Evolutionary Ecology Research, 2007, **9**: 1–17

Assessing patterns of senescence in *Drosophila mojavensis* reared on different host cacti

Luciano M. Jaureguy* and William J. Etges

Program in Ecology and Evolutionary Biology, Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA

ABSTRACT

Questions: Can senescence observed under laboratory conditions predict senescence under conditions thought to contribute to expression of intrinsic mortality rates in nature? Is senescence, under contrasting environmental conditions, described by alternative mortality models or by a single one with different parameter values?

Organism: *Drosophila mojavensis*. Many populations of this cactophilic species use one of two principal hosts, pitaya agria cactus (*Stenocereus gummosus*) or organ pipe cactus (*Stenocereus thurberi*) to carry out their life cycles.

Methods: Flies were grown on both cactus hosts and standard laboratory food over their entire life cycle. Adult mortality rates and mean longevity were calculated in all adult cohorts. We employed maximum likelihood procedures to determine which of four statistical models best described the mortality trajectories of these flies.

Conclusions: Mortality rates of flies grown on cacti were best described by Gompertz and Gompertz-Makeham models, whereas flies grown on laboratory media were best described by Logistic and Logistic-Makeham models. Rates of mortality decelerated at older ages in individuals grown on laboratory media, but not in cactus-reared flies. Models commonly used in *Drosophila* laboratory studies may be inadequate to accurately assess the shape of natural mortality risk functions.

Keywords: ageing, *Drosophila*, ecology, host cacti, mortality models.

INTRODUCTION

Ageing is one of the most striking processes in biology. Multiple mechanisms have been implicated in causing senescence in different species, so a general understanding of the evolution of ageing patterns remains unclear (reviewed in Harshman, 2003). Patterns of age-specific reproduction are thought to be caused by antagonistic pleiotropy (Gasser *et al.*, 2000; Rose and Charlesworth, 1980; Rose, 1991), due to genetic trade-offs between early and late life patterns of

* Author to whom all correspondence should be addressed. e-mail: ljaureg@uark.edu
Consult the copyright statement on the inside front cover for non-commercial copying policies.

© 2007 Luciano M. Jaureguy

mortality, and/or accumulation of late-life deleterious mutations (Hughes *et al.*, 2002). The extent to which each one of these processes governs the evolution of ageing is not well understood.

Experimental manipulations of diet (Mair *et al.*, 2003), genotype (Tu *et al.*, 2002), and mating status (Chapman and Partridge, 1996), as well as selection experiments showing how natural selection causes evolution in alternative environments (Mueller, 1987), have attempted to uncover relevant mechanisms of ageing by estimating simple parameters such as changes in mortality rates or early death rates. Exponential increases in mortality rates with age revealed by fitting the Gompertz model had been considered common to most organisms (Comfort, 1964; Finch, 1990). Here, mortality is described by baseline mortality (i.e. early mortality) and the rate at which it increases over time (exponential mortality). However, the existence of mortality rate deceleration (logistic and two-stage Gompertz models) and therefore mortality plateaus were reported in *Ceratitis capitata* and *Drosophila melanogaster* (Carey *et al.*, 1992; Curtsinger *et al.*, 1992; Vaupel *et al.*, 1998). These findings challenged the ideas of constant and exponential increases in mortality rates, and that all species follow the same pattern of mortality (Carey *et al.*, 1992).

Factors that affect the degree to which extrinsic mortality and mortality deceleration influence the shape of mortality curves are poorly understood. An important issue in studies of senescence is the choice of the environment(s) in which longevity and ageing are measured. Expression of any quantitative trait, such as ageing, is expected to be a function of the environment in which phenotypes are measured, making laboratory studies of ageing in many organisms especially difficult to interpret if environmental influences are large or these conditions are very different from those in nature. For example, most studies of senescence in *D. melanogaster* have been performed with laboratory-adapted strains grown on artificial culture media containing very different nutrients than the fermenting substrates typically used in the wild. In *Caenorhabditis elegans*, long-lived *daf-2* mutants grown in laboratory cultures have been studied intensively to help understand why such mutations prolong ageing. Under more natural soil conditions, these mutants died faster than wild-type worms (Leslie, 2005; Van Voorhies *et al.*, 2005). Similarly, mouse lines founded with wild-trapped progenitors lived longer than mice adapted to laboratory conditions (Miller *et al.*, 2002). Thus, it is critical for genetic analysis to assess relevant environmental influences on ageing (Partridge, 1997), including conditions thought to contribute to expression of intrinsic mortality rates in nature.

