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CORRELATED RESPONSES IN LIFE-HISTORY TRAITS TO ARTIFICIAL SELECTION FOR BODY WEIGHT IN *DROSOPHILA MELANOGASTER*

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Abstract.—*Drosophila melanogaster* that had been successfully selected on rich and poor larval medium for increased and decreased fresh weight at eclosion were tested on an intermediate medium for correlated responses in longevity, fertility, and hatchability. Larger flies laid more eggs early in life and lived shorter lives than smaller flies, which not only lived longer but also laid more eggs later in life. This supports the notion of a mortality cost of reproduction in *Drosophila*. The total number of eggs laid per lifetime did not differ between the two groups. The percentage of offspring hatched started at normal levels (about 50% of eggs laid), then declined rapidly in large flies. In small flies, hatchability started at a lower level early in life (40–65%), but declined less rapidly, and later in life was higher than the hatchability of eggs laid by larger flies.

Key words.—Body size, correlated responses, fertility, longevity, viability.

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Body weight is an important phenotypic component of fitness in fruit flies. Robertson (1957) found a phenotypic correlation between thorax length and egg production but no additive genetic correlation. Tantawy and Vetukhiv (1960) and Tantawy (1961) showed in female *D. pseudoobscura* that larger females lived longer and laid more eggs than smaller females. Larger males are more successful in competitive mating experiments than smaller males (Ewing, 1961; Partridge and Farquhar, 1983; Bijlsma and Trapman, 1989); heavier flies may also resist starvation better if they are heavier because they contain more fat (Zwaan et al., 1991). Male body weight is moderately well correlated with fitness (Mackay, 1985). Both genes and environment influence body weight in *Drosophila melanogaster,* which responds to artificial selection (e.g., Robertson, 1960; Bos and Scharloo, 1973; Scheiner and Lyman, 1989; Hillesheim and Stearns, 1991), increases phenotypically with decreasing temperature (David et al., 1983), and decreases above a certain threshold of larval density (Scheiring et al., 1984; Zwaan et al., 1991).

It would appear that selection should favor larger flies across a broad range of environmental conditions, but results differ on how selection on body weight affects lifetime egg production and longevity. Partridge and Fowler (1992) found significant differences in lifetime egg production among lines selected for longevity and for late fecundity; the long-lived lines were heavier and produced more eggs but were older at eclosion. Rose (1984) found neither a difference in the total number of eggs laid nor a size difference in flies selected in one treatment for early and in the other for late fecundity and longevity (Rose et al., 1984). Luckinbill et al. (1984, 1988), who also used a selection protocol like that of Partridge and Fowler, found no correlation of body size with longevity.

This paper asks, were there correlated responses in longevity and fecundity in lines selected for increased and decreased fresh weight at eclosion on rich and poor larval medium (Hillesheim and Stearns, 1991)? Selection for increased longevity can result in increased body size, but will selection for increased body size result in increased longevity? In this experiment, we measured the longevity of virgin males, virgin females, and reproductive females, and we counted representative samples of the number of eggs laid per female and the number of offspring hatched. Flies selected on both rich and poor medium were assayed on intermediate medium.

MATERIALS AND METHODS

All experiments were done in an incubator at 25°C, 80% relative humidity.

Flies.—The flies tested were descended

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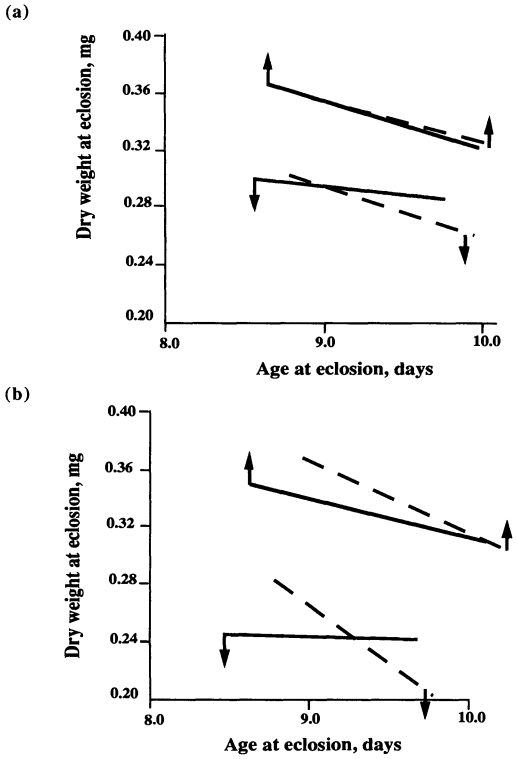

