Quantitative genetic tests of recent senescence theory: age-specific mortality and male fertility in *Drosophila melanogaster*

MS Snoke¹ and DEL Promislow

Department of Genetics, University of Georgia, Athens, GA 30602-7223, USA

Quantitative genetic models of aging predict that additive genetic variance for fitness components should increase with age. However, recent studies have found that at very late ages, the genetic variance components decline. This decline may be due to an age-related drop in reproductive effort. If genetic variance in reproductive effort affects the genetic variance in mortality, the decline in reproductive effort at late ages should lead to a decrease in the genetic variance in mortality. To test this, we carried out a large-scale quantitative genetic analysis of age-specific mortality and fertility in virgin male *Drosophila melanogaster*. As in earlier studies, we found that the additive variance for age-specific mortality and fertility declined at late ages. Also, recent theoretical developments provide new predictions to distinguish between the mutation

accumulation (MA) and antagonistic pleiotropy (AP) models of senescence. The deleterious effects of inbreeding are expected to increase with age under MA, but not under AP. This prediction was supported for both age-specific mortality and male fertility. Under AP, the ratio of dominance to additive variance is expected to decline with age. This prediction, too, was supported by the data analyzed here. Taken together, these analyses provide support for both the models playing a role in the aging process. We argue that the time has come to move beyond a simple comparison of these genetic models, and to think more deeply about the evolutionary causes and consequences of senescence.

Heredity (2003) 91, 546–556, advance online publication, 17 September 2003; doi:10.1038/sj.hdy.6800353

Keywords: senescence; mutation accumulation; antagonistic pleiotropy; mortality; fertility; *Drosophila melanogaster*

Introduction

Beginning with Fisher and Haldane, a distinguished line of evolutionary biologists have argued that the root cause of senescence lies in the age-related decline in the force of selection (Fisher, 1930; Haldane, 1941; Williams, 1957; Hamilton, 1966; Charlesworth, 2000). But the underlying genetic mechanisms that shape the patterns of senescence are far from agreed upon (Wachter and Finch, 1997). Two competing mechanisms have been proposed – mutation accumulation (MA) and antagonistic pleiotropy (AP). Under MA, the declining force of selection leads to the accumulation of late-acting deleterious mutations from one generation to the next (Medawar, 1952; Charlesworth, 1990). Alternatively, under the AP model, late-acting deleterious mutations may be favored by selection if they have early-acting beneficial (or ‘antagonistically pleiotropic’) effects (Williams, 1957).

Theoreticians have been able to derive explicit quantitative genetic predictions for each of these models. For example, an early prediction by Charlesworth (1990) was that MA should lead to an age-related increase in the additive genetic variance for fitness components. This is based on the recognition that the degree to which fitness

is reduced by a deleterious mutation (ie, ‘sensitivity’) decreases as the age at onset of the mutation’s effects increases (Hamilton, 1966). Charlesworth’s model shows that genetic variance components have an inverse relationship with sensitivity due to mutation–selection balance. As the sensitivity decreases with age, the additive variance is predicted to increase. This prediction was supported in a study of mortality rates in the fruit fly, *Drosophila melanogaster* (Hughes and Charlesworth, 1994). But subsequently, Charlesworth and Hughes have shown that a similar pattern could also be seen if AP underlies senescence (Charlesworth and Hughes, 1996). In this light, they have derived two new predictions that allow us to distinguish between MA and AP. According to their theoretical analysis, the effects of inbreeding depression on age-specific fitness traits should increase with age under the MA model, but be age independent under the AP model. Inbreeding depression is predicted to increase under MA because it is inversely related to sensitivity. In contrast, under AP, inbreeding depression is unrelated to sensitivity, so inbreeding depression is expected to influence fitness in a manner irrespective of age.

Additionally, Charlesworth and Hughes (1996) predict that the age-related change in the ratio of dominance to additive variance (V_D/V_A) for fitness would differ under the effects of MA versus AP. Under MA, the dominance variance is inversely related to sensitivity squared, while the additive variance is inversely related to sensitivity. As sensitivity decreases with age, the ratio of V_D/V_A would be expected to increase. However, under AP,

Correspondence: D Promislow, Department of Biology, Jordan Hall, Indiana University, Box 34B, 1001 East Third Street, USA.

E-mail: promislow@uga.edu

¹Current address: Department of Biology, Indiana University, Jordan Hall, Box 34B, 1001 East Third Street, Bloomington, IN 47401, USA.

Received: 1 July 2002; accepted: 7 July 2003

while the additive variance is inversely proportional to sensitivity, all other genetic variance components are independent of sensitivity. Over time, the additive variance should increase, but the dominance variance may not. Thus, if senescence is due to genes with antagonistic pleiotropic effects, V_D/V_A should decrease with age. These predictions were tested recently by Hughes *et al* (2002) using measures of mortality rates and reproductive success in flies. Again, they found strong support for MA, but not for AP.

Another traditional test of MA and AP relies on the estimates of covariance for fitness parameters between ages. The models of AP predict that early- and late-age fitness traits will exhibit negative genetic correlations (Rose and Charlesworth, 1980), while at least some models of MA predict positive genetic correlations between ages (Charlesworth, 2001). Many of the attempts to test these theoretical predictions empirically have focused on analyses of age-specific mortality (eg, Hughes and Charlesworth, 1994; Promislow *et al*, 1996; Shaw *et al*, 1999). While the mortality rates are obviously of direct relevance to studies of aging, quantitative genetic analyses of mortality rates present a unique set of challenges (Promislow *et al*, 1996, 1999; Promislow and Tatar, 1998; Shaw *et al*, 1999). Mortality rates are binomially or beta-binomially distributed within genetically identical, same-aged cohorts, log-normally distributed across genotypes, and increase exponentially with age (Promislow *et al*, 1996) (for further discussion of these statistical issues, see Promislow and Tatar, 1998; Vaupel *et al*, 1998; Pletcher and Geyer, 1999; Promislow *et al*, 1999; Shaw *et al*, 1999). Moreover, the error variance of mortality rates reaches minima both early in life, when the intrinsic mortality is low so few individuals die, and late in life, when few individuals are alive so few individuals die (Promislow *et al*, 1999; Pletcher and Curtsinger, 2000).

