Age-specific mortality rates of reproducing and non-reproducing males of *Drosophila melanogaster*

Takahiro Miyo* and Brian Charlesworth

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3JT, UK

The proximate and evolutionary causes of the levelling of mortality rates at late ages, observed in several species, remain obscure. To investigate the causes of mortality levelling late in life in *Drosophila melanogaster*, we examined the effect of reproduction on mortality patterns, by conducting population cage experiments with a total of more than 45 000 individuals. Several different genotypes of reproducing and non-reproducing males from F₁ crosses among isogenic lines were studied. Our results suggest that significant mortality levelling can occur even in non-reproducing males, but that reproduction also significantly affects mortality patterns. The results show that mortality levelling is strongly affected by the Gompertz initial mortality rate and exponential rate of increase parameters, probably through the effects of heterogeneity in mortality risks.

Keywords: ageing; mortality levelling; *Drosophila melanogaster*; cost of reproduction; Gompertz function

1. INTRODUCTION

Senescence, the decline in a wide range of bodily functions with age, is an almost universal phenomenon and has been observed in most species of animals and plants that have been studied (Charlesworth 1994). Senescence can be characterized in terms of the linear increase with age in the logarithm of the age-specific mortality rate, described by the Gompertz model (Gompertz 1825; Gavrilov & Gavrilova 1991; Vaupel 1997; Charlesworth 2001). Recent demographic studies using large cohorts of individuals have revealed that this linearity is often violated late in life, when the graph of log mortality rate against age exhibits flattening at advanced ages (Carey *et al.* 1992; Promislow *et al.* 1996). This phenomenon has been observed in several species, including humans, medflies, fruitflies, beetles, and nematodes (Curtsinger *et al.* 1995; Vaupel 1997).

The causes of this mortality levelling late in life are controversial. It may be caused in part by genetic or environmental heterogeneity in the rate of ageing among individuals within a cohort (Promislow *et al.* 1996; Service 2000), although genetically homogeneous cohorts still exhibit mortality plateaux late in life (Curtsinger *et al.* 1992). Another hypothesis proposes that mortality levelling late in life reflects a corresponding plateau in the force of natural selection, which remains at zero after the end of reproduction (Rose & Mueller 2000). Because the flattening in mortality curves approximately coincides with the age at which reproduction ends, it seems reasonable to assume that mortality levelling reflects the corresponding plateau in the force of natural selection (Rose *et al.* 2002). However, a survival cost of reproduction in *Drosophila melanogaster* has been well documented: for example, the effect of reproductive activity on longevity of males (Partridge & Farquhar 1981; Partridge & Andrews 1985), and the effect of egg production on female longevity

(Partridge *et al.* 1987; Sgró & Partridge 1999). The cessation of reproduction may, therefore, itself reduce risk and damage, which could contribute to mortality rate levelling after the cessation of reproduction (Charlesworth & Partridge 1997; Charlesworth 2001). In medflies and *D. melanogaster*, mortality curves from large cohorts of genetically homogeneous individuals have mostly been obtained from actively reproducing individuals, so there is a possibility that levelling could be associated with reduced mortality after the cessation of reproduction. The results of Sgró & Partridge (1999) suggest that this may be the case for females.

To explore this possibility further, it is necessary to examine the mortality patterns of large non-reproducing cohorts. In this study, we have investigated the effects of reproduction on mortality patterns of males, by conducting population cage experiments with large samples of reproducing and non-reproducing *D. melanogaster* males. The mortality of more than 45 000 flies was followed, to obtain accurate mortality curves for non-reproducing and reproducing males.

2. MATERIAL AND METHODS

(a) *Drosophila stocks*

To obtain mortality curves, three pairs of isogenic lines, IS-5, IS-24 and IS-25 of *D. melanogaster* (Meigen) were used for collecting virgin male flies from all six F₁ reciprocal crosses among them. F₁ flies were used to minimize contributions to mortality levelling from genetic and environmental causes of variation (Promislow *et al.* 1996; Service 2000). In addition, the genotypes of F₁ flies are similar to those of individuals from natural populations, and generally have much greater longevity than inbred lines (Fukui *et al.* 1996).

