Age-Specific Patterns of Genetic Variance in *Drosophila melanogaster.*
II. Fecundity and Its Genetic Covariance With Age-Specific Mortality

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ABSTRACT

Under the mutation accumulation model of senescence, it was predicted that the additive genetic variance ($V_A$) for fitness traits will increase with age. We measured age-specific mortality and fecundity from 65,134 *Drosophila melanogaster* and estimated genetic variance components, based on reciprocal crosses of extracted second chromosome lines. Elsewhere we report the results for mortality. Here, for fecundity, we report a bimodal pattern for $V_A$ with peaks at 3 days and at 17–31 days. Under the antagonistic pleiotropy model of senescence, it was predicted that negative correlations will exist between early and late life history traits. For fecundity itself we find positive genetic correlations among age classes $>$3 days but negative nonsignificant correlations between fecundity at 3 days and at older age classes. For fecundity vs. age-specific mortality, we find positive fitness correlations (negative genetic correlations) among the traits at all ages $>$3 days but a negative fitness correlation between fecundity at 3 days and mortality at the oldest ages (positive genetic correlations). For age-specific mortality itself we find overwhelmingly positive genetic correlations among all age classes. The data suggest that mutation accumulation may be a major source of standing genetic variance for senescence.

Patterns of genetic variance and covariance for fitness traits play a crucial role in theories of life-history evolution (ROFF 1992; STEARNS 1992; CHARLESWORTH 1994), and knowledge of the genetic variance-covariance matrix can shed light on three key issues. First, how and why is genetic variance for fitness traits maintained (LANDE 1976; BULMER 1989; HOULE 1989; BARTON 1990)? Second, can we use the genetic variance-covariance matrix to predict the direction of short-term evolutionary change (e.g., LANDE 1982; HOULE 1991)? Third, and the focus of this paper, what is the evolutionary basis for senescence? In particular, can we use age-dependent changes in additive genetic variance ($V_A$) and covariance to test two specific models for the evolution of aging, mutation accumulation (MEDAWAR 1952) and antagonistic pleiotropy (WILLIAMS 1957)?

Both mutation accumulation and antagonistic pleiotropy theories grew out of the recognition that in an age-structured population, the force of natural selection declines with age (FISHER 1930; HALDANE 1941; MEDAWAR 1952). In the first case, MEDAWAR pointed out that deleterious mutant alleles with late-acting effects can increase in frequency since they are not strongly opposed by selection. Senescence then results from the age-related increase in the frequency of mutations with late expressed deleterious effects (PARTRIDGE and BARTON 1993; CHARLESWORTH 1994). Models of this process suggest that it can lead to an age-related increase in additive variance for fitness traits (CHARLESWORTH 1990).

In the second case, antagonistic pleiotropy, the fitness costs of late-acting deleterious mutations could be more than compensated for if those alleles have beneficial pleiotropic effects on fitness early in life. For example, increases in late-life mortality may be balanced by increases in early fecundity or rate of development. Most models of this process have predicted negative genetic covariance between at least some pairs of early- and late-age fitness traits (LANDE 1980; ROSE 1982, 1985; CHARLESWORTH 1994; but see PARTRIDGE and BARTON 1993). However, while negative genetic correlations can be taken as support for antagonistic pleiotropy, failure to find negative correlations does not exclude the possibility that antagonistic pleiotropic alleles cause senescence (PEASE and BULL 1988; CHARLESWORTH 1990; HOULE 1991).

Several quantitative genetic studies have estimated standing genetic variance to test the predictions of an age-related increase in $V_A$ for demographic fitness components, including fecundity and male mating ability (ROSE and CHARLESWORTH 1981a; ENGSTRÖM et al. 1992; HUGHES 1995). Taken together, the results are equivocal at best. ROSE and CHARLESWORTH (1981a) used a sib-mating design to estimate age-specific additive variance for fecundity in *Drosophila melanogaster.* They failed to find an age-related increase and suggested that mutation accumulation did not account for senescence in their population. However, any real age-
related increase in additive variance in their study may have been biased downward by the differential loss, due to mortality at later ages, of genotypes with relatively high fecundity (Clark 1987; Engström et al. 1992; Partridge and Barton 1993). To avoid this difficulty, Engström et al. (1992) restricted their analysis of age-specific variance components of fecundity to females that survived for the duration of the assay period. Although Engström et al. (1992) found a dramatic increase in $V_a$, their result may be confounded by the logarithmic transformation that they used on fecundity data (G. Engström, personal communication). This transformation can lead to an artificial increase in variance with age for a trait that is actually Poisson distributed and declines with age, as is the case for fecundity in Drosophila. Kosuda (1985) also reports an increase in total genetic variance for male mating ability in D. melanogaster, but this analysis, as with Hughes (1995), does not account for the increase in binomial sampling variance that accompanies the decrease in trait mean with age.