Unfortunately, studies of longevity in *Drosophila* using natural hosts are scarce. Ideally, rates of intrinsic mortality can be estimated most reliably under natural conditions such that phenotypic expression of age-specific mortality rates is unaffected by genotype × environment interaction. Some ecologically tractable species of *Drosophila* use fermenting mushrooms, flowers, sap, or cactus substrates to carry out their life cycles (Filchak *et al.*, 2005). In some cactophilic *Drosophila*, adult flies live in and around cactus 'rot pockets' of fermenting tissues, so metabolism of the volatile by-products of fermentation has been studied in detail (Starmer *et al.*, 1977; Ganter *et al.*, 1989) because these substrates lack appreciable concentrations of free carbohydrates (Fogleman and Abril, 1990). In *Drosophila mojavensis*, Etges and Klassen (1989) found that components of fitness such as longevity, fecundity, and age at first reproduction were significantly influenced by atmospheric ethanol due to direct assimilation, metabolism, and storage of this nutrient and its metabolites (Etges, 1989a). Etges and Heed (1992) showed that male contributions to female fitness caused by re-mating decreased female longevity in *D. mojavensis* feeding on fermenting cactus and ethanol vapour, conditions thought to approximate more natural conditions. However, estimates of

mortality rates or effects of different host cacti on rates of senescence have not been made. Thus, cactophilic *Drosophila* represent an attractive system to assess patterns of senescence under realistic environmental conditions, and in particular the effects of free carbohydrates on rates of mortality.

Here we use *D. mojavensis* to assess ageing patterns under natural conditions using fermenting cactus tissues. This species is endemic to the Sonoran and Mojave Deserts of the southwestern USA, and northwestern Mexico. Most populations use one of two principal host cacti, pitaya agria cactus (*Stenocereus gummosus*) in Baja California and organ pipe cactus (*Stenocereus thurberi*) in most mainland Mexico populations (Heed and Mangan, 1986; Ruiz and Heed, 1988). Mainland Sonora populations are evolutionarily derived from Baja California and have shifted their main breeding and feeding resource from agria to organ pipe cactus. This host plant shift generated many changes in life-history traits, such as longer development times and larger thorax sizes in mainland populations (Etges, 1993) and increased adult longevity under stress conditions (Starmer *et al.*, 1977; Etges and Klassen, 1989).

All life stages are dependent on fermentation by-products resulting from the interaction of several key factors in this cactus–microorganism–*Drosophila* system (Fogleman and Danielson, 2001). Fermenting agria and organ pipe tissues typically contain higher concentrations of volatile compounds, particularly ethanol, than *Opuntia* or other Sonoran Desert columnar cacti, because of the high tissue concentrations of fermentable simple and complex sugars (Fogleman and Heed, 1989). Fermenting cactus tissues are complex environments, containing degraded phytochemicals, bacteria, and yeasts that serve as sources of energy for developing larvae as well as adults, but contain negligible free carbohydrates. Since *D. mojavensis* can be cultured on fermenting cactus tissues in the laboratory (Etges and Heed, 1987; Ruiz and Heed, 1988), estimates of adult longevity and senescence were made under conditions similar to those in nature.