These lines are homozygous for all three major chromosomes, which were originally isolated from flies collected from a natural population at Beltsville, MD (Charlesworth & Lapid 1989; Charlesworth *et al.* 1992). The three lines were chosen solely on the basis of having sufficient productivity for reliable stock

* Author for correspondence (tmiyo1@staffmail.ed.ac.uk).

maintenance. The fourth chromosome of these lines was made homozygous for the recessive marker *spa^{pol}*, to detect any accidental contamination. The details of the mating programme for the construction of these lines were described by Charlesworth *et al.* (1994). The stocks were maintained in three-quarters of a pint (ca. 300 mL) milk bottles with standard Lewis cornmeal medium, in an incubator at $25 \pm 1^\circ\text{C}$ with a photoperiod of 12 L: 12 D (07.30–19.30).

(b) Population cages and mortality assays

The mortality of a cohort of ca. 800 adult flies was followed in a population cage. Population cages were made from plastic fish tanks (20 cm wide by 30 cm long by 20 cm high), with an opening in the side for collecting dead flies and supplying food and water for flies. The opening was covered with ca. 30 cm of nylon stocking, truncated at both ends and fastened with two rubber bands.

Virgin male flies from F_1 reciprocal crosses among the three isogenic lines were collected from bottles using gentle CO_2 anaesthetization. After collection, male flies were stored in small glass vials (24 mm diameter by 74 mm high). For experiments involving 'non-reproducing' males, ca. 800 virgin males that had newly emerged over a 72 h period were released into a population cage (mean 807.04, s.e.m. 16.67; $n = 27$). For experiments involving 'reproducing' males, the same genotypes were provided with approximately equal numbers of females, yielding the same total density of flies (435.87 ± 4.73 for males, and 437.93 ± 6.41 for females).

Dead flies were collected from each cage three times a week, using a paintbrush. Flies stuck on the medium were first removed, and were collected if they were dead. In common with most published studies, we did not control cohort densities by supplying visible mutant flies, because it has been shown that age-specific mortality trajectories are generally similar in both density-controlled and naturally declining cages (Khazaeli *et al.* 1996). After collecting the dead flies from the cage, a Petri dish containing food medium was inserted, replacing the old one. About every 10 days, a vial containing distilled water and plugged with an absorbent cotton ball was exchanged for a fresh vial. Population cages were stored in an incubator at $25 \pm 1^\circ\text{C}$ with a photoperiod of 12 L: 12 D (07.30–19.30), and were frequently rotated around the incubator to reduce the effect of micro-environmental variation within the incubator.

The dead flies that had been collected were sexed when necessary, based on observation under a stereomicroscope, and counted. Although we manipulated the population cages as gently as possible and tried to prohibit flies from escaping, some flies escaped, and some dead flies had decayed too severely to determine their sex. These flies were discarded from the analyses.

Three to five replicate cages were set up for each genotype-treatment combination. The mortality of a total of 46 283 flies was followed to obtain mortality curves for non-reproducing males and reproducing males and females (774.37 ± 16.09 for non-reproducing males ($n = 27$); 420.33 ± 4.99 for reproducing males ($n = 30$); 425.50 ± 6.68 for reproducing females ($n = 30$)). These sample sizes should be large enough to detect mortality levelling late in life, based on previous studies (Pletcher 1999).

