DIRECT SELECTION ON LIFE SPAN IN DROSOPHILA MELANOGASTER

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Abstract.—An important issue in the study of the evolution of aging in Drosophila melanogaster is whether decreased early fecundity is inextricably coupled with increased life span in selection experiments on age at reproduction. Here, this problem has been tackled using an experimental design in which selection is applied directly to longevity. Selection appeared successful for short and long life, in females as well as males. Progeny production of females selected for long life was lower than for short-lived females throughout their whole life. No increase of late-life reproduction in long-lived females occurred, as has been found in selection experiments on age at reproduction. This discrepancy is explained in terms of the inadequacy of the latter design to separate selection on life span from selection on late-life fecundity. Moreover, starvation resistance and fat content were lower for adults selected for short life. In general, the data support the negative-pleiotropy-disposable-soma theory of aging, and it is hypothesized that the pleiotropic allocation of resources to maintenance versus to reproduction as implicated in the theory might involve lipid metabolism. It is argued that further research on this suggestion is urgent and should certainly comprise observations on male reproduction because these are for the greater part still lacking. In conclusion, the longevity of D. melanogaster can be genetically altered in a direct-selection design, and such an increase is accompanied by a decreased general reproduction and thus early reproduction.

Key words.—Aging theory, Drosophila, early and late fecundity, selection on longevity.

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On the basis of population genetic theory, evolutionary biologists have tried to explain the origin of aging (for reviews, see, e.g., Kirkland 1989). The key element of this explanation is the observation that any population will encounter mortality even in the absence of age-dependent mortality (i.e., aging). Consequently, the force of natural selection will decline with age for any genotype and, as a result, “senescence will tend to creep in” (Hamilton 1966, p. 25). Two scenarios that are not mutually exclusive have been described.

First, natural selection can hardly act against mutations with deleterious late effects in life because most, if not all, of the original individuals carrying such mutations will be deceased at the time of gene expression (selection shadow; Hoekstra 1993). Thus, such mutations will be able to accumulate during evolution (mutation-accumulation theory; Medawar 1952). Second, mutations with favorable effects early in life, but with pleiotropic negative effects late in life, can nevertheless be selected for if these late effects are delayed until the selection shadow (negative-pleiotropy theory; Williams 1957). Therefore, aging is the result of the expression of deleterious mutations late in life, with or without pleiotropic effects. In adopting Williams' concept of pleiotropy, specific pleiotropic genes were suggested by Kirkwood and Holliday (1979) in the disposable-soma theory of aging, namely, genes that control the level of somatic maintenance versus the level of reproductive output (Kirkwood 1985). Important aspects of the evolutionary theories of aging have been supported by mathematical analysis (Williams 1957; Hamilton 1966; Charlesworth 1980; Kirkwood 1987).

Although gerontologists have pointed out that the issue of the evolutionary account of aging is by no means settled (Sacher 1982; Finch 1990), the central ideas of the theory outlined above show promise for experimentally unraveling the evolution and thus the genetics of aging (Bell 1984; Kirkwood 1991; Rose 1991).

Several such attempts have been made using Drosophila as a model organism. On the basis of both a sib analysis of adult females and selection experiments, Rose and Charlesworth (1980, 1981a,b) concluded that senescence in D. melanogaster is due to late-acting deleterious effects of genes that have pleiotropic beneficial effects early in life. It was demonstrated in selection experiments on age at reproduction that senescence could be postponed in the “old” lines but only at the expense of a loss in early fecundity relative to the “young” lines (Rose 1984), thus confirming the results of a comparable pioneer experiment of Wattiaux (1968) using D. subobscura. Total fecundity did not differ between the lines, because, “old” lines had higher late-life fecundities. The importance of these results and conclusions were emphasized by the findings of Luckinbill and coworkers (1984, 1987), who confirmed the observations in an independent experiment using the same selection procedure. However, several observations signify that the action of negative pleiotropic genes alone cannot be the whole story.

Results on other adult fitness characters like desiccation resistance, indicated that accumulation of mutation had played a role in the establishment of increased life span in the Rose population (Service et al. 1988). Moreover, an interspecific comparison (Schnebel and Grossfield 1988) and a study using recombinant extracted lines (Hughes and Clark 1988) at best showed only weak consistency with the model of antagonistic pleiotropy in Drosophila. The basis of this discrepancy might be that the factual selection in the age-at-reproduction design is applied to increased late fecundity, which is however contingent on selection for increased longevity (Clark 1987). Therefore, the differences in longevity of the lines need not be the consequence of early fecundity differences. This was also noticed by Partridge and Fowler...
(1992), who demonstrated in their selection experiment on age at reproduction that a comparatively small increase in longevity was observed without a concomitant decrease in early reproduction. This corroborated the findings of Mueller (1987) in his r- and K-selected lines. Furthermore, it is essential to note that the progeny-production pattern was altered in lines selected for body weight (Hillesheim and Stearns 1992) and for developmental time (Zwaan et al. 1993), without, at least in the latter case, the longevity effects predicted by the pleiotropy model.

