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Genetics

Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence

(mutation accumulation/antagonistic pleiotropy/aging)

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ABSTRACT **Two major theories of the evolution of senescence (mutation accumulation and antagonistic pleiotropy) make different predictions about the relationships between age, inbreeding effects, and the magnitude of genetic variance components of life-history components. We show that, under mutation accumulation, inbreeding decline and three major components of genetic variance are expected to increase with age in randomly mating populations. Under the simplest version of the antagonistic pleiotropy model, no changes in the severity of inbreeding decline, dominance variance, or the genetic variance of chromosomal homozygotes are expected, but additive genetic variance may increase with age. Age-specific survival rates and mating success were measured on virgin males, using lines extracted from a population of *Drosophila melanogaster*. For both traits, inbreeding decline and several components of genetic variance increase with age. The results are consistent with the mutation accumulation model, but can only be explained by antagonistic pleiotropy if there is a general tendency for an increase with age in the size of allelic effects on these life-history traits.**

Senescence is an almost universal feature of the life histories of species in which a clear distinction can be made between parent and offspring (1–3). Senescent decline in survival and reproductive performance appears to result from the deterioration of many different biological parameters with age, and it seems unlikely that there is a single proximate cause of this deterioration (1–3). The standard evolutionary theory of aging asserts that the ultimate cause of senescence is the decline with age in the net effect on Darwinian fitness of age-specific changes in survival or reproductive output (4–6). This means that, other things being equal, natural selection places a greater relative weight on changes in early survival or reproduction than on changes at later ages. Provided that the appropriate genetic variability is available, this will lead to the evolution of a life history in which mortality increases and reproductive performance declines with age (4–8).

There are two major models of the paths by which this age-specific selection pressure can cause the evolution of senescence. The mutation accumulation theory of senescence postulates that there are numerous loci subject to mutation to deleterious alleles, whose effects on survival or other components of fitness are restricted to narrow bands of ages (3, 4, 7–9). The equilibrium frequencies of such deleterious alleles will be higher the later in life in which they act (8) because of the decline in the sensitivity of net fitness to changes in survival or reproduction with age (6). This will cause both a decline in mean performance with age (senescence) and a corresponding increase in genetic variance (7, 9). The alternative path involves "antagonistic pleiotropy," according to which genes that increase early performance are likely to become established in a population even if they have adverse affects on late performance (3, 5, 7, 8, 10, 11).

Although there is widespread agreement that the general evolutionary theory of senescence provides a powerful tool for interpreting the genetics and comparative biology of aging, the relative contributions of mutation accumulation and antagonistic pleiotropy to senescence have not been elucidated (2, 3, 7, 12). The two theories are clearly not mutually exclusive, but it is legitimate to ask whether there is evidence for or against either of them. Recent advances in the genetics of Alzheimer disease suggest that both mechanisms may contribute to this aspect of senescence in humans (13). In *Drosophila*, there is evidence that both supports and casts doubt on the tradeoffs postulated by the antagonistic pleiotropy model (3, 14, 15). There is also conflicting evidence on the importance of mutation accumulation (16–20). In this paper, we show that inbreeding depression is expected to increase with age under the mutation accumulation model but not under antagonistic pleiotropy (unless the effects of segregating alleles themselves increase with age). We present data on inbreeding effects on survival and male mating success in *Drosophila melanogaster* that are consistent with this prediction of the mutation accumulation model. We also provide theoretical results on the expected behavior of components of genetic variance under the two alternative models and compare the models with the available data. Overall, the results are consistent with a role for mutation accumulation in the evolution of senescence, but antagonistic pleiotropy is not ruled out.

THEORETICAL ANALYSIS

In this section, we will develop some simple models of mutation accumulation and antagonistic pleiotropy and use them to predict the age-related patterns of inbreeding depression and genetic variance components. A randomly mating population at equilibrium under mutation–selection balance (mutation accumulation) or heterozygote advantage (antagonistic pleiotropy) will be assumed.

