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DIRECTIONAL AND STABILIZING DENSITY-DEPENDENT NATURAL SELECTION FOR PUPATION HEIGHT IN *DROSOPHILA MELANOGASTER*

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Abstract.—Six populations of *Drosophila melanogaster* have been kept at extreme population densities, three high and three low, for 175 generations. Larvae from the high density populations pupate 50%–100% higher than larvae from the low density populations. At high larval test densities there is both a directional and a stabilizing component to selection, with viabilities ranging from 0.14 to 0.992, depending on the choice of pupation site. The directional component is stronger on the populations which have evolved at low densities, while the stabilizing component is stronger on the populations which have evolved at high densities. There is no indication that the evolution of this trait, in response to density, has altered its phenotypic plasticity.

Key words.—Behavior, density-dependent natural selection, *Drosophila melanogaster*, *K*-selection, pupation height, *r*-selection, stabilizing selection.

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Pupation height, the height above the medium at which individuals pupate, is a behavioral trait that affects fitness in laboratory populations of *Drosophila* species, especially at high densities. Significant levels of genetic variation for pupation height have been detected in *D. melanogaster*, and this genetic variation appears to be largely additive and autosomal (Sokolowski and Hansell, 1983; Bauer and Sokolowski, 1985). Markow (1979) and Garcia-Florez et al. (1989) were able to select successfully for both high and low pupation height, and Mensua (1967) for the former. Pupation height has a considerable effect on fitness in high density cultures because: pupae on the surface of the medium suffer increased mortality with increasing moisture content of the medium (Sameoto and Miller, 1968), and larvae pupating on the sides of vials, near the surface of the medium, are likely to be dislodged and drowned by other larvae (Chiang and Hodson, 1950).

Increasing the density of larvae tends to markedly increase pupation height in *D. melanogaster* (Sokolowski and Hansell, 1983) and *D. simulans* (Ringo and Wood, 1983). Sameoto and Miller (1968) reported an increase in the proportion of larvae pupating on the walls of vials, rather than the surface of the medium, in response to increased moisture content of the medium. Mensua (1967) observed an increase in pupation height with larval density, diameter of the culture vessel and aeration deficiency. Temperature effects (Sokal et al., 1960; Mensua, 1967) and light-darkness regime effects (Markow, 1979; Schnebel and Grossfield, 1986) have also been reported. Thus, there is also a substantial environmental component to pupation height.

Mueller and Sweet (1986) demonstrated that *D. melanogaster* populations, maintained at high densities for many generations, had a higher mean pupation height compared to low density populations initially derived from the same source. The study reported here was a continuation of their work, and assessed the strength and direction of selection at low and high densities, acting on individuals from these populations that had different pupation height phenotypes.

MATERIALS AND METHODS

Selection Regimes

This study used three *r*-selected and three *K*-selected populations that had been maintained for 175 generations by methods previously described in detail (Mueller and Ayala, 1981). The *r*-selected populations were maintained by allowing 50 adults, three to six days old, to lay eggs in a standard half-pint culture bottle for 24 hr. After 14 days, a sample of 50 was chosen from the

300–500 progeny to repeat the process. For the last 10 generations the procedure had been slightly modified to forestall any further effects of random genetic drift. This modification increased the total breeding population tenfold (Mueller, 1991).

The *K*-selected populations were maintained by the serial transfer method (Mueller and Ayala, 1981) at carrying capacity, which is 800–1,200 adults per culture. Resources were renewed weekly and adults permitted to reproduce indefinitely. All cultures consisted of 40 ml of cornmeal-flour-sugar-agar medium in a half-pint milk bottle with one facial tissue inserted to control moisture. Cultures were maintained at 23°C on a 12 hour light : dark cycle.

Both larvae and adults in the *K*-selected populations undergo extreme crowding unlike the *r*-selected lines. In the *K*-selected cultures the medium becomes rather loose and fluid while in the *r*-selected cultures the medium remains relatively solid.