Our aim here was to estimate mean longevity, mortality rates, and assess adult survivorship using a population of *D. mojavensis* grown on different host cacti and laboratory food containing simple carbohydrates. We employed maximum likelihood procedures to determine which of four statistical models describes the mortality trajectories of these flies. These four models are commonly used in ageing studies, including those with *Drosophila* (e.g. Promislow and Haselkorn, 2002). Parameter estimates including baseline mortality and rate of increase in mortality were obtained for each treatment under the best fitted model. Mortality deceleration rate and age-independent mortality estimates were found to depend on whether models that include these parameters were appropriate to describe mortality for a specific treatment. The importance of comparing ecologically relevant conditions in studies of ageing is discussed.

MATERIALS AND METHODS

Flies from mainland Mexico (Punta Onah, Sonora) collected in November 2003 were maintained in shell vials using banana-Karo-malt-brewer's yeast-agar food. Large numbers of flies were maintained in Plexiglas cages (12,720 cm^3) and were fed with lab food (see recipe below) in screw-on cups. Eggs laid on these cups were transferred to bottles containing lab food and emerging adults were added to the cages. Eggs were sampled from screw-on cups containing oviposition medium (300 ml fermented cactus juice, 20 g dextrose, 3 g agar) for 12 h each day. Eggs were washed with 70% ethanol and sterile water and counted onto 1-cm^2 pieces of filter paper and placed on either fermenting cactus (agria,

organ pipe) or lab food in half-pint bottles (Etges, 1998; see below), at a density of 250 eggs per bottle. Culture bottles were placed in an incubator set at a 14 h : 10 h light/dark cycle and rotated every day to different shelves to avoid temperature stratification. To obtain age-synchronized experimental flies, bottles were emptied when most of the bottles had newly emerged individuals. Then, three-day adult cohorts (approximately 400 flies) were sorted by sex and placed in 900-cm^3 plastic cages with one side replaced by nylon netting. Cactus-agar food in Petri dishes of 5 cm diameter (Etges, 1993; see below) or lab food were fitted to each cage. Twelve cages (3 food types × 2 sexes × 2 replicates) were randomly assigned to sealed desiccators containing 1 litre of 4% ethanol in the bottom of each container. Food cups were replaced every 7–8 days and ethanol solutions were replaced when deaths were scored. Dead flies were aspirated daily from the cages.

Culture conditions

Cactus agar medium for feeding adults was prepared by mixing cactus, water, and agar homogenized in a blender in the following proportions: 953 g of cactus, 486 ml of deionized water, and 5 g of agar. This mixture was autoclaved, cooled, poured in Petri dishes (5 cm diameter), cooled, and inoculated with cactophilic yeasts and bacterium described below. Lab food consisted of: 20 g of agar, 60 g yeast, 57 g malted barley, 125 ml corn syrup, one banana and 1 litre of deionized water. Fifteen millilitres of propionic acid, 0.5 g penicillin, and 0.5 g streptomycin were added before pouring to reduce mould and bacterial contamination (Brazner and Etges, 1993).

Cactus cultures for growing larvae were prepared using 60 g of thawed agria or organ pipe cactus tissue placed in sterilized half-pint bottles with 75 g of aquarium gravel in the bottom. These cultures were briefly autoclaved, allowed to cool, and then inoculated with seven species of yeasts (*Dipodascus starmeri*, *Candida sonorensis*, *Starmera amethionina*, *Candida valida*, *Pichia cactophila*, *Pichia mexicana*, *and Sporopachydermia cereana*) and a pectolytic bacterium (*Erwinia cacticida*) common to agria and organ pipe rots in nature (Starmer, 1982; Fogleman and Starmer, 1985).

Statistical analyses

Mortality rates were estimated as described in Promislow *et al.* (1996). For each cage a d_x value was assigned, the number of death at age x. At the end of the experiment, the number of flies in the initial cohort (N_o) for each cage was calculated. From N_o and d_x it was possible to estimate the number of flies alive at any age, N_x. The probability of surviving from age x to age $x + 1$ is $P_x = N_{x+1}/N_x$. The mortality rate, μ_x, is defined as: $\mu_x \approx \ln (P_x)$ (Elandt-Johnson and Johnson, 1980; Promislow *et al.*, 1996).