Partridge and Fowler (1992) suggested that early fecundity differences might have increased life span, not by causing accelerated or delayed senescence but by pleiotropically causing instantaneous age-independent effects (see also Finch 1990, p. 638). Indeed, Partridge and Andrews (1985) have shown that reproduction reduced survival probability to later ages without accelerating mortality rates. Selection, therefore, could have resulted in the elimination of alleles that increase the overall risk of mortality attributable to reproduction early in life (Partridge and Andrews 1985). According to Partridge and Fowler (1992), these genes would have pleiotropic, but not age-specific, effects in the sense of Williams’ theory. The fact that longevity differences between the lines were still present in tests using virgin flies (Service 1989) does not necessarily challenge this view because fecundity differences were also maintained; in addition, the increase in risk could be consequential to divergence in general activity (e.g., Service 1987). Interestingly, mortality curves of the Luckinbill population (Arking 1987) indicated a progressive decrease in the age-independent mortality rates in the course of selection.

The experimental data collected thus far clearly show that it is possible to genetically prolong life in Drosophila. However, the issue in the evolution of aging in Drosophila has now become whether decreased early fecundity is inextricably coupled to selection for increased life span (Zwaan et al. 1995). In other words, are genes with pleiotropic effects on longevity and fecundity or genes with late-life deleterious effects responsible for aging in this species? This issue can only be settled by using alternative selection schedules (see also Partridge and Andrews 1985). Ideally, those schedules should abolish the confounding effects of different parental ages at breeding (Lints 1988b) and of different (early) reproductive history (Partridge and Fowler 1992) occurring between the selection lines. To our knowledge, until now no experiments have been described avoiding both these obscuring effects, although Lints et al. (1979) circumvented the parental age and Engström et al. (1992) the reproductive-history effect. The experimental setup presented in this paper is an attempt to pave alternative ways in selection experiments on postponed senescence in D. melanogaster. Taking advantage of the dependence of adult life span on temperature (e.g., Zwaan et al. 1992) in combination with family selection made it feasible to select directly on life span. The direct responses to this selection, as well as the correlated responses in developmental time, body weight, starvation resistance, and fat fraction, mated female and male longevities, and lifet ime progeny production of females are discussed in relation to the previous studies mentioned above.

**Materials and Methods**

**Selection Procedure**

The outbred Groningen 83 wild-type Drosophila melanogaster strain (Zwaan et al. 1991, 1992, 1995) was used as the base population for the selection lines. This population is and has been maintained since its capture in high numbers in uncrowded half-pint bottles (40 ml standard medium; Zwaan et al. 1995). In the reported experiments, development was completed at 25°C and 45% RH.

In October 1991, two replicate lines were started for selection for long (L₁, L₂) as well as for short (S₁, S₂) adult life span, starting from approximately 3200 individuals raised in vials (50 eggs/vial). In addition, two internal control (C₁, C₂) lines were set up. Such control lines are indispensable to accurately record the response to selection, allowing correction of the data for possible inbreeding or drift effects and fluctuations between generations (Zwaan et al. 1995). Each line comprised 30 vials, each with a pair of adults, including 5 reserve vials (fig. 1). All pairs were allowed to lay eggs for 1 wk in three consecutive vials (8 ml standard medium plus 0.8 mg ampicillin). A lump of live yeast was added to the first of these vials. Generally, density never exceeded 100 eggs per vial. At eclosion, flies were collected as virgins, and the sexes were stored separately. If, for any reason, a pair did not yield sufficient progeny, it was replaced by a progeny-producing reserve pair. Despite this precaution, the number of pairs could not always be maintained at 25 throughout the experiment.

In all lines, progeny from each pair was divided into two groups. The first group consisted of 15 virgin females and 15 virgin males who were placed in a 29°C climate room for the longevity assay (fig. 1). We used 29°C because the flies are relatively short-lived at this temperature; in this strain, the relative timing of the aging events was not affected the same at 20°C compared with 25°C (Zwaan et al. 1992). Typically, these flies were collected in a 2-d interval from the first laying vial. These adults were stored in groups of five per vial, sexes separated (i.e., six vials/pair). Thus, for each generation, a total of 900 vials were stored in the 29°C climate room. Flies were transferred to fresh vials once a week, and, since no reserve flies were collected, fly numbers were occasionally reduced by accidental deaths and escapes (at worst three flies/sex/pair). Twice a week (on Tuesday and Friday), the vials were scored for deceased individuals, until all the flies had died. Median longevity could be calculated using linear extrapolation of survival percentages on time in the interval including the LT₅₀ point. This was done for the progeny of both sexes of each pair, and, for selection, the phenotypic value was taken as the longevity mean over both sexes. Thus, ideally, selection line means were the average over 25 data points per sex.