Measurement of Pupation Height

Pupation heights were measured on three *r*-selected populations (*r*-1, *r*-2, *r*-3) and three *K*-selected populations (*K*-1, *K*-2, *K*-3) at two different density levels. Since pupation heights may vary over time for the same population (Bauer and Sokolowski, 1985), pairs of *r*- and *K*-selected populations, matched by indices, were run simultaneously at both density levels. These indices have been assigned randomly to each population with respect to these phenotypes. The high density treatment had 150, and the low density treatment 20 larvae per 8-dram vial (22 × 95 mm). Each vial contained 10 ml of the culture medium. The high density treatment was replicated twice, and the low density treatment five times, for each *r*- and *K*-population. The number of larvae used for the high density treatment was determined by a pilot study observing pupation behavior and mortality with 100, 150, 200, 300, and 400 larvae per vial. At densities of 200 larvae per vial, or more, a large number of larvae pupated all the way up on the cotton plug, a problem for taking accurate measurements of pupation height. Larval mortality at those densities was also very high.

Collection of larvae for the experiment was done by a method similar to Nunney (1983). For each population, 100 adults from the running cultures were allowed to lay eggs in a half-pint bottle for 24 hr. This process was repeated for four consecutive days, using fresh adults for laying eggs in the same culture bottle every time. After 14 days, the newly emerged adults were kept in a fresh culture bottle, with an abundant supply of dry yeast, for 3 days. They were then permitted to lay eggs on non-nutritive agar in watch-glasses. First instar larvae were collected from the watch-glasses after 24 hr and shifted to vials.

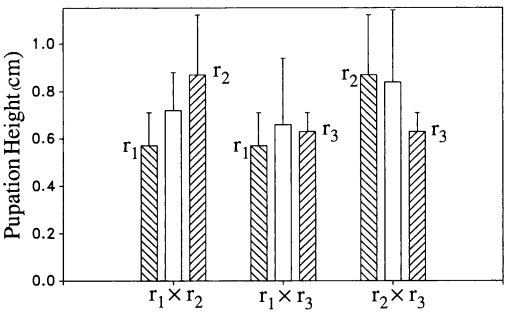

FIG. 1. The pupation height plus 95% confidence interval for the three *r*-populations and their F_1 hybrid populations. A one way of analysis of variance shows no significant effect of population and thus supports the conclusion that none of the hybrid populations has a pupation height significantly greater than the mean of the two parental populations.

Pupation heights were measured by the technique described by Mueller and Sweet (1986). Twenty-five days after the setting up of the vials, 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 was given a pupation height of zero. The experiments were run for 26 days after egg laying. Any individuals unemerged at that time were scored as deaths, since adults usually emerge in two to three weeks even at high densities.

RESULTS

Inbreeding Depression in the r*-Populations*

We note here that only some traits had been adversely affected by small population size. For instance, female fecundity late in life had apparently decreased in the *r*-popu-