Inbreeding Effects Under Mutation Accumulation. Consider an age-specific trait z—e.g., survival from age x to $x + 1$ in a discrete age-class model. Assume for simplicity that mutations at several loci affect only this trait and have no effect on performance at any other age. Let the mutation rate of the wild-type allele at the ith locus that affects z be u_i and the homozygous effect of a mutation at this locus be δz_i (measured as the reduction in z relative to the value for wild-type). The sensitivity of fitness to a change in trait z is $S(z)$, such that the reduction in fitness due to a homozygous mutation at locus i is $S(z)\delta z_i$. $S(z)$ is the partial derivative of net fitness with respect to z, setting z equal to the value for wild-type homozygotes (8).

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Under most circumstances, $S(z)$ is a decreasing function of age (6). It will thus be highest for traits expressed early in the reproductive portion of the life history and lowest for traits expressed late in life (it is zero for postreproductive traits). We assume that mutant alleles are partially recessive, such that the reduction in trait value in heterozygotes is $h_i\delta z_i$, where h_i is the dominance coefficient and $0 < h_i < 0.5$. There is good evidence from *Drosophila* to support this last assumption (21, 22).

Under these conditions, the equilibrium allele frequency at the ith locus is $q_i \approx u_i/S(z)h_i\delta z_i$ (8). We assume that mutant alleles are always rare, so that $q_i \ll 1$. Because the population mean is then mainly affected by the heterozygous carriers of the mutations, the mean trait value relative to the value for wild-type homozygotes is reduced below one by approximately $2q_ih_i\delta z_i = 2u_i/S(z)$. With multiplicative interactions between alleles at different loci, the mean trait value for the equilibrium population is

$$\bar{z}_O \approx exp\ -\frac{2}{S(z)}\sum_i u_i. \qquad [1]$$

This result shows that, as expected intuitively, age-specific mutational effects lead to senescent decline in a randomly mating population, because $S(z)$ declines with age (7, 9). If inbreeding leads to the production of individuals with inbreeding coefficient F, without any change in allele frequencies, the ith locus causes a reduction in mean trait value for the inbred population below that for the wild-type homozygote of approximately $\{2(1 - F)h_i + F\}q_i\delta z_i$ (23). With multiplicative effects, the net trait value for the inbreds is then

$$\bar{z}_I \approx exp\ -\frac{1}{S(z)}\sum_i u_i\left\{2(1 - F) + \frac{F}{h_i}\right\}. \qquad [2]$$

The inbreeding depression in a trait z associated with inbreeding coefficient $F > 0$ is defined as the difference between the means of outbred and inbred indviduals, relative to the outbred mean (22). In the present case, it is given by

$$\frac{(\bar{z}_O - \bar{z}_I)}{\bar{z}_O} \approx 1 - exp\ \frac{2F}{S(z)}\sum_i u_i\left\{1 - \frac{1}{2h_i}\right\}. \qquad [3a]$$

If $h_i < 0.5$, as assumed here, the inbreeding depression is always positive and increases with F. An alternative measure of the effect of inbreeding on fitness components is the regression coefficient of the natural logarithm of trait value on $-F$, which is usually denoted by B (23). This is equivalent to the value of $\ln(\bar{z}_O/\bar{z}_I)$ for $F = 1$, which is sometimes referred to as the inbreeding load (24). The use of balancer chromosomes in *Drosophila* enables direct measurements of this for the effects of single chromosomes on fitness components (25). We have

$$B(z) = \frac{2}{S(z)}\sum_i u_i\left\{\frac{1}{2h_i} - 1\right\}. \qquad [3b]$$

Provided that there are no systematic differences in mutation rate or dominance coefficients among genes affecting different ages, these results shows that inbreeding depression and inbreeding load should increase as $S(z)$ declines with age.