To test predictions arising from antagonistic pleiotropy, a second set of studies has used analyses of pedigree data to estimate genetic covariance between reproduction and mean age of death. Again, when considered as a whole, no clear pattern emerges from the results. In Drosophila, genetic covariance between reproduction and mean age of death may be positive (Giesel 1986; Hughes and Clark 1988; Engström et al. 1992), negative (Rose and Charlesworth 1981a; Tučíć et al. 1988; Service 1993), or not significantly different from zero (Scheiner et al. 1989; Hughes 1995). In other invertebrates, the genetic covariances are predominantly positive, e.g., milkweed bug (Hegmann and Dingle 1982), bean beetle (Nomura and Yonezawa 1990; Tanaka 1993), and Daphnia (Spitze et al. 1991).

Unfortunately, a host of factors may detract from the interpretation of these studies. First, with one notable exception (Hughes and Clark 1988), adult life expectancy was used to measure mortality. But if we find a negative correlation between early fecundity and life expectancy (i.e., mean age at death measured from sexual maturity), we cannot tell whether life expectancy declined due to an increase in mortality late in life (as predicted by antagonistic pleiotropy) or due to changes in mortality at early ages (perhaps due to immediate physiological costs of reproduction, e.g., Partridge and Andrews 1985; Chapman et al. 1995). Furthermore, important differences in mortality late in life may only produce negligible differences in life expectancy from sexual maturity. For each of these reasons, the use of life expectancy weakens our ability to ascribe genetic correlations between early fecundity and late-age mortality.

Second, various factors can lead to spurious positive genetic correlations between traits and age classes. For example, we might observe positive genetic correlations if genetic lines become inbred and previously masked deleterious recessive alleles are expressed, which could depress values for fitness related traits at all ages (Rose 1984a; Bell and Koufopanou 1986; Clark 1987). Similarly, if a population is placed in a novel environment (e.g., from a natural to a laboratory setting) in those genotypes that are poorly adapted to the novel environment, some or all fitness-related traits could decline (Service and Rose 1985; Fry 1993). Again, this could lead to positive genetic correlations that do not reflect the correlations existing in the natural population.

Finally, in a companion paper (Promislow et al. 1996), we pointed out several statistical pitfalls for those who wish to study the genetics of demographic characters. At all but the largest sample sizes, we will likely underestimate the true additive variance for mortality rates at early and late ages and so may also underestimate the additive genetic covariance between fecundity and mortality. Further, for both mortality (Promislow et al. 1996) and fecundity (as noted above), if the data are incorrectly transformed one may draw erroneous conclusions.

Bearing these methodological and statistical problems in mind, we set out to estimate genetic variance components for fecundity as a function of age, as well as patterns of genetic covariance both within and between age-specific fecundity and mortality. Our aim was to test predictions arising from models for the evolution of senescence, but at the same time to minimize the impact of potentially confounding factors discussed above. To that end, we estimated age-specific patterns of fecundity and mortality throughout the life span of the cohort. We used flies from a large laboratory-adapted population and a breeding design that eliminated the risk of inbreeding effects. Finally, we used a large number of flies (~35,000 females and 30,000 males) to minimize sampling error in our mortality estimates.

Taken together, these data on age-specific mortality and fecundity provide us with a powerful tool for a fine-scale examination of the structure of genetic variances and covariances, their causes, and their consequences.