FIG. 1. Direct and correlated responses to selection on weight at eclosion in weight and age at eclosion in both larval food environments. The arrow indicates the direction of selection. Solid lines indicate that selection was carried out on rich larval medium; dashed lines indicate that selection was carried out on poor larval medium. Flies were tested on both rich and poor larval medium. (a) Flies were taken for the assay directly from the selection lines after 11–12 generations with no control for maternal effects. (b) Flies were taken for the assay after another four to five generations of selection and then after having been held for two generations on intermediate (1%) larval medium to control for maternal effects.

from selected lines. Fresh body weight was mass selected in both males and females—four replicate lines upwards and four replicate lines downwards on rich food (4% yeast—40 g dead yeast/1,000 g medium) and on poor food (0.5% yeast—5 g dead yeast/1,000 g medium). Details on the origin of the flies and the selection regime are given in Hillesheim and Stearns (1991).

The flies were measured in the 18th generation. The flies on rich food had 10 generations of selection, no selection in the 11th generation, selection again in the 12th and 13th generations, no selection in generations 14 through 16, then again selection in generation 17. The flies on poor food were selected for 11 generations initially, then according to the same pattern. The flies tested were taken directly from the selection regime, were not held for one generation on the same type of food to reduce maternal effects, and were tested on a larval medium with a yeast concentration (1%) intermediate between rich (4%) and poor (0.5%). Therefore we did not test for differences between rich and poor media, only for differences between lines selected upwards and downwards on the same medium.

Fecundity and Longevity of Mated Females.—Within each food regime of flies selected on rich food (R-lines) and on poor food (P-lines), three replicate lines selected upwards and three replicate lines selected downwards were tested (2 food regimes × 2 selection regimes × 3 replicates per treatment = 12 lines total). Fresh body weight was measured in two- to three-day-old flies. For each replicate, 30 pairs of three-day-old flies were established separately in small plastic tubes. Every 24 hr the laying substrate (15 mm diameter) was replaced with a fresh one with a drop of fresh yeast. Flies were checked for survival once a day, and dead males were replaced with virgin males from the same replicate. The number of eggs per individual female was counted every second day until she died.

Viability.—The eggs counted for one 24 hr period were transferred at two, four, or six day intervals to large vials (12–14 ml medium for the hatched larvae). Offspring of each of 15 females per replicate were kept separately and females of the same age were used to replace females that died. After 14 days, the number of hatched flies and the percentage of hatched flies per female per test interval were determined. Thus the viability results represent a sample of about one-fourth of the eggs laid per lifetime.

Longevity of Virgin Flies.—For each replicate, two vials (12–14 ml medium with fresh yeast) were started with 20 virgin males and two other vials with 20 virgin females. Every five days they were transferred to new vials and the dead flies were counted until the last fly died.

Statistical Methods.—Survival curves were analyzed with a Wilcoxon rank sum test for two groups and Kruskal Wallis test

TABLE 1. Mean fresh body weight (mg) of 30 females and 30 males per line ± 1 SE.

Replicate	Males		Females	
	Upwards	Downwards	Upwards	Downwards
	Lines selected on rich larval food			
1	1.09 ± 0.01	0.78 ± 0.01	2.11 ± 0.03	1.34 ± 0.02
2	1.06 ± 0.02	0.70 ± 0.01	2.01 ± 0.02	1.31 ± 0.02
3	1.11 ± 0.02	0.75 ± 0.01	2.18 ± 0.02	1.25 ± 0.02
Mean	1.09 ± 0.01	0.74 ± 0.02	2.10 ± 0.05	1.30 ± 0.03
	Lines selected on poor larval food			
1	1.02 ± 0.02	0.74 ± 0.01	1.83 ± 0.02	1.33 ± 0.06
2	0.95 ± 0.01	0.75 ± 0.01	1.73 ± 0.03	1.26 ± 0.03
3	1.13 ± 0.02	0.75 ± 0.02	1.95 ± 0.04	1.22 ± 0.02
Mean	1.03 ± 0.05	0.75 ± 0.00	1.84 ± 0.06	1.27 ± 0.03

for more than two groups (Pyke and Thompson, 1986). Fertilities and viabilities were analyzed with two-way ANOVAs with sex and direction of selection as main effects and with lines nested within the direction of selection (upwards and downwards).