Genetic Variance Components Under Mutation Accumulation. The above model can also be used to predict variance components. Its application to the standard formula for additive genetic variance (26) implies that the additive genetic variance, V_A, in a trait z contributed by a single locus is $2q_i(h_i\delta z_i)^2$ (27). If the genetic variance from all sources is small, as is usually the case, the multiplicative model can be well approximated by an additive one. Substituting the equilibrium gene frequency under mutation selection balance into this expression and summing over loci, yields the expression in Table 1 for V_A, scaled relative to the outbred mean value. Similar expressions for the dominance variance, V_D (26), and the genetic variance among completely inbred lines, V_G (22), are also given in Table 1. It will be seen that the variance components other than V_D are inversely proportional to $S(z)$, as is the inbreeding load, whereas V_D is inversely proportional to $S(z)^2$. V_D is also proportional to the square of the mutation rate, whereas the other variances are proportional to the mutation rate. This suggests that V_D may be expected to increase from a very low level early in life, relative to the other variance components, to a much larger value very late in life, when $S(z)$ approaches zero. The assumption that mutations affect only one age-class is, of course, oversimplified, but the qualitative expectation of an increase in inbreeding depression and genetic variance components with age will hold, provided there is some degree of age specificity in the effects of mutant genes.

Inbreeding Effects Under Antagonistic Pleiotropy. Antagonistic pleiotropy can be studied with the following simple model, based on that of Rose (10, 11). Consider two traits z_1 and z_2, which are both controlled by the same polymorphic locus, segregating for two alleles A_1 and A_2, with frequencies p and q. The alleles have partially recessive but opposing small effects on the two traits (see Table 2). The sensitivities of net fitness to changes in traits 1 and 2 are $S(z_1)$ and $S(z_2)$ (evaluated

Table 1. Mutation accumulation model

	Formulation of the model
Genotypes at ith locus	$A_{i1}A_{i1}$ $A_{i1}A_{i2}$ $A_{i2}A_{i2}$
Value of trait z	1 $1 - h_i\delta z_i$ $1 - \delta z_i$
Outbred mean due to ith locus	$\bar{z}_{iO} \approx 1 - 2q_ih_i\delta z_i \approx 1 - 2u_i/S(z)$
Inbred mean due to ith locus	$\bar{z}_{iI} \approx 1 - (1 - F)(1 - \bar{z}_{iO}) - Fq_i\delta z_i \approx 1 - 2(1 - F)\{u_i/S(z)\} - Fu_i/\{h_iS(z)\}$
Additive variance (scaled)	$\frac{V_A(z)}{\bar{z}_O^2} \approx \frac{2}{S(z)}\sum_i u_ih_i\delta z_i$
Dominance variance (scaled)	$\frac{V_D(z)}{\bar{z}_O^2} \approx \frac{4}{S(z)^2}\sum_i \frac{u_i^2\left(\frac{1}{2} - h_i\right)^2}{h_i^2}$
Homozygous genetic variance (scaled)	$\frac{V_G(z)}{\bar{z}_I^2} \approx \frac{1}{S(z)}\sum_i \frac{u_i\delta z_i}{h_i}$

