THE EVOLUTION OF DEVELOPMENT IN DROSOPHILA MELANOGASTER SELECTED FOR POSTPONED SENESCENCE

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Abstract.—The role of development in the evolution of postponed senescence is poorly understood despite the existence of a major gerontological theory connecting developmental rate to aging. We investigate the role of developmental rate in the laboratory evolution of aging using 24 distinct populations of Drosophila melanogaster. We have found a significant difference between the larval developmental rates of our Drosophila stocks selected for early (B) and late-life (O) fertility. This larval developmental time difference of approximately 12% (O > B) has been stable for at least 5 yr, occurs under a wide variety of rearing conditions, responds to reverse selection, and is shown for two other O-like selection treatments. Emerging adults from lines with different larval developmental rates show no significant differences in weight at emergence, thorax length, or starvation resistance. Long-developing lines (O, CO, and CB) have greater survivorship from egg to pupa and from pupa to adult, with and without strong larval competition. Crosses between slower developing populations, and a variety of other lines of evidence, indicate that neither mutation accumulation nor inbreeding depression are responsible for the extended development of our late-reproduced selection treatments. These results stand in striking contrast to other recent studies. We argue that inbreeding depression and inadvertent direct selection in other laboratories’ culture regimes explain their results. We demonstrate antagonistic pleiotropy between developmental rate and preadult viability. The absence of any correlation between longevity and developmental time in our stocks refutes the developmental theory of aging.

Key words.—Aging, developmental rate, Drosophila, life-history evolution, natural selection, pleiotropy, survival, trade-offs.

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At least 45 trade-offs between life-history characters are known (Stearns 1992), most of which can be classified as costs of current reproduction for future reproduction or survival. The trade-off between early fecundity and longevity in outbred laboratory populations of Drosophila melanogaster is one especially well-documented and well-replicated result (Rose and Charlesworth 1981a; Rose 1984; Luckinbill et al. 1984; Tucić et al. 1988; Zwaan 1993), lending support to Williams’ (1957) pleiotropy theory for the evolution of senescence and much other life-history theory based on a cost to reproduction (Stearns 1992).

Recently, Partridge and Fowler (1992) appeared to have found an exception to this result. Partridge and Fowler selected for later-life fertility and found no cost in early fertility. Finding slower development, larger body size, and lower viability, they suggested that there is a trade-off between larval viability and developmental time, on one hand, and adult body size and survival, on the other. However, other reports on the size of flies selected for postponed senescence have revealed no consistent weight differences between selection treatments and controls (Rose et al. 1984; Luckinbill et al. 1988; Zwaan 1993). To further muddy the waters, upward selection on fresh weight at eclosion by Hillesheim and Stearns (1991, 1992) produced flies that were shorter-lived than unselected controls. The heavier flies also had higher early-life fecundity and lower late-life fecundity, with no advantage in lifetime fecundity. This baffling array of findings raises some potentially severe problems for the study of life-history trade-offs, problems that call out for empirical clarification.

Another point of interest in the relationship between development and adult life history is the developmental theory of aging. This theory posits that aging is a part of development, after the fashion of differentiation or growth (Lints 1978; 1988). The main experimental evidence for the developmental theory comes from a study by Lints and Lints (1969), who observed increases in both adult lifespan and larval developmental time when Drosophila larvae were reared at high densities. From this observation, they suggested that developmental time is important in
determining the rate of senescence of the adult. Recent work has failed to corroborate this finding (Zwaan et al. 1991,1992; Zwaan 1993). Zwaan et al. suggested that the effects of larval crowding on adult aging are more likely to be the result of viability selection than any meaningful connection between development and longevity. In any case, such phenotypic manipulations skirt around the critical issue of genetic correlation. If developmental time and longevity were positively geneti- cally correlated, there might be some evolu- tionary rationale for the developmental theory of aging. But laboratory selection experiments on developmental time and longevity by the same group (Zwaan 1993) failed to reveal evidence for a genetic correlation between developmental time and longevity. It would be useful if the developmental theory of aging could be supported by some reproducible experiments, or disposed of by further experimental refutation.

Nonetheless, there is, in general, the potential for trade-offs to arise between distinct life-cycle stages, such as larva and adult in holometabolous insects. Selection for adult survival and late-life fertility thus could, in principle, elicit extended larval growth to increase the size and provisions available to the emerging imago. But later maturation will reduce fitness, all other things being equal (Charlesworth 1980). Insects with holometabolous life cycles, like Drosophila may, therefore, face trade-offs among fitness components imposed by the timing of metamorphosis. In particular, even when direct selection is restricted to one life stage, there is potential for trade-offs to impinge on other stages.

The principal goal of the present communication is an intensive investigation of the role of development in the laboratory evolution of aging, with a view to clarifying or refuting extant confusions. Using 24 distinct populations of D. melanogaster selected for different adult demographic parameters, we examine stage-specific developmental time, viability, size and weight, starvation resistance, and response to limited larval food. We test experimentally contradictory findings from other laboratory selection experiments, and argue that inbreeding depression and inadvertent selection can explain these findings. We determine the preadult response to selection for postponed senescence in our stocks, and demonstrate antagonistic pleiotropy between developmental rate and viability. We also use our selected populations to refute the developmental theory of aging.

Materials and Methods

Origin and Handling of Selection Treatments

The experimental Drosophila melanogaster popula-tions used in these experiments were all derived from a South Amherst, Massachusetts, population first studied by P. T. Ives (Ives 1970). The population was sampled in 1975 and has been maintained as an outbred population in 2-wk discrete generations with abundant food to the present time. In 1980, after over 120 genera-tions of laboratory culture, 10 populations were derived from the basal IV stock. Five replicate B populations were maintained on the same 2-wk schedule and thus selected for early-life net fertility, and 5 replicate O populations were selected for late-life fertility by progressively extending the total generation time to 10 wk, as outlined in Rose (1984). Additionally, a set of populations called RU were reverse-selected from the O populations back to the B-type culture regimen starting in 1985 (Service et al. 1988). At the time of most experiments reported here (1992), the Bs had undergone about 250 generations, the Os about 60 generations, and the RUs had been reverse selected for about 120 generations. RU replicate number 5 was lost during selection; thus, only 4 populations exist at present. Replicates are maintained independently at population sizes greater than 1000 adults per generation.

Two other selection treatments are relevant to this paper. In 1989, two starvation-resistance selected treatments, SB and SO, were created, each consisting of five replicate populations founded from the same-numbered replicate of each B and O population (Rose et al. 1992). As controls, two lines, named CB and CO, were also derived. The CB and CO lines are maintained in demographic parallel to the S lines, though provided with food throughout all phases of the life cycle. The life cycles of CB and CO lines are identical to the O life cycle in all ways except for the duration of the selection phase. The total (egg-to-egg) generation time of these lines is about 5 wk at writing: intermediate between B and O, though slightly variable in length depending upon the duration of selection of the starvation lines. The S lines and their controls had undergone about 40 generations of selection when these assays were done.

