differences in larval ability to compete for limiting food resources.

MATERIALS AND METHODS

Modes of Selection

The study utilized three *r*-selected and three *K*-selected populations, maintained for over 140 generations by methods previously described in detail (Mueller and Ayala, 1981). The *r*-selected populations are generated by allowing 50 adults, 3–6 days old, to lay eggs in a standard half-pint culture bottle for 24 hours. The adults are then discarded. After 14 days, 50 adults from among the 300–500 progeny are randomly chosen, and the process is repeated. The *K*-selected populations are maintained by the serial-transfer system, resulting in a population size of about 800–1,200 adults per culture, which is close to the carrying capacity. Resources are renewed weekly, and the adults are permitted to reproduce throughout the lifespan. All culture bottles contain 45 ml cornmeal-flour-sugar-agar medium with a folded facial tissue inserted to control moisture. The cultures are kept at 23°C with a 12L: 12D cycle.

The *r*-selected and *K*-selected populations differ in several respects. There is no indication of crowding in the *r*-selected populations, whereas extreme crowding occurs in the *K*-selected populations, both in the adult and larval stages (Mueller and Sweet, 1986). The *r*-selected populations are much more likely to undergo genetic drift. The two types of populations also have different age-structures, though this should not significantly affect the evolution of feeding rate.

Measurement of Sclerite-Retraction Rate

Rates of sclerite retraction were measured on three *r*-selected (*r*-1, *r*-2, *r*-3) and three *K*-selected (*K*-1,

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TABLE 1. The mean rates and standard errors of sclerite retraction for each population. The difference in mean retraction rates for each matched pair of populations is significant at the 0.01 level.

Population	Mean number of sclerite retractions per minute (SE)	
K-1	158 (4.7)	*
r-1	139 (3.3)	
K-2	164 (3.5)	*
r-2	145 (3.5)	
K-3	155 (3.2)	*
r-3	131 (3.8)	
K-F_1	166 (4.3)	*
r-F_1	135 (4.8)	
K-pooled	159 (2.3)	*
r-pooled	138 (2.2)	

* $P < 0.01$.

K-2, *K*-3) populations. Pairs of *r*- and *K*-selected lines, matched by indices, were run simultaneously, and these pairs are compared in the statistical analysis. Measurements were also made on F_1 hybrids of the *r*-selected (*r*-F_1) and *K*-selected (*K*-F_1) lines. Comparison of the results from the *r*-selected lines and the *r*-F_1 indicates the effect of genetic drift on the evolution of competitive ability in the *r*-selected flies (Mueller, 1988). The *K*-F_1 larvae provide a matched pair for the *r*-F_1 larvae.

The *r*-F_1 populations were obtained by crossing ♂ *r-i* × ♀ *r-j* (*i*, *j* = 1, 2, 3; $i \neq j$). For each cross, the reciprocal cross was also carried out. The *K*-F_1 populations were analogously derived. Measurements were made simultaneously on equal numbers of the progeny of all these crosses, and the results for the *r*-F_1 and *K*-F_1 populations were pooled for the analysis.

For all selected lines, about 150 adults from the population bottles were shifted to a fresh bottle and allowed to lay eggs for 24 hours before being shifted back to the original cultures. After 14 days, the emergents were transferred to fresh bottles containing medium that had live yeast added to its surface. After three days, the flies were removed from the bottles and allowed to lay eggs for three hours on watch glasses containing nonnutritive Kalmus Agar. After 24 hours, 50 larvae from each watch glass were shifted onto fresh watch glasses containing nonnutritive Kalmus Agar with 158 mg live yeast in the form of a dense paste on the surface. After having fed for 70 hours on these watch glasses, 20 larvae from each line were used for the actual measurements. For the *r*-F_1 and *K*-F_1 hybrids, four larvae from among the progeny of each heterotypic cross were measured, i.e., 24 larvae of each hybrid type.

For measuring the feeding rate, one larva at a time was shifted to a petri dish containing 3% agar coated with a 10% live yeast suspension on its surface. After allowing the larva 90 seconds to adjust to the new surroundings, it was observed under the microscope for 60 seconds, and the number of sclerite retractions was counted. The procedure for measuring retraction rates is similar to that of Sewell et al. (1975). After measurement, each larva was placed in a separate vial, and its sex was recorded upon emergence.