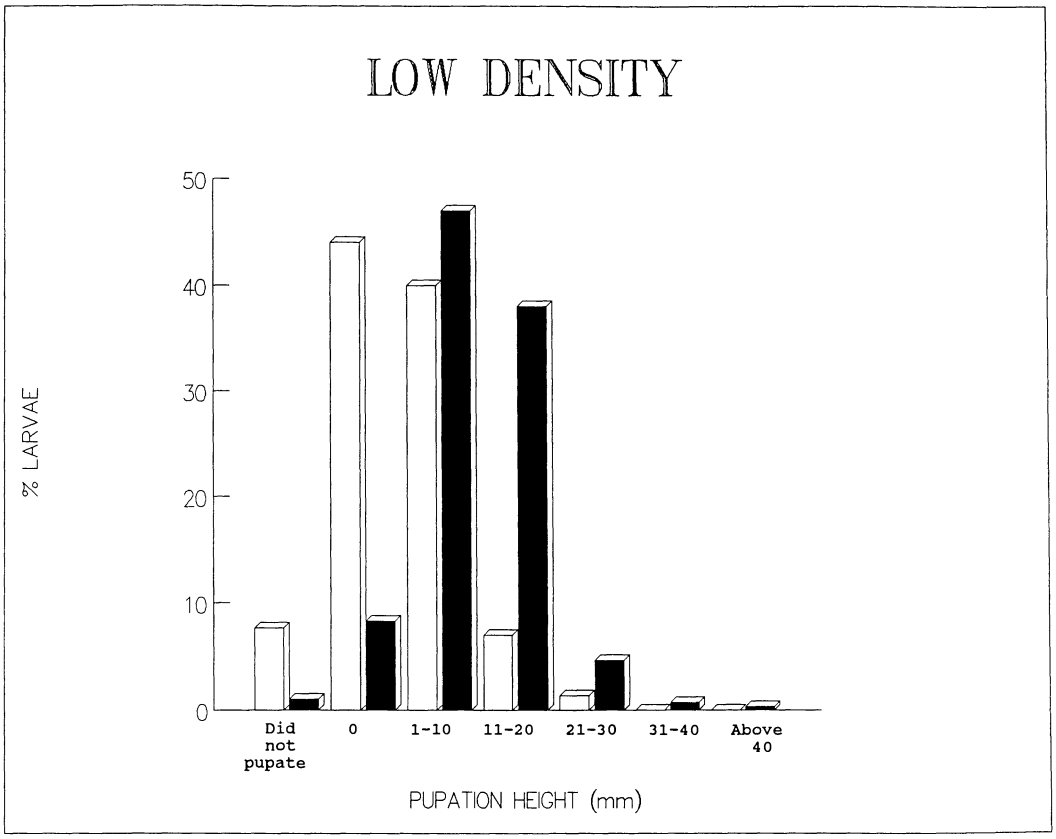

FIG. 2. Percentage of larvae from *r*-selected (open bars) and *K*-selected (solid bars) populations pupating in different height intervals under different density regimes (data pooled for the *r*- and *K*-selected lines).

lations relative to the *K*-populations as a result of genetic drift, although early fecundity remained unaffected (Mueller, 1987). Larval behavioral traits that responded to density-dependent selection showed no signs of inbreeding depression in the *r*-populations. These traits included pupation height (Mueller and Sweet, 1986), larval feeding rates (Joshi and Mueller, 1988) and larval competitive ability for limited food (Mueller, 1988). These conclusions were based on observations of the F_1 progeny of the three independent *r*-populations which had intermediate phenotypes relative to the parental population (data for pupation height in Fig. 1).

TABLE 1. Analysis of variance (ANOVA) of pupation height data over populations (*r* versus *K*) and larval density (20 versus 150). Population and density are treated as fixed effects while subpopulation is nested within population and vial is nested within the interaction of subpopulation and density. Since subpopulation shows a significant effect, the *F*-ratio for population is calculated as the ratio of the population mean square to the subpopulation mean square.

Source	*df*	*MS*	*F*	*P*
Population	1	15,790	34.4	0.005
Density	1	43,148	376	<0.001
Population × Density	1	18	0.16	0.694
Subpopulation(Population)	4	459	4.00	0.003
Density × Subpopulation(Population)	4	403	3.51	0.007
Vial (Subpopulation × Density)	30	123	1.07	0.358
Error	2,227	115		