The second part of the progeny of each pair was foreordained in family selection as potential parents for the next generation and stored at 15°C (fig. 1). Females and males were stocked separately in groups of 10 to 15 (one vial per sex; fig. 1), and vials were renewed every week. As a result, 300 vials were stored in the 15°C climate room every generation. Generally, low ambient temperature greatly increases adult life span in D. melanogaster (David 1988), and, in this
strain, mean longevity at 15°C (>120 d) is still substantially greater than maximum longevity at 29°C (<60 d). Moreover, reproductive abilities of females and males appeared unchanged even after 60 d at low temperature. In addition, this parental “cold-storage” had no effect on longevity of the offspring at 29°C (pilot experiment, data not shown).

The factual selection was carried out after all flies in the longevity test had died and median life span had been calculated. In the lines selected for increased longevity, the six groups of progeny of individual parents were chosen from the 15°C pool whose brothers and sisters had the highest median life span in the longevity assay (family selection; Falconer 1989). In contrast, the six shortest-living families were chosen as parents for the next generation in the short life-span selected lines. In the control lines, six families were chosen at random by drawing numbers. Subsequently, within each selection line, the six families were randomly paired; this could imply brother-sister matings. Each of the six families contributed 5 pairs of adults, thus resulting in the necessary 30 pairs per line (fig. 1). All the pairs were then allowed to produce progeny, whereupon the next selection cycle could be initiated, as outlined above. It appeared that it took approximately 60 d to complete one round of selection; in total, there were six generations of selection.

Several disadvantages of this experimental setup are obvious. (1) Inbreeding will be quite severe. Effective population numbers will be much lower than 25 pairs per line because only six families were selected per generation. (2) Longevity at 29°C may not be representative for life span outside the 20°C to 29°C temperature range of D. melanogaster. However, the use of this temperature was nearly unavoidable in order to achieve reasonable selection progress. More importantly, by using 29°C as the adult temperature, the longevity values of the flies were known before their brothers and sisters stored at 15°C themselves reached ages at which senescent decline became visible. Nevertheless, this selection procedure provides some remedies to the experimental problems delineated in the introduction. (1) The actual phenotypic value for longevity is determined on virgin individuals. Thus, mating-activated reproductive physiology and behavior proven to be unrelated to aging (Partridge and Andrews 1985; Partridge et al. 1986) will not confound the outcome of selection. (2) No differences between the selection treatments in parental age at reproduction will be present, because all lines were propagated at the same time. Moreover, such age effects will probably be very small because, from a physiological perspective, 60-d-old virgins previously stored at 15°C should be regarded as relatively young adults at 25°C.

**Correlated Responses**

In the fourth generation of selection, flies were taken for measurements of correlated responses. In order to make those measurements more reliable, crosses were performed between the replicates within a treatment, and two generations of outcrossing occurred. These crosses were produced in the following manner. After the proper families were selected for founding the fifth generation, six families per line were discarded from the 15°C virgin pool. These were the six shortest-lived families for the long-lived lines and the six longest-lived families for the short-lived lines; the remaining “extremes” in the selected lines were thus removed. Six families were removed at random in the control lines. Furthermore, all remaining families were split in two in order to make the four possible crosses within each treatment (e.g., L1 × L1, L2 × L2, L1 × L2 and L2 × L1). Each cross consisted of 50–70 pairs, divided over two half-pint bottles. All crosses were grown according to standard procedures (see below) in vials containing 100 eggs. At eclosion, the resulting flies were stored at 15°C. After a week, they were allowed to mate within their group in bottles containing an extra lump of live
yeast to stimulate egg production. Four days later, eggs were collected at 3-h laying intervals and seeded into vials (Zwaan et al. 1995). Each of the possible 12 groups (4 for each treatment) consisted of 5 vials with 100 eggs. The following traits were measured on the emerging adults (F2).

**Developmental Time and Viability.**—Developmental time and viability (egg-to-adult survival) were determined as described in Zwaan et al. (1995). The latter data were angular transformed before statistical analysis.