Although the adult-selection treatments of stocks discussed in this paper differ radically in timing of reproduction, the larval rearing and
early adult environments are identical. Populations are reared at regulated moderate larval densities of 50–150 eggs per 8-dram vial on approximately 5 ml (excess) banana-molasses medium and transferred to their selection treatment at 2 wk from egg collection. The larval culture environment is 25°C with a 24:0 light-dark cycle. The lack of a light-dark cycle may be a particularly important feature of our system, because it means that the evolution of differences in developmental time has been freed from a modality that might either mute or exaggerate the response to selection if emergence is rhythmically dependent on light conditions (Brett 1955).

All experiments reported on in this communication were performed in our laboratory at the University of California, Irvine, with the exception of the first (1988) assay, which was done at the University of Kentucky.

**Statistical Methods**

The selection scheme employed by our laboratory entails identical treatment of five independent replicate populations per selection treatment (four in the case of the RU treatment). For statistical comparisons, the population mean is the primary datum. This technique is conservative, because even large experiments reduce to a small number of degrees of freedom in the analysis, as tests involving treatment means are conducted using only the five replicate population means. Distributional nonnormality of data for individuals within populations is therefore not a factor in our analysis. Tests for the normality in distribution of the replicate means were not conducted; with four or five data points, it is very unlikely that any test could demonstrate nonnormality. Normality of the replicate means is expected, particularly as our sample sizes and replication levels are high. For this reason, we have also not performed any transformations on the data, as is often done, particularly with viability data.

The B and O lines were founded en masse from the IV population and therefore have no special pairwise associations of numbered replicate populations caused by ancestry (e.g., B1 to O1). Statistical comparisons of B and O lines are therefore always unpaired with respect to replicate number. For all other selection treatments described, there is a special historical relationship between replicates of the same number: subsequently derived stocks have been founded in a paired manner (e.g., CB1 and SB1, were both derived from B1); analyses have been pairwise by population number for these selection lines.

All statistical analyses were performed using Statview II and SuperAnova on Macintosh computers.

**Standard Procedures for Developmental Time Assays**

Before experimentation, all selection treatments were put through two full generations of 2–3 wk common culture as a standardizing procedure. Standardization was performed to synchronize selection treatments on previously different schedules, bolster population sizes, and remove any parental and grandparental age effects potentially arising from the selection itself. Standard developmental time experiments were performed with exactly 60 eggs per 8-dram vial and excess banana-molasses food (5 ml/vial), except where noted. About 2000 adult flies of each replicate were given approximately 2 d of fresh, yeasted food in a cage to increase egg output before experimental collection. This procedure was employed to minimize the egg-laying period (1–4 h, depending on the experiment) and to ensure that eggs laid were not advanced in embryonic development owing to retention of eggs by the females. Ten replicate vials were collected for each population sample by gentle brush-transfer of eggs to thin squares of standard medium, which were then placed in the vial. Exactly 60 eggs were counted into each vial. When several people assisted in setting up an assay, each population sample of ten vials was split between two egg collectors (five vials each), so that no whole sample was taken by one person, and each egg collector handled a range of selection treatments.

Flies were collected at 6-h intervals (at 6:00, 12:00, 18:00, and 24:00 h each day). Checks were continued until no adults had emerged for 72 h. A week after the termination of emergence checks, the vials were reexamined. In the postmortem analysis we checked for very late emergent flies, total numbers of pupae, and numbers of flies dead in puparium (these are easily discerned from empty pupal cases using transmitted light), as well as adults that had died during emergence from pupa. Inevitably one or two adults were found stuck in the culture medium. These flies were counted in the postmortem check, but not considered in the egg-to-adult viability estimate (i.e., they were considered inviable in the transition from pupa to adult; the food medium being
a natural hazard in their laboratory environment).

Because small differences in incubator position and proximity to the light can have an effect on developmental time, in all experiments controlled interspersion of selection treatments with random daily repositioning of blocks was employed. For example, in the B/O/RU experiment, B/O/RU, were housed together in the same experiment rack as one block. Racks were randomly repositioned vertically and horizontally on a daily basis within the same incubator.

All assays of egg-to-adult developmental time and viability followed the standard procedures outlined above, except where noted and in the earliest study of B versus O developmental time. The first assay, conducted in the summer of 1988, differed substantially in several ways from the protocol later adopted. This pilot experiment was smaller, using only four of the five replicates (B2.5 and O2.5) for each selection treatment and four vials for each population. It employed a cornmeal/brewer's yeast medium with dextrose and sucrose sweeteners, and each vial was inoculated with a live yeast suspension the day following the collection of eggs for the experiment.

**Hatching Time and Hatchability of B and O Treatments**

In the same experiment and at the same time that eggs were collected for the assay of egg-to-adult developmental time in the Bs, Os, and RUs, a subset of eggs was separated for an egg-hatching-time assay on B and O lines. Eggs were collected from population cages over one hour and exactly 100 eggs were counted onto a food-colored agar plate for each of the populations. Hatching was scored every hour for 37 h, when it appeared that the hatching period was over. As a precaution, the assay plates were recounted about two days later to make sure that unhatched eggs were indeed inviable; no eggs scored as unhatched at the end of the timed checks hatched subsequently.

**Pupation Time and Height of B and O Treatments**

To measure pupation height (Mueller and Sweet 1986) and time from egg to pupa, eggs were collected in the standard manner. The vials were wrapped in clear plastic sleeves 48 h after egg collection, and the distance between the surface of the medium and the bottom of the sponge stopper was standardized to $\pm 1$ mm. Pupae were distinguished from larvae according to the criteria of Ashburner (1989) for a P1 pupa: immobile, without visible larval segments, and with a smooth tanning of the pupal coat visible to the unaided eye. Each 6-h check was scored with a different color of marking pen. When all pupae had formed, the plastic sleeves were removed from the vials and data on spatial and temporal formation of the pupae were taken. We used a Summagraphics digitizing pad and Macintosh computer driven by MacMeasure II to measure pupation height. Adults emerging from this assay were sorted and sexed as in the standard assay (see above).

**Crosses within the O-Selection Treatment**

Crosses among O populations were performed to test for mutation-accumulation as a mechanism for the evolution of extended developmental time of the O populations. We performed crosses between different population replicates and compared them to pure-breeding samples to try to detect F1 heterosis in developmental time. To balance the design, we did not run reciprocal crosses (e.g., O1 male × O2 female and O2 male × O1 female), and each true-breeding sample was run twice from independently derived and sampled flies. Accordingly, ten crossed samples were compared to ten true-breeding samples handled identically but independently. Egg-to-adult developmental time was scored in the standard manner (see above).

**Developmental Time and Viability with Varied Food Levels**

Two experiments involving (1) B and O selection treatments, and (2) B, CB, and CO treatments were conducted to determine developmental time and viability at different larval food levels. We varied the food level while keeping the number of eggs constant. Exactly 60 eggs were counted into each vial on a small piece of non-nutritional agar. Food levels of 1.0, 1.5, 2.0, and 4.0 ml food per vial were employed in the first experiment; these translate into a standardized density series of 16.7, 25.0, 33.3, and 66.7 µl of food per larva. In the second experiment, a fifth food level of 0.5 ml (8.3 µl/larva) was added. The 4-ml food treatment approximates normal food levels in selection.