Table 2. Antagonistic pleiotropy model

	Formulation of the model		
Genotypes at polymorphic locus	A_1A_1	A_1A_2	A_2A_2
Value of trait 1	1	$1 - h\delta z_1$	$1 - \delta z_1$
Value of trait 2	$1 - \delta z_2$	$1 - h\delta z_2$	1
Fitnesses (neglecting second-order terms)	$1 - S(z_2)\delta z_2$	$1 - hS(z_1)\delta z_1 - hS(z_2)\delta z_2$	$1 - S(z_1)\delta z_1$
Ratio of equilibrium gene frequencies	$\frac{p}{q} \approx \frac{S(z_1)(1-h)\delta z_1 - hS(z_2)\delta z_2}{S(z_2)(1-h)\delta z_2 - hS(z_2)\delta z_1}$		
A nontrivial stable equilibrium exists if	$S(z_1)(1-h) - hS(z_2) > 0$, $S(z_2)(1-h) - hS(z_1) > 0$		
If $\delta z_1 = \delta z_2$ and $h = 0$	$\frac{p}{q} \approx \frac{S(z_1)}{S(z_2)}$		
Outbred trait means	$\bar{z}_{1O} = 1 - q(q + 2hp)\delta z_1$ $\bar{z}_{2O} = 1 - p(p + 2hq)\delta z_2$		
Inbred trait means	$\bar{z}_{1I} = 1 - (1 - \bar{z}_{1O})(1 - F) - Fq\delta z_1$ $\bar{z}_{2I} = 1 - (1 - \bar{z}_{2O})(1 - F) - Fp\delta z_2$		
Additive variances	$V_A(z_1) = \frac{1}{2}pq\{1 + (1-2h)(q-p)\}^2(\delta z_1)^2$ $V_A(z_2) = \frac{1}{2}pq\{1 + (1-2h)(p-q)\}^2(\delta z_2)^2$		
Dominance varianced	$V_D(z_i) = 4\left\{pq\left(\frac{1}{2} - h\right)\delta z_i\right\}^2$ (i = 1, 2)		
Homozygous genetic variances	$V_G(z_i) = pq(\delta z_i)^2$ (i = 1, 2)		

at the relevant trait values for A_1A_1 and A_2A_2, respectively). If traits 1 and 2 are components of fitness for early and late parts of the adult life history, respectively, then $S(z_1) > S(z_2)$ (6). With sufficiently recessive effects, this model can result in heterozygote advantage with respect to net fitness and hence in the maintenance of polymorphism (Table 2 and refs. 10 and 11). The mean values of the two traits for the outbred equilibrium population and a completely inbred population are given in Table 2. As with mutation accumulation, this model accounts for a decline in outbred trait mean with age. If $S(z_1) > S(z_2)$ and $\delta z_1 = \delta z_2 = \delta z$, it is easily seen that $p > q$, because the adverse effect on net fitness of allele A_1 through its homozygous effect on trait 2 is smaller than the adverse homozygous effect of A_2 (10). This yields

$$\bar{z}_{1O} - \bar{z}_{2O} = (p - q)\delta z > 0. \quad [4]$$

We also have the following approximate general expression for the inbreeding load for trait i:

$$B(z_i) \approx pq(1 - 2h)\delta z_i \quad (i = 1, 2). \quad [5]$$

This demonstrates that, under the antagonistic pleiotropy model, the inbreeding load is independent of the fitness sensitivity for the trait in question.

Genetic Variance Components Under Antagonistic Pleiotropy. The genetic variance components can be obtained by the same methods as for the mutation accumulation model and are given in Table 2. Similar formulae for V_A and V_D have been derived previously (10, 11, 28). In contrast to the results for inbreeding depression and the other variance components, there can be a consistent difference between the additive variances of the two traits, which is related to differences in their fitness sensitivities. It is easily seen from Table 2 that $V_A(z_1) < V_A(z_2)$ if $p > q$, provided that the homozygous effects of the two alleles are of similar magnitude. The most extreme situation is when $h = 0$ (i.e., there is complete recessivity). If $\delta z_1 = \delta z_2$, we then have

$$\frac{V_A(z_2)}{V_A(z_1)} = \frac{S(z_1)^2}{S(z_2)^2}, \quad [6]$$

showing that the trait with the lower fitness sensitivity has a much higher additive genetic variance at equilibrium.

It is also evident from Table 2 that, in contrast to the behavior of the additive genetic variance, the other genetic variance components are independent of the $S(z_i)$ values. In addition, the dominance variance may be substantially smaller than the additive variance, as shown by Rose (10). Assuming that $S(z_1) > S(z_2)$ and $\delta z_1 = \delta z_2 = \delta z$, the ratio of dominance to additive variance will be largest for trait 1. We have

$$\frac{V_D(z_1)}{V_A(z_1)} = \frac{8pq\left(\frac{1}{2} - h\right)^2}{\{1 + (1-2h)(q-p)\}^2}. \quad [7]$$