**Starvation Resistance.**—Twenty-five females and males, previously aged for 21 d (five virgins/vial) at 25°C, were put singly into vials containing agar. Twice a day, the vials were checked for dead individuals and starvation resistance (in hours) was taken as the midpoint of two successive scorings (Zwaan et al. 1991).

**Body Weight and Fat Fraction.**—Simultaneously, fat content was determined using ether extraction on individuals from the same 21-d-old virgin pool. As a result, adult body weight and fat weight could be assessed (for details, see Zwaan et al. 1991). Consequently, fat content was calculated as the ratio of fat weight to live body weight. For each sex and cross, five replicates of five flies were incorporated, and the data on fat content were angular transformed before analysis.

**Virgin Longevity at 25°C.**—At eclosion, flies were collected as virgins and distributed into vials. Each vial contained five flies, female or male, and five vials were taken per group and sex. Vials were renewed once and scored for deceased imagos three times a week. Longevity was taken as the mean day of two successive scorings. As a result of accidental deaths and escaped flies, numbers occasionally fell below 25. On the whole, longevity means were based on 22 to 25 data points. Moreover, these means also served as control values for the longevity of mated flies (mated longevity).

**Mated Longevity and Female Lifetime Progeny Production.**—For each group and sex, 25 individuals were used. Each female was placed into a vial with two males and each male with two females that were 4-7-d old and derived from the Groningen 83 base stock. These base-stock flies were removed and replaced by fresh flies on days 18 and 35 after eclosion of the test flies. Vials were checked for deaths three times a week. Fresh vials were provided to males once a week. In contrast, vials for females were renewed three times a week, and all progeny eclosing from these vials was counted (about 10-14 d later), until the female had died. At the death of the female, wing length was determined as a measure of body size (Zwaan et al. 1992). Assessment of life span was the same as in the virgin test. Mated longevity and total progeny production means were based on 21 to 25 data points.

### Table 1. ANOVA on adult longevity for the selected lines in terminal generation of selection.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>F-test</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>9018.6</td>
<td>2</td>
<td>26.8†</td>
<td></td>
</tr>
<tr>
<td>Replica within treatment (A)</td>
<td>504.9</td>
<td>3</td>
<td>10.8***</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>229.5</td>
<td>1</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Treatment by sex</td>
<td>163.7</td>
<td>2</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Replica within treatment by sex (B)</td>
<td>157.2</td>
<td>3</td>
<td>3.4†</td>
<td></td>
</tr>
<tr>
<td>Error (C)</td>
<td>3821.2</td>
<td>244</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† P < 0.025; *** P < 0.001.

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Fig. 2. A. Adult female and male longevity at 29°C in the course of selection in L-, C- and S-lines. B. The directional selection (L and S) lines are shown relative to the mean of the control lines.
TABLE 2. Realized heritabilities over six generations of selection for longevity. Females and males are treated separately (SE in parentheses; n = 6).

<table>
<thead>
<tr>
<th></th>
<th>L lines</th>
<th></th>
<th>S lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td>0.430**</td>
<td>0.436†</td>
<td>-0.009</td>
</tr>
<tr>
<td></td>
<td>(0.078)</td>
<td>(0.115)</td>
<td>(0.089)</td>
</tr>
<tr>
<td>Males</td>
<td>0.511**</td>
<td>0.517**</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td>(0.064)</td>
<td>(0.088)</td>
<td>(0.118)</td>
</tr>
</tbody>
</table>

† P < 0.025; ** P < 0.01.

Data Analysis

The direct response to selection on life span was tested for significance in the terminal generation of selection using an analysis of variance (ANOVA), with replicates nested within the treatments (factors: treatment, replicates within treatment and sex). The two reciprocal crosses in the correlated-response experiment did not statistically differ from one another in any trait under determination. Therefore, these reciprocal crosses were treated as replicates, which allowed the construction of an ANOVA with treatment, replicates within treatment, crossing, and sex as possible factors. In addition, differences between groups were tested for significance using the Student-Newman-Keuls procedure (SNK test).

RESULTS

Direct Response

The applied selection on life span has caused significant divergence in longevity among the lines in the course of the generations (table 1; fig. 2). Although there was considerable variation among the replicates within one treatment and from generation to generation (table 1; fig 2A), the responses to selection became clear when the data were plotted relative to the control values (fig. 2B). For both females and males, the response to selection for increased life span was high and yielded significant and large realized heritabilities (table 2). Moreover, it appeared that continuing selection would have elicited a further response, as no slackening of the response was yet observed (fig. 2B). In contrast, selection for short longevity was less successful; after three generations of selection, the lines were leveling off (fig. 2B), and female lines showed a tendency to return to control values (see below). These observations were reflected in the low realized heritabilities, which were significant only for S2 males (table 2).