To eliminate humidity as a factor in these experiments, we provided an agar base (at the same concentration as the agar in normal food) under
the low-food treatments to make them up to the same total volume as the control (4 ml) treatment. Pupae from low-food treatments were therefore not exposed to desiccating conditions. Five vials were collected for each food level for each replicate population. Developmental time and viability data were collected in the same manner as the "standard" assay (see above), except that a postmortem analysis was not performed on the second (B, CB, CO) experiment; thus, no data on stage-specific survivorship of preadult phases were available in the second minimum-food experiment.

**Body Size**

Body size of B and O flies was estimated in two different ways: wet weight and thorax length. Adult wet-weight data were obtained by weighing flies at 24 h to 48 h from emergence, using offspring from parents at five ages (4, 9, 14, 19, 24 d from emergence). Eggs were collected from parents over an 8-h period, counted to exactly 60 per 8-dram vial, and reared in the standard manner at 25°C. After emergence, males and females were placed on standard banana food for approximately 24 h, ether-killed, and weighed in groups of five using a Cahn analytical balance. In total, 9897 flies were weighed. Although the holding period between emergence and weighing varied between 24 and 48 h, we interspersed selection treatments (B and O) in a regimented fashion to control against generating age-specific differences that might be misleading. This design does not address the issue of age-specific weight differences between selection treatments, which may have some role in the inconsistent results for weight found by Rose et al. (1984).

As another index of body size, we measured thorax lengths of B and O flies from the density experiment. The thorax was measured antero-posteriorly along the dorsal side on flies preserved in 70% ethanol. Measurements were taken using a Wild M5 microscope fitted with a camera lucida aligned over a Summagraphics digitizing tablet connected to a Macintosh computer; the software was MacMeasure II (a highly modified version of W. Rasband's MacMeasure by A. R. Palmer, University of Alberta). This digital measuring system allowed high precision without the error associated with hand-recording of data. Three independent repeat measures of each thorax were made for 20 individuals of each sex for each food treatment; the mean of the three repeat measures was used as a single datum in the analysis. The total number of measurements was 4800 (4 food treatments × 2 selection treatments × 5 replicate populations × 2 sexes × 20 individuals × 3 repeat measures). Because measurements took a week to complete with three people working, sampling error was controlled by always pairing a B and an O treatment in any measurement session, with food levels randomized per measurement session separately for B and O replicates within each selection treatment.

**Adult Starvation Resistance**

Starvation resistance was measured on B and O flies at two adult ages: immediately upon emergence and after 4 d of feeding on standard Rose Lab banana-molasses medium. Flies were collected at 6-h intervals from the estimated peak of emergence and split into immediately starved (3 ± 3 h of adult life) and 4-d (96 ± 3 h) fed groups. The flies immediately starved were sexed and sorted into sealed and humidified vials (following Service et al. 1985) in groups of four, and mortality was scored at 6-h intervals. The flies treated with 4 d of feeding and mating were placed on standard banana medium in groups of eight (four males, four females) before removal to starvation vials. Forty flies of each sex were tested for each B or O population/treatment. The assay therefore involved 1600 flies in total.

**Longevity**

Longevity data reported here are a subset of data collected in 1990 (Nusbaum et al. in prep) in which longevity was assayed simultaneously on all laboratory selection treatments reported on in this communication, except the RU treatment. Briefly, longevity was measured by daily scoring of mortality of flies kept in mixed-sex vials (four male, four female). Flies were maintained on standard medium with transfer to fresh medium three times per week.

**RESULTS**

**Long-Term Evolution of the O-B Difference in Developmental Time and Viability**

The difference in egg-to-adult development time and viability between B and O selection treatments over the last five years (1988–1993) is given in table 1. The difference between selection treatments was analyzed in two different ways: factorial ANOVA and simple regression. In these analyses, we excluded the 1988 data because they were collected under a different ex-
Table 1. Long-term evolution of the B/O difference in developmental time and viability. Mean egg-to-adult developmental times and viabilities for the five B and five O populations from nine experimental assays over 5 yr of selection are shown. Os have shown consistently longer developmental times throughout the period of sampling. The earliest assay was conducted on an unconventional and lower nutrition culture medium, and this is reflected in lower viabilities; these data were excluded from long-term analyses. No temporal trends were detected in the difference between B and O, in either developmental time or viability, suggesting stability in both characters.

<table>
<thead>
<tr>
<th>Date</th>
<th>Developmental time (h) Males</th>
<th>Developmental time (h) Females</th>
<th>Egg-to-adult viability (%) (Males and females combined)</th>
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<tr>
<td></td>
<td>B</td>
<td>O</td>
<td>O-B</td>
</tr>
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Experimental protocol and with lower replication levels (see Materials and Methods).

Analysis of variance on developmental time, with selection treatment, sex, and assay date as factors, incorporating replicates, revealed highly significant differences between B and O treatments (B < O; P < 0.001), sexes (male < female; P < 0.001), and assay (P < 0.05). No interaction terms were significant. A two-factor ANOVA on viability (selection × assay date) showed a significant difference between stocks (B < O; P < 0.01) and assay date had a significant effect (P < 0.05). The interaction term was not significant.

We tried to determine whether the significance of the assay date factor reflected a secular trend in the difference between the selection treatments over evolutionary time by linear-regression analysis. The difference values were regressed against time. Regression slopes for developmental time and viability were not significantly different from zero for either the averaged male/female developmental time data or egg-to-adult viability. It appears that any trends in differentiation are more subtle than the between experiment variability, if they are present at all. A slight upward trend in overall viability of both selection lines likely has resulted from improvements in the assay technique, rather than any sort of adaptation.

The 1988 data were analyzed separately using the two-tailed t-distribution with n − 2 = 6 df. Using this test, the developmental time difference between B and O was statistically significant (P < 0.01), but the viability difference was not. The viabilities in this first experiment were considerably lower than those reported for later experiments (Table 1). This probably reflects the lower nutritional content of the cornmeal medium used and that the stocks were not adapted to that medium. We do not invest much confidence in these results because of the differences in assay technique, but we have included them for sake of completeness. It is surprising, then, that the developmental time data compare very favorably, in both absolute magnitude and relative values, with the data from later experiments. Note, however, that the difference between O and B developmental times remains stable across a variety of food levels in the minimum food experiments and may be a generally more robust finding.

Developmental Time and Viability of Reverse-Selected (RU) Populations

Figure 1 summarizes the egg-to-pupa and egg-to-adult viability data from our experimental assay of B, O, and RU (reverse-selected) treatments. Figure 2 shows the frequency distributions for the emergence of females (the pattern for males is very similar). Developmental time has responded to reverse-selection by returning to values statistically indistinguishable from the Bs. The mean developmental times in this assay...
were (male/female, ±SD): B = 234.8 ± 2.65/229.52 ± 3.33; O = 250.28 ± 4.26/246.32 ± 4.6; RU = 236.05 ± 1.58/231.33 ± 2.70. A one-tailed, unpaired t-test with \( n - 2 = 8 \) df showed a significant difference between B and O selection treatments (B < O; \( P < 0.01 \)). A paired t-test on RU and O selection treatments (using only replicates 1–4, as there is no RU,2) also revealed a significant difference (RU < O; \( P < 0.05 \)) in developmental time. B and RU treatments were not significantly different.