To alleviate at least part of this problem, in their test of the MA model of senescence, Promislow *et al* (1996) use very large sample sizes to estimate the genetic variance components for mortality. They find that additive genetic variance for mortality rates increases early in life, but then, contrary to the theoretical expectation, shows a significant decline later in life.

Promislow *et al* (1996) propose several alternatives to account for this unexpected decline in the additive variance at late ages. One suggestion is that the observed decline in the additive variance could have been due to the age-related decline in reproductive investment seen in *Drosophila* (Partridge and Fowler, 1992). In contrast to Hughes and Charlesworth's (1994) original study, Promislow *et al* (1996) measured mortality in mixed-sex cohorts. If variation among genotypes in mortality rates was due to genetic variation in reproductive effort, then as reproductive effort declined late in life, so too would the genetic variation in mortality rates.

Here, we test the hypothesis that the age-related decline in reproductive effort leads to a decline in the genetic variance for mortality rates by estimating quantitative genetic variance components for age-specific mortality rates in 33 221 virgin male *D. melanogaster*, including both inbred and outbred genotypes. The use of virgin flies minimizes investment in reproductive effort, and so should greatly reduce any genetic variation for survival that is due to costs of reproduction. By

comparing these results with previous work (Promislow *et al*, 1996), we can examine the influence of variation in reproductive effort on variation in mortality. In addition, we also measure age-specific fertility in a subset of these males. Our measurements of mortality and fertility give us the opportunity to test not only earlier predictions regarding age-related changes in additive variance and genetic covariance, but also Charlesworth and Hughes's (1996) more recent predictions for both MA and AP models.

Materials and methods

Stocks

We created a large, outbred population of *D. melanogaster* from flies caught in August of 1998 from a peach orchard in Watkinsville, GA, USA (referred to hereafter as GA98). We maintained lines derived from these flies both in $40 \times 25.5 \times 30$ cm³ plexiglass population cages and in bottled populations for approximately 1 year. To maintain cage populations, we kept 14 half-pint plastic bottles (Applied Scientific) with 50–60 ml of standard yeast–agar–molasses–cornmeal medium, with two bottles replaced biweekly. The densities were consistently greater than 3000 flies per cage. We waited 1 year until beginning the experiments described here to allow for acclimation to the lab culture. We kept the other wild caught flies (referred to hereafter as TW1) in half-pint plastic bottles (Applied Scientific) with 60 ml of standard yeast–agar–molasses–cornmeal food per bottle, and reapportioned flies between bottles every 2–3 weeks. All flies were kept at 24°C on a 12L/12D schedule.

To estimate genetic variance components, we carried out a North Carolina II partial diallel design (Comstock and Robinson, 1952), using lines that were homozygous for different second chromosomes. We extracted second chromosomes from the GA98 caged population, using the *SM1/Bl* balancer stock, which had been crossed with GA98 to place the balancers on a GA genetic background. We crossed *SM1/Bl* males from this balancer stock to females from GA98 and then used standard procedures to generate F₃ progeny that were homozygous for the second chromosome (eg, Hughes, 1995). In all, we created 22 lines homozygous for the second chromosome. Ideally, one would place the balancer stock on the genetic background from which the chromosome will be extracted. In the present case, the genetic variation at chromosomes X, III and IV includes alleles from both the GA98 and balancer stock backgrounds, but is distributed randomly within and among genotypes, so it should not bias our overall estimates of genetic variance components for the second chromosome.

We selected 10 of these lines at random and crossed them using the North Carolina II partial diallel design (Comstock and Robinson, 1952). In this design, males and females of each of the five lines were crossed reciprocally with males and females of each of the other five lines. These crosses generated 25 heterozygous genotypes, with each cohort of flies identically heterozygous for the second chromosome. We also carried out within-line crosses to generate 10 lines that were fully homozygous for chromosome II, for a total of 35 lines. Offspring from each cross were raised in 12 half-pint plastic bottles. As part of a separate experiment, we also

generated a series of lines identically heterozygous for the second and third chromosome and homozygous for the dominant brown eye color mutation (*bw^D*) to assay sperm competitive ability (see below). We obtained these flies from a cross between two chromosome extraction lines, C57 and C288, derived from our GA98 population, each of which was homozygous for both the second and third chromosome and the brown dominant mutation. We maintained these flies in half-pint plastic bottles as described above in a 12L/12D schedule.

Assays

Mortality: To measure mortality in the 35 lines described above, we collected an average of 949 ± 132 (means $\pm$ SD) males per line. We apportioned virgin males from each line into five mortality cages, with an average of 203 ± 25 flies per cage. We created the mortality cages from 32 oz. plastic containers with mosquito netting in the center of the snap-on lid, which allowed for airflow. In the side, we cut a hole 2 cm in diameter near the bottom of the container. We fit a piece of plastic pipe approximately the circumference of an 8-dram vial, into which we inserted vials with 3 ml of standard yeast–agar–molasses–cornmeal food. Opposite the plastic pipe, we cut a small hole and fit it with a gusset through which we removed dead flies. Every other day, we replaced the food and removed and counted all dead flies. We maintained a cage until five or fewer flies remained.

To calculate the age-specific mortality rates for each genotype, we grouped the number of dead flies into 10-day bins. We calculated the instantaneous mortality at age x (μ_x) as

$$\mu_x = (-\ln(N_x + \Delta x / N_x)) / \Delta x \quad (1)$$

where N_x is the number of flies alive at day x and Δx is the bin size (in days) over which mortality is calculated (Lee, 1992). For the variance component analyses, we calculated mortality separately for each cage. For the inbreeding load analysis (see below), we pooled all cages within a genotype to calculate the mortality.

We used *Winmodest* (Pletcher, 1999), a maximum-likelihood estimator of parametric mortality models, to fit age at death data to the Gompertz model

$$\mu_x = Ae^{Bx} \quad (2)$$

the Gompertz–Makeham model,

$$\mu_x = Ae^{Bx} + M \quad (3)$$

and the Logistic–Makeham model,

$$\mu_x = \frac{Ae^{Bx}}{1 + S \cdot A/B(e^{Bx} - 1)} + M \quad (4)$$

Under the Gompertz model, on a natural log scale, $\ln(A)$ is the intercept and B is the slope of $\ln(\text{instantaneous mortality})$ versus age, x . The Makeham parameter, M , (equation (3)) provides an estimate of the age-independent extrinsic mortality. Large-scale demographic analyses have found that at very late ages, the age-related rate of increase in the mortality rates may slow (Carey *et al.*, 1992; Curtsinger *et al.*, 1992; Vaupel *et al.*, 1998). The values of S in equation (4) measure the degree to which mortality rates decelerate at late ages. *Winmodest* provided log-likelihood (LL) estimates for each of these models. We used equations (3) and (4) to test for

significant effects of deceleration using the LL ratio test (see Statistical analysis, below).