Although the sexes did not respond differently to selection (sex and treatment by sex, NS; table 1), the fading female response in the short life-span experiment demands a critical survey of the data. The longevity of the control lines fluctuated between generations but generally decreased in the time interval of selection (fig. 2A). This was particular true for the C2 line, and C2 females showed a significant decrease in longevity over generations (r = -0.736, P < 0.05). Although apparently random directional changes in longevity occur in Drosophila melanogaster (Lints et al. 1989; also generation 2, fig. 2A), the general deterioration of the C2 line might signify inbreeding depression. As a result, the responses to selection for increased life span may have been exaggerated relative to selection for decreased life span in figure 2B. Nevertheless, as these remarks apply only to the C2 line, they cannot place any doubt on the observation that the L- and S-lines successfully diverged in longevity. They only suggest that conclusions about the absolute responses must be drawn with caution.

The relatively poor response to selection for short life span may be explained in an alternative way. Lints et al. (1979, p. 195) argued that downward selection for longevity would probably result “in the collection of a series of more or less deleterious, semilethal or conditional lethal genes,” which do not, however, have any bearing on the genes involved in aging. It is unlikely that, in the design presented in this paper, such genes would be solely responsible for the observed decreased longevity in the short lines, since the selected families need to have appropriately high reproductive abilities to establish the next generation.

Correlated Responses

Developmental Time and Body Weight.—The correlated responses in developmental time and body weight are shown in figures 3A and B. Generally, mean S-line developmental time was higher than mean C- and L-line developmental time (treatment: F = 1303.6; df = 2, 3; P < 0.001; SNK test). It seemed that this observation could be attributed primarily to the S1 line (fig. 3A; SNK test), whose effect, however, disappeared in the crosses (treatment by cross: F = 10.3; df = 2, 3; P < 0.05). The sexes responded in a similar way to selection (treatment by sex: F = 2.7; df = 2, 3; NS), but, as
4). Mean viability was about 68%, with the SI line having a significantly affect starvation resistance (cross: $F = 0.3; \text{df} = 1, 3; \text{NS}$). Starvation resistance for females was greater than for males (sex: $F = 115.9; \text{df} = 1, 3; P < 0.01$). Crossing the replicates did not significantly influence the viability (cross: $F = 0.2; \text{df} = 1, 3; \text{NS}$).

Adult body weight was not significantly affected by selection (treatment: $F = 6.6; \text{df} = 2, 3; \text{NS}$), nor by crossing the replicates (cross: $F = 0.4; \text{df} = 1, 3; \text{NS}$). However, for females, mean body weight was relatively less for short life selection; however, male short-line body weight was at least equal to control and long-line weights (fig. 3B; treatment by sex: $F = 30.7; \text{df} = 2, 3; P < 0.01$). The SI line is again an exception, with the flies, especially females, being significantly lighter, (fig. 3B; SNK test). For females, this probably also affected body weight in the crosses (treatment by cross: $F = 0.8; \text{df} = 2, 3; \text{NS}$). On the whole, in the SI line, a longer larval period was accompanied by smaller adults, which is in contrast to the positive correlation between body size and developmental time normally observed in Drosophila (see Zwaan et al. 1995). It is probable that an unusual combination of alleles has become fixed in this line as a result of genetic drift caused by very low effective population sizes (see Materials and Methods). This genetic combination is disrupted in the crosses for developmental time and, to a lesser extent, for body weight (fig. 3). Therefore, the above-described correlated responses in developmental time and body weight may not be causally related to selection on life span.

Viability.—No significant statistical effect on viability could be observed (treatment: $F = 0.2; \text{df} = 2, 3; \text{NS}$; fig. 4). Mean viability was about 68%, with the SI line having a significant higher egg-to-adult survival (SNK test), which confirmed the atypical behavior of this line. Crossing of the replicates did not significantly influence the viability (cross: $F = 0.5; \text{df} = 1, 3; \text{NS}$).

Starvation Resistance and Fat Fraction.—Starvation resistance was significantly lower for S-lines than for C- and L-lines, for both females and males (treatment: $F = 62.6; \text{df} = 2, 3; P < 0.01$; treatment by sex: $F = 5.1; \text{df} = 2, 3; \text{NS}$; SNK test; fig. 5A). Crossing the replicates did not significantly affect starvation resistance (cross: $F = 0.3; \text{df} = 1, 3; \text{NS}$). Starvation resistance for females was greater than for males (sex: $F = 281.0.; \text{df} = 1, 3; P < 0.001$), which is in agreement with previous findings (e.g., Zwaan et al. 1991). There were no indications of differences in starvation resistance between control and long lines.