### MATERIALS AND METHODS

We estimated genetic variance components for fecundity and mortality in D. melanogaster using a reciprocal partial diallel cross (North Carolina II cross, Comstock and Robinson 1952; also see Hughes 1995) of second chromosome balancer lines (Dobzhansky and Spassky 1953; details for these stocks in Fukui et al. 1996). We have described the results for mortality in Promislow et al. (1996), where details of the genetic crosses and maintenance procedures are elaborated. Briefly, 58 balancer second chromosome lines were extracted from a large, outbred, laboratory population from which we selected two sets of five lines at random. We then paired males from lines 1–5 to mate with females from lines 6–10, and reciprocally, we paired females from lines 1–5 with males from lines...
6–10; these crosses yielded 25 lines, plus reciprocals, of second chromosome heterozygote flies. The adult offspring of these final heterozygous lines were used to measure age-specific mortality and fecundity. Two cohort cages, each of ∼650 flies (mixed sex), were set up for each of the heterozygous lines and their reciprocals for a total of 100 cages. Cohorts were collected over a 48-hr period.

Experimental stocks were derived from LF350 (Weber and Digges 1990), a laboratory population founded in 1981 and maintained in population cages at 24°, constant light and 55–65% relative humidity on agar-yeast-molasses-cornmeal media. In the experiments reported here, the environmental conditions and media are the same as those of LF350. Balancer lines were derived from the LF350 base populations and in the process were maintained in either 8-dram vials or half-pint milk bottles for a total of 12 generations. At the 12th generation, these lines were crossed and the adults of the next generation were placed in 3.84 cages made of inverted plastic jars. These cages were designed in such a way that we could remove dead flies daily to estimate age-specific mortality; a screen covered the jar mouth, a screen window on the side provided air flow, and a gasket aperture provided a way to aspirate dead flies out of the cage. To feed flies, dishes of food (68 cm², fashioned from the jar lids) were placed against the screen at the bottom of the cage. Females laid eggs through the screen onto the food medium.

Fresh food was provided four times each week through the end of the study; on Sunday and Wednesday afternoons (between 1600 and 1700 hr), and on Monday and Thursday mornings (between 0900 and 1000 hr). With this schedule, between Sunday night and Monday morning, and between Wednesday night and Thursday morning, females were exposed to fresh food for a set period of 17 hr. In the first five weeks, starting at age 3 days, we counted eggs on these “17-hr” dishes to estimate age-specific fecundity. We stopped censusing eggs in the middle of the fifth week when no cage produced more than five eggs, even though all cages had at least 18 females alive at this time.

We estimated the number of eggs laid on each dish by counting all the eggs within each of six pie-shaped radial sections. Each section covered 1/48 of the total dish area and the six sections were distributed uniformly around the dish. The radial sections were superimposed on the medium by placing over the food dish a plastic petri plate with etched polar coordinates. Eggs were counted within 48 hr of collection. Dishes were stored at 4° until counted to prevent hatching.

We calculated average per capita fecundity for each cage at each age by dividing the total number of counted eggs by the number of females present at the census. Fecundity, consisting of counts, is Poisson distributed and will naturally show a correlation between mean and variance. Accordingly, all estimates of fecundity were square-root transformed (Sokal and Rohlf 1995).

**Genotype component analysis:** The genetic analyses were conducted using Quercus, a package of maximum likelihood analysis of quantitative genetic data (Shaw 1987; Shaw and Shaw 1992). With Quercus we can analyze quantitative traits from individuals of known pedigree and evaluate the significance of variance components with log-likelihood ratio tests (see Promislow et al. 1996). We used the restricted maximum likelihood (REML) option to find unbiased estimates of components of variance (V_A, additive; V_D, dominance; V_E, environmental) and maternal effects (V_M) for age-specific fecundity. As in the companion paper on mortality (Promislow et al. 1996), here we treat each cage as an individual in the model, since per capita fecundities are known for each cage but not for each fly within a cage. In the genetic analysis then, cages are treated as individuals with known pedigree based on their maternal and paternal stocks, and our estimates of variance components reflect the variation among cohorts and not among individuals within cages. This approach is appropriate since all the individuals within a given reciprocal cross are genetically identical at the second chromosome. However, our interpretation of environmental variation is consequently unconventional since it refers only to cage effects and overlooks the among-individual variation within cages; our measures of V_E are underestimates and should not be used to calculate heritability.