Fertility: We set up a separate set of identically constructed cages for male fertility estimates, with one cage of approximately 200 flies per genotype. Food for these cages was changed three times per week. For each fertility trial, we collected approximately 350 virgin female flies from the TW1 stock from 24 to 48 h before placing them individually in vials with a single virgin male. We collected 10 males from each of the 35 genotypes and maintained each group in an 8-dram vial with medium for 12–24 h. At this time, we anesthetized the flies on ice and placed females singly into the vials with single males. After the female had mated and laid approximately 30 eggs or 24 h had passed, whichever came first, we removed the adults and counted all the eggs. Vials were then placed at 24°C. At 14 days after the eggs were laid, we cleared the eclosed progeny into eppendorf tubes and froze them for later counting. Fertility was calculated as the percentage of eggs that eclosed into adults. We measured male fertility at ages 4, 9, 15, 22, 29, 36, 41, 51 and 61 days.

For one assay, at 15 days, we saved the mated females after they had laid eggs to test for female genetic variance in male sperm competitive ability. At 4 days after the original mating, we remated each female to a male with the dominant eye color mutation *bw^D*. After 24 h, we transferred the female to a new vial to lay eggs. After 4 days, we transferred the female to another vial and allowed her to lay eggs for 7 more days, after which the female was discarded. At 17 days after the eggs were laid, we collected the progeny from all the vials. We scored the progeny for eye color and counted them. We estimated the sperm competitive ability of the wild-type flies as P_1 , the proportion of the total progeny of each female that were wild type (Boorman and Parker, 1976).

Statistical analysis

Mortality rates typically increase exponentially with age after an initially declining or flat period (eg. Promislow *et al.*, 1996). This exponential increase leads to a positive mean-variance correlation among mortality estimates at different ages. To determine the relative contribution of additive and dominance genetic variance to mortality rates at different ages, we first need to remove this mean-variance correlation, which can lead to a spurious increase in the genetic variance components even if the true relative additive genetic variance is constant (Lynch and Walsh, 1998). One previous approach has been to transform the data such that each age class has a mean mortality of 1 (eg. Hughes and Charlesworth, 1994). Given that the mortality rates are log-normally distributed among genotypes within an age class, and that the genetic and experimental manipulations appear to act additively on the logarithm of mortality (Promislow and Tatar, 1998), we log transformed the mortality data to remove the significant mean-variance correlation (Promislow *et al.*, 1996).

The genetic variance components for mortality (μ) were estimated using the *Quercus* computer package, written and developed by Shaw and Shaw (1992, 1994) at the University of Minnesota. We used the *nf6* restricted maximum-likelihood program to estimate the variance and covariance components for the mortality data, using

a three-parameter model with additive variance, dominance variance and environmental variance (V_A , V_D and V_E). In the few cases where the three-parameter model would not converge to a solution for the maximum likelihood, we added a fourth parameter (maternal variance, V_M) to the model, after which the model converged. We tested for the significance of each parameter using the LL ratio test, where twice the absolute value of the difference in LL between models with and without the k parameters of interest is distributed as χ^2 with k degrees of freedom.

Even with very large sample sizes, low numbers of death at early and late ages can lead to biased estimates of genetic variance (Promislow *et al.*, 1996, 1999; Pletcher and Cutsinger, 2000). To correct for this sampling bias, Shaw *et al.* (1999) developed a new statistical approach. The challenge is to come up with a model that accounts for the fact that (a) mortality rates among genotypes are roughly log-normally distributed (Promislow *et al.*, 1996); (b) within a specific age cohort of a given genotype, the sampling variance for mortality is binomial or beta-binomial (Searle *et al.*, 1992); and (c) among age classes, the mortality rates increase exponentially with age, but the increase decelerates at late ages (Carey *et al.*, 1992; Cutsinger *et al.*, 1992; Vaupel *et al.*, 1998).

The model developed by Shaw *et al.* (1999) addresses these concerns by fitting a parametric model to the genotype-specific mortality data (the Gompertz–Logistic model, which is equivalent to equation (4) with $M=0$; Vaupel, 1990). The fitting process itself uses a Markov chain Monte-Carlo (MCMC) maximum-likelihood approach. The MCMC model provides continuous estimates of means and genetic variances for mortality rates, along with 95% confidence limits for the variance estimate. The MCMC approach facilitates finding maximum-likelihood solutions for complex models. The power of the model lies in its ability to account for sampling bias in the mortality data. However, as it currently stands, the MCMC method is limited to estimates of additive and environmental variance, so we also provide variance component estimates from *Quercus*. The use of both the models allows us to present unbiased and perhaps more biologically correct estimates of additive and environmental variance from MCMC, as well as the more complete model, including V_D and V_M , provided by *Quercus*, at the cost of some sampling bias.

To test for age-related changes in the ratio of V_D to V_A , we fit $\log(1 + V_D/V_A)$ versus age using first-order linear regression, where V_D and V_A were measured with *Quercus* using 10-day bins. The V_D/V_A ratio was log transformed to ensure that residuals from the model were normally distributed.

We also used longevity data to calculate the mean life expectancy. We used Winmodest to correct for censored data (ie, where known-age flies escaped during handling). As with mortality data, we estimated the genetic variance components for life expectancy in *Quercus* using a full six-parameter model (V_A , V_D , V_M , V_P , V_I and V_E), where V_P is the paternal variance and V_I is the variance due to nuclear by extranuclear epistatic interactions, as well as subsets of this model.