RESULTS

Body weight responded to selection in both directions in both food environments, and these differences were still significant after four generations without selection (Table 1). Both age and weight at eclosion responded to selection on weight at eclosion, and they did so both in the larval medium on which selection occurred and in the other larval medium (Fig. 1; detailed description of the method is given in Hillesheim and Stearns, 1991). Selection for heavier weight at eclosion in one food environment produced similar responses in flies tested on the other larval food environment, i.e., the reaction norms for age and size at maturity shifted upward and remained nearly parallel (upper pairs of lines in Fig. 1a and 1b). Selection for lighter weight at eclosion in one food environment produced much less of a response in the other food environment, i.e.,

TABLE 2. ANOVAs on fresh body weight of lines selected on rich and poor larval food. 2-way analysis with direction of selection and sex as main effects, with replicate lines nested within direction of selection (30 males and females per replicate line).

Source	*df*	*MS*	*F*
	Rich larval food		
Direction of selection	1	29.65	273.15***
Replicates within direction of selection[1]	4	0.11	9.66***
Sex	1	55.47	1,117.06***
Sex × direction of selection	1	4.69	94.36***
Sex × replicates[2]	4	0.05	4.42**
Error[3]	348	0.01	
	Poor larval food		
Direction of selection	1	16.30	48.90**
Replicates within direction of selection[1]	4	0.33	14.77***
Sex	1	39.57	1,149.80***
Sex × direction of selection	1	1.69	48.99**
Sex × replicates[2]	4	0.04	1.53
Error[3]	347	0.02	

[1] Denominator for F (direction of selection); [2] denominator for F (sex) and for MS (sex × direction of selection); [3] denominator for F (replicate lines within direction of selection) and for MS (sex × replicated lines within direction of selection).
** $P < 0.01$; *** $P < 0.001$.

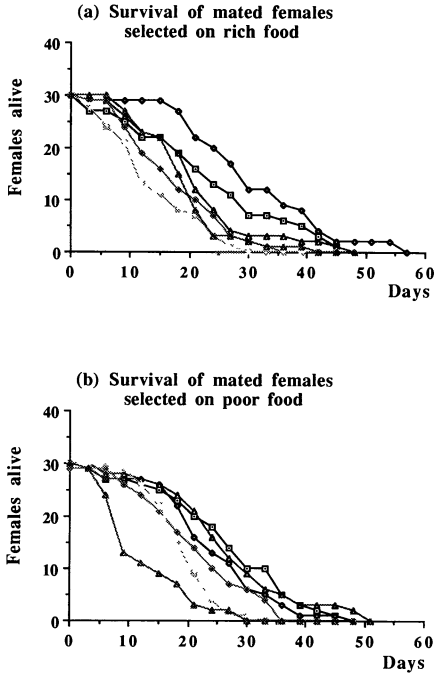

FIG. 2. Survival curves of reproductive females selected on rich larval food (a) and on poor larval food (b). Open symbols are used for the means of flies selected downwards (small), closed symbols for those selected upwards (large). Flies selected on both rich and poor medium were assayed on intermediate medium.

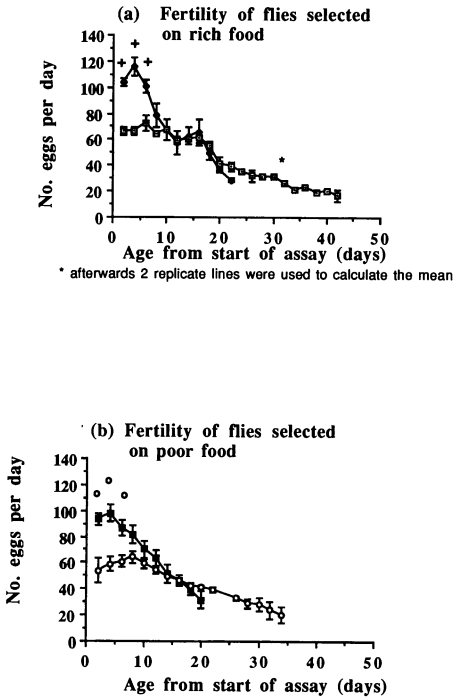

FIG. 3. Number of eggs laid averaged across the three lines selected upwards and the three lines selected downwards, ±1 SE. Means were plotted so long as at least four females per replicate were alive. Closed symbols = mean of lines selected upwards; open symbols = mean of lines selected downwards; + = $P < 0.001$; o = $P < 0.05$. Other differences were not significant. Flies selected on both rich and poor medium were assayed on intermediate medium.

the reaction norms for age and size at maturity did shift downward, but not nearly as much in the food environment in which selection did not occur as in that in which it did occur, with the result that the reaction norms crossed (lower pairs of lines in Fig. 1a and 1b).