To statistically assess patterns of mortality among treatments, we fitted four different models (Pletcher *et al.*, 2000) to each combination of sex and food type estimating the maximum likelihood for each model with *Winmodest* (Pletcher, 1999a, 1999b). Instead of mortality rates, this method uses age at death to fit model distributions. Maximum likelihood significantly reduces bias due to demographic sampling error (Promislow *et al.*, 1999). The most common model is the Gompertz model (Gompertz, 1825), which assumes mortality rates increase exponentially and depends on two parameters: (a) baseline mortality rate and (b) the increase in that rate or rate of ageing. If mortality rates level off in older individuals (Curtsinger *et al.*, 1992; Fukui *et al.*, 1993), the Logistic model (L) is more appropriate since it includes

a parameter that accounts for the degree of mortality deceleration (s) (Pletcher, 1999a). Gompertz-Makeham (GM) and Logistic-Makeham (LM) are extensions of previous models, but they also account for extrinsic causes of death (c) (see Pletcher, 1999b; Pletcher *et al.*, 2000). It is important to keep in mind that these four models are nested (i.e. Gompertz-Makeham with $c = 0$ reduces to the Gompertz model). Therefore, if maximum likelihoods are the same for both models, adding one parameter will not fit the data significantly better (Pletcher, 1999a). Thus, when maximum likelihood estimates were the same for two models within the same treatment, we chose the model with the fewest parameters. We used log-likelihood ratio tests to determine whether parameters were significantly different between treatments (Pletcher, 1999a). This test compares the log-likelihood of the null hypothesis model, where a parameter is constrained to be equal in both treatments, with the log-likelihood of the alternative hypothesis, where parameters are unconstrained and can have unique parameter values. Twice the difference between these two log-likelihoods has a chi-square distribution with degrees of freedom equal to the number of additional parameters constrained in the null hypothesis (Pletcher, 1999a, 1999b). We corrected for multiple comparisons by using Bonferroni's sequential test (Rice, 1989).

Additionally, longevity was subjected to analysis of variance (ANOVA) with sex and food type as fixed effects (SAS Institute, 1989). Two replicate cages were included for each treatment to increase statistical power when assessing rates of ageing (Service *et al.*, 1998; Pletcher, 1999a). The magnitude of food effects was assessed by performing pair-wise comparisons (contrasts) among food types. For model fitting, replicates were pooled since they responded similarly within each treatment and because higher sample sizes allowed improved and unbiased parameter estimation.

RESULTS

Longevity

Significant effects of both sex and food type were detected for mean longevity (Table 1). Further analyses revealed significant differences among the three different food types (AG-LF: $t_6 = -5.82$, $P = 0.0011$; LF-OP: $t_6 = 3.089$, $P = 0.0214$; AG-OP: $t_6 = -2.762$, $P = 0.0328$), showing that flies grown on lab food had higher mean longevity than flies grown on cactus (Fig. 1). Females lived, on average, longer than males when reared on either organ pipe (females = 17.3 days, standard error = 0.56; males = 13.2, days, standard error = 0.43; $P = 0.038$) or lab food (females = 21.1 days, standard error = 0.24; males = 16.3 days, standard error = 0.20; $P = 0.0214$). Agria-reared flies did not exhibit a significant difference between the sexes (females = 12.0 days, standard error = 0.28; males = 12.5 days, standard error = 0.32; $P = 0.7498$).

Patterns of mortality

Contrasting patterns of mortality resulted in survivorship curves crossing between cactus- and lab food-reared flies (Fig. 2). Males grown on lab food had higher survival rates in the first 15 days than cactus-reared flies, but females showed higher survivorship than cactus-reared flies up to 23 days (Fig. 2). Interestingly, organ pipe flies had a higher late survival than flies in the other treatments. However, this effect was more apparent in females as it was reflected by the highest longevity of 50 days. After 15 days, a mid-life survivorship

Table 1. Analysis of variance of mean longevity for flies growing on three different food types (agria, organ pipe, or lab food)