Both egg-to-pupa and egg-to-adult viabilities of the RU lines were intermediate between B and O treatments (fig. 1). The t-tests showed significant differences (\( P < 0.05 \)) for both egg-to-adult and egg-to-pupa viability between B and O lines. RUs, however, were not significantly different from either B or O for either viability character.

**Stage-Specific Developmental Time and Pupation Height of B and O Lines**

Table 2 combines data from our experimental assay of hatching time and hatchability of eggs with data from our assay of pupation time, time in pupa, time to adulthood, and pupation height for the B and O treatments.

The average times from oviposition to hatching were 20.48 h (B) and 21.12 h (O). The average hatchabilities were 95.4% (B) and 93.8% (O). One-factor ANOVA of population means shows that the two selection treatments do not differ significantly in either hatching time or hatchability (\( P = 0.10 \) and \( P = 0.38 \), respectively). Note that this measurement of the percentage of hatching does not distinguish between hatchability of fertilized eggs and the laying of infertile eggs. The purpose of the experiment was to test whether hatching percentage played a role in determining egg-to-adult viability differences; thus, in this us-

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**Fig. 1.** Viabilities of B (early-reproduced), O (late-reproduced), and RU (reverse-selected from late-to early-reproduced) selection treatments. Each bar represents the mean of the replicate populations within a selection treatment (±SE). The O treatment displayed significantly higher egg-to-adult viability than either early-reproduced treatment. B and RU treatments were not significantly different.

**Fig. 2.** Egg-to-adult developmental times of B, O, and RU selection treatments. The bold lines show the sum of the replicate populations, which are shown with fine lines. Laboratory selection for postponed senescence has significantly increased developmental time of the O treatment over their control B treatment, which is maintained in the same manner as the ancestral population. Reverse selection (from late- to early-reproduced) in the RU treatment has restored fast development to statistical identity with the Bs.
TABLE 2. Timing and survivorship of major preadult stages in the B (baseline) and O (old) treatments. The O-B difference is reported at the bottom. Data for egg-hatching time come from the “hatching-time” experiment. All other data are from the “pupation-time” experiment. Each cell represents ten observations of 60 larvae. Statistical significance of the O-B difference is based on the one-tailed t-distribution with n = 5 – 1 df.

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<th></th>
<th>Egg to adult</th>
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<th>Egg to pupa</th>
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<td>95.0</td>
<td>149.12</td>
</tr>
<tr>
<td>3</td>
<td>236.82</td>
<td>92.8</td>
<td>21.48</td>
<td>88.0</td>
<td>128.88</td>
</tr>
<tr>
<td>4</td>
<td>259.10</td>
<td>92.9</td>
<td>21.32</td>
<td>93.0</td>
<td>149.30</td>
</tr>
<tr>
<td>5</td>
<td>241.80</td>
<td>89.0</td>
<td>19.98</td>
<td>98.0</td>
<td>136.16</td>
</tr>
<tr>
<td>Mean</td>
<td>246.03</td>
<td>91.4</td>
<td>21.12</td>
<td>93.8</td>
<td>138.36</td>
</tr>
<tr>
<td>SD</td>
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<td>1.5</td>
<td>0.7</td>
<td>3.7</td>
<td>10.4</td>
</tr>
<tr>
<td>SE</td>
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<td>0.7</td>
<td>0.3</td>
<td>1.7</td>
<td>4.6</td>
</tr>
<tr>
<td>O-B difference</td>
<td>18.13</td>
<td>6.4</td>
<td>0.63</td>
<td>−1.6</td>
<td>15.99</td>
</tr>
</tbody>
</table>

** P < 0.05; ** P < 0.01.

age, the distinction between “percentage of hatching” and “hatchability” is not important. All other differences in the timing of major ontogenetic events were significant statistically when tested using the one-tailed t-distribution at 95% confidence, with O > B in all cases; the one-tailed test was considered appropriate because of the long-standing observation and a priori expectation of greater developmental time of the Os. The average times from egg to pupa were B = 122.37 h and O = 138.36 h; the difference (O - B) was 15.99 h (P < 0.001). The average times from pupa to adult were 105.52 h and 107.67 h (B and O, respectively; P < 0.05); the difference was 2.15 h.

Thus, the overall developmental time difference between B and O selection treatments is predominantly due to a difference in the duration of the larval phase: Time in the egg accounts for about 2% of the difference; time in pupa accounts for about 11% of the difference; time as larva accounts for about 87% of the difference in total developmental time from egg to adult.

Pupation heights of B and O selection lines did not differ significantly when compared using a two-tailed t-test (table 2). A failure to find a difference in pupation heights confirms the results of Mueller et al. (1993) for these selection stocks. It should be noted that, in the both studies, pupation heights were restricted to vials considerably shorter than the potential maximum height of pupation. Pupation height may reflect larval energetic expenditure on wandering and pupation site selection, but pupation in a relatively confined container may not allow differences to be observed.

Developmental Time, Viability, and Longevity in Intermediate Generation-Time Selection Treatments

Because all slower developing treatments display increased developmental times, higher preadult viabilities, and greater longevities, we have incorporated data from an assay (Chippindale et al. MS) that simultaneously surveyed developmental time and viability in B, CB, O, and CO treatments. Data from this assay are presented in figure 8. CBs were significantly slower developing than Bs (P < 0.05; two-tailed t-test) and showed significantly higher egg-to-adult viability (P < 0.05; two-tailed t-test). On the other hand, the CO treatment showed no significant
evolution of either developmental time or viability from its O ancestor treatment. Longevity data are a subset of the data in Nusbaum et al. (in prep.). CB longevity had increased significantly in females (B = 40.97 d, CB = 45.84 d; P < 0.01; two-tailed t-test) but not males (B = 49.62 d, CB = 48.90 d), as previously reported by Rose et al. (1992). CO longevity had decreased significantly (P < 0.01) in both males (O = 86.83 d, CO = 63.18 d) and females (O = 74.34 d, CO = 58.62 d). Selection for intermediate generation time of CB from B and CO from O has therefore elicited some convergence in the two treatments but is asymmetrical with respect to the characters concerned: CBs have evolved longer development and greater viability without a change in longevity of both sexes; COs have evolved downward in longevity without any change in developmental time or viability from the Os. Apparently longevity can evolve without any impact on developmental characters, as shown in figures 8a and 8b.