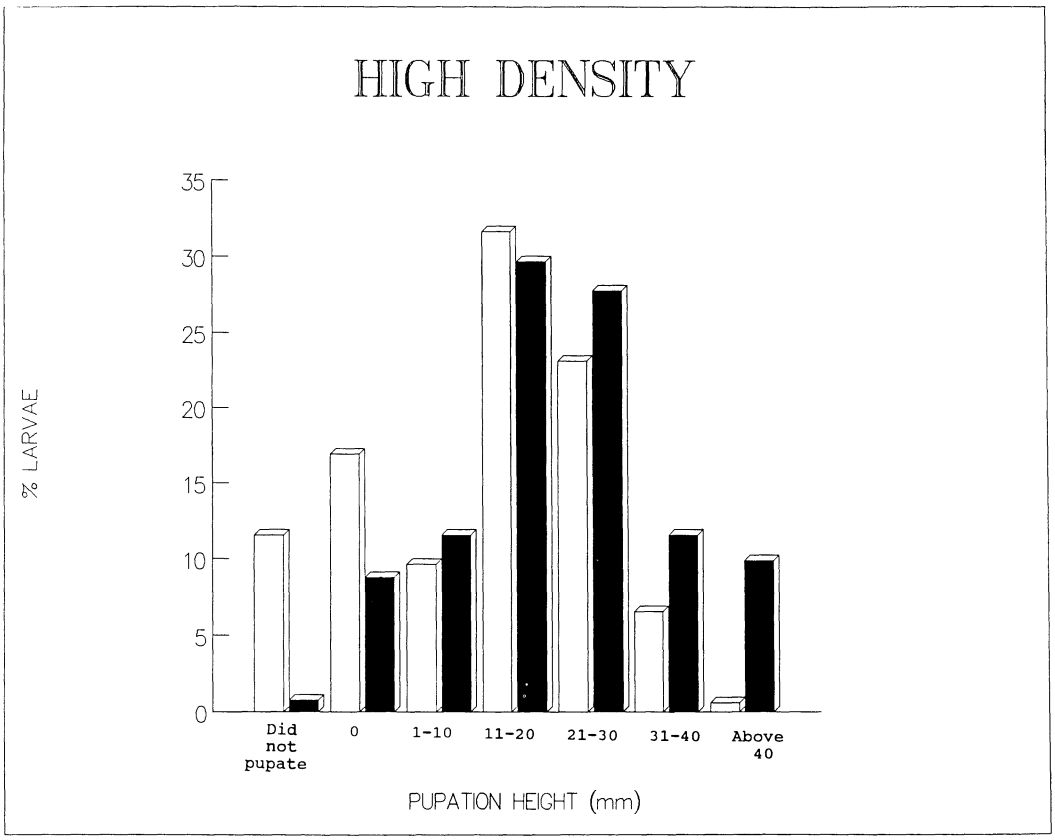

FIG. 2. Continued.

Pupation Height Differences between r- *and* K-*Populations*

An analysis of variance on pupation heights of the *r*- and *K*-populations showed that there is a significant effect of both population and density (Table 1). Pupation height increased at higher density (Fig. 2) and was generally greater in *K*-populations than *r*-populations (Fig. 2).

Larvae from the *r*-selected populations tend to pupate on the surface of the medium to a much greater extent than their *K*-selected counterparts. This difference is especially marked in the low density treatments. However, the differences between *r*- and *K*-populations are significant even if larvae pupating on the surface are excluded from the analysis (data not shown). Thus, the difference in mean pupation heights is not merely a consequence of the fact that a greater proportion of *r*-selected larvae pupate on the surface of the medium. The *r*-selected populations also suffer higher larval mortality prior to pupation. This may be an effect of inbreeding depression.

Mortality versus Pupation Height

Mortality of larvae pupating in different height intervals for both low and high density treatments showed similar profiles for both *r*- and *K*-selected populations (Table 2). The large variation in percent mortality of *r*-selected pupae at heights above 40 mm is due to random error associated with the extremely low numbers of larvae pupating at those heights. In the high density treatments there was high mortality for larvae pupating on the surface, or above 40 mm. In the low density experiments few larvae pupate above 40 mm; mortality is solely due to pupation on the surface. Formally, a 2 × 6 (alive versus dead and six pupation height intervals) contingency table of the pooled

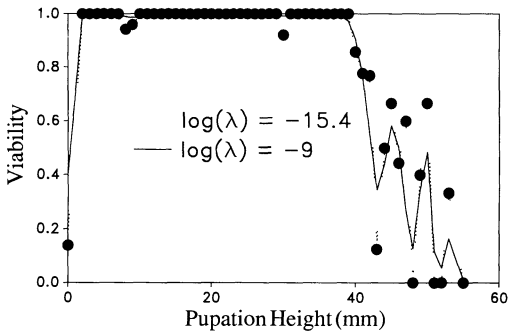

FIG. 3. Viability of the pooled *K*-population data at high density, as a function of pupation height. The lines represent cubic splines fit to these data with the indicated weighting function (λ). Each point on the graph represents the average viability of all larvae pupating at that particular height. Some points are the average of many individuals while others may represent only a single observation.