For the variance component analysis of fertility, the data tended to be left skewed. To normalize these data, we transformed them using the equation $\phi'_i = \ln(-\ln(\phi_i))$, where ϕ_i is the proportion of eggs that survive to

adulthood for male i . For samples where all eggs survived to adulthood, fertility equals one, and $\phi'_i = \ln(0)$ is undefined. Thus, if there was no egg–adult mortality, we arbitrarily set $\phi_i = 0.98$. The male fertility data sets (which were substantially larger than the mortality data sets, since data were obtained from individual flies, rather than aggregate cohorts) would not arrive at convergent solutions in *Quercus*. Accordingly, we estimated the additive variance for fertility based on the among-sire component from type III sums of squares using the PROC GLM procedure in SAS (SAS Institute, 2000). The additive variance was calculated as four times the sire mean square.

We tested for age-related changes in inbreeding depression for both mortality and fertility. Following Charlesworth and Hughes (1996), we calculated inbreeding depression as

$$(x) = (Z_{O,x} - Z_{I,x})/Z_{O,x} \quad (5)$$

where $z_{O,x}$ and $z_{I,x}$ are the average values at age x among all outbred lines and all inbred lines, respectively, for the negative of log mortality and absolute fertility ($-\ln(\mu_x)$ and ϕ_x , respectively). However, these estimates are biased by the fact that the denominator (z_O) decreases with age for both $-\ln(\mu_x)$ and ϕ_x . Thus, we may expect $I(x)$ to increase with age for mortality and fertility. Accordingly, we also estimated inbreeding depression as $z_O - z_I$. For the mortality analysis, we only included data up to 72 days, as only one of the 10 inbred lines had high survivorship beyond that age. We included fertility measures up to age 63 days. For both mortality and fertility, we tested for significant relationships between inbreeding load and age using least-squares regression.

Finally, to determine whether the variation in male fertility among genotypes could be explained by the variation in sperm competitive ability, we used *Quercus* to calculate the additive genetic covariance between fertility and sperm competitive ability for the data from 15-day-old males. We also tested for genotypic correlation using linear regression to compare fertility and sperm competitive ability across mean values for the 25 heterozygous genotypes, and across mean values for the 10 inbred genotypes.

Results

Age-specific mortality

Mortality rates followed a pattern similar to that found in previous studies of mortality in flies (Figure 1). The data are well described by the Gompertz–Makeham model (equation (3)), albeit with a slight initial decrease in mortality. Based on the LL ratio tests in Winmodest, there is only moderate evidence for mortality deceleration late in life. Significant deceleration was more common among inbred lines (5/10) than outbred lines (6/25). There appeared to be a strong genetic component to the leveling off (Table 1), as the six outbred lines that showed deceleration were products of crosses involving two particular lines (lines 8 and 10). This result suggests a strong influence of dominant alleles. Furthermore, in some cases where lines 8 and 10 were crossed with lines 34 and 47, no deceleration was seen. Lines 34 and 47 were among the few inbred lines that did not show deceleration. Among the pooled outbred lines, there is

Figure 1 Log-transformed mortality versus age for inbred (dashed lines) and outbred (solid lines) genotypes. Curves represent mortality smoothed over 6 days, with rates based on 947 ± 23 virgin male flies (means $\pm$ SE) per line. Note that most curves follow a standard Gompertz trajectory with little evidence of deceleration late in life (see text for discussion). The inset shows unsmoothed mortality curves per 2 days for pooled inbred flies (dashed lines, $n = 9163$ flies) and outbred flies (solid line, $n = 23987$ flies).

little evidence of deceleration (Figure 1, inset), although there does appear to be some deceleration among inbred lines. In contrast to previous studies with laboratory lines (eg, Pletcher and Curtsinger, 1998), late-age mortality rates are relatively high, with μ_x between 0.3 and 0.5 (–1.2 to –0.7 on a natural log scale) (Figure 1).

Based on the *Quercus* analysis, we found that the additive genetic variance (V_A) for age-specific mortality rates increased to a maximum value at age 50 days, after which it declined steadily (Figure 2). Maternal variance

Table 1 Crosses that showed significant mortality deceleration

Line	25	32***	34	42	47
8***	NS	***	NS	***	***
10***	***	***	NS	***	NS
12***	NS	NS	NS	NS	NS
13	NS	NS	NS	NS	NS
22***	NS	NS	NS	NS	NS

Row and column numbers refer to each of 10 chromosome extraction lines (see text). ***Significant evidence for deceleration ($P < 0.0001$ in all cases after sequential Bonferroni correction of 35 comparisons) based on the LL ratio test and row headings refer to inbred lines, while interior values refer to crosses between inbred lines.

showed a similar pattern, with no detectable variance in most early ages, and then highly significant variance from ages 40 to 80 days, although the levels were somewhat lower than for the additive variance. Dominance variance was significant at the earliest age, and also at middle ages, although V_D explained a much lower proportion of the total genetic variance than did V_A . Heritabilities range as high as 40% at age 50 days. After correcting for the fact that we are analyzing variation only for the second chromosome, this gives a heritability of close to 100%. The meaning of this heritability is questionable, given that the mortality rate is an aggregate measure of a large number of individuals, so we greatly underestimate the variance due to environmental effects and measurement error among individuals.

In contrast to the *Quercus* analysis of mortality, results based on Shaw *et al.*'s (1999) MCMC approach showed a monotonic decline in additive variance across all age classes (Figure 3). Overall, the V_A estimates were high. However, the MCMC model does not provide estimates for the dominance variance. The differences between the *Quercus* and MCMC models are explored further below.

In our test of inbreeding load using equation (5), we found an overall age-related increase in the difference

Figure 2 Genetic variance components for log-transformed mortality in virgin males at 10-day intervals. Error bars are standard deviations. Figures show environmental, additive, dominance and maternal variance (V_E , V_A , V_D and V_M , respectively). Tests for significance based on the LL ratio test (see text). Significance values after sequential Bonferroni correction (Holm, 1979) ($^{\dagger}P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$).