Effects of Larval Food Manipulation on Developmental Time and Viability

Food-level manipulations produced a general pattern of reduced viability and increased developmental time with increased crowding in all selection treatments assayed. These results are summarized in figure 3 (B and O) and figure 4 (B, CB, CO). We have used some caution in equating these food-level manipulations with the terms “density” or “crowding” because some components of a “crowded” environment may be absent in our manipulations. L. D. Mueller (pers. comm. 1993) has argued that a truly crowded environment for *Drosophila* development would consist of intense scramble competition for food, as well as high waste-product build up, space limitations, and so on. Insofar as we have modulated the volume of food allowed per larva, we have performed a “density experiment,” but we have also controlled humidity, surface area available for larval spiracular access and normal space for pupation, and perhaps provided an environment in which waste products would not build up to extreme levels in any of the food treatments. The present results do, however, closely resemble results from a true density experiment (in the sense of Mueller 1988) done on B and O lines (unpubl. data).

In the B versus O experiment, three-factor ANOVA with selection treatment, sex, and food level as fixed effects revealed highly significant effects of stock (B < O; P < 0.01), sex (P < 0.05) and food level (P < 0.01) for developmental time. No interaction terms were statistically significant. Both egg-to-adult and egg-to-pupa viabil-
FIG. 4. Norms of reaction for (a) developmental time and (b) egg-to-adult viability of B, CB, and CO selection treatments across a gradient of larval food levels. Each point is the mean (±SE) of the five replicate populations within a selection treatment. CB and CO treatments showed longer developmental time and higher egg-to-adult viability than the Bs and were indistinguishable from one another.

ities showed highly significant effects of selection (B < O; P < 0.01) and food level (P < 0.01) with no significant interaction effects.

In the B, CB, CO experiment, three-factor ANOVA on developmental time, with selection treatment, food level, and sex as factors, showed significant effects of selection (P < 0.001) and food (P < 0.001) and a significant (P < 0.01) interaction between these factors. The sex factor was not significant, in contrast to the B versus O experiment, and this was probably due to the addition of the 8.33μl/larva treatment. At this (lowest) food level, males developed faster than females in 12 of 15 populations. This reversal of relative developmental times between sexes is shown in figure 6. The three-way interaction of factors was also significant at P < 0.05. When selection treatments were paired to test differences between means, CB and CO were both significantly slower developing than B (P < 0.001; two-tailed t-distribution). CB and CO were not significantly different from each other in developmental time. A two-factor ANOVA on egg-
to-adult viability revealed significant effects of selection treatment (P = 0.0014) and food (P < 0.001) with no significant interaction. The paired comparisons showed no significant differences between CB and CO, but both CB and CO were significantly higher than B in viability (P < 0.05).

Body Size of B and O Lines

Body size was estimated by both thorax length and wet-weight measures. Thorax lengths were measured over a gradient of larval food levels from the food-level experiment on B and O selection treatments. The norms of reaction for thorax length across a larval density gradient are shown in figure 5. Analysis of variance with selection treatment, sex, and food level as fixed effects revealed highly significant (P < 0.01) effects of sex (male < female) and food level (low < high). The sex × food interaction was significant (P = 0.004). No other interaction terms were significant. Selection treatment was not statistically significant (O > B; P = 0.10). When sexes were analyzed separately (ANOVA: stock × food), the O males were significantly larger than the B males (P = 0.018), though the females were not significantly different (P = 0.77). An important consideration is the difference between the two selection treatments at normal culture densities alone; at the 66.7μl per larva food level, there were no significant differences between B and O for either sex.

Wet weights were taken on young flies approximately 48 h from adult emergence. We weighed progeny from the same populations after the parents had been aged to 4, 9, 14, 19, and
24 days of age; we wanted to make sure that parental age did not affect the data. Table 3 shows the results of this experiment, in which we weighed a total of 4,962 B flies and 4,961 O flies in groups of five. We found no significant difference in wet weight between the two selection treatments (ANOVA, with selection, sex, and parental age as factors). The difference between sexes was highly significant ($P < 0.01$) as was the parental age ($P < 0.01$). No interaction term was statistically significant, although the sex $\times$ age term was very close ($P = 0.059$).

Interestingly, the significance of the parental age factor in the ANOVA reflected a general decline in offspring size with parental age of the B treatment. Simple regression of the mean offspring weights against parental age for each replicate population and sex revealed a negative correlation for all tests. Regression-slope values were then used in a two-factor ANOVA, with selection treatment and sex as factors. Bs had a significantly more negative mean slope than Os ($p = 0.006$), and the interaction term was not statistically significant. When sexes were analyzed separately, the B male and female slopes were both significantly negative ($P < 0.05$), although both male and female slopes for the O treatment group were not significantly different from zero ($P = 0.34$ [males] and $P = 0.194$ [females]). Thus, it is apparent that parental age does affect offspring weight in these stocks: B offspring weight declines relative to O offspring weight with the age of the parents. This effect was not sufficient to generate an overall difference in weight between the Bs and Os and does not explain the inconsistent results of Rose et al. (1984), since the parents were of same age in their experiments.

**Starvation Resistance of B and O adults**

Starvation resistance was measured for both newly emergent and 4-d-old flies of the B and O treatments, as shown in figure 7. A three-factor ANOVA with selection, sex, and age as factors, showed significant effects of selection (O $>$ B; $P < 0.05$), sex (f $>$ m; $P < 0.01$), and age from emergence ($P < 0.05$). No interactions were significant. When the two different age classes were tested separately (two-tailed t-test with sexes pooled; df = 8), there were no significant differences between B and O at emergence. After 4 d of adult feeding, the B and O treatments differed significantly (B $<$ O; $P < 0.01$).

**Crosses within the O-Selection Treatment**

Crosses between O populations were performed to test for mutation accumulation slowing their development relative to the Bs. These crosses failed to show any difference between
TABLE 3. Wet weights of B and O flies at 24 h from emergence. Means for the five replicates at five parental ages; "N" is the number of individuals weighed. Two-factor ANOVA with stock and sex as factors and populations as replicates reveals no significant difference between the stocks or interaction between stock and sex. The difference between the sexes is highly significant (P < 0.01). Regression of population means against parental age showed a significantly negative slope for the Bs but not the Os with age.

<table>
<thead>
<tr>
<th>Parental age (d)</th>
<th>Male weight (mg)</th>
<th>Female weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>n</td>
</tr>
<tr>
<td>4</td>
<td>0.879</td>
<td>500</td>
</tr>
<tr>
<td>9</td>
<td>0.867</td>
<td>500</td>
</tr>
<tr>
<td>14</td>
<td>0.751</td>
<td>500</td>
</tr>
<tr>
<td>19</td>
<td>0.733</td>
<td>490</td>
</tr>
<tr>
<td>24</td>
<td>0.742</td>
<td>496</td>
</tr>
<tr>
<td>Mean</td>
<td>0.794</td>
<td>2486</td>
</tr>
<tr>
<td>SD</td>
<td>0.072</td>
<td>0.056</td>
</tr>
<tr>
<td>SE</td>
<td>0.032</td>
<td>0.025</td>
</tr>
</tbody>
</table>

purebred and crossed groups of Os for egg-to-adult developmental time (table 4). The crossed samples had a mean developmental time of 234.9 ± 2.2/231.92 ± 2.3 (male/female, ±SE), although the pure samples had a mean developmental time of 233.4 ± 0.77/230.28 ± 0.84 (male/female, ±SE). An unpaired, two-tailed t-test, revealed no significant difference between crosses and purebred experimental treatments. Because it might be argued that deriving two samples of the purebred populations to balance the design might be pseudoreplication, we also tested the two groups of purebred populations separately. This did no modify the conclusion that no differences existed for developmental time between pure and crossed groups.