Source	d.f.	Sum of squares	F-ratio	Prob > F
Food	2	83.270	17.14	0.003
Sex	1	23.653	9.74	0.021
Food × sex	2	16.849	3.47	0.100
Error	6	14.575		

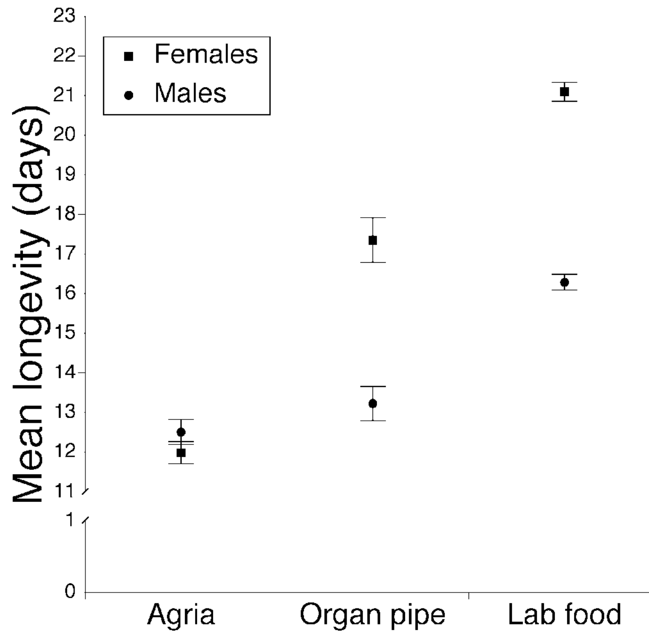

Fig. 1. Mean longevity (± standard error) of adult flies feeding on agria, organ pipe cactus, and lab food. Mean longevity is the average of two cohorts of flies grown under the same environmental conditions.

plateau appeared in organ pipe-reared females that extended to the end of the experiment. Male survivorship also diverged, showing the same patterns as females but maximum life span did not surpass that of lab food-reared females. Survivorship of agria-reared flies decreased over time as compared to lab food-reared flies, suggesting there was little change in survival from early to late ages.

Because of handling, some flies escaped or were stuck to the food. However, there were no significant differences between treatments in cohort size (ANOVA, cactus × sex interaction $P = 0.761$).

Model fitting

Based on maximum likelihood estimation, food treatments were described by different models (Table 2, Fig. 3). Gompertz-Makeham was the best model for describing mortality

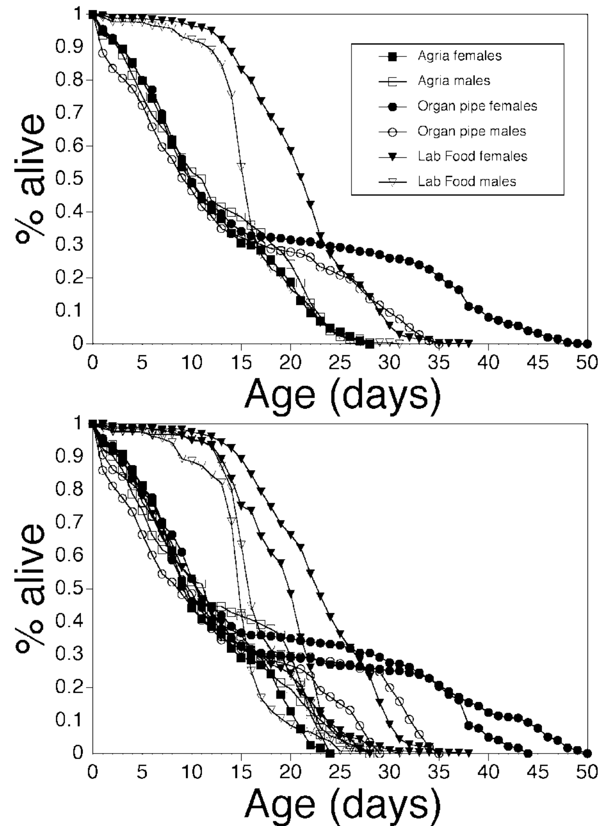

Fig. 2. Survivorship curves of adult flies feeding on agria, organ pipe cactus, and lab food. (Top panel) Two replicate cages were pooled to estimate survival. (Bottom panel) Individual cages.