We also used Quercus to estimate the additive genetic correlations from the data on fecundity presented in this paper and from the mortality data presented in Promislow et al. (1996). Mortality data include estimates for weeks 1–8 for males and weeks 1–7 for females. We found the genetic correlations between fecundities across each pair of age classes, between mortality rates across each pair of age classes and between fecundity and mortality rate across each pair of age classes. We analyzed mortality rates as in Promislow et al. (1996) with the use of logarithm-transformed values of instantaneous mortality, that is, ln(μ_I), where μ_I ≈ ln(P) and P is the probability of surviving from age x to age x + 1 (Elandt-Johnson and Johnson 1980). For some pairs of traits, the REML algorithm would not converge within our iteration limits. In these cases, we present point estimates of additive genetic correlation (marked by parentheses in the tables and figures). The point estimates were calculated from the mean square and cross products estimated from a general linear model (Cockerham and Weir 1977). Since our design lacks block replicates, we cannot conduct hypothesis tests with these point estimates.

In all analyses with Quercus, we estimated variance components assuming an inbreeding coefficient of 1.0 in parents, which accounts for the identity by descent of the second chromosome among individuals within each of the parental lines.

**RESULTS**

Fecundity: Daily per capita fecundity reached a maximum of eight to 16 eggs (Figure 1). The levels of egg production we observe are smaller than those we find for females housed singly in vials in our laboratory (A.A. Khazaeli, unpublished data). This difference is...
due partly to the behavior of females in cohort cages relative to vials and to the fact that some eggs stick to the screen. However, this bias in the mean should have little effect on the relative differences in the number of eggs laid among genotypes. The square-root-transformed fecundity data are approximately normal [Shapiro-Wilks test (Shapiro and Wilks 1965) on the residual values determined from the genetic variance component analyses: only Days 21 and 31 show a significant difference from normality, respectively, $P = 0.037$ and $P < 0.0001$]. After transformation there is no mean-variance correlation ($r^2 = 0.05, F_{1,0} = 0.426, P = 0.53$); the phenotypic variance of the square-root-transformed values appears roughly constant across all ages.

We calculated genetic variance components for gross fecundity (the within-cage sum of daily per capita fecundity) for comparison with previous studies. Additive genetic variance for gross fecundity is marginally greater than zero ($\chi_A^2 = 3.39, P = 0.07$), and the dominance variance is small but significant ($\chi_D^2 = 3.98, P < 0.05$). We can compare the additive variance estimate to those of other studies, scaled for trait means, with the coefficient of additive genetic variance, $CV_A = \sqrt{V_A}/\text{mean}$. For gross fecundity, $CV_A = 6.46$, which is similar to the value for Drosophila male mating ability reported in Hughes (1995), but somewhat lower than the values for female fecundity calculated by Charlesworth (1987).