It is easily seen that, holding p constant, this ratio is largest when $h = 0$, when it reduces to $p/\{2q\}$. Thus, unless there is a high degree of asymmetry in the allele frequencies ($q < 1/3$), there will be less dominance than additive variance (10), although V_D/V_A is always ≥ 0.5 for the early life-history trait 1 when $h = 0$, and ≥ 0.18 when $h = 0.2$, a value suggested by data on *Drosophila* life-history traits (21, 22). The reverse is true for the late-life trait 2: V_D/V_A is always ≤ 0.5 when $h = 0$ and ≤ 0.18 when $h = 0.2$. With highly asymmetric allele frequencies, as is necessary for a large decline in outbred mean (Eq. 4), there can be a substantial reduction in V_D/V_A between early and late life under the antagonistic pleiotropy model.

EXPERIMENTAL PROCEDURES AND RESULTS

We now compare these theoretical expectations with the results of measurements of components of genetic variance and the effects of inbreeding for two fitness components of virgin male *D. melanogaster*: age-specific survival and age-specific mating success. Virgins were used in order to minimize the effects of tradeoffs involving reproductive effort on patterns of variation produced by mutation accumulation.

Experimental Procedures. The experimental methods have been described in detail elsewhere (20, 22). Forty different third chromosomes were isolated from the large, randomly mating laboratory population *IV* of *D. melanogaster* by the use of a genetically marked balancer chromosome, and crossed

onto the same *IV*-derived genetic background. The *IV* population has been maintained in large numbers since 1977 (29) and has therefore had ample time to equilibrate under laboratory conditions. Third chromosome heterozygotes and homozygotes were assayed in five blocks of eight lines each.

Within each block, crosses between lines were used to produce the heterozygous individuals in a North Carolina II breeding design (30) as follows. Let the lines used in a given block be labeled 1–8. These are divided into two sets, 1–4 and 5–8. Both reciprocal crosses are made between all pairs of lines in the different sets (1 × 5, 1 × 6, etc.); in addition, the homozygous genotypes from crosses 1 × 1, 2 × 2, etc., are produced. Over all blocks, 80 heterozygous and 30 homozygous genotypes were assayed. Four replicates of each heterozygous genotype (two from each reciprocal cross) and two replicates of each homozygous genotype were scored, and the performance of a genotype was averaged over all replicates. The mean performances of the heterozygous and homozygous lines in a block were used to estimate outbred and inbred trait means, respectively. In each block, we calculated the inbreeding load for male mating success and for survival as $B = \ln(\bar{z}_O/\bar{z}_I)$. We have previously shown for our data that this method of calculation is not biased by nonrandom loss of homozygous lines (22).

Survival of virgin males was measured for three disjunct 3-week periods over the course of the adult life span (weeks 1–3, 5–7, and 9–11), and the mating success of virgin males was measured in a standard assay for competitive mating ability at 3 days and at 21 days after eclosion (20, 22). Different individuals were assayed at the different ages. This procedure yielded five independent estimates of outbred and inbred mean performance for each trait, because the experiment involved five independent blocks. Variance components were obtained by the methods described (19, 20, 22). Because the mean survival during weeks 9–11 was anomalously low in block 1, we have omitted this block from the analyses of age-specific patterns of inbreeding load and genetic variances for survival. If this block is included, the increase in inbreeding load with age is much larger than that reported below.

Age-Specific Inbreeding Loads. The inbreeding load for survival over intervals of three weeks increases with age in every block of the experiment (Table 3). The load for early-life survival (weeks 1–3) is not significantly greater than zero, by a one-tailed *t*-test of the mean over all blocks against its standard error (one-tailed $t = 1.01$, $P > 0.3$). In contrast, the loads for mid-life (weeks 5–7) and late-life survival (weeks 9–11) are significantly greater than zero ($t = 2.81$, $P < 0.05$ and $t = 4.16$, $P < 0.02$, respectively). The inbreeding load for late-life survival is significantly more severe than that for early survival (paired $t = 4.55$, $P < 0.02$) and is also significantly larger than that for mid-life survival (paired $t = 4.38$, $P < 0.02$). Also, the load for mid-life survival is significantly larger than for early survival (paired $t = 2.71$, $P < 0.05$). Estimates of survival at the three ages are not independent, so a repeated-measures ANOVA was used to determine if the trend for inbreeding load to increase with age is significant. The increase in inbreeding load is significant by this test (univariate $F_{2,6} = 18.5$, $P < 0.01$; Mauchly criterion met at $P = 0.25$). Very similar results are obtained if the data are analyzed in terms of inbreeding depression instead of inbreeding load.