(c) Mortality rate estimation

We collected, sexed and counted dead flies in each cage three times a week, to estimate age-specific mortality rates on a weekly basis. For example, if a cage was set up on a Tuesday, dead flies were collected on Tuesday, Thursday and Saturday, until all flies had died. Consequently, census intervals varied, depending on the

day of observation and the day on which the population cage was set up. We have therefore based our statistical analyses on weekly mortality rates, which also reduces random fluctuations (Pletcher *et al.* 1998). After the last death was observed in a cage, the number of flies in the initial cohort (N_0) was then calculated as the sum of the deaths over all census periods (Promislow *et al.* 1996). Because dead flies were collected on the same days of each week, the number of individuals in a cohort could be calculated every 7 days. The age-specific weekly mortality rate, μ_x , was then estimated as

$$\mu_x = -\ln(P_x), \quad (2.1)$$

where $P_x = N_{x+1}/N_x$ represents the fraction surviving from week x to week $x+1$ (Promislow *et al.* 1996).

(d) Mortality curves

The natural logarithm of mortality rate, $\ln(\mu_x)$, was plotted against age. Mortality curves are often described by the Gompertz equation:

$$\mu_x = ae^{bx}, \quad (2.2)$$

where a represents the initial mortality rate at birth, and b represents the rate of exponential increase in mortality with age (Gavrilov & Gavrilova 1991; Pletcher *et al.* 1998). This model assumes a linear increase with age in the logarithm of the age-specific mortality rate. However, recent studies on the demography of large cohorts of animals have shown that this linearity tends to be violated late in life, when the increase in mortality rate slows down (Carey *et al.* 1992; Curtsinger *et al.* 1992).

To evaluate the mortality levelling late in life, we used a maximum likelihood software package, WINMODEST (Pletcher 1999) to fit the logistic frailty model for the mortality data:

$$\mu_x = ae^{bx} / [1 + (as/b)(e^{bx} - 1)], \quad (2.3)$$

where a represents the initial mortality rate at birth, b represents the rate of exponential increase in mortality with age, and s indicates the degree of deceleration in mortality at older ages (Pletcher 1999). The Gompertz model (equation (2.2)) is a special form of the logistic frailty model (equation (2.3)), for $s = 0$. Large s values indicate more rapid mortality levelling at advanced ages (Pletcher 1999).

For fitting the logistic frailty model, the mortality data obtained for the first three weeks were truncated from our analyses, because of the sharp decreases in mortality after initiating the cages (see figure 1). Time was rescaled so that each population cage started at day 21. Rather than fitting a logistic-Makeham model, which assumes age-independent mortality rates early in life (Pletcher 1999), we truncated the data for the first three weeks and fitted a logistic model, as was done by Promislow *et al.* (1996). After this time, mortality rates tended to increase with age, as assumed by the logistic model.

(e) Statistical procedures

A two-way analysis of variance (ANOVA) was conducted for each parameter, taking two independent variables, genotypes and reproductive status, into account (PROC GLM) (SAS Institute 1985; Cody & Smith 1991). As is often found in large-scale experiments, we had two unusual cages, which showed rapid increases in mortality rates early in life. As a result, these two cages showed extremely high b and s parameters. We therefore treated these cages as significant outliers ($p < 0.01$, on both a t -test and Dixon's test (Grubbs 1969)) and removed them from the analyses. Even after removing these two cages, the mortality rate levelling parameter, s , still showed somewhat high variation and was

Figure 1. Mortality rate trajectories, plotted on a log scale against age, for (a) reproducing and (b) non-reproducing males. Each mortality curve is based on average of 420 reproducing males or 774 non-reproducing males.

distributed non-normally. In the case of the levelling parameter s , therefore, the significance of the F -values was also tested by permutation tests, in which 1000 permutation replications were created from the original data by resampling without replacement (Efron & Tibshirani 1993). Each mortality rate levelling parameter s was randomly assigned to one of the genotype-treatment combinations, using a random number generator, RANUNI(0) (SAS Institute 1985; Cody & Smith 1991). The procedure for a two-way ANOVA was then applied to each of the permutation replications (PROC GLM) (SAS Institute 1985). Achieved significance levels (ASL) were estimated as the proportion of the replications that produced larger F -values than the observed F -values out of 1000 replicate permutations.