We find a bimodal pattern for age-specific $V_A$ for fecundity (Figure 2). Additive genetic variance for fecundity is significantly greater than zero at age 3 days, is not measurably different from zero at ages 7, 10 and 14 days, and is greater than zero from ages 17–31 days, excluding age 21 days (each age, log-likelihood ratio test, d.f. = 1, $P < 0.01$). The bimodal pattern remains after we correct for multiple comparisons; the $V_A$ at ages 3, 28 and 31 days retain significance ($\alpha = 0.05$, Dunn-Sidak correction, Sokal and Rohlf 1995). We also tested for differences in additive variance between ages using $perfI$, a two sample version of Quercus that compares the variance components among independent populations (see Promislow et al. 1996). These tests support the suggestion that $V_A$ for fecundity is bimodal with respect to age: $V_A$ for fecundity at age 14 days is significantly less than at age 3 days and at ages 17–31 days exclusive of age 21 days (each pair of days: log-likelihood ratio test, d.f. = 1, $P < 0.03$), and the variance for fecundity at age 7 days is less than the variance at ages 28 and 31 days (each pair of ages: log-likelihood ratio test, d.f. = 1, $P < 0.05$). However none of these tests retain significance after correction for multiple comparisons ($\alpha = 0.05$, Dunn-Sidak correction, Sokal and Rohlf 1995). We also note that these data are consistent with a linear age-specific increase in $V_A$ with age (across ages, $r^2 = 0.46, P < 0.05$). In this case the $V_A$ at age 3 days may simply reflect a positive deviation from this regression. To resolve whether $V_A$ at age 3 days is biologically real, we would need to conduct genetic analyses of fecundity on a finer scale. Finally, dominance variance is small in magnitude relative to the additive variance (Figure 2) and different from zero at ages 14, 28 and 31 days, but only when we do not correct for multiple comparisons (log-likelihood ratio test, d.f. = 1, $P < 0.05$). Maternal effects ($V_M$) were negligible and not significantly different from zero in any age class. Measures of $V_A$ at advanced ages may be depressed by the early extinction of high fecundity genotypes, but this bias is unlikely here since fewer than 5% of females died by the age at which we observed a significant increase in $V_A$ (age 17 days). Furthermore, in all the cages at least 18 females were still alive by the age when reproduction was essentially completed (Day 35). Finally, we note that these age-dependent patterns for genetic variance may be confounded with temporal changes in the laboratory environment. For instance, the genetic variance components for age-specific mortality show differences in the amount of genetic variation among blocks run at different times (Hughes and Charlesworth 1994). To control for
such period effects, it will be necessary that future studies on the genetics of demographic traits examine replicate cohorts that are initiated at different times.

**Genetic correlation:** We estimate additive genetic covariance between three sets of age-specific traits: (1) fecundity, (2) fecundity and mortality in females, and (3) mortality in females and in males. We estimate covariances even when the additive genetic variance of one trait is not significantly greater than zero, because this failure to reject the null hypothesis does not imply that the variance actually is zero.

(1) The genetic correlation between fecundity at different ages shows two distinct patterns (Figure 3). First, there is no statistically significant relationship between fecundity at age 3 days and any other age. However six of eight correlations are negative in value, which may suggest a trade-off between early and late fecundity, although this proportion of negative values is not significantly different from random (sign test, exact probability = 0.109). Second, we find positive genetic correlations among all fecundities at ages >3 days. Each of the pair-wise correlations involving fecundities after age 14 days are significantly greater than zero (each pair, log-likelihood ratio test, d.f. = 1, \( P < 0.05 \)). Since we are most interested in the overall sign of the correlations among age-specific fecundities, we note that the table-wide sign is significantly positive (sign test, normal approximation, \( t = 3.77, n = 34, P < 0.001 \)).

(2) As with fecundity alone, we find that the patterns of correlation between fecundity and age-specific mortality are qualitatively different for early vs. later ages (Figure 4). Fecundity at age 3 days has a positive genetic correlation with mortality in 6 of the 7 weeks, and the correlation was greatest and statistically significant with mortality estimated at week 7 (log-likelihood ratio test, d.f. = 1, \( P < 0.01 \), for all pairs of mortality at weeks 5 and 6 vs. fecundity at ages 21–31 days; log-likelihood ratio test, d.f. = 1, \( P < 0.05 \), for mortality week 4 vs. fecundity age 28 days and for mortality week 7 vs. fecundity age 31 days). The table-wide pattern of correlations taken over all ages is significantly negative in sign (sign test, normal approximation, \( t = 4.41, n = 63, P < 0.001 \)); overall, the fitness components of age-specific fecundity and survival are positively correlated.

(3) Last we present the genetic correlations among age-specific mortalities (Table 1). For males, we find a nearly uniform pattern of positive correlations (26 of 27 cases) and \( P < 0.10 \) in 12 individual cases (Table 1). While none of the individual pairs are significantly different from zero, the table-wide sign of the correlations is significantly positive (sign test, normal approximation, \( t = 4.81, n = 27, P < 0.001 \)). For females, we
TABLE 1