The fat fraction of the adults was significantly affected by selection ($F = 15.9; \text{df} = 2, 3; P < 0.025$; fig. 5B), in agreement with its physiological relation with starvation resistance. For females, S-lines had lower fat contents relative to C- and L-lines (SNK test). As a consequence, a good positive correlation between starvation resistance and fat fraction was found ($r = 0.807, P < 0.001, n = 12$). In males, no statistical differences could be found between the lines (SNK test), whether crossed or not (treatment by sex: $F = 36.9; \text{df} = 2, 3; P < 0.01$). The sex difference in fat fraction (sex: $F = 508.1; \text{df} = 1, 3; P < 0.001$) largely explains the starvation-resistance differences between females and males ($r = 0.930, P < 0.001, n = 12$). Finally, no significant differences were observed between the lines and the crosses ($F = 0.0; \text{df} = 1, 3; \text{NS}$).

Virgin and Mated Longevity at 25°C.—Artificial selection for life span at 29°C also exerted its influence at 25°C. In general, mean S-line life span was lower than C-line life span, which was lower than L-line life span (fig. 6). These observations were significant for virgin flies (treatment: $F = 17.5; \text{df} = 2, 3; P < 0.025$; SNK test; fig. 6A), but not for mated flies (treatment: $F = 5.5; \text{df} = 2, 3; \text{NS}$; only for males, $L_1$ and $L_1 \times L_2 > S_2$, SNK test; fig. 6B). Significant sex differences were found only in the mated test (sex: $F = 599.1; \text{df} = 1, 3; P < 0.001$); females were shorter-lived than males, resulting in a significant "sex-by-mating status" interaction for both the lines (sex: $F = 50.8; \text{df} = 1, 3; P < 0.01$; sex by mating status: $F = 9.4; \text{df} = 1, 3; P < 0.025$).
by mating status: $F = 12.2; \text{df} = 1, 3; P < 0.05$) and the crosses (sex: $F = 189.5; \text{df} = 1, 3; P < 0.001$; sex by mating status: $F = 22.0; \text{df} = 1, 3; P < 0.025$). This corroborates our earlier results (Zwaan et al. 1995) and those obtained by others (Luckinbill et al. 1988; Service 1989). Crossing replicates did not significantly alter adult longevity (virgin, cross: $F = 1.9; \text{df} = 1, 3; \text{NS}$; mated, cross: $F = 1.0; \text{df} = 1, 3; \text{NS}$). Generally, females were somewhat more consistent in showing differences in longevity concordant to their applied selection than males (fig. 6). Longevity differences between the selection treatments were smaller for mated flies than for virgins (fig. 6).

In addition to mean longevity, we also fitted the virgin data to a logistic survivalship curve (see also Zwaan et al. 1992). It appeared that the differences in mean longevity found between the long and the short-lived lines were reflected in an identical way in the $LT_{10}$, $LT_{50}$, and $LT_{90}$ values (data not shown). This clearly indicates that the increased life span in the L-lines resulted from a delay in the onset of senescence (see also Arking 1987).

**Progeny Production of Females.**—Females of the L-lines produced about 35% fewer total offspring during their lives than C- and S-line females (fig. 7). This was statistically significant for the lines (treatment: $F = 23.9; \text{df} = 2, 3; P < 0.025$; SNK test), but not for the crosses (treatment: $F = 3.4; \text{df} = 2, 3; \text{NS}$). The latter result might be solely due to the exceptional high production of the $C_1 \times C_2$ cross (SNK test; fig. 7). However, no statistical effects were produced by crossing the replicates ($F = 1.6; \text{df} = 1, 3; \text{NS}$). Generally, the total production of C and S females did not differ, suggesting that the latter females invested relatively more in reproduction per unit time than the C females, in view of their shorter life span. The differences in total progeny production between the females of the selected lines remained almost unchanged during their lifetimes (fig. 8), with statistically significant differences in the aforementioned direction during the first 3 wk (ANOVAs; SNK test; fig. 8). Presumably, the failure to detect significant effects in weeks 4 and 5 could have been the result of (1) diminishing differences, and/or (2) low statistical power because of fewer surviving females.

Body size is known to be an important factor in determining total lifetime progeny production (e.g., Partridge et al. 1986; Zwaan et al. 1995). However, in this case, it cannot explain the observed variations in productivity in these experiments, because wing length did not differ between the groups (data not shown; treatment: $F = 4.9; \text{df} = 2, 3; \text{NS}$; cross: $F = 3.0; \text{df} = 1, 3; \text{NS}$). Therefore, it can be concluded that increased (virgin) life span is apparently accompanied with general decreased reproductive activity.