It has been demonstrated that, under the logistic frailty model, the mortality levelling parameter s in *D. melanogaster* is negatively correlated with the initial mortality rate parameter a and positively correlated with the exponential increase parameter b (Promislow *et al.* 1996; Service 2000). We tested for these effects by obtaining correlation coefficients among these variables, using genotypic mean values and residual deviations from the genotypic mean values, respectively (PROC CORR) (SAS Institute 1985). Furthermore, the effect of reproductive status on the mortality levelling parameter s , when both the a and b parameters were held constant, was evaluated by comparing residuals from the expected s values, obtained by a multiple regression analysis, PROC REG (SAS Institute 1985), using a t -test with unequal variances, PROC TTEST (SAS Institute 1985).

3. RESULTS AND DISCUSSION

Weekly mortality rates on a log scale were plotted against age for 30 cages of reproducing males and 25 cages of non-reproducing males, respectively (figure 1). Most cages showed relatively high mortality rates during the initial phase, just after the flies were released into population

cages. This was followed by low mortality rates. After about week 3, the mortality rates tended to increase linearly during most of adult life. This phenomenon, relatively high mortality rates during an early phase of life, is widely documented, and is known as the 'bathtub effect' (Gavrilov & Gavrilova 1991, p. 226). It has been observed in other population cage experiments using *D. melanogaster* (Promislow *et al.* 1996; Pletcher *et al.* 1998; Sgró & Partridge 1999). In some cases, it may reflect genetic variation in mortality rates during early life (Promislow *et al.* 1996; Pletcher *et al.* 1998), but in our study it is likely to reflect a response to the drastic environmental changes experienced on transfer to the cages.

Although the Gompertz model (equation (2.2)) has been commonly used for describing mortality patterns, most mortality trajectories obtained in this study appeared to show levelling-off of mortality rates late in adult life (figure 1). We therefore adopted the logistic frailty model (equation (2.3)), which takes into account mortality levelling late in life, for analysing the mortality data. The logistic frailty model fitted significantly better than the Gompertz model, in 20 out of 30 cages (67%) for reproducing males and in 11 out of 25 cages (44%) for non-reproducing males ($p < 0.05$; likelihood ratio test (Pletcher 1999)). Maximum likelihood estimates of mortality rate parameters, obtained by fitting the logistic frailty model (equation (2.3)), are listed in table 1. Reproducing males tended to have a lower initial mortality rate a , a higher exponential increase rate b , and higher levelling parameter s , than non-reproducing males.

To evaluate the effect of genotype and reproductive status, a two-way ANOVA was conducted for each mortality rate parameter (table 2). There were significant effects of genotypes on both b ($F_{5,43} = 2.52$, $p = 0.044$) and s ($F_{5,43} = 3.51$, $p = 0.010$), whereas Promislow *et al.* (1996) found significant additive genetic variation in all three parameters. Reproductive status affected all of the three parameters significantly ($F_{1,43} = 40.85$, $p < 0.0001$ for a ; $F_{1,43} = 28.63$, $p < 0.0001$ for b ; $F_{1,43} = 6.51$, $p = 0.014$ for s). No significant interactions between genotype and reproductive status were detected.

Significant s parameters were obtained for non-reproducing males, demonstrating that mortality rate levelling could occur even under non-reproducing conditions, which should minimize any survival costs of reproduction. However, the 'non-reproducing' males displayed various mating behaviours in the cages (T.M.'s observations). They were, therefore, not completely reproductively inactive. There were, however, significant effects of reproductive status on s (table 2), such that higher levelling parameters s were obtained for reproducing males than non-reproducing males (table 1). At first sight, this suggests that cessation of reproduction by reproducing flies contributes to the mortality levelling late in life because of the associated reduction in mortality costs of reproduction.