K-population high density data showed a significant ($\chi^2_5 = 619$, $P < 0.001$) interaction between pupation height and survival to emergence. For the pooled *r*-population data at high density there is also a significant interaction ($\chi^2_4 = 538$, $P < 0.001$).

Form of the Selection Function

The viability of pupae shows a sharp discontinuity when moving from the surface to the side of the vials just above the surface (Fig. 3). There appears to be a gradual decline in viability at heights >40 mm, while nearly all pupae in the interval 1–40 mm survive to become adults. It is clear that the function describing viability versus pupation height is neither Gaussian or quadratic. We have attempted to fit the data to a cubic spline following the procedures outlined by Schluter (1988) and the lines shown in Figure 3 are two estimated fitness functions.

The "best" fitting cubic spline is the one which minimizes the generalized cross-validation score and will depend on a choice of smoothing parameter, λ. Very large values of λ will produce curves with small variance but large bias, whereas very small values of λ will produce curves that track every point closely and have quite high variance. In Figure 3, the curve corresponding to log(λ) = −15.4 is the best fit. This curve fits the data below 41 mm quite will but is overly sensitive to the points above 41 mm. If splines with larger values of λ[log(λ) = −9] are used, the curve is less noisy above 41 mm but fails to predict well the pupation height on or very close to the surface. Since many larvae pupate on the surface (Table 2) models that inaccurately predict these observations are undesirable.

Estimates of the Magnitude of Directional and Stabilizing Selection

The most direct way to examine the magnitude and direction of selection would be to determine the phenotypic mean and variance of the dead and surviving populations of pupae (Endler, 1986, pp. 172–173). From these quantities inferences about selection may be made. All these tests ultimately re-

TABLE 2. Percent mortality (rounded to the nearest percent) of larvae from *r*- and *K*-selected lines pupating in different height intervals. Total numbers of pupae at each height interval are shown in parenthesis.

Density	Population	Pupation height (mm)					
		0	1–10	11–20	21–30	31–40	Above 41
High	*r*-1	83 (58)	4 (19)	1 (98)	0 (65)	0 (23)	100 (2)
	K-1	81 (20)	0 (37)	0 (86)	0 (86)	0 (41)	47 (28)
	r-2	80 (54)	0 (49)	1 (101)	0 (52)	5 (14)	—
	K-2	77 (23)	6 (37)	0 (99)	1 (73)	0 (91)	63 (21)
	r-3	73 (40)	0 (20)	1 (86)	0 (91)	9 (22)	67 (3)
	K-3	97 (36)	0 (29)	0 (80)	1 (91)	5 (22)	51 (41)
Low	*r*-1	73 (41)	0 (53)	0 (2)	—	—	—
	K-1	83 (6)	0 (59)	0 (32)	0 (1)	—	—
	r-2	44 (43)	0 (51)	0 (1)	—	—	—
	K-2	81 (16)	0 (49)	0 (33)	0 (1)	—	—
	r-3	46 (48)	0 (17)	0 (18)	0 (4)	—	—
	K-3	33 (3)	0 (33)	0 (49)	0 (12)	0 (2)	0 (1)

—: No pupae in this height interval.

quire some estimate of the variance in the two distinct populations. From Table 2 it can be inferred that at low density the only dead pupae were on the surface. Consequently, we cannot estimate the variance in pupation height of the dead pupae. In lieu of these formal tests, we can determine whether the pupation height of the surviving larvae in the low density experiments overlaps zero (the height of all dead pupae). The mean height of surviving larvae for all three K-populations at low density is 11.3 mm $\pm$ 0.6 (95% confidence interval). The mean height of surviving larvae for all three r-populations at low density is 5.5 mm $\pm$ 0.7. Based on these results we conclude the average pupation height of the dead larvae is significantly less than the surviving larvae giving rise to directional selection for increased pupation height.