TABLE 3. Mean longevities (days) for 30 mated females per replicate ± 1 SE. Flies selected on both rich and poor medium were assayed on intermediate medium.

Replicate	Upwards	Downwards
Females selected on rich larval food		
1	17.2 ± 1.6	30.9 ± 2.2
2	13.7 ± 1.4	23.6 ± 2.4
3	18.6 ± 1.4	20.9 ± 1.9
Mean	16.5 ± 1.4	25.1 ± 3.0
Females selected on poor larval food		
1	21.7 ± 1.6	28.7 ± 1.7
2	18.8 ± 1.0	23.9 ± 1.7
3	12.5 ± 1.3	27.4 ± 2.1
Mean	17.7 ± 2.7	26.7 ± 1.4

There were significant differences among replicates, between the sexes, and significant interactions between sex and direction of selection (Table 2). These are discussed in Hillesheim and Stearns (1991). As reported previously (Hillesheim and Stearns, 1991), the mean fresh body weight of the flies tested in the fertility experiment differed significantly between the selection treatments in both sexes for both poor and rich larval foods (Table 1).

In both R- and P-groups, the mated reproductive females that had been selected for large body size had significantly shorter lives than those that had been selected for small body size (Fig. 2 and Table 3, Wilcoxon rank-sum test, $z = 5.06$ for lines selected on rich food; $z = 6.02$ for lines selected on poor food; $P < 0.001$ for both).

TABLE 4. Mean lifetime egg production per replicate (SE = standard error). Flies selected on both rich and poor medium were assayed on intermediate medium. An ANOVA with replicate lines nested within direction of selection yielded, for rich food, $F_{1,4} = 0.43$, NS, and for poor food, $F_{1,4} = 0.00$, NS. SE = 1 standard error.

Replicate	Upwards SE		Downwards SE
	Lines selected on rich larval food		
1	1,300 ± 116.5		1,565 ± 102.0
2	1,205 ± 102.4		1,302 ± 128.3
3	1,165 ± 92.5		1,087 ± 101.6
Mean	1,224 ± 59.9 ($N = 88$)	NS	1,318 ± 67.0 ($N = 87$)
	Lines selected on poor larval food		
1	1,521 ± 123.3		1,201 ± 107.1
2	1,144 ± 88.8		1,251 ± 123.4
3	806 ± 87.7		1,030 ± 132.6
Mean	1,153 ± 65.3 ($N = 89$)	NS	1,159 ± 70.2 ($N = 88$)

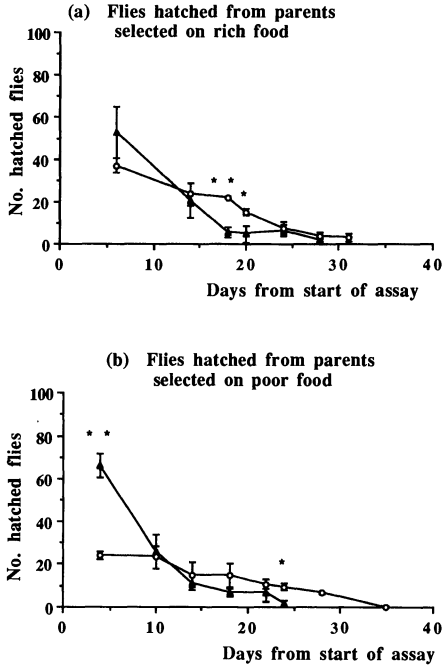

FIG. 4. Number of hatched flies averaged across the three replicate lines ± 1 SE. For the legend see Figure 2; $*P < 0.05$; $**P < 0.01$. Other differences were not significant.

The early fecundity of females selected upwards on both rich (Fig. 3a) and on poor (Fig. 3b) medium was higher than that of females selected downwards, which, however, survived longer and had higher fecundity later in life (Fig. 3). In both the R and the P groups, large flies laid significantly more eggs per day than did small ones during the first week. This difference disappeared nine days after the start of the assay.

Table 4 reports estimates of the total number of eggs laid per lifetime. Large and small flies did not differ in the total number of eggs laid. The longer life of the smaller flies compensates for their lower egg production early in life.