However, significant effects of reproduction on the other two parameters, a and b , were also detected (table 2), which may in fact be primarily responsible for the patterns we observe. Promislow *et al.* (1996) found that the mortality levelling parameter s was negatively correlated with a and positively correlated with b . The genetic correlation between b and s has been estimated as 0.77 across second chromosome lines of male *D. melanogaster* (Shaw *et al.*

Table 1. Maximum likelihood parameter estimates (mean $\pm$ s.e.m.) using the logistic frailty model, $\mu_x = ae^{bx}/[1 + (as/b)(e^{bx}-1)]$. (a , the initial mortality rate at birth; b , the rate of exponential increase in mortality with age early in life; s , the degree of deceleration in mortality at older ages.)

cross			reproducing			non-reproducing			
F	M	n	$\ln(a)$	b	s	n	$\ln(a)$	b	s
5	24	5	-6.10 ± 0.34	0.89 ± 0.15	0.39 ± 0.22	4 ^a	-4.85 ± 0.37	0.58 ± 0.05	0.08 ± 0.04
24	5	5	-5.98 ± 0.40	1.20 ± 0.20	0.87 ± 0.27	5	-4.99 ± 0.59	0.94 ± 0.07	0.63 ± 0.11
5	25	5	-6.28 ± 0.51	0.93 ± 0.06	0.29 ± 0.09	4	-4.47 ± 0.37	0.57 ± 0.05	0.14 ± 0.08
25	5	5	-5.63 ± 0.45	0.90 ± 0.11	0.34 ± 0.11	3	-5.27 ± 0.42	0.81 ± 0.18	0.43 ± 0.36
24	25	5	-6.37 ± 0.30	0.97 ± 0.08	0.23 ± 0.15	4 ^a	-3.98 ± 0.30	0.53 ± 0.06	0.00 ± 0.00
25	24	5	-7.22 ± 0.51	1.10 ± 0.11	0.65 ± 0.25	5	-4.57 ± 0.40	0.55 ± 0.06	0.08 ± 0.08
grand mean		30	-6.26 ± 0.18	1.00 ± 0.05	0.46 ± 0.08	25	-4.67 ± 0.18	0.66 ± 0.04	0.23 ± 0.07

a : the initial mortality rate at birth; b : the rate of exponential increase in mortality with age early in life; s : the degree of deceleration in mortality at older ages.

^a One of the replicates was removed from the analysis.

1999; Service 2000). Correlations between a and s were consistently negative and significant across isogenic lines of male *D. melanogaster* (Fukui *et al.* 1996; Service 2000), and a and b also tend to be negatively correlated. The non-genetic correlation between b and s was estimated as 0.58 for male *D. melanogaster* (Promislow *et al.* 1996; Shaw *et al.* 1999). In this study, the same pattern was obtained for the correlations among the three parameters of the logistic model, especially for non-reproducing cohorts of males. The correlation using genotypic mean values between $\ln(a)$ and s was -0.74 (one-sided $p = 0.046$; $n = 6$), and was 0.99 between b and s (one-sided $p < 0.0001$; $n = 6$). The environmental correlation between b and s , based on residual deviations from genotypic mean values, was 0.79 (one-sided $p < 0.0001$; $n = 25$). It is thus possible that the effect of reproduction on the a and b parameters is the main cause of mortality levelling late in life.

In fact, when s was multiply regressed against $\ln(a)$ and b , there was a significant linear relationship between s and the other two parameters ($F_{2,52} = 76.38$, $p < 0.0001$, $r^2 = 0.746$; figure 2). The residuals of s , when both a and b are held constant, do not show a significant effect of reproduction: the difference between reproducing and non-reproducing males in the mean residual value was 0.079 ($t = 1.48$, d.f. = 47, $p = 0.147$). It is, therefore, likely that the effects of reproduction on the initial mortality rate parameter a and the exponential increase parameter b indirectly contribute to mortality levelling late in life, rather than this being a direct effect of the cessation of reproduction.