Genetic correlations between age-specific mortality estimates

<table>
<thead>
<tr>
<th>Week</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(2.71)</td>
<td>0.654</td>
<td>0.937</td>
<td>0.777</td>
<td>0.778</td>
<td>0.723</td>
<td>0.898</td>
</tr>
<tr>
<td>2</td>
<td>0.861</td>
<td>1.249</td>
<td>0.888</td>
<td>0.808</td>
<td>0.838</td>
<td>0.364</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5815</td>
<td>0.4244</td>
<td>0.1914</td>
<td>0.0943</td>
<td>-0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.941</td>
<td>0.825</td>
<td>0.791</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.969</td>
<td>0.904</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.008</td>
<td>0.9967</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8308</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(0.68)</td>
<td>0.228</td>
<td>-0.396</td>
<td>-0.248</td>
<td>0.5759</td>
<td>1.3195</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.7611</td>
<td>0.1143</td>
<td>0.009</td>
<td>0.0322</td>
<td>-0.554</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.857</td>
<td>0.656</td>
<td>0.582</td>
<td>0.2635</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.862</td>
<td>0.749</td>
<td></td>
<td>0.4008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1.004</td>
<td></td>
<td>0.866</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>0.973</td>
<td></td>
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</tr>
</tbody>
</table>

Entire entries represent genetic correlations. Log-likelihood ratio test, \( \chi^2 \), of unconstrained covariance relative to covariance constrained to zero. Bold entries, \( P < 0.01 \); italic entries, significance could not be determined due to lack of convergence of the maximum likelihood algorithm when the covariance was constrained to zero; parentheses entries, point estimates based on linear model, no error estimates available (see text).

also find a tendency toward positive genetic correlations among age-specific mortalities, but the case is not as strong as for males. We find positive correlations in 17 of 20 cases, and \( P < 0.10 \) for three pairs (Table 1). As with males, the table-wide pattern of correlation is significantly positive (sign test, normal approximation, \( t_s = 3.13, n = 20, P < 0.01 \)). Overall, these results indicate that there is a strong genetic association among mortality rates across ages in both males and females; again, age-specific fitness components are positively correlated.

DISCUSSION

The results described here bear on two models for the evolution of senescence: mutation accumulation (MEDAWAR 1952) and antagonistic pleiotropy (WILLIAMS 1957). In the introduction to this paper, we discussed two expectations that have been derived from MEDAWAR's and WILLIAMS' proposals, an age-related increase in \( \nu_A \) for fitness components under mutation accumulation and a negative genetic correlation between early-age and late-age fitness components for antagonistic pleiotropy.

We designed the present study to test the mutation accumulation model by estimating age-specific genetic variance components for fecundity, and to test the antagonistic pleiotropy model by testing for negative genetic correlations between early-age and late-age fitness components. The experiment was designed to overcome many of the problems encountered by previous studies, including inbreeding, small genetic and demographic sample size, and the effects of novel environments (ROSE 1984a; BELL and KOUPANOU 1986; CHARLESWORTH 1987; CLARK 1987; PARTRIDGE and BARTON 1993). Our results indicate that both mechanisms may contribute to the senescence observed in our laboratory population of \( D. melanogaster \), but they also suggest that mutation accumulation may be the dominant source of standing genetic variance for late-age fitness traits.

Fecundity: In a companion paper, we showed that \( \nu_A \) for age-specific mortality increased early in life and then declined at later ages (PROMISLOW et al. 1996). In marked contrast, for fecundity we find significant \( \nu_A \) very early in life, at age 3 days. At ages 7–14 days, there was no detectable additive variance for fecundity, but after age 17 days \( \nu_A \) became significant once again. The late rise in \( \nu_A \) is consistent with the prediction for mutation accumulation as a cause of senescence in \( D. melanogaster \). However, since egg laying stops after week 5 in these stocks and in many wild-type stocks, we can make no inferences about patterns of variance at ages as late as those observed for mortality rates.