Figure 3 Age-specific additive genetic variance with 95% upper and lower confidence limits. These estimates are based on an MCMC maximum-likelihood model, which fits a parametric Gompertz–Logistic to the mortality data for all genotypes (Shaw *et al.*, 1999). Only estimates for additive genetic variance are available (see text).

between inbred and outbred mortality rates ($F_{1,11} = 85.9$, $r^2 = 0.89$, $P = 1.6 \times 10^{-6}$, Figure 4). This pattern was also seen when we defined inbreeding load as the difference between $\ln(\mu_x)$ for the average of inbred and outbred lines, $I = \ln(\mu_x)_{\text{inbred}} - \ln(\mu_x)_{\text{outbred}}$ ($F_{1,11} = 19.76$, $r^2 = 0.64$, $P = 0.00099$). Note that at later ages, there was no evidence of an effect of inbreeding on mortality (see Figure 1, inset). To test predictions from the AP model, we estimated the age-related change in the ratio of V_D/V_A . We plot the log-transformed ratios of $(1 + V_D/V_A)$ (to increase linearity of the fit), which show a highly significant decline as a function of age (Figure 5) (least-squares regression, $r^2 = 0.58$, $P = 0.0103$). A non-parametric analysis gives similar results (Kendall's $\tau = -0.629$, $P = 0.013$). This decline is predicted by Charlesworth and Hughes' (1996) model of AP.

AP models also predict a negative correlation between early- and late-age fitness traits, but we found no such pattern. Of 35 pair-wise comparisons across all ages, only three were negative and none significantly so. Statistically significant covariances tended to be positive, and clustered across older age classes, suggesting that genes that decrease mortality late in life often have effects across several age classes (Figure 6). While only two comparisons remained significant after correction for multiple comparisons (Holm, 1979), this is probably an overly conservative test, because 12 of the 35 comparisons were significant at least at the 5% level before correction. The observed positive correlations are consistent with the findings from previous analyses of covariance for mortality rates across ages (Promislow *et al.*, 1996).

We also estimated genetic variance components for life expectancy. The full six-parameter *Quercus* model would not provide a convergent solution. However, we were able to fit two smaller models, one with five parameters including additive, dominance, environmental, maternal

Figure 4 Inbreeding load for male mortality, based on the difference between average mortality among all inbred and all outbred lines for each of thirteen age-classes (equation (5)). Inbreeding load increases significantly with age ($P = 1.6 \times 10^{-6}$).

Figure 5 Ratio of the dominance variance to additive variance for mortality as a function of age. We plotted data on the Y-axis as $\log(1+x)$, where $x = V_D/V_A$. The log transformation linearized the relationship, and we added 1 to eliminate the problem of log-transforming zero values at ages 30 and 90 days, when $V_D = 0$. Values for dominance variance were obtained from a four-component analysis of genetic variance, –as shown in Figure 2. Based on least-squares regression, $r^2 = 0.58$, $P = 0.0103$.

and paternal variance (A, D, E, M and P) and one with four parameters, including additive, dominance, environmental and interaction variance (A, D, E and I). In the five-parameter model, neither maternal nor paternal variance is significant ($P > 0.15$, based on an LL ratio test). The four-parameter model provided evidence for significant additive variance ($\chi^2_1 = 5.38$, $P = 0.020$) and significant nuclear $\times$ extranuclear interaction variance ($\chi^2_1 = 15.1$, $P = 0.0001$).

Figure 6 Additive genetic covariance for log(mortality) across ages. Note that most covariances are positive, and values are highest for late-age mortality rates.

Figure 7 Average of male fertility for inbred (open squares) and outbred (solid squares) genotypes from ages 4 to 63 days. Data based on 100 males (10 inbred strains) or 250 males (25 outbred strains) for each age.

Fertility

The mean fertility levels were very high, with averages close to 90% for males at most early ages (Figure 7). The data are non-normally distributed, with a strong left skew, due to the high number of males with relatively high fertility. This situation was improved substantially by the $\ln(-\ln(\phi_x))$ transformation. In our *Quercus* analysis, we found no significant variance components for male fertility at any age (data not shown). However, the high fertility rates led to relatively low statistical power in *Quercus*, and the likelihood analysis frequently failed to converge. Based on type III sums of squares analysis using PROC GLM in SAS, we found significant additive variance at both early (9 days) and later (36 and 51 days) ages, with a general trend of initial decline followed by subsequent increase to age 61 (Figure 8).

Figure 8 Variance (open squares) and coefficient of additive variance (solid squares) for age-specific male fertility. * $P < 0.05$, ** $P < 0.01$.

Figure 9 Inbreeding load for male fertility, based on the ratio of asin (square-root)-transformed mean male fertility values for inbred and outbred genotypes (see text for details). Inbreeding load increases significantly with age ($P = 0.00018$).

However, we cannot test for a decline at very late ages, as was seen in our mortality analysis, because males were no longer fertile after about 9 weeks of age.

Inbreeding depression (equation (5)) for fertility was positively correlated with age ($F_{1,7} = 47.8$, $r^2 = 0.87$, $P = 0.00023$) (Figure 9), as predicted by MA theory. This pattern is also seen if we define inbreeding depression, $I = \phi_0 - \phi_1$ ($F_{1,7} = 24.3$, $r^2 = 0.78$, $P = 0.0017$).

Finally, although we only measured the sperm competitive ability for males at 15 days, we found a significant positive correlation between 15 days fertility

and P1, the ability of males to 'defend' against insemination from a subsequent male. This correlation appeared to be due to dominance covariance (likelihood ratio test: $\chi^2_1 = 8.90$, $P = 0.0028$).

Discussion

Mortality rates typically approximate a Gompertz or Gompertz–Makeham function (Finch *et al*, 1990; Promislow, 1991; Ricklefs, 1998). Consistent with these previous studies, the mortality curves in this study show a clear Gompertz–Makeham pattern, although we see a slight dip in mortality early in life (Figure 1). This initial drop in mortality at early ages, giving the so-called 'bathtub shape' mortality pattern, is common among many species, including humans (eg, Promislow, 1991). Larger-scale studies of age-specific mortality rates have found that at very late ages, the increase in mortality begins to decelerate (eg, Curtsinger *et al*, 1992; Vaupel *et al*, 1998) and late-age mortality rates can sometimes even decline with age (Carey *et al*, 1992). Numerous explanations have been put forward to explain these observations. In this study, we found some evidence for mortality deceleration in inbred lines, but very little evidence for this phenomenon in outbred lines.

Most studies that have found leveling off of mortality at late ages have used lab-adapted lines of flies (Promislow and Tatar, 1998). It may be that deceleration at late ages is somehow caused by the genetic architecture that evolves under laboratory culture, although we also see deceleration in other types of populations, including nematodes and humans (Vaupel *et al*, 1998). Others have suggested that deceleration may be related to the costs of reproduction (Sgró and Partridge, 1999; Charlesworth, 2001). Our results are consistent with this latter hypothesis, as the flies analyzed here were held as virgins and did not show extensive deceleration. However, we cannot determine whether this is causally related to the lack of leveling off, as we did not maintain control cages with reproductively active flies.