**DISCUSSION**

**Extended Developmental Time Has Evolved in Late-Reproduced Populations**

The ten B and O lines are long-established, outbred populations of *Drosophila* selected for early- and late-life fertility, respectively. Despite extreme differences in the adult demography, both selection treatments have been handled identically with respect to the preadult conditions. Nonetheless, our assays of egg-to-adult developmental time have shown that the O line develops approximately 5% slower than the B line. This difference has been shown here for two different rearing media as well as with and without larval crowding or food limitation. The difference between O and B developmental times has also persisted throughout 5 yr of continued selection on the adult phase for the timing of reproduction, with no detectable directional trend.

The difference between B and O selection treatments in overall developmental time is mostly (87%) accounted for by the duration of the larval stage. The Os have a larval stage approximately 15 h longer than that of the Bs. It is probable that the O populations have increased in developmental time (rather than the Bs having decreased) for several reasons. First, the B-type selection treatment is identical to the ancestral maintenance regimen. The IV popu-

![Fig. 7. Differentiation of starvation resistance in B and O selection treatments. Starvation resistance is strongly genetically correlated with longevity and widely evidenced to be a product of stored lipid reserves in *Drosophila melanogaster*. Adult O flies have repeatedly been shown to have higher levels of lipids and higher starvation resistance when assayed at 4 + d of age. Here we show that this starvation advantage is not present in the newly emerged imago. Differences between sexes and selection treatments occur as a function of adult age and are not present at eclosion. This result is consistent with the notion of antagonistic pleiotropy between early reproduction and aging-related characters and inconsistent with a connection between extended development and postponed senescence.](image-url)
Inbreeding, from which both lines were derived, had gone through more than 120 generations of 2-wk propagation before selection was imposed on B and O, and should have been close to evolutionary equilibrium for those conditions. Second, reverse selection of the RU treatment from the Os to a B-type schedule returned developmental time to the faster pace of the Bs. Third, two other demographically selected treatments that have a lengthened adult period (CB, CO; 4–5-wk generations) have developmental times greater than the Bs, and statistically indistinguishable from the Os. Therefore, we conclude that the O stocks have a longer developmental time that is derived from that of the IV or B stocks, rather than the reverse.

**Why Does Extended Development Evolve?**

Inbreeding depression of the slower developing selection treatments is a highly unlikely explanation for the evolution of extended development in the O stocks because of the large O population sizes (in the range of 10^3). Inbreeding would be more likely to afflict the B populations than the Os, as the B treatment has undergone five times as many generations as the Os. Yet it is the Os that show slowed development. Inbreeding also should be accompanied by depression of other performance characters, which we have not observed here, or in other contemporaneous experiments on these lines (Chippindale et al. 1993; Leroi et al. 1994a). Furthermore, experimental hybridization between replicates within selection treatments has been performed periodically in the past (Hutchinson and Rose 1991) and in the present study for the O treatment, without any evidence of F1 superiority, and thus hybrid vigor. Therefore, we conclude that inbreeding depression is not a plausible explanation of longer O development.

Another possible explanation is indirect selection. Early emergence will, all other things being equal, enhance early reproduction, because it provides more time for adults to mature sexually, both anatomically and physiologically, and more time to feed and mate in preparation for egg laying. Both B and O selection treatments have an absolute deadline for emergence from pupa at 2 wk, since any individuals that have not emerged by 2 wk are discarded. Bs are then immediately reproduced, whereas Os are transferred to cages for a further 8 wk before egg collection for the next generation. The 2-wk emergence deadline places a limit on the degree to which developmental time can evolve upward, and the emergence-distributions for adults of all populations examined in this study are entirely completed before the 2-wk deadline under normal culture densities. But the absence of direct (truncation) selection on developmental time does not preclude direct selection on early adult characters, which are correlated to developmental time for the aforementioned reasons. Thus, B stocks, for example, may be subject to indirect selection on developmental time because of selection on females in these stocks to lay fertile eggs at 14 d of age from their own oviposition as eggs, about 4 d from pupal eclosion. Later-

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**Table 4. A comparison of egg-to-adult developmental time of purebred versus crossed O populations from an experiment run to test a mutation accumulation hypothesis for slower O development. Restoration of faster development in the crossed samples would point towards mutation accumulation or hybrid vigor (caused by inbreeding). The data indicate no such pattern.**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Purebred group</th>
<th>Crossed group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (h)</td>
<td>Female (h)</td>
</tr>
<tr>
<td>1 x 1 (1)</td>
<td>233.83</td>
<td>231.28</td>
</tr>
<tr>
<td>2 x 2 (1)</td>
<td>229.69</td>
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</tr>
<tr>
<td>3 x 3 (1)</td>
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<td>230.28</td>
</tr>
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<td>4 x 4 (1)</td>
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<td>229.74</td>
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</tr>
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</tr>
<tr>
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</tr>
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<tr>
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reproduced selection treatments (O, CB, and CO) have, by contrast, experienced a relaxation of selection for early maturation and mating as a result of their extended adult maintenance, and thus they may be free of indirect selection on developmental time. They may have thereby evolved increased developmental time as a result of the removal of the long-standing selection pressure for earlier development imposed on the Bs. This conclusion does not, however, explain the evolutionary-genetic mechanism responsible for this increased developmental time.

When selection is relaxed, mutation accumulation is a reasonable expectation. Could this be the mechanism that produced the increased O, CB, and CO developmental times? The degree of mutation accumulation should depend on the number of generations elapsed since the relaxation of selection. However, selection treatments such as CB and CO have undergone different numbers of generations since derivation from 2-wk stocks (CB = 51, CO = 111 generations) but are statistically equivalent to the Os in developmental time. The strikingly uniform pattern of occurrence both between and within selection treatments is not consistent with random accumulation of selectively neutral mutations. In turn, the Os have been repeatedly assayed over the last 5 yr, yet have not shown a detectable upward trend in developmental time. These arguments do not preclude very limited action of mutation accumulation. It is perhaps most important, then, that crosses between O populations did not restore rapid development to the F1 hybrids (table 4), as a mutation accumulation hypothesis would usually require.