The age-specific inbreeding depression for male mating success has been reported (22). If inbreeding loads are calculated from these data, it is found that the mean inbreeding load at 3 days is 0.147 (SE = 0.026) and at 21 days it is 0.450 (SE = 0.100). The increase in load with age is significant (one-tailed paired $t = 2.83$, $P < 0.05$). This increase in the effect of inbreeding on male mating success is particularly striking, because it occurs over a short time period (18 days). A possible explanation for the relatively rapid increase in inbreeding load for mating success is that mating success was scored under highly competitive conditions, whereas the conditions for survival assays were quite benign (abundant food, low density of males, and no females present). There is evidence that genotypic differences in fitness components may be magnified under harsh conditions (31).

Age-Specific Variance Components. We have estimated V_A and V_D for age-specific survival from the data on the heterozygous genotypes, and we have also estimated V_G from the homozygous lines (Table 4). Variance components are presented both for the raw trait values, and for traits scaled relative to block means. The theory described above relates directly to scaled variance components. But survivorship decreases markedly with age, so that scaling relative to the mean may cause artifactual increases in variance components with age (16). Unscaled genetic variance components therefore provide a more conservative test of the prediction that variance components should increase with age.

All genetic variance components increase with age on both scales. Unscaled V_A and V_D for late-life survival are significantly greater than zero but do not differ significantly from zero at the earlier ages. V_D for late-life survival is significantly larger than V_D for early survival ($t = 2.41$, $P < 0.05$), and V_G for late-life survival is significantly greater than V_G for mid-life survival ($t = 2.77$, $P < 0.05$). Also, V_G for mid-life survival is significantly larger than V_G for early survival ($t = 2.57$, $P < 0.05$). It should be noted that these results are different from those reported previously for age-specific mortality of heterozygous genotypes (19). Due to an error in calculation, these values were for 1-week mortality at ages 5, 7, and 10 weeks, rather than for mortality over 3 weeks. This correction does not change the conclusions of that paper, however.

For male mating success, we have reported a significant increase in V_A between ages 3 and 21 (20). No increases in V_D or V_G were observed over this period (20, 22).

DISCUSSION

An increase with age in the deleterious effects of inbreeding has been detected in the same two traits that have previously

Table 3. Mean age-specific survival rates of third chromosome homozygotes and the associated inbreeding loads for each block of the experiment

	Weeks 1–3			Weeks 5–7			Weeks 9–11		
Block	Heterozygotes	Homozygotes	Load	Heterozygotes	Homozygotes	Load	Heterozygotes	Homozygotes	Load
1	0.993	1.000	−0.007	0.861	0.640	0.297	0.049	0.000	∞
2	0.999	0.993	0.006	0.979	0.887	0.099	0.550	0.435	0.234
3	0.987	0.951	0.037	0.885	0.798	0.104	0.584	0.406	0.374
4	0.994	1.000	−0.006	0.988	0.910	0.082	0.712	0.521	0.313
5	0.995	0.994	0.001	0.970	0.974	−0.004	0.616	0.561	0.094
Mean	0.994	0.984	0.010	0.956	0.892	0.070	0.615	0.479	0.254
SE	0.003	0.011	0.010	0.024	0.036	0.025	0.035	0.037	0.061

The standard errors of the overall means (SEs) are calculated from the between-block variances in block means for each variable. Means and standard errors are calculated from blocks 2 through 5.