**Discussion**

**Response to Selection**

The use of family selection to directly select on life span appears to be a successful alternative to selection on age at reproduction in producing significant differences in longevity among lines. The response to short life-span selection appeared to be less successful than selection for long life, at least for females (fig. 2B). However, after the lines were allowed to recombine freely for two generations, with or without being crossed within one treatment, selection for decreased life span was also successful, for both mated and virgin female and male longevity at 25°C. Moreover, all other characters were measured in the F$_2$ generation, which lessens the effect of possible deleterious alleles generally unrelated to the expression of the characters under consideration. In the S$_1$ line, a negative correlation between developmental time and body weight was found, which contradicts the life-history configuration repeatedly found in Drosophila (e.g., Zwaan et al. 1995). This was presumably caused by genetic-
drift effects and is therefore not likely the result of selection for short life span, which is corroborated by its failure to occur in the S2 line. For all other correlated responses, construction of crosses between the replicates had no significant effect on the studied character, indicating that, within the replicates of one treatment, approximately the same genes were selected for. The reported observations must therefore be considered real (correlated) genetic effects in the strains.

**Selection Design and Suboptimal Condition**

It has been shown previously that the life span of *Drosophila melanogaster* could be changed genetically (Rose and Charlesworth 1981; Luckinbill et al. 1984; Mueller 1987; Engström et al. 1992; Partridge and Fowler 1992), but this clearly can also be done using an experimental design completely different from selection on age at reproduction. In the age-at-reproduction experiments, suboptimal conditions—that is, high larval densities—were generally required to produce a selection response (Clare and Luckinbill 1985; Luckinbill and Clare 1986; Service et al. 1988), and the failure to produce responses to selection in other studies (Lints and Hoste 1974; Lints et al. 1979) has been attributed to the absence of such conditions (Arking and Clare 1986). As a condition for successful artificial selection, destabilized developmental processes were considered necessary in order to show phenotypically the underlying genetic variation (Clare and Luckinbill 1985; Luckinbill and Clare 1986; Arking and Clare 1986). Although it has been established that larval density affects longevity (Zwaan et al. 1991 and references therein), our results show that longevity can be genetically altered without high larval densities. One could argue, however, that an adult temperature of 29°C should be interpreted as a suboptimal condition similar to crowding. However, unlike crowding (Luckinbill and Clare 1986), high temperature is not required to express the selected differences, since the longevity effects at 29°C were also observed in adults measured at 25°C using controlled egg densities. Lints (1985; 1988a) has seriously questioned the experimental evidence in support of evolutionary theories of aging (Rose 1984), because the results could at least partially be explained by parental age effects (Lints 1988b). However, the decanalization interpretation cited above, in addition to the absence of parental age effects on longevity in the Luckinbill population (Luckinbill and Clare 1986) and the findings reported here (involving equal parental ages), strongly suggest that Lints’ doubts are not confirmed. Moreover, the lack of response to direct selection on life span in the experiment of Lints et al. (1979) might have resulted from using pairs of flies as the unit of selection. Partridge (1986) reported that reproductive activity reduces longevity, without necessarily interfering with pathways causally related to senescence. Therefore, the short-lived pairs in the experiment of Lints et al. (1979) may have been the fittest flies showing high reproduction, and the long-lived pairs may have been consisted of “crippled” adults showing low reproduction. This would make selection very ineffective indeed and, presumably, even impossible. This interpretation has been confirmed by observations on the same selection design in our laboratory (unpubl. data), which was why we abandoned it and replaced it with the design reported here.

**Longevity and Early and Late Fecundity**

The results on life-time progeny production in our experiments showed that the reproductive output of the long-lived females was lower at any age than that of short-lived females. This appears at odds with previous publications that reported either decreased early and increased late reproduction (Rose 1984; Luckinbill et al. 1984) or only increased late reproduction (Mueller 1987; Partridge and Fowler 1992). Two arguments can be advanced to resolve this apparent contradiction.