RESULTS

In Table 1, the mean rates of sclerite retraction for all populations are given. In all cases, the mean of the *K*-selected population is greater than that of the corresponding *r*-selected population. The mean of the three *K*-selected populations, taken together, is also greater than that of the *r*-selected populations. In all cases, the difference is statistically significant at the 0.01 level. The mean of the *K*-F_1 hybrids is also significantly greater than that of the *r*-F_1 hybrids. When the observations for the three *r*-selected lines are pooled and the mean compared to that of the *r*-F_1 hybrids, it is seen that the means are not significantly different.

The variances for all populations were also computed, and no discernible trend appears. The variance of the hybrids is more than the corresponding parental populations, but the difference is not statistically significant.

Table 2 shows the results of a two-way analysis of variance for the four matched pairs of *r*- and *K*-selected populations. In all cases, there is evidence for the existence of a distinct genetic component in the observed variation in the rates of sclerite retraction. The exact proportion of the genetic and environmental components varies from pair to pair though, probably reflecting unavoidable differences arising because the different pairs of populations were raised and measured at different times.

TABLE 2. Least-squares two-way analysis of variance for matched pairs of *r*- and *K*-selected populations.

Population pair	Source	SS	*d.f.*	MS	*F* ratio	*P*
r-1, *K*-1	population	2,549.734	1	2,549.734	7.238	0.011
	sex	0.052	1	0.052	0.000	0.990
	population × sex	57.178	1	57.178	0.162	0.689
r-2, *K*-2	population	1,882.089	1	1,882.089	8.523	0.006
	sex	1,189.224	1	1,189.224	5.385	0.026
	population × sex	11.427	1	11.427	0.052	0.821
r-3, *K*-3	population	4,915.485	1	4,915.485	18.858	0.000
	sex	126.354	1	126.354	0.485	0.491
	population × sex	5.267	1	5.267	0.020	0.888
r-F_1, *K*-F_1	population	6,957.038	1	6,957.038	16.562	0.000
	sex	6.003	1	6.003	0.014	0.905
	population × sex	2,663.719	1	2,663.719	6.341	0.016

On the whole, sex does not appear to have any significant effect on feeding rate. Population-by-sex interactions seem to contribute significantly to the observed variation in the case of the two hybrid populations, but this is probably a chance occurrence due to sampling, as no such general trend is discernible.

Discussion

The results obtained from these experiments clearly indicate the existence of significant genetic variation for feeding rate in *D. melanogaster*. It is also evident that the *r*- and *K*-selected populations have diverged significantly with respect to rates of sclerite retraction, the mean rates of the *K*-selected lines being higher than those of the *r*-selected ones in all cases. This is in accordance with earlier work on the evolution of competitive ability in the same populations (Mueller, 1988). The results also support the view (Bakker, 1961) that larval feeding rate is an important component of competitive ability under high-density conditions in *Drosophila*.

The results also indicate that both *r*- and *K*-selected lines have a fair amount of variability with regard to feeding rate; the variance in both types of populations is roughly the same. The demonstration of a genetic basis for variation in feeding rate, together with the consistently greater mean feeding rate of the *K*-selected populations, strongly implicates density-dependent natural selection as the causal agent behind the higher feeding rates of the *K*-selected flies. Earlier studies (Buzzati-Traverso, 1955; Ayala, 1965, 1968) document an increase in the carrying capacity of *Drosophila* populations upon being shifted to a laboratory environment. This would imply that *Drosophila* populations rarely exist under high-density conditions outside a laboratory. The base population from which the *r*- and *K*-selected lines used in this study were derived was never exposed to very high density conditions. Thus, it is plausible to assume that a large part of the observed divergence between the two types of lines is due to an increase in the retraction rates of the *K*-selected flies as a response to being kept at a high population density.