In their analysis of age-specific genetic variance for mortality, Promislow *et al* (1996) found that the additive genetic variance declined at late ages. As one explanation for this unexpected decline, they suggested that variation in mortality may simply be a reflection of variation in reproduction. If reproduction leads to a direct and immediate increase in the mortality rate, genotypes with relatively high reproductive effort at a given age will have relatively high mortality rates at that age. However, as individuals reach postreproductive ages, investment in reproduction declines, and so variation in mortality declines. To test this hypothesis, we replicated the large-scale approach used by Promislow *et al* (1996), but filled our mortality cages with only virgin males. As with earlier large-scale demographic studies, we found that the additive variance for mortality rates still declined at late ages. Thus, it is unlikely that the decline in the genetic variance is due to decreasing costs of reproduction at late ages. We assume here that virgin males pay a considerably lower cost of reproduction than males in a mixed sex environment. However, as the males in this experiment still had testes and produced sperm and accessory gland proteins, not all costs of reproduction were eliminated.

Two separate statistical approaches, *Quercus* and MCMC, detected a significant decline in the additive variance late in life. Surprisingly, the MCMC analysis revealed a pattern of a steady decline in V_A throughout life. The high initial additive variance that we observed was also seen in Shaw *et al*'s (1999) reanalysis of data from Promislow *et al* (1996) and from one of the five independent experiments carried out by Hughes and Charlesworth (1994). There are fundamental differences between these two types of analyses, each with its own strengths and weaknesses. The *Quercus* analysis estimates genetic variance components at discrete and independent time intervals, making no assumption about the underlying age-specific trajectory of mortality rates or variance components. However, it fails to account for differences in error variance due to age-related changes in the sample size. Shaw *et al*'s (1999) MCMC method controls for sampling effects, but relies on fitting a family of parametric curves to the data, thus smoothing over differences among age classes.

The fact that the *Quercus* and MCMC methods both show a decline in late-age additive variance gives us confidence that this pattern is real. Whether early additive variance is relatively high, as suggested by the MCMC method and consistent with some earlier studies (eg, Tatar *et al* 1996), or low, as suggested by the *Quercus* approach, remains an open question.

Nevertheless, results from both *Quercus* and MCMC analyses should be considered with due caution. Pletcher and Curtsinger (2000) point out that in a heterogeneous population, the decline in the additive variance could be a sampling artifact, as individuals with intrinsically high mortality rates die off the soonest. While traditional methods have been subject to extensive simulation analysis to test for bias (Pletcher and Curtsinger, 2000), no such tests have been carried out on the newer MCMC method. Furthermore, the MCMC method has only been implemented to detect environmental and additive genetic variance.

As a final note regarding survival, it was of some interest to find significant variation for nuclear $\times$ extranuclear interaction. This pattern has not been explored in previous quantitative genetic assays of mortality. To the extent that this term is influenced by interactions between a father's nuclear genotype and a mother's mitochondria, this result is consistent with the suggestions from diverse fields that mitochondria may play an important role in the aging process (Harman, 1972; Avise, 1993; Kann *et al*, 1998).

In our analysis of additive variance for male fertility, we found an initial decline, followed by a steady increase. This pattern was similar to that observed in earlier studies of female fecundity (eg, Tatar *et al*, 1996). The high early additive variance could be due to variation not in fertility *per se*, but in the age at maturity of males. Moreover, while the steady increase in additive variance at later ages is consistent with MA, we were not able to collect data on fertility at very late ages, when the males were still alive but no longer fertile. These results are limited by the fact that egg sample sizes per vial were typically on the order of 30 eggs. Thus, we could not observe egg mortality levels lower than 1/30, or approximately 3%. In the cases where no egg mortality was observed, we arbitrarily set the level at 2%. Future

studies of fertility will need to use substantially larger sample sizes to increase the statistical power.

Until recently, an age-related increase in the additive genetic variance was taken as evidence for MA, although AP could also give rise to this pattern. Recent theoretical advances offer a pair of novel tests that might allow us to distinguish between the MA or AP models of senescence. First, the ratio V_D/V_A should decrease with AP and increase with MA (Charlesworth and Hughes, 1996). Our results show that even at late ages, when V_A decreases to near 0, the V_D/V_A ratio still decreases, consistent with the AP model. This suggests that the decline in this ratio is not simply due to an age-related increase in V_A as expected under MA. Unfortunately, dominance estimates are not provided by the unbiased MCMC model, so we need to bear in mind that variance component estimates at late ages for both additive and dominance components may be biased by sampling error.

Interestingly, at the same time that we found strong evidence for AP from V_D/V_A ratios, we also found support for the MA model of senescence. Charlesworth and Hughes' (1996) second prediction is that inbreeding depression should increase with MA but not with AP. For both mortality and fertility, age-specific inbreeding load increased, offering strong support for the MA model. These results are in line with Charlesworth and Hughes' (1996) earlier study. We should note that our analysis calculated the inbreeding load for log-transformed measures of mortality, although the original model (Charlesworth and Hughes, 1996) was based on the analysis of age-specific survival rate. Given that the mortality rates increase linearly with age on a logarithmic scale, we would expect the inbreeding load to appear to increase for survival for scaling reasons alone (see Promislow *et al.*, 1999). In fact, the inbreeding load for survival rates increases exponentially with age (data not shown). In any case, the male fertility rates, which do not suffer from this scaling problem, also show an age-specific increase in inbreeding depression, lending further support to the model.

These scaling issues are central to how we interpret results from quantitative genetic studies of mortality. The scale of mutational effects can influence the expected pattern of variance under MA. Consider Charlesworth's (2001) model for the evolution of senescence. In his model, the age-specific mortality rate at age z , $\mu(z)$, is described by

$$\mu(z) \approx \gamma + v + ve^{\gamma(z-b)} \quad (6)$$

where γ is a measure of the rate of aging, v is the density of new mutations for each age (arbitrarily set to 1 in Charlesworth (2001)) and b is approximately the age at onset of demographic senescence. Charlesworth goes on to show that the age-specific variance for the mortality rate is a monotonically increasing function of age,

$$V(\mu(z)) \propto \delta\mu e^{\gamma(z-b)} \quad (7)$$

(from equation (A.2) in Charlesworth, 2001), where $\delta\mu$ is the age-independent effect of mutations on mortality.