First, it is by no means surprising that in all studies on selection on age at reproduction, fecundity in late lines increased relative to early lines, since this was exactly the trait to which selection was applied (see also Clark 1987). The decrease in early fecundity in two of the populations (Luckinbill et al. 1984; Rose 1984) could have resulted from a negative genetic correlation between early and late fecundity (Partridge and Fowler 1992). The existence of such a correlation in *Drosophila melanogaster* has been supported (Rose and Charlesworth 1981a) but also denied (Engström et al. 1992) by experimental data. The increase of adult life span in the late lines then would not have been due to early fecundity genes with pleiotropic action on life span, but rather to the selective elimination of accumulated deleterious mutants because the age at reproduction was progressively de-
layed. However, the observations in this study strongly indicate that decreased general reproductive activity, and thus decreased early fecundity, is genetically correlated to increased longevity in *D. melanogaster*. This would suggest that the increased late fecundity in the Rose and Luckinbill populations may have been caused by the inability of the age-at-reproduction design to separate selection for increased life span from selection for increased late fecundity, implying genetic variation for late reproduction independent of early reproduction. This has been reported for recombinant extracted lines (Hughes and Clark 1988) but not for the Rose population (Rose and Charlesworth 1981a). Partridge and Fowler (1992, p. 78), however, have argued that the increased late fecundity could be explained by "Medawar's (1952) theory of low selection intensity against mutants with a deleterious effect on the capacity to produce surplus eggs."

Second, the observation that early fecundity was left unchanged in lines with increased life span (Mueller 1987; Partridge and Fowler 1992) might be explained by other correlated responses to selection in these populations. In these cases, both body weight and developmental time of the long-lived females and males was increased (Bierbaum et al. 1989; Partridge and Fowler 1992). The increased body weight of the flies most probably resulted from this increased developmental period, since these traits show a strong genetic correlation (e.g., Hillesheim and Stearns 1991; Zwaan et al. 1995). Variations in these traits can explain the precocious reproductive activity of the long-lived lines since body size is, in general, related to fecundity (e.g., Partridge et al. 1986) and, more specifically, females of lines selected for increased body weight (Hillesheim and Stearns 1992) and for increased developmental time (Zwaan et al. 1995) both exhibited enhanced early reproduction relative to control-line females (for more details, see Zwaan et al. 1995). Moreover, in an earlier study (Zwaan et al. 1995), we have shown that no genetic relation exists between developmental time and longevity, which is confirmed by the results in this study. This does not support the suggestion of Partridge and Fowler (1992) that the increased longevity of their late lines was due to a superior adult soma resulting from a prolonged period of growth. The alternative explanation of coevolutionary selection for developmental time (Partridge and Fowler 1992; Roper et al. 1993) is therefore much more likely.

Partridge and Andrews (1985) argued that early fecundity differences in lines selected for age at reproduction could be due to elimination of alleles that increase the temporary risk of mating early in life. According to Partridge and Fowler (1992), this would fit the mutation-accumulation theory (Medawar 1952) rather than the negative-pleiotropy theory of aging (Williams 1957), since the age specificity of the effects of these alleles would not differ. However, as pointed out by Kirkwood (1988), this interpretation of the mutation-accumulation theory is somewhat atypical as the alleles in question exert their effects very early in life. Therefore, the lines of reasoning are probably correct but should rather be put in terms of the disposable-soma theory of aging (Kirkwood and Holliday 1979; see also Kirkwood 1988). It is very important to recognize that the allocation trade-offs between reproduction and maintenance, as suggested by the theory, will be present through all systems and levels of organization.

This would yield complex interrelationships between life-history traits, which has indeed been confirmed by theoretical analysis of resource allocation models (e.g., Houle 1991; de Laguerie et al. 1991). Nevertheless, our study is consistent with the possibility that increased longevity arose as a consequence of decreased allocation of resources to reproduction at all ages, especially because the longevity variation resulted from selection on virgin longevity.

**A Possible Mechanism Underlying Increased Life Span**

It is remarkable that altered starvation resistance (Service 1985) and thus changed fat content (Service 1987) previously found to be associated with selection on age at reproduction, was also observed in this study, despite the short selection span (only 4 generations as compared to more than 20; Service et al. 1987). Moreover, Service et al. (1988) clearly demonstrated this to be a genetic association, because reversed selection resulted in reduced starvation resistance and increased early fecundity, in accordance with previous observations (Service and Rose 1985). Fat has proven to be important both for maintenance and reproductive activities (e.g., Geer et al. 1970). Therefore, variation in starvation resistance and fat content versus variation in reproduction might reflect the physiological phenotype resulting from genes concurrently allocating fat to these traits (see also Service et al. 1985; Service 1989). The observation that the adult female fat body is a site of synthesis of yolk-polypeptides is plausible support for such mechanism (e.g., Service 1987).

Our data suggest that female reproduction changes in relation to fat content, but this remains to be affirmed for males. It has been shown that protein metabolism in male accessory glands is greatly increased upon copulation (e.g., Schmidt et al. 1985; Service 1989). The observation that the adult male fat body is a site of synthesis of yolk-polypeptides is plausible support for such mechanism (e.g., Service 1987).

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LITERATURE CITED


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