The observation that the feeding rates of the *r*-F_1 hybrids are not significantly greater than the parental *r*-selected lines further strengthens this view, as it rules out the possibility of drift having played a significant role in the evolution of feeding rate. It is important to rule out the possibility that the slower feeding rate of the *r*-selected larvae is due to fixation of alleles that reduce feeding rate as a result of drift. If such alleles are neutral in low-density populations, then their conditional expected time until fixation is $4N$ generations, where N is the effective population size (Ewens, 1979). Thus the *r*-selected populations used in this study could undergo fixation of such alleles by drift in 200 generations or fewer. Studies of female fecundity on the same populations have revealed that drift was a major factor causing the *r*-selected females to have low fecundity late in life (Mueller, 1987). However, other traits that have diverged significantly in the *r*- and *K*-selected populations, such as pupation height (Mueller and Sweet, 1986) and competitive ability (Mueller, 1988) show no evidence of inbreeding depression in the *r*-selected populations.

The results are interesting in the light of the observed increase in competitive ability in *Drosophila* populations subjected to artificial selection for high retraction rates (Burnet et al., 1977). Our experimental procedure subjected the *K*-selected populations to natural selection for increased competitive ability; this resulted in a correlated increase in retraction rates. The study thus supports the prediction that, under high-density conditions, natural selection acts to favor the evolution of traits conferring enhanced competitive ability for limiting resources.

Furthermore, given the nature of the formal models of "scramble"-type larval competition in *Drosophila* (Nunney, 1983), our results and those of Burnet et al. (1977) indicate that retraction rates are apparently good indicators of the ability of *Drosophila* larvae to compete for limiting food resources. This observation is of value for experimental studies, as it is much easier to measure sclerite-retraction rates than competitive ability.

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TWO CONSEQUENCES OF HOMOSEXUAL COURTSHIP PERFORMED BY *DROSOPHILA MELANOGASTER* AND *DROSOPHILA AFFINIS* MALES

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Sexually mature *Drosophila melanogaster* males perform an elaborate series of courtship behaviors in response to conspecific virgin females (Spieth, 1952; Bastock and Manning, 1955). Mature males will also perform vigorous courtship, including attempted copulation, toward young, sexually immature males. This behavior is performed by males from long-established laboratory strains (Cook and Cook, 1975; Jallon and Hotta, 1979), isofemale lines recently derived from natural populations (McRobert and Tompkins, 1983), and males in the field (McRobert and Tompkins, unpubl.).

The evolutionary significance of this homosexual courtship is unclear. It has been suggested that young males that are exposed to courtship stimuli may subsequently perform better courtship themselves when they become sexually mature. Alternatively, mature males that "practice" courting young males may court females more effectively (Tompkins et al., 1980; Siegel et al., 1984). However, neither of these hypotheses has been tested. Accordingly, we observed older *D. melanogaster* males that had courted young conspecific males and young *D. melanogaster* males that had elicited courtship to see whether either performance or elicitation of homosexual courtship affected the males' courtship of females, as indicated by their success in copulating. Our findings provide the first evidence that homosexual courtship benefits the young male.

Mature males, on the other hand, do not benefit from homosexual courtship; their ability to copulate with females is not affected by prior experience with males. Therefore, it is not surprising that a mechanism for minimizing the amount of time that a male spends performing homosexual courtship has evolved in *D. melanogaster*. Briefly, a mature male that courts an immature male will subsequently perform less courtship in response to a second young male. This phenomenon is known as experience-dependent modification of male courtship (Gailey et al., 1982; Vaias and Tompkins, unpubl.).

Drosophila affinis is another species in which mature males court young, sexually immature males (McRobert and Tompkins, 1987). Recently, we have shown that *D. melanogaster* and *D. affinis* interact sexually (McRobert and Tompkins, 1986*a*). Mature *D. melanogaster* males perform courtship in response to young *D. affinis* males that is quantitatively and qualitatively indistinguishable from the courtship that young conspecific males elicit. In addition, *D. affinis* males court young *D. melanogaster* males as vigorously as they court young conspecific males.

If homosexual behavior is disadvantageous to the courting male, it seems reasonable to assume that a mechanism should have evolved to minimize this behavior in all situations in which courtship of males occurs. Accordingly, we have analyzed the courtship behavior of *D. affinis* to determine whether experience-dependent courtship modification (EDCM) occurs after mature males court young, conspecific males. In addition, we have analyzed the cross-species interactions of *D. affinis* and *D. melanogaster* males to see whether interspecific homosexual courtship is modified by experience.

MATERIALS AND METHODS

Stocks

All flies were maintained on a standard cornmeal-agar-molasses medium, at either 22° or 25°C in a 12:12 LD cycle with lights on at 8 A.M. The *D. mela-*