We can use the Delta method to determine the expected age-related change in the variance for log (mortality):

$$V(\log(\mu(z))) \approx V(\mu(z))/(\mu(z))^2 \quad (8)$$

If we assume that mutations act additively on mortality (ie, $\delta\mu$ is independent of age), then variance increases initially, reaching a maximum at approximately b , and then declines. This pattern is similar to what we observed in our data. However, previous studies suggest that mutations act additively on the logarithm of mortality (Promislow and Tatar, 1998). In this case, we note that $\delta\mu$ is a function of age, such that $\delta\mu(z) = K\mu(z)$. Substituting $\delta\mu(z)$ into equations (7) and (8) and taking the first derivative of $V(\log(\mu(z)))$ with respect to z , it is straightforward to show that the variance of log(mortality) is a monotonically increasing function of age. Thus, it is premature to conclude that our results are truly inconsistent with MA models for the evolution of senescence. We need further evidence for the effects of novel mutations on mortality rates, and a formal model that assumes multiplicativity rather than additivity of mutational effects.

Even for traits other than age-specific mortality rates, scaling effects continue to dog our efforts to test aging theory using quantitative genetic assays. In a recent test of Charlesworth and Hughes's (1996) model, Hughes *et al.* (2002) used a crossing design similar to the one used here to estimate genetic variance components for reproductive success (measured as the number of offspring produced by five males and five females). Reproductive success is approximately Poisson distributed, so as the mean declines with age, so does the total variance. To remove the mean-variance relationship that could confound their analysis, Hughes *et al.* (2002) square root transformed the data, and then standardized the data so that each block and age had a mean of 1.0. For Poisson-distributed data over a broad range of values ($0.1 < \bar{x} < 50$), this procedure of transformation and standardization creates a negative correlation between the variance and the mean, with $\sigma^2 = \frac{1}{2}\bar{x}^{-5/4}$. Given that the mean declines with age, the variance will thus appear to increase for the transformed, standardized data, even if no such increase actually occurs biologically.

Taken together, our results lead us to consider two distinct possibilities. First, we might conclude that aging in some fitness traits is due primarily to MA, while aging in other traits is due primarily to AP. For example, the genetic variance in traits related to male reproductive fitness (in particular, sperm competitive ability) appears to be due primarily to dominance variance (Hughes, 1995; Mack, 2001), while the genetic variation for mortality appears to be due primarily to additive variance (eg, Hughes and Charlesworth, 1994; Promislow *et al.*, 1996).

What might lead to such differences among traits? In the case of fertility-related traits, their underlying genetic structure may be shaped in part by the conflict of interest between males and females (Svensson and Sheldon, 1998). This conflict could lead to the maintenance of variation for fitness traits through the effect of genes with sexually antagonistic pleiotropic effects (Clark *et al.*, 1995; Chippindale *et al.*, 2001). At the same time, segregating variation may be due in part to alleles that are maintained through mutation-selection balance. In the case of mortality rates in this study, we find evidence for the influence of both MA and AP. Quantitative traits such as survival are likely to be influenced by an enormous number of genes (Price and Schluter, 1991), and many of these are likely to affect aging (Rose, 1991). It is unlikely

that all these aging-related genes act in the same manner, or have arisen due to identical selective pressures in the past.

The last 20 years have seen continued attempts to determine which of these two basic evolutionary genetic models can explain the origin and maintenance of senescence (Rose and Charlesworth, 1980; Hughes and Charlesworth, 1994; Charlesworth and Hughes, 1996; Promislow *et al.*, 1996; Tatar *et al.*, 1996). The work we have presented leads us to conclude that both processes may well play a role in the origin and maintenance of genetic variation for aging. These conclusions should not lead us to stop our inquiry into the evolutionary genetics of aging. Rather, we suggest that the time has arrived for alternative approaches to the standard tests of AP and MA. First, as we apply the power of modern molecular genetics to identify specific genes or gene regions that influence aging (eg, Johnson, 1990; Lin *et al.*, 1998, 2000; Rogina *et al.*, 2000; Clancy *et al.*, 2001; Tatar *et al.*, 2001), we may be able to examine directly whether MA or AP maintains variation at individual loci. Second, we need to explore variation in patterns of aging in natural populations. Finally, as we focus so much of our energy on the genetic causes of aging, so too should we consider the evolutionary and ecological consequences of aging. Using the strengths of all these areas will allow us to more completely understand the underlying genetic architecture and evolution of aging.

Acknowledgements

We thank the Promislow lab for logistical support, and especially Louise Pearse and Jackie Kerr for untiring help. We received valuable comments on a previous version of this manuscript from W Anderson, D Gomulkiewicz, T Haselkorn, D Hoyt, A Keyser, P Mack, N Priest, C Spencer and two anonymous referees. This work was funded by National Institute on Aging Grant AG14027 to DP.