Could the evolution of longer O developmental time reflect some general relationship between longevity and development? The "developmental theory of ageing" (Lints 1978, 1988) predicts the existence of a positive genetic correlation between developmental time and longevity. Superficial inspection of the present data seems to support the developmental theory: The longer-lived O selection treatment does exhibit longer developmental time than its B control. At least three lines of reasoning suggest that this pattern has little to do with any profound connection between developmental time and aging. First, the continued upward selection response of O populations for longevity (Leroi et al. 1994a) is incongruent with the developmental theory of aging, since these populations have not displayed any detectable increase in developmental time over the last 5 yr or more. Second, the results of selection of lines with life-cycle times intermediate between B and O show no evidence for such a correlation (fig. 8a). CB populations, which have been demographically selected for reproduction at 2 to 3 wk from emergence have shown only a slight increase in longevity over their ancestor Bs, but are equal to the long-lived Os in developmental time. The COs, which have been back-selected demographically from the O populations (from 8 wk to 2 to 3 wk of adult selection before reproduction), have retained a developmental time equal to the Os, despite a substantial decline in longevity. Third, we failed to find any evidence for a direct contribution of extended development to either adult size or resistance to starvation stress. Rather, all the available evidence suggests that adults of demographically selected treatments (B, O, CB, CO) begin with essentially the same resources at emergence. The combination of these selection results is strong evidence that the larval phase has evolved independently from the aging phenotype. The developmental theory of aging is therefore refuted by our results, as it has been before (Zwaan et al. 1991, 1992; Zwaan 1993).

Another connection might be forged between preadult and adult survival, since all longer-lived treatments (CB, CO, O) also displayed higher preadult viabilities than the short-lived Bs. (This is the reverse of the pattern found by Partridge and Fowler [1992], who argued for a negative association between preadult and adult survival.) But such a hypothesis is easily refuted by consideration of intermediate generation-time selection treatments. Figure 8b illustrates the independence of longevity and egg-to-adult viability in these demographically selected populations. The CB treatment has evolved substantially higher viability with only a slight increase in average adult longevity, whereas the CO treatment has declined markedly in longevity from its O ancestor treatment but has not given up any preadult viability. Furthermore, in the second "minimum food" experiment, CB and CO treatments behaved identically in developmental time and viability across food levels despite considerable differences in longevity between the two treatments. We have failed to find any evidence for a connection between viability and longevity under conditions directly comparable to normal selection conditions, and with the stress of larval food limitation. It therefore appears that preadult viability and adult longevity can evolve in-
Developmental time, viability, and longevity: What correlates with what? In this triptych, we summarize the results of demographic selection on B (2-wk), CB and CO (4–5-wk), and O (10-wk) treatments. Arrows emphasize the convergent selection applied to CB (2–5-wk generations) and CO (10–5-wk generations) from their B and O ancestor treatments. Part (A) shows a search for the "developmental theory of aging", which posits that developmental time and aging are causally correlated. Clearly, however, developmental time and longevity can evolve quite independently, refuting this theory. In part (B), we show the independent evolution of viability and longevity. Although populations which display greater longevity also have higher preadult viabilities, the two characters are not genetically correlated. In part (C), the relationship between developmental time and viability is illustrated. Slower developing treatments (CB, CO, O) have higher egg-to-adult viabilities than fast developing (B) populations. The pattern supports the conclusion that antagonistic pleiotropy between developmental time and viability has driven the evolution of extended development in these stocks.

Over the 24 populations we have assayed, slower development is consistently associated with an increase in preadult viability. Although these differences seem small (e.g., O > B by 4.5% in egg-to-adult viability; table 1), preadult viability is a fitness character of enormous weight. The preadult period is subject to the full force of natural selection for survival (Charlesworth 1980). It is notable that there is an inverse relationship between preadult viability and developmental time across the stocks, over multiple experiments. This pattern suggests that there is antagonistic pleiotropy among loci affecting both developmental time and preadult viability. When O, CB, and CO stocks were removed from selection for early reproduction, they had relaxed indirect selection for early developmental time. This should have shifted the balance between selection on developmental time and preadult viability during the larval stage, causing a consistent shift in favor of alleles that slowed development, but enhanced viability. Because this effect is one of a shift in selective equilibrium during a life-cycle stage under strong selection, it is not surprising that stocks with very different periods of reduced selection on developmental time, such as the CBs and COs, should nonetheless have similar developmental times and viabilities. Since we have shown that developmental time was altered by selection without any change in body size or apparent provisioning of the imago, it is apparent that lower developmental rate confers higher preadult survivorship. Thus, despite the general perception that body size and adult fitness are critically associated in Drosophila (e.g., Robertson 1957; Tantawy and
Vetukhiv 1960; Partridge and Fowler 1992), selection has, in this instance, acted on preadult viability rather than adult size. With the hypothesis of antagonistic pleiotropy between developmental time and viability, all the significant experimental results can be accounted for.

**Phenotypic Manipulations of Larval Food Level Are Incongruent with Selection Results**

Phenotypic manipulations have been presented as a good means of deducing evolutionary relationships among characters (e.g., Partridge 1992; Bell and Koufopanou 1986; but see Reznick 1992). But, unless genetic information is available in the context of selection or quantitative genetic results, this assertion is difficult to test. In an earlier paper (Chippindale et al. 1993), dietary manipulation of adults was compared with selection results for several fitness characters. Both methods produced a qualitatively similar trade-off between survival and reproduction, which may reflect a fundamental physiological relationship mediated by discrete allocative pathways. However, in that study, considerable differences between selection response and phenotypic manipulation were also identified. Leroi et al. (1994 b) extended this line of questioning and demonstrated that phenotypic trade-offs are easily altered by selection.

In the present context of larval growth and survival, we may also draw a comparison between the two methodologies. Among selection treatments, we have found a negative association between developmental rate and juvenile survival, whereas adult body size remained unaffected. Our manipulations of larval food level produced a strikingly different pattern among the same three traits. Food-limitation increases developmental time while at the same time leading to a smaller adult and reducing preadult viability. There is no correspondence between the selection result and phenotypic plasticity for these characters, suggesting that the physiological mechanisms producing longer-developing flies under selection are different from those highlighted by food limitation. Considering the evidence from controlled laboratory selection experiments, phenotypic manipulations seem to be poor predictors of evolutionary response.

**Sexual Dimorphism in Developmental Time**

A puzzle in the evolution of *D. melanogaster* is the coincidence of sexual dimorphism in body size (females are much larger than males) and developmental time (females develop faster than males). The timing of pupation may be related to attaining a critical minimum size for survival to adulthood. Males, which are about half the weight of females at emergence, should be expected to achieve this critical mass earlier, all other things being equal. Why do female larvae have such vastly higher growth rates? Our data offer little help in answering this question. However, in this context, an interesting feature of the crowding experiments was the reversal of relative developmental times of males and females under extreme food limitation in the second experiment (see fig. 6). In early-reproduced populations, it might be argued that the longer time to peak fertility after emergence in females (Ashburner 1989) would lead to greater selection for early emergence in females than males (Roper et al. 1993). In late-reproduced populations, such differential selection on the sexes should be removed. Sexual dimorphism in developmental time has persisted in later-reproduced populations in our laboratory nonetheless. The density dependence of this dimorphism is a curiosity that deserves further investigation.