Table 4. Components of variance for age-specific survival

Variance component	Weeks 1–3			Weeks 5–7			Weeks 9–11		
	Estimate	SE	t value	Estimate	SE	t value	Estimate	SE	t value
				Heterozygotes					
V_A	-3×10^{-5}	4×10^{-5}	0.87	0.003	0.001	0.91	0.101*	0.015	4.09
	-3×10^{-5}	4×10^{-5}	0.87	0.002	0.002	0.96	0.025**	0.004	5.50
V_D	1×10^{-4}	2×10^{-4}	0.91	0.002	0.002	1.16	0.021	0.009	2.10
	1×10^{-4}	2×10^{-4}	0.90	0.002	0.002	1.19	0.007*	0.003	2.44
V_E	6×10^{-4}	3×10^{-4}	1.66	0.005	0.003	1.64	0.072**	0.015	4.98
	5×10^{-4}	3×10^{-4}	1.68	0.004	0.002	1.79	0.026**	0.005	5.51
				Homozygotes					
V_G	0.002	0.004	0.99	0.012	0.006	2.08	0.409*	0.139	2.98
	0.002	0.002	0.99	0.009	0.004	2.26	0.064*	0.026	3.03
V_E	0.003	0.003	1.12	0.033	0.016	1.60	0.099*	0.039	2.52
	0.003	0.003	1.13	0.019	0.010	1.80	0.016*	0.004	4.25

The estimates of variance components are obtained from the means of the within-block estimates for blocks 2–4 (18, 19); the standard errors are obtained from the between-block variances of these estimates. The upper member of each pair of rows is for the variances standardized relative to the block means; the lower member is for the unstandardized values. Statistical significance is indicated as follows: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. The significance of the heterozygous genetic variance components and the environmental variances were obtained by t-tests of the estimates against their standard errors (18, 19); the significance of the genetic variance of homozygotes was obtained from the F ratio from an ANOVA.

shown evidence of increasing genetic variation with age in virgin males (19, 20, 22, 32). Both sets of observations are qualitatively consistent with the predictions of the mutation accumulation theory of senescence. The extent to which they agree quantitatively can be partially assessed as follows. Equations (Eq. **3b**) and the results in Table 1 imply that the relative values of the additive genetic variance at different ages should match those for the inbreeding load B, if the model of complete independence between mutational effects at different ages is correct. Table 4 shows that the ratios of scaled V_A and V_G for late-life survival to the values for mid-life survival are both 34, while Table 3 implies a corresponding ratio of B values of $\approx$3.6. The ratios for V_A and V_G are of uncertain meaning, however, because the mid-life values do not differ significantly from zero at 5 weeks. Scaled V_D for survival also increases significantly from mid-life to late-life by a factor of 10, although again mid-life V_D is not significant (Table 4). V_D/V_A decreases from 0.67 to 0.21 from mid-life to late-life. The ratio of scaled V_A for male mating success at 21 days to that at 3 days is 3.4 (20), and the ratio of B values is 3.1 (see above), which is in close agreement. There is no evidence for significant V_D for male mating success at either age, and the homozygous genetic variance for male mating success does not apparently change between 3 and 21 days (20, 22).

Because of our ignorance of the degree of age specificity of the effects of deleterious mutations and of the nature of the dependence of the fitness sensitivity function on age under selectively relevant conditions, there are considerable difficulties in using Eqs. **3a** and **3b** to make further quantitative tests of the agreement between the predicted and observed relation between inbreeding effects and age. We note, however, that males aged 9 weeks or more are completely infertile (unpublished data). Hence, $S(z)$ for flies of these age should be zero, and inbreeding depression should approach its maximal value of 1 (Eq. **3a**) if there were completely independent effects of mutations on survival at different ages. In fact, the mean inbreeding depression for survival is only about 0.22 for flies aged 9 weeks, suggesting some degree of dependence between genetic effects on survival at different ages. In the case of male mating success, there is a nonsignificant genetic correlation of 0.04 between early and late mating success in flies heterozygous for their third chromosome, but there is a borderline significant genetic correlation of 0.97 for chromosomal homozygotes (20). Because the effects of deleterious mutations on genetic correlations are more likely to be manifest in highly inbred populations (9, 33, 34), this difference provides an indication that mutational effects at the two ages may not be completely independent of each other. This possibility is supported by the general tendency for large positive mutational correlations to be observed between different fitness components, including early and late female fecundity (18, 21). If true, it would mean that the rate of increase of inbreeding decline with age would be much smaller than predicted by Eqs. **3a** and **3b**. The same is obviously true of the variance components. The quantitative effects are hard to predict without making assumptions about the patterns of correlation of gene effects across age, for which we currently have very poor information.