Figure 4 shows the number of flies hatched, which was significantly greater at the start for the large flies in the P-group. However, it decreased rapidly, and on day 18 and 20 for the R-groups and day 24 for the P-groups the smaller mothers produced more hatched offspring. The mean number of hatched offspring produced per female per lifetime (which would be about four times the values reported in Fig. 4) did not depend on the direction of selection to which the ancestors had been subjected (upwards or downwards) for either type of larval medium on which they had been selected (rich and poor—Table 5).

The percentage of eggs that hatched was "normal"—about 50–65% (Kaufmann and Demerec, 1942)—at the beginning of adult life for the large flies but decreased strongly after two weeks. The small flies started with a lower hatching percentage than the large flies, and theirs also decreased as the flies aged, but the decrease was much less rapid, and later in life the hatchability of eggs produced by small flies was significantly higher than that of large flies (Fig. 5). The slopes of linear regressions of mean percentage hatched on age of mother differed for large and small flies only on poor larval medium (rich food: $t = 1.38$, $df = 4$, NS; poor food: $t = 3.486$, $df = 4$, $P \leq 0.05$).

The survival curves of virgin males and females selected upwards and downwards on rich larval food (Fig. 6) did not differ (Wilcoxon rank-sum test, $P < 0.93$ for males, $P < 0.19$ for females). The survival curves of virgin males and females selected upwards and downwards on poor larval food differed significantly (Fig. 7). Virgin males selected upwards lived longer lives ($z = 2.93$; $df = 1$; $P < 0.003$), but virgin females selected upwards lived shorter lives ($z = 3.69$;

TABLE 5. Estimates of progeny production for females based on the sum of the mean values over 15 females per replicate measured at each of seven or eight 24 hr intervals (cf. Fig. 3). To convert to an estimate of lifetime progeny production, these values should be multiplied by 4, for only ca 25% of the reproductive lifespan was present in the sample. SE = 1 standard error. Flies selected on both rich and poor medium were assayed on intermediate medium. A two tailed t-test between upwards and downwards selected flies was done for each larval food: rich food, $df = 4$, $t = 0.79$, NS; poor food, $df = 4$, $t = 0.67$, NS.

Replicate	Upwards		Downwards
Lines selected on rich larval food			
1	98		112
2	131		137
3	47		92
Mean	92 ± 24.4	NS	114 ± 13.0
Lines selected on poor larval food			
1	117		73
2	137		96
3	100		140
Mean	118 ± 10.7	NS	103 ± 19.6

$df = 1$; $P < 0.001$). Virgins lived about twice as long as mated females.

DISCUSSION

Whereas Partridge and Fowler (1991) found that flies selected for greater longevity were larger at eclosion and laid more eggs per lifetime, the flies selected here to be larger at eclosion had shorter lives and laid more eggs early in life but did not lay more eggs per lifetime. This result held for both R- and P- groups. The contradiction is apparent, not real. In selecting for heavier flies, we were also selecting for flies that had larger ovaries, laid more eggs at the start of life, and—perhaps therefore—died younger. In selecting for flies that lived longer, Partridge and Fowler were selecting flies that grew more slowly, were larger at eclosion, did not lay more eggs early in life, but had invested more in a durable physiological infrastructure.

Whereas the flies selected here to be large at eclosion had higher fecundities early in life, they did not differ from the small flies in total number of eggs laid per lifetime. These results are in line with Rose's (1984). The selection criterion used here—light or heavy fresh body weight at day two—implied selection of females for fast ovary development. The total lifetime egg production was the same for large and small females because the small females with longer life had relatively high egg production later in life.

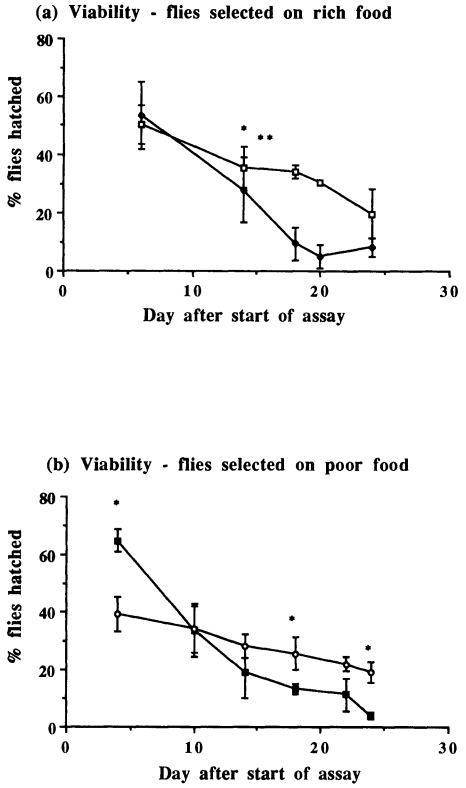

FIG. 5. Hatching percentages in the lines selected upwards and downwards on rich (a) and poor (b) food. Flies selected on both rich and poor medium were assayed on intermediate medium. Open symbols are lines selected downwards (small flies) and closed symbols are lines selected upwards (large flies) ± 1 SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. Other differences were not significant. Differences tested by Mann Whitney U-test.