References

- Awise JC (1993). The evolutionary biology of aging, sexual reproduction, and DNA-repair. *Evolution* **47**: 1293–1301.
- Boorman E, Parker GA (1976). Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol Entomol* **1**: 145–155.
- Carey JR, Liedo P, Orozco D, Vaupel JW (1992). Slowing of mortality rates at older ages in large medfly cohorts. *Science* **258**: 457–461.
- Charlesworth B (1990). Optimization models, quantitative genetics, and mutation. *Evolution* **44**: 520–538.
- Charlesworth B (2000). Fisher, Medawar, Hamilton and the evolution of aging. *Genetics* **156**: 927–931.
- Charlesworth B (2001). Patterns of age-specific means and genetic variances of mortality rates predicted by the mutation accumulation theory of aging. *J Theor Biol* **210**: 47–65.
- Charlesworth B, Hughes KA (1996). Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. *Proc Natl Acad Sci* **93**: 6140–6145.
- Chippindale AK, Gibson JR, Rice WR (2001). Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc Natl Acad Sci USA* **98**: 1671–1675.
- Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E *et al.* (2001). Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* **292**: 104–106.
- Clark AG, Aguade M, Prout T, Harshman LG, Langley CH (1995). Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. *Genetics* **139**: 189–201.
- Comstock RE, Robinson HF (1952). Estimation of average dominance of genes. In: Gowen JW (ed), *Heterosis*. Iowa State College Press Ames: IA. pp 494–516.
- Curtis JW, Fukui HH, Townsend DR, Vaupel JW (1992). Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*. *Science* **258**: 461–463.
- Finch CE, Pike MC, Witten M (1990). Slow mortality rate accelerations during aging in some animals approximate that of humans. *Science* **249**: 902–906.
- Fisher RA (1930). *The Genetical Theory of Natural Selection*. Clarendon Press: Oxford.
- Haldane JBS (1941). *New Paths in Genetics*. Allen and Unwin: London.
- Hamilton WD (1966). The moulding of senescence by natural selection. *J Theor Biol* **12**: 12–45.
- Harman D (1972). The biologic clock: the mitochondria? *J Am Geriatr Soc* **20**: 145–147.
- Holm S (1979). A simple sequential rejective multiple test procedure. *Scand J Stat* **6**: 65–70.
- Hughes KA (1995). The evolutionary genetics of male life-history characters in *Drosophila melanogaster*. *Evolution* **49**: 521–537.
- Hughes KA, Alipaz JA, Drnevich JM, Reynolds RM (2002). A test of evolutionary theories of aging. *Proc Natl Acad Sci USA* **99**: 14286–14291.
- Hughes KA, Charlesworth B (1994). A genetic analysis of senescence in *Drosophila*. *Nature* **367**: 64–66.
- Johnson TE (1990). Increased life-span of age-1 mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. *Science* **249**: 908–912.
- Kann LM, Rosenblum EB, Rand DM (1998). Aging, mating, and the evolution of mtDNA heteroplasmy in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **95**: 2372–2377.
- Lee ET (1992). *Statistical Methods for Survival Data Analysis*. 2nd edn.. John Wiley & Sons, Inc.: New York.
- Lin SJ, Defossez PA, Guarente L (2000). Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* **289**: 2126–2128.
- Lin YJ, Seroude L, Benzer S (1998). Extended life-span and stress resistance in the *Drosophila* mutant methuselah. *Science* **282**: 943–946.
- Lynch M, Walsh B (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Inc.: Sunderland, MA.
- Mack PD (2001). Sperm competition and the role of females in *Drosophila melanogaster*. PhD, University of Georgia.
- Medawar PB (1952). *An Unsolved Problem in Biology*. H.K. Lewis: London.
- Partridge L, Fowler K (1992). Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* **46**: 76–91.
- Pletcher SD (1999). Model fitting and hypothesis testing for age-specific mortality data. *J Evol Biol* **12**: 430–440.
- Pletcher SD, Curtis JW (1998). Mortality plateaus and the evolution of senescence: why are old-age mortality rates so low? *Evolution* **52**: 454–464.
- Pletcher SD, Curtis JW (2000). The influence of environmentally induced heterogeneity on age-specific genetic variance for mortality rates. *Genet Res* **75**: 321–329.
- Pletcher SD, Geyer CJ (1999). The genetic analysis of age-dependent traits: modeling the character process. *Genetics* **153**: 825–835.
- Price T, Schluter D (1991). On the low heritability of life-history traits. *Evolution* **45**: 853–861.

- Promislow DEL (1991). Senescence in natural populations of mammals: a comparative study. *Evolution* **45**: 1869–1887.
- Promislow DEL, Tatar M (1998). Mutation and senescence: where genetics and demography meet. *Genetica* **102/103**: 299–314.
- Promislow DEL, Tatar M, Khazaeli A, Curtsinger JW (1996). Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. *Genetics* **143**: 839–848.
- Promislow DEL, Tatar M, Pletcher S, Carey JR (1999). Below-threshold mortality: implications for studies in evolution, ecology and demography. *J Evol Biol* **12**: 314–328.
- Ricklefs RE (1998). Evolutionary theories of aging: confirmation of a fundamental prediction, with implications for the genetic basis and evolution of life span. *Am Nat* **152**: 24–44.
- Rogina B, Reenan RA, Nilsen SP, Helfand SL (2000). Extended life-span conferred by cotransporter gene mutations in *Drosophila*. *Science* **290**: 2137–2140.
- Rose MR (1991). *Evolutionary Biology of Aging*. Oxford University Press: Oxford.
- Rose MR, Charlesworth B (1980). A test of evolutionary theories of senescence. *Nature* **287**: 141–142.
- SAS Institute (2000). *SAS/STAT User's Guide*. SAS Institute: Cary, NC.
- Searle SR, Casella G, McCulloch CE (1992). *Variance Components*. Wiley: New York.
- Sgró CM, Partridge L (1999). A delayed wave of death from reproduction in *Drosophila*. *Science* **286**: 2521–2524.
- Shaw FH, Promislow DE, Tatar M, Hughes KA, Geyer CJ (1999). Toward reconciling inferences concerning genetic variation in senescence in *Drosophila melanogaster*. *Genetics* **152**: 553–566.
- Shaw RG, Shaw FH (1992, 1994) Quercus: programs for quantitative genetic analysis using maximum likelihood. Published electronically on the Internet, available via anonymous ftp from ecology.umn.edu: directory path pub/quercus and from ftp.bio.indiana.edu; directory path biology/quantgen/quercus.
- Svensson E, Sheldon BC (1998). The social context of life history evolution. *Oikos* **83**: 466–477.
- Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS (2001). A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**: 107–110.
- Tatar M, Promislow DEL, Khazaeli A, Curtsinger J (1996). Age-specific patterns of genetic variance in *Drosophila melanogaster*. II. Fecundity and its genetic correlation with age-specific mortality. *Genetics* **143**: 849–858.
- Vaupel JW (1990). Relatives' risks: frailty models of life history data. *Theor Popul Biol* **37**: 220–234.
- Vaupel JW, Carey JR, Christensen K, Johnson TE, Yashin AI, Holm NV *et al* (1998). Biodemographic trajectories of longevity. *Science* **280**: 855–860.
- Wachter KW, Finch CE (1997). *Between Zeus and the Salmon: The Biodemography of Aging*. National Academy Press: Washington, DC.
- Williams GC (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**: 398–411.