Mutation accumulation may account for the increase in \( \nu_A \) for fecundity observed after age 17 days, but how can we account for the significant \( \nu_A \) that we find at age 3 day? We consider two potential explanations. First, the early \( \nu_A \) may be maintained through balancing selection based on pleiotropic effects between mortality and
are associated with senescence from those that are reproduction, as appears to be the case for male mortality may be increased only at or near the time of the critical distinction between effects on mortality that compare fecundity to longevity cannot make where the fitness effects of death will be negligible. Selection both on life span and on the schedule of fecundity. Thus, we cannot isolate the correlated from mortality in classes of fecundity may provide a basis for maintaining \( V_A \) for fecundity at age 3 days, and we observe a tendency toward such negative genetic correlations, as have other pedigree-based analyses of genetic covariance in \( D. \) melanogaster (Rose and Charlesworth 1981a; Tucić et al. 1988; Scheiner et al. 1989). However, substantial dominance variance is expected to arise if selection maintains \( V_A \) for life history traits through antagonistic pleiotropy (Curtsinger et al. 1994; Hughes 1995), and we find little evidence for dominance variance for either age-specific fecundity or mortality (Promislow et al. 1996). Second, \( V_A \) for early fecundity may be a consequence of selection that maintains variation in larval developmental rate. Variation in larval development rate could, in turn, lead to variation in the age at first reproduction or peak reproduction (e.g., Hiraiizumi 1961; Hudak and Gromko 1989; Roper et al. 1993). Thus, when same-aged females are assayed, variation in the rate of ovarian maturation in adults will appear as genetic variation for the rate of early egg production. Further empirical and theoretical work is required to evaluate this hypothesis.

Covariance among fitness components: Models of senescence based on antagonistic pleiotropy predict negative genetic correlations between early-age fecundity and late-age mortality (Rose 1991; but see Partridge and Barton 1993). Studies of standing genetic covariance in \( D. \) melanogaster, both through sib-analysis and selection, claim to support this prediction (Rose and Charlesworth 1981a,b; Rose 1984b; Luckinbill et al. 1984; Tucić et al. 1988; Service 1993; Zwaan et al. 1995; but see Engström et al. 1992; Partridge and Fowler 1992; Stearns and Kaiser 1995). However, these reports are each limited in some crucial way (Curtsinger et al. 1995). For instance, selection studies typically increase the longevity of a strain by restricting reproduction to specific ages, but this practice produces direct selection both on life span and on the schedule of fecundity. Thus, we cannot isolate the correlated from the direct selection responses (Curtsinger et al. 1995; Zwaan et al. 1995). Only recently has Zwaan et al. (1995) selected on life span without direct pressure on fecundity.

Furthermore, for both selection and sib-analysis, studies that compare fecundity to longevity cannot make the critical distinction between effects on mortality that are associated with senescence from those that are independent of aging. Reproduction may affect longevity because it is “risky” (Partridge 1987); age-specific mortality may be increased only at or near the time of reproduction, as appears to be the case for male \( D. \) melanogaster (Partridge and Andrews 1985). Reproduction may also increase mortality by an equal amount at all ages in which case longevity is decreased but the rate of senescence is unaffected as occurs in the beetle \( Callosobruchus \) maculatus (Tatar et al. 1993; Tatar and Carey 1995). Finally, reproduction may affect late-age mortality alone or it may increase the rate of change of mortality with age. In these cases, reproduction would affect senescence since the changes reflect a progressive decline in fitness as a function of age (Rose 1991).

We report a positive genetic correlation between early fecundity and late-age mortality or, in the usual framework, a negative correlation with late-age survival. To our knowledge, this is the first demonstration of a trade-off between these demographic parameters, and it supports the notion that early fecundity has a genetic trade-off explicitly with senescence.

Although we find at least one set of negative fitness correlations, the fitness correlations in this study are dominated by positive values, not only between age-specific fecundity and mortality, but also between age classes within fecundity and within mortality. Positive fitness correlations have often been observed in genetic studies (Reznick 1985; Bell and Koufopanou 1986; Giesel 1986; Hughes and Clark 1988; Engström et al. 1992; Hughes 1995) and are sometimes explained as the result of inbreeding or of novel environments (Rose 1984a; Service and Rose 1985). Neither of these factors are likely to account for our results since our demographic analysis involved second chromosome heterozygote flies that originated from a large laboratory-adapted population, and we conducted our experiments under the same environmental conditions of this base population.