For the data at high density, let x be the measured pupation height of an individual from either the surviving class (s) or the dead class (d) of pupae. We can then compute the following statistics (Endler, 1986, pp. 171–173),

$$i = \frac{\bar{x}_s - \bar{x}_d}{\sqrt{\text{var}(x_d)}},$$

$$j = \frac{\text{var}(x_s) - \text{var}(x_d)}{\text{var}(x_d)},$$

$$j' = j + i^2.$$

The statistic i provides a measure of directional selection and j and j' measure changes in the variance of the trait. If selection is stabilizing the variance of the selected population it is expected to be less than the variance of the unselected (dead) population and hence the statistics j and j' should be negative.

To test for differences in the mean pupation height in the selected and unselected populations a t-statistic has been calculated (see Table 3 in Endler, 1986, p. 171). The large sample sizes used in this study give these t-statistics effectively infinite degrees of freedom. Consequently, we report the value of this statistic as t_∞ (Table 3). To test for significant differences in the variance of the two groups we used Levene's test (Levene, 1960, Table 3).

TABLE 3. Estimates of directional (i) and stabilizing (i and j') selection in the pooled r- and K-data at high larval density. Significant differences between the mean pupation height of the selected and nonselected populations are indicated by t_∞; significant differences between the variances of these populations are indicated by Levene's test.

Population	i	t_∞	j	j'	Levene
r	1.66	18.3	−0.12	2.6	228**
K	0.13	2.29	−0.75	−0.73	382**

** $P < 0.0001$.

There appears to be significant selection for increased pupation height in both populations based on the significant difference between the selected and nonselected populations (see Table 3, t_∞). Selection certainly is much greater in the r-population than the K-population.

The magnitude of j provides evidence for stabilizing selection in both populations. For both the r- and K-populations the variance of the surviving population is less than the dead population. Since the distribution of pupation heights in both these populations departs substantially from normal, the most appropriate test for the equality of variance is the Levene test which yielded significant differences for both r- and K-populations (Table 3).

Empirical Estimates of the Fitness Function

Examination of Figure 3 suggests that the selection function may in fact be best approximated as follows,

$$w = \begin{cases} w_1 & \text{if } x = 0 \\ w_2 & \text{if } 0 < x < 41 \\ w_3 & \text{if } x \geq 41, \end{cases}$$

where w is the probability of sucessful eclosion. If there is a stabilizing component to selection then $w_2 > w_1, w_3$. This has been examined by using a bootstrap resampling scheme to estimate the w_i and put confidence intervals on w_2–w_1 and w_2–w_3 (Tables 4, 5). It is clear that at the 95% level of confidence, selection is more stringent in the tails of the pupation height distribution and thus selection has a stabilizing component for both the r- (Table 4) and K-populations (Table 5).

TABLE 4. The empirical fitness function with 95% and 99% confidence intervals for the pooled *r*-population data.

Parameter	Estimate	95% confidence interval	99% confidence interval
w_1	0.21	(0.15, 0.27)	(0.13, 0.30)
w_2	0.989	(0.981, 0.996)	(0.978, 0.997)
w_3	0.20	(0.0, 0.67)	(0.0, 1.0)
w_2-w_1	0.79	(0.71, 0.84)	(0.69, 0.85)
w_2-w_3	0.79	(0.33, 0.99)	(−0.01, 0.996)

Environmental Sensitivity of r- *versus* K-*Populations*

Differential responses to density by each population may be tested by examining the population × density interaction in the ANOVA (Table 1). This interaction term is not significant even though the density × subpopulation interaction is significant. This latter interaction indicates that not all subpopulations respond in a similar manner to density.

DISCUSSION

These results confirm earlier observations of Mueller and Sweet (1986). They examined pupation heights at only one larval density (100 larvae/vial) but varied food levels. The current study shows that the differences between the *r*- and *K*-populations are manifest over a range of larval densities. Data presented here show that the differences between the *r*- and *K*-populations cannot be explained by inbreeding depression in the *r*-populations. Hence, the differentiation is most likely due to natural selection.