These results support the view that aging is in part a byproduct of selection for reproductive performance (Rose et al., 1984; Rose, 1991) and that mating imposes a cost of reproduction on females that is realized in higher mortality rates for mated than for unmated females (Partridge et al., 1987; Service, 1989).

Under natural conditions, the life expectancy of *Drosophila* is probably on the order of one to two weeks. If the results found here can be applied to the field, then selec-

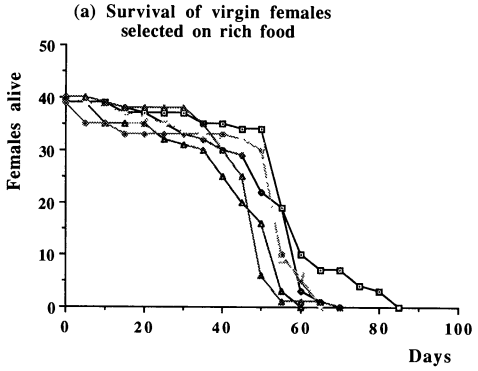

FIG. 6. Survival curve of virgin males (top) and virgin females (bottom) selected on rich larval food (R-group). Open symbols are lines selected downwards (small flies) and closed symbols are lines selected upwards (large flies). Flies selected on both rich and poor medium were assayed on intermediate medium.

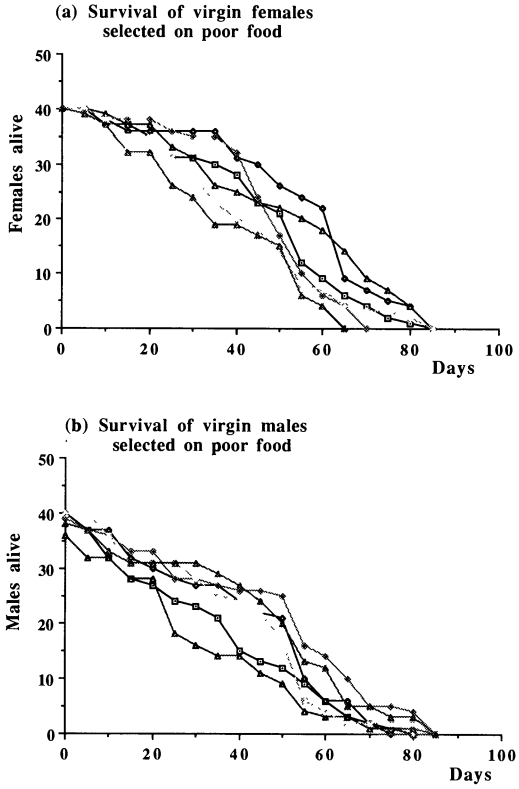

FIG. 7. Survival curve of virgin males (top) and virgin females (bottom) selected on poor larval food (P-group). Open symbols are lines selected downwards (small flies) and closed symbols are lines selected upwards (large flies). Flies selected on both rich and poor medium were assayed on intermediate medium.

tion should favor larger flies, which have higher egg production and the same hatchabilities as smaller flies over the first one to two weeks. There would be no advantage for flies modified by the sort of selection applied by Partridge and Fowler, for although their flies were larger and lived longer, they did not have higher fecundities over the first two weeks of life.

On the other hand, the smaller flies would gain in natural circumstances that permitted a life expectancy of three to four weeks or more, for they live longer and produce eggs that have higher hatchability later in life. Hatchability is a complex trait, for it depends on the percentage of eggs that are fertilized as well as the percentage of fertilized eggs that survive both larval and pupal stages. The percentage of eggs fertilized is in part a male trait that depends on male courtship behavior and sperm quality and in part a female trait that depends on willingness to mate and quality of sperm storage. To tease these apart, one should mate large females with large and small males and small females with large and small males, then measure the hatchability of the eggs produced.

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