The effects of reproduction on survival have been well documented previously (Partridge & Farquhar 1981; Partridge & Andrews 1985; Partridge *et al.* 1986, 1987; Tatar *et al.* 1993; Sgró & Partridge 1999). However, the reasons for changes in survival in terms of the underlying age-specific mortality parameters have not always been carefully analysed. In this study, reproducing males tended to have a lower initial mortality rate a , a higher exponential increase rate b , and higher levelling parameter s than non-reproducing males (table 1). Tatar *et al.* (1993) also demonstrated that egg production and mating affect age-specific mortality rates in the female beetle *Callosobruchus maculatus*. They found that egg production and mating increased the rate of change of age-specific mortality rates, indicating acceleration of senescence. More importantly, their data indicate that age-specific mortality trajectories of egg-laying and mating females tended to level off earlier than those of unmated females (fig. 4 of Tatar *et al.* 1993), suggesting that reproducing females tended to have a higher exponential increase rate b and a higher levelling parameter s , consistent with our results. Sgró & Partridge (1999) showed that the declining impact of early egg production contributes to the mortality rate levelling late in life in female *D. melanogaster*. Their results also seem to indicate that females of a young-selection line, which has higher fecundity in early life, had a higher exponential increase rate and an earlier mortality levelling late in life, than females of the old-selection line (fig. 3b of Sgró & Partridge 1999). Because their experimental conditions and methods of analysis were considerably different, comparisons between their results and ours should not be made without caution. However, our results seem to be broadly consistent with theirs.

Table 2. Effects of genotype and reproductive status on each mortality rate parameter.

parameter	$\ln(a)$				b				s			
	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P ^a
genotype	5	0.37	0.42	0.829	5	0.14	2.52	0.044	5	0.46	3.51	0.010
reproduction	1	35.44	40.85	<0.0001	1	1.58	28.63	<0.0001	1	0.86	6.51	0.014
interaction	5	1.66	1.91	0.112	5	0.05	0.98	0.442	5	0.10	0.76	0.581
residual	43	0.87			43	0.06			43	0.13		

^a Achieved significance levels (ASL) of each effect on the s parameter, obtained from permutation tests, were as follows: ASL_{genotype} = 0.014; ASL_{reproduction} = 0.016; ASL_{interaction} = 0.560.

Figure 2. Partial regression of parameter s on parameter b . The regression line (solid line) was plotted for pooled data on cages containing both reproducing males (crosses) and non-reproducing males (circles). Under the hypothesis that mortality levelling is a result of a reduced survival cost of reproduction at advanced ages, reproducing males should produce larger mortality levelling parameters, s , than non-reproducing males, when the effects of other variables are removed. Positive deviations from the regression line in non-reproducing males, and negative deviations in reproducing males were detected, opposite to expectation. However, this difference was not significant when the effects of both a and b were taken into account.

The conclusion that the effects of reproduction on the initial mortality rate and the exponential increase rate indirectly contribute to mortality rate levelling late in life is consistent with the results of Service (2000, 2004), who conducted mortality simulation studies in which each individual was assumed to have its own Gompertz function. He demonstrated that mortality rate levelling at older ages could be produced by heterogeneity in individual mortality risks, even though mortality curves for each individual was assumed to follow the Gompertz function. For a given mean value of b , the height of the mortality plateau was negatively correlated with the coefficient of variation of b (Service 2000; table 2). For a given coefficient of variation of b , its mean value was negatively correlated with the age of onset of mortality plateaux, and positively correlated with the height of the mortality plateau (Service 2000; table 2). Because higher values of s indicate more rapid mortality levelling (Pletcher 1999), these results parallel the environmental correlation between b and s detected by us. Environmental influences on age-specific mortality rates in *D. melanogaster* involve various reproductive activities (Sgró & Partridge 1999; Service 2000), so that heterogeneity among individual flies within a cohort may be greater in reproducing male cohorts, causing enhanced mortality levelling late in life associated with higher values of b and lower values of a .