As discussed above, an increase in additive genetic variance with age for a life-history trait is not necessarily a unique prediction of the mutation accumulation theory; it is also expected under antagonistic pleiotropy. In addition, a fairly small dominance variance relative to additive variance for late adult life-history traits is compatible with antagonistic pleiotropy (ref. 10 and Eq. **7**). The apparent paradox that V_D is often smaller than V_A for fitness components (20, 35) can thus be reconciled with the maintenance of polymorphism by heterozygote advantage and antagonistic pleiotropy, as far as late-life traits are concerned. The low ratio of V_D to V_A for late-life survival (Table 4) is thus compatible with antagonistic pleiotropy. But traits that show a sharp decline in mean with age should have moderate to high dominance variances relative to additive variances early in life (Eq. **7**), because of the effects of high frequencies of polymorphic alleles with deleterious late effects. The lack of evidence for dominance variance in early life-history traits, such as egg-to-adult viability, early female fecundity, and early male survival and mating success (Table 4; refs. 16, 27, 20, and 35) is thus rather hard to reconcile with the antagonistic pleiotropy model. In addition, in contrast to what is observed for both survival and male mating success, this theory does not predict any increase in inbreeding effects with age, unless there there are consistent differences between genetic effects at different ages. The same applies to the increase in dominance variance and homozygous genetic variance with age, which was observed for survival.

An increase with age in the effects of segregating alleles on life-history traits would seem to imply that senescence is, at least in part, caused by factors that make the organism intrinsically more sensitive to deleterious gene effects later in life. It is interesting in this context to note that there is a significant increase in the environmental variance in survival between mid- and late-life for chromosomal heterozygotes (paired $t = 3.65$, $P < 0.05$ for the unstandardized variances). This is not due simply to increased binomial sampling variance

for late-life survival estimates because of smaller numbers of survivors. There is a highly significant excess variance of late-life survival over binomial expectation when the error sums of squares are compared with the expected binomial sampling variances ($\chi^2 = 283$ for 184 df, $P < 0.001$), indicating that there is real environmental variance in survival late in life. There is no such excess over binomial expectation earlier in life.

This suggests that old flies are more sensitive than young flies to environmental variation in causes of death; an increase in environmental variation has also been observed for female fecundity (16). Similarly, older flies could also be inherently more sensitive to genetic variation. Increased sensitivity to both environmental and genetic factors with advancing age could be caused by genes with deleterious effects on homeostasis late in life. Such genes could accumulate by either of the two evolutionary mechanisms discussed in this paper. Examination of the mutational variances of traits at different ages is one way to test this possibility, but adequate data are not yet available (18).

One further point is worth making. Antagonistic pleiotropy does not necessarily require the maintenance of genetic variation in the population—e.g., if the dominance coefficient h in Table 2 is too large, then fixation of allele A_1 will occur if $S(z_1) > S(z_2)$ and the homozygous allelic effects on the two traits are similar. Thus, it is perfectly possible for antagonistic pleiotropy to have contributed to the evolution of senescence without leaving its trace on the pattern of age-specific inbreeding depression and genetic variances (7). Experiments that fail to demonstrate genetic tradeoffs thus do not falsify the antagonistic pleiotropy hypothesis. In contrast, the mutation accumulation theory entails the existence of genetic variability maintained by mutation pressure and will be falsified if the requisite properties of this variation are not observed.

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