Positive fitness correlations could arise as a consequence of the functional structure of physiological trade-offs that underlie the relationship between traits (Pense and Bull 1988; Charlesworth 1990; Houle 1991; de Jong and van Noordwijk 1992). Houle (1991) and Charlesworth (1990) developed mutation-selection balance models that incorporated such functional structure in terms of independent loci for resource allocation and acquisition. Both authors found that at least some positive genetic correlations result when mutations affect a large number of loci for acquisition relative to allocation. The positive genetic correlations that we observed, therefore, may be due to a predominance of genetic variance for acquisition despite the occurrence of genetic trade-offs between traits. However, none of these models specify across which ages we should expect to find positive rather than negative genetic correlations. The nature of the age structure of genetic correlations observed in our data suggests a need for greater specificity of such models.

In a more general way, positive fitness correlations may arise as a consequence of accumulated mutations...
with pleiotropic deleterious late-age effects. The genetic lines analyzed in our study originated from population cages and were then passed through two-week discrete culture for 12 generations. In discrete culture there is great potential for the accumulation of de novo mutations because, in practice, flies older than 1 week contribute very little to the next generation. Since life history mutations are likely to have substantial positively correlated effects (Mukai and Yamazaki 1971; Simmons et al. 1980; Houle et al. 1994), deleterious effects on age-specific fecundity and mortality will tend to cosegregate at older ages. In this way, mutations with age-specific effects could lead to positive fitness correlations among traits at ages as early as 7 days post-eclosion and lead to senescence marked by the decline in demographic fitness traits.

The feasibility of this explanation is tempered by two issues. First, 12 generations in discrete culture are not likely to produce substantial mutational variance (Charlesworth 1987). Potentially, mutation accumulation at ages beyond 7 days also occurred in the LF350 stock, but the actual age-dependent intensity of selection is unknown for the demography of population cages. Second, when the deleterious effects of mutations act across early and late ages, selection at the early ages may forestall their accumulation (Partridge and Barton 1993; Houle et al. 1994). The extent to which mutational effects correlate across ages is a critical but largely unresolved question (Houle et al. 1994; Clark and Guadalupe 1995).

We tentatively conclude that the patterns of genetic variance for age-specific fecundity and of covariance for age-specific mortality and fecundity are consistent with the simultaneous action of both antagonistic pleiotropy and mutation accumulation. This agrees with Service et al.'s (1988) interpretation of the evolution of postponed senescence in D. melanogaster. Service et al. (1988) applied reverse selection for early reproduction in Rose's (1984b) late reproducing lines and found that starvation resistance decreased as early reproduction increased. They interpret this correlated response as evidence that antagonistic pleiotropy played a role in the original increase of starvation resistance in the late reproducing lines. At the same time, they found no change in two other traits, ethanol resistance and desiccation resistance, that also increased in Rose's (1984b) late-reproducing lines. Service et al. (1988) interpret this stasis as evidence that mutation accumulation was the source of the original variation of ethanol and desiccation resistance in the base population.

Our study advances in two important ways the notion that both mutation accumulation and antagonistic pleiotropy may play an important role in the evolution of senescence. First, Service et al. (1988) did not report how life span responded to reverse selection on early fecundity, even though an increase in life span was the central demographic trait used by Rose (1984b) to infer postponed senescence in these lines. Here, we explicitly analyze the genetic correlates of age-specific mortality. We suggest that mutation accumulation is a major source of genetic variance for life span and senescence.

However, we also find a genetic basis for trade-offs across age-specific traits, which is consistent with antagonistic pleiotropy as a source of variation for senescence. This observation relates to our second point. Partridge and Barton (1993) emphasized that to fully explain the evolution of senescence, we need to understand the relative contribution of antagonistic pleiotropy and mutation accumulation to observed patterns of physiological and demographic deterioration. In our population of D. melanogaster, we suggest that mutation accumulation plays the dominant role since the fitness correlations among age-specific traits beginning as early as age 7 days were overwhelmingly positive. While the analysis of standing genetic variation will not tell the whole story about the evolution of senescence (Parridge and Barton 1993), our analysis provides a clear implication for how we interpret experiments that select for postponed senescence. If mutations with late-age effects accumulate in laboratory-adapted populations before they are subjected to selection, then selection for late-age fitness components will produce a rapid response for improvement across many late-age life-history traits. Even if antagonistic pleiotropy makes a contribution to the selection response, much of the phenotypic difference between selected and control lines will reflect the culling of late-acting deleterious mutations and not the effects of changes in gene frequency for loci with alternative fitness effects across age classes.

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