Selection at High Larval Densities

Various laboratory and field studies have led to an understanding of the genetic basis of differences in *Drosophila* larval pupation behaviors. Yet, as Sokolowski and Bauer (1989) note, studies linking these behavioral differences to fitness are lacking. This study is the first to explicitly investigate the relationship between pupation height and fitness in different environments. In fact, as a practical matter this study has only been able to measure the viability of individuals with different pupation phenotypes. We have measured the size of pupae at various heights and found no correlation between pupa (and therefore adult) size and pupation height (Mueller, unpubl. data). Since adult size is correlated with female fecundity and male mating success these data would indicate that pupation height is not closely correlated with these other fitness components, but at this time no direct measurements have been made.

TABLE 5. The empirical fitness function with 95% and 99% confidence intervals for the pooled *K*-population data.

Parameter	Estimate	95% confidence interval	99% confidence interval
w_1	0.14	(0.068, 0.22)	(0.049, 0.24)
w_2	0.992	(0.985, 0.997)	(0.982, 0.999)
w_3	0.5	(0.40, 0.60)	(0.37, 0.64)
w_2-w_1	0.85	(0.79, 0.91)	(0.75, 0.94)
w_2-w_3	0.49	(0.39, 0.60)	(0.35, 0.62)

The experiments reported here clearly show stabilizing selection for pupation height under high density conditions. The value of j' for the *K*-populations was −0.73. In a survey of the literature Endler (1986, p. 212) found only 5 out of 420 studies had values of j' less than −0.7. Larvae pupating on the surface of the medium have high mortality for reasons discussed earlier. The sudden transition from almost zero mortality in the 31–40 mm height range to over 50% mortality at pupation heights above 40 mm is surprising. This may be due to a humidity gradient in the vials but at this time remains unexplained.

The exact form of the selection function is difficult to express in a simple functional form. The data from high densities clearly identify three different pupation height ranges: the surface, a large range just above the surface, and a very high region with approximately constant viabilities w_1, w_2, and w_3 respectively. The assumption that these w's are constant appears to be fairly robust except possibly region 3, where viability may be declining with increasing pupation height (Fig. 3).

Selection at Low Larval Densities

Selection pressure in the low density situation would be largely directional, for increased pupation height. Surprisingly, larvae from *r*-selected populations, which

should have experienced such selection, still have a pronounced tendency to pupate on the surface of the medium. Mortality of larvae pupating on the surface is lower in the low density experiments, but is still considerable. In the culture bottles in which they are maintained, about 90% of the *r*-selected larvae pupate on the paper tissue which remains upright and relatively dry; in the *K*-selected cultures the tissue is usually partially buried in the medium and is extremely soggy. Thus, it is possible that the *r*-selected larvae in the culture bottles are not experiencing selection for increased pupation height to the degree that is indicated by the experiments described here which utilized vials without tissue paper.

It is clear from this study that if the *r*-population were introduced into a crowded culture there would be substantial selection for increased pupation height. The value of *i* in these populations at high density is 1.66 which is greater than most published studies of directional selection differentials (Endler, 1986, p. 209). In fact we have recently tested six derivatives of the *r*-populations which had been kept at high population density for 25 generations and they have exhibited substantial increases in pupation height relative to the parental *r*-populations (Guo et al., 1991).

Phenotypic Plasticity

The non-significant interaction between population and density indicates there is no difference between the *r*- and *K*-populations' response to changing density. The response of the *K*-selected larvae is more extreme, presumably a consequence of having been under strong selection for increased pupation height. It appears that these environments have not resulted in a differentiation of this measure of phenotypic plasticity but rather have simply increased the magnitude of the response at all densities.

Conclusion

The experiments reported here, along with previous studies on these *r*- and *K*-selected populations (Mueller and Ayala, 1981; Mueller and Sweet, 1986; Joshi and Mueller, 1988; Mueller, 1988; Mueller et al., 1991) provide unequivocal experimental validation of the predictions of density-dependent natural selection theory. This study clearly shows that not only the magnitude, but even the nature of selection acting on a metrical trait can vary based upon differences in population density. This lends credence to the idea that changing the population density can change the kinds of selective forces acting on a population not only due to changes in the magnitude or sign of the selection coefficient, but more subtly by changing the type of selection on a particular trait.

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