Rose *et al.* (2002) conducted selection experiments using *D. melanogaster* and compared the ages of onset of mortality plateaux between populations that had different last ages of reproduction. The times of onset of mortality plateaux approximately coincided with the days when reproduction ceased. In addition, the onset of mortality plateaux quickly responded to reverse selection for earlier reproduction, demonstrating a genetic basis for mortality levelling late in life. In our study, a significant effect of genotype on the mortality

levelling parameter s was also detected (table 2). In addition, significant genetic correlations among all three parameters of the logistic model were detected. The changes in mortality levelling observed by Rose *et al.* (2002) may, therefore, also reflect indirect effects of changes in a and/or b .

In conclusion, our results show that significant mortality levelling late in life can occur even under non-reproducing conditions. In these conditions, there is a limited opportunity for trade-offs between reproduction and mortality, so that mortality patterns may approximate those predicted by mutation-accumulation models of ageing (Charlesworth 2001). However, reproduction affected mortality levelling, such that reproducing males showed greater levelling than non-reproducing males. This may largely reflect the effect of reproduction on the Gompertz mortality rate parameters, a and b , which are environmentally correlated with the levelling parameter s , probably through the effects of heterogeneity in mortality risks (Service 2000, 2004). There were considerable differences among different genotypes in b and s , indicating a genetic basis for mortality rate variation; these two parameters were strongly positively genetically correlated.

We thank Helen Borthwick for technical support and Helen Cowan for fly media preparation. We thank Leonid Gavrilov and Natalia Gavrilova for discussions. We also thank three anonymous reviewers for comments. T.M. was supported by a Royal Society–Daiwa Foundation fellowship; B.C. is supported by The Royal Society.