**Comparison with the Recent Literature**

Partridge and Fowler (1992) showed extended development of their “old” lines compared with their “young” lines, which is echoed in our observation of longer developmental times of O (Rose “old”) over B (Rose “young”) lines. Partridge and Fowler speculated that longer development in their “old” flies leads to larger body size, reflecting increased investment in the “durability of the soma.” The apparent penalties that the “olds” pay are exacted in preadult survivorship as well as later time of emergence. These results differ substantially from ours, as we find higher preadult survival rates in selection treatments with longer developmental times and no difference in body size. A slower rate of growth in the Os seems to be associated with greater preadult survival. Thus, the present study and that of Partridge and Fowler seem to be contradictory.

But the Partridge and Fowler (1992) study appears to have a number of problems that may preclude direct comparisons with our work. Roper et al. (1993, p. 455) discussed some of these problems, specifically, inbreeding depression and inadvertent selection: “Our results point to the need to avoid inadvertent direct selection on characters other than those intended. In the pres-
ent context, standardization of larval density and avoidance of selection pressure for rapid development are clearly important." It is crucial to any claim for a correlated response that there be no direct selection on the character supposed to be correlated with the character under direct selection—in this case, no direct selection on larval competitive ability or developmental time when selecting on the adult. Yet, it is clear from Partridge and Fowler that their "young" and "old" lines differ substantially in the preadult conditions as well. Densities apparently were regulated by the duration of egg laying. The "young" lines, with more females laying at roughly peak fertility and before substantial mortality selection has acted, are likely to outlay the "olds" many times over. This difference in egg laying between "young" and "old" stocks probably leads to differences in effective population size and more rapid inbreeding depression of the "old" treatment (Roper et al. 1993). This difference in larval density is indicated in Partridge and Fowler: on average, about 69% (or about 3500 flies) of their "young" lines and about 83% (or about 2500) of "old" adults have emerged at day 17. In other words, the "old" lines developed faster than the "young" lines in normal culture and had higher survivorship under normal culture conditions. However, in their controlled developmental-time experiments, the mean time to emergence is only about 10.9 d ("young") and 11.8 d ("old"), both considerably less than 17 d. The two lines are reversed in rank order from the observations obtained from normal lab culture, for both speed and survival. Since developmental time is increased and viability is decreased by crowding of larval cultures (Bakker 1961; Roper et al. 1993; present data), it is probable that the Partridge and Fowler stocks are very crowded indeed. Moreover, direct truncation selection on the distribution of adult emergences will have acted more strongly on "young" than "old" treatments, since the former were normally more subject to crowding. Thus, the basic selection procedures used by Partridge and Fowler were not at all analogous to the usual late-fertility Drosophila selection procedures (e.g., Rose 1984).

In contrast to Partridge et al.'s results, Hillesheim and Stearns (1991, 1992) reported that flies selected for greater fresh weight at eclosion had reduced longevity. Inadvertent direct selection and differential inbreeding are likely to account for the two laboratories' disparate results as well. Indeed, in explaining the contradiction between their own results and those of Partridge and Fowler, Hillesheim and Stearns (1992, p. 750) stated: "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." Again, it appears that multiple types of selection were imposed on these stocks, making the distinction between direct and indirect responses to selection obscure. An additional problem with this study may be inbreeding depression. The authors used very small population sizes ($n_e \leq 10$) throughout selection on weight at emergence. Some attempt to counter inbreeding depression by crossing replicates was made at two points in selection, but selection was imposed again with small population size for about ten generations. Such crosses are likely to lead to linkage disequilibrium and also confound the replicate system for the purpose of statistical inference.

The problem of inbreeding depression is also raised by Roper et al. (1993). They presented evidence that both "young" and "old" selection treatments suffer from inbreeding depression, and that the "old" lines seemed to be more strongly afflicted. Here we look in greater detail at the data from Partridge and Fowler (1992) and contrast them with our selection treatments. Figure 9 shows a comparison of viability data from our B and O lines and the "young" and "old" stocks of Partridge and Fowler. The data on viability in the "young" and "old" Dahomey lines were calculated by combining their tabled data from the "competitive ability" experiment with their reported proportions of total flies emerging, to extract the wild-type viabilities. One thing that stands out in the comparison is the reversed ranking of B and O and "young" and "old" with respect to viability. The Partridge "old" lines had exceedingly low viabilities: even at high food levels, the olds have less than 50% average survivorship to adulthood (44.3%, 18.1%, and 82.0% for old populations 1–3, respectively). At the low food levels, it is particularly surprising to see the poor performance of the old treatment against a mutant stock, which might be expected to be highly inbred in its derivation. The low survivorship of old flies and the high variance between replicate old populations immediately raises the concern that inbreeding depression alone could account for the preadult differences between lines, since all developmental performance characters are depressed in the old lines.
Roper et al. (1993) attempted to recover the populations from inbreeding depression by crossing replicates within selection treatments. But it is questionable whether a single-generation cross is adequate to remove the confusion that inbreeding depression can engender for life-history investigation. Such crosses will lead to the arbitrary juxtaposition of components of inbred genomes, and are no substitute for crossing and then maintaining selection for a number of generations to reduce the frequency of deleterious alleles fixed by genetic drift in individual lines. The experiments of Roper et al. fail to support some of the earlier results (Partridge and Fowler 1992). In particular, incorporation of hybrid data and comparison with the base stock did not support the notion of a trade-off in performance between the two life stages.

Conclusions

Although some of the present results seem to corroborate the studies of Partridge and Fowler (1992) and Roper et al. (1993), the evolutionary mechanisms involved in the extension of development in their and our late-reproduced stocks are quite different. In their studies, considerable inbreeding depression may have arisen, and some selection treatments appear to have experienced strong, inadvertent, direct selection on developmental rate. It is a routine finding that selection alters a character mean, and a mundane one that inbreeding depression can have pervasive effects on performance characters. Small differences in culture regime have apparently given rise to inadvertent selection and inbreeding in the work of Partridge and colleagues. Our selection system has evolved by different mechanisms. In our selection treatments, reduced developmental speed is apparently selectively favored because of beneficial effects on preadult viability. When selection for early reproduction has been relaxed in our late-reproduced treatments, the primary target of selection has been preadult viability rather than increased body size or any other adult attribute. Thus, the antagonistic pleiotropy by which slower larval growth confers increased survivorship of the larval and pupal stages deserves attention, rather than any supposed trade-off between larval and adult survival.

The evolutionary relationship between development and adult life history is still poorly understood, despite some ambitious claims. For example, optimization models often assume a trade-off between developmental time and size of the adult (e.g., Werner 1986; Rowe and Ludwig 1991; Sibly et al. 1991), yet here we have found that adult body size and other early adult characters are conserved despite differences in larval developmental time. Differences in the adult life histories of our stocks have come about because of differences in adult selection, although preadult characters have evolved separately. There is no good evidence for the hypothesis of Partridge and Fowler (1992) that postponed senescence has evolved in laboratory-selected Drosophila by preadult investment or trade-offs between developmental rate and adult fertility and longevity. Preadult adaptation does not explain the laboratory evolution of postponed senescence in D. melanogaster, though it may be secondarily involved in such evolution.

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