REFERENCES

- Carey, J. R., Liedo, P., Orozco, D. & Vaupel, J. W. 1992 Slowing of mortality rates at older ages in large medfly cohorts. *Science* **258**, 457–461.
- Charlesworth, B. 1994 *Evolution in age-structured populations*, 2nd edn. Cambridge University Press.
- Charlesworth, B. 2001 Patterns of age-specific means and genetic variances of mortality rates predicted by the mutation-accumulation theory of ageing. *J. Theor. Biol.* **210**, 47–65.
- Charlesworth, B. & Lapid, A. 1989 A study of ten families of transposable elements on X chromosomes from a population of *Drosophila melanogaster*. *Genet. Res. Camb.* **54**, 113–125.
- Charlesworth, B., Lapid, A. & Canada, D. 1992 The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. I. Element frequencies and distribution. *Genet. Res. Camb.* **60**, 103–114.
- Charlesworth, B. & Partridge, L. 1997 Aging: levelling of the grim reaper. *Curr. Biol.* **7**, R440–R442.
- Charlesworth, B., Jarne, P. & Assimacopoulos, S. 1994 The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. III. Element abundances in heterochromatin. *Genet. Res. Camb.* **64**, 183–197.
- Cody, R. P. & Smith, J. K. 1991 *Applied statistics and the SAS programming language*, 3rd edn. Englewood Cliffs, NJ: Prentice-Hall.
- Curtsinger, J. W., Fukui, H. H., Townsend, D. R. & Vaupel, J. W. 1992 Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*. *Science* **258**, 461–463.
- Curtsinger, J. W., Fukui, H. H., Khazaeli, A. A., Kirscher, A., Pletcher, S. D., Promislow, D. E. L. & Tatar, M. 1995 Genetic variation and aging. *A. Rev. Genet.* **29**, 553–575.
- Efron, B. & Tibshirani, R. J. 1993 *An introduction to the bootstrap*. Boca Raton, FL: Chapman & Hall.
- Fukui, H. H., Ackert, L. & Curtsinger, J. W. 1996 Deceleration of age-specific mortality rates in chromosomal homozygotes and heterozygotes of *Drosophila melanogaster*. *Exp. Gerontol.* **31**, 517–531.
- Gavrilov, L. A. & Gavrilova, N. S. 1991 *The biology of life span: a quantitative approach*. Chur, Switzerland: Harwood Academic Publishers.
- Gompertz, B. 1825 On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Phil. Trans. R. Soc. Lond.* **115**, 513–583.
- Grubbs, F. E. 1969 Procedures for detecting outlying observations in samples. *Technometrics* **11**, 1–21.
- Khazaeli, A. A., Xiu, L. & Curtsinger, J. W. 1996 Effect of density on age-specific mortality in *Drosophila*: a density supplementation experiment. *Genetica* **98**, 21–31.
- Partridge, L. & Andrews, R. 1985 The effect of reproductive activity on the longevity of male *Drosophila melanogaster* is not caused by an acceleration of ageing. *J. Insect Physiol.* **31**, 393–395.
- Partridge, L. & Farquhar, M. 1981 Sexual activity reduces lifespan of male fruitflies. *Nature* **294**, 580–582.
- Partridge, L., Fowler, K., Trevitt, S. & Sharp, W. 1986 An examination of the effects of males on the survival and egg-production rates of female *Drosophila melanogaster*. *J. Insect Physiol.* **32**, 925–929.
- Partridge, L., Green, A. & Fowler, K. 1987 Effects of egg-production and of exposure to males on female survival in *Drosophila melanogaster*. *J. Insect Physiol.* **33**, 745–749.
- Pletcher, S. D. 1999 Model fitting and hypothesis testing for age-specific mortality data. *J. Evol. Biol.* **12**, 430–439.
- Pletcher, S. D., Houle, D. & Curtsinger, J. W. 1998 Age-specific properties of spontaneous mutations affecting mortality in *Drosophila melanogaster*. *Genetics* **148**, 287–303.
- Promislow, D. E. L., Tatar, M., Khazaeli, A. A. & Curtsinger, J. W. 1996 Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. *Genetics* **143**, 839–848.
- Rose, M. R. & Mueller, L. D. 2000 Ageing and immortality. *Phil. Trans. R. Soc. Lond. B* **355**, 1657–1662. (doi:10.1098/rspb.2000.0728)
- Rose, M. R., Drapeau, M. D., Yazdi, P. G., Shah, K. H., Moise, D. B., Thakar, R. R., Rauser, C. L. & Mueller, L. D. 2002 Evolution of late-life mortality in *Drosophila melanogaster*. *Evolution* **56**, 1982–1991.
- SAS Institute 1985 *SAS user's guide: statistics*. Cary, NC: SAS Institute.
- Service, P. M. 2000 Heterogeneity in individual mortality risk and its importance for evolutionary studies of senescence. *Am. Nat.* **156**, 1–13.
- Service, P. M. 2004 Demographic heterogeneity explains age-specific patterns of genetic variance in mortality rates. *Exp. Gerontol.* **39**, 25–30.
- Sgró, C. M. & Partridge, L. 1999 A delayed wave of death from reproduction in *Drosophila*. *Science* **286**, 2521–2524.
- Shaw, F. H., Promislow, D. E. L., Tatar, M., Hughes, K. A. & Geyer, C. J. 1999 Toward reconciling inferences concerning genetic variation in senescence in *Drosophila melanogaster*. *Genetics* **152**, 553–566.
- Tatar, M., Carey, J. R. & Vaupel, J. W. 1993 Long-term cost of reproduction with and without accelerated senescence in *Callosobruchus maculatus*: analysis of age-specific mortality. *Evolution* **47**, 1302–1312.
- Vaupel, J. W. 1997 Trajectories of mortality at advanced ages. In *Between Zeus and the salmon: the biodemography of longevity* (ed. K. W. Wachter & C. E. Finch), pp. 17–37. Washington, DC: National Academy Press.