in males grown on either agria or organ pipe. Organ pipe female mortality was not described by any of the models analysed in this study. This was probably due to a drastic change in mortality rate after day 17, when there was a drop in mortality that was even lower than mortality in the first few days. Like agria-reared flies, there were also differences between males and females for which model best described mortality of lab food-reared flies. Mortality of lab food females was best described by the Logistic model, whereas male mortality was best described by the Logistic-Makeham model.

Differences in mortality parameters

Both rate of mortality (b) and age-independent mortality differed among lab food- and agria-reared females (both $P < 0.0001$), but comparisons with organ pipe-reared females could not be included because these mortality rates did not fit any of the models. For males, baseline mortality was not significantly different between lab food and cactus-reared flies (agria vs. lab food, $\chi^2_1 = 0$, $P = 1$; organ pipe vs. lab food, $\chi^2_1 = 3.54$, $P = 0.06$). Therefore, differences in this mortality parameter were not responsible for differences in mean longevity between cactus- and lab food-reared males. Similarly, rate of mortality (b) and

Table 2. Maximum likelihood parameter estimates and 95% confidence intervals (in parentheses) for flies grown on agria or organ pipe cacti and lab food

	a	*b*	*s*	*c*	Best fit model
Agria					
Females	0.035	0.088	—	—	G
	(0.030–0.041)	(0.077–0.100)			
Males	0.00009	0.353	—	0.056	GM
	(0.00002–0.0004)	(0.295–0.422)		(0.050–0.063)	
Organ pipe					
Females	—	—	—	—	—
Males	1.29×10^{-07}	0.465	—	0.065	GM
	(4.13×10^{-9} to 4.03×10^{-6})	(0.368–0.587)		(0.059–0.071)	
Lab food					
Females	0.001	0.259	0.677	—	L
	(0.0005–0.002)	(0.224–0.300)	(0.438–1.047)		
Males	2.06×10^{-9}	1.332	4.480	0.007	LM
	(0 to 3.72×10^{-8})	(1.137–1.559)	(3.636–5.559)	(0.005–0.010)	

a = baseline mortality, *b* = rate of ageing, *s* = rate of mortality deceleration, *c* = age-independent mortality. See text for details. G = Gompertz-Makeham, GM = Gompertz-Makeham, L = Logistic, LM = Logistic-Makeham.

age-independent mortality (c) were not significantly different between agria and organ pipe males (both $P > 0.05$). Rates of mortality in males grown on organ pipe cactus were approximately 25% higher, whereas baseline mortality was 85% lower. However, mean longevities of these two groups of flies were not significantly different. This suggests that mortality rates at early ages were high enough to counteract the effect of baseline mortality, offsetting any significant differences in longevity among groups. Males and females exhibited significant differences in all mortality parameters when grown on agria or lab food (all $P < 0.001$).

DISCUSSION

Different environmental conditions caused by contrasting larval and adult diets produced significant effects on patterns of mortality of flies reared on cacti versus lab food for the population of *D. mojavensis* studied. Mortality rates of males grown on cacti increased after approximately 17 days, whereas females showed an exponential increase, at least in agria, that was described by the Gompertz model. The observed rates of mortality of lab food-reared flies were similar to other *Drosophila* studies showing mortality rate plateaus (Curtsinger *et al.*, 1992). Mortality of cactus-reared flies was best described by Gompertz and Gompertz-Makeham models, whereas rates of mortality of lab food-reared flies were best described by Logistic and Logistic-Makeham curves. Environmental effects could not be described by differences in parameter values for a single model. Thus, different survival functions were necessary to describe differences among treatments (Wilson, 1994). Such contrasting changes of mortality over time showed significant influences of rearing environments on mortality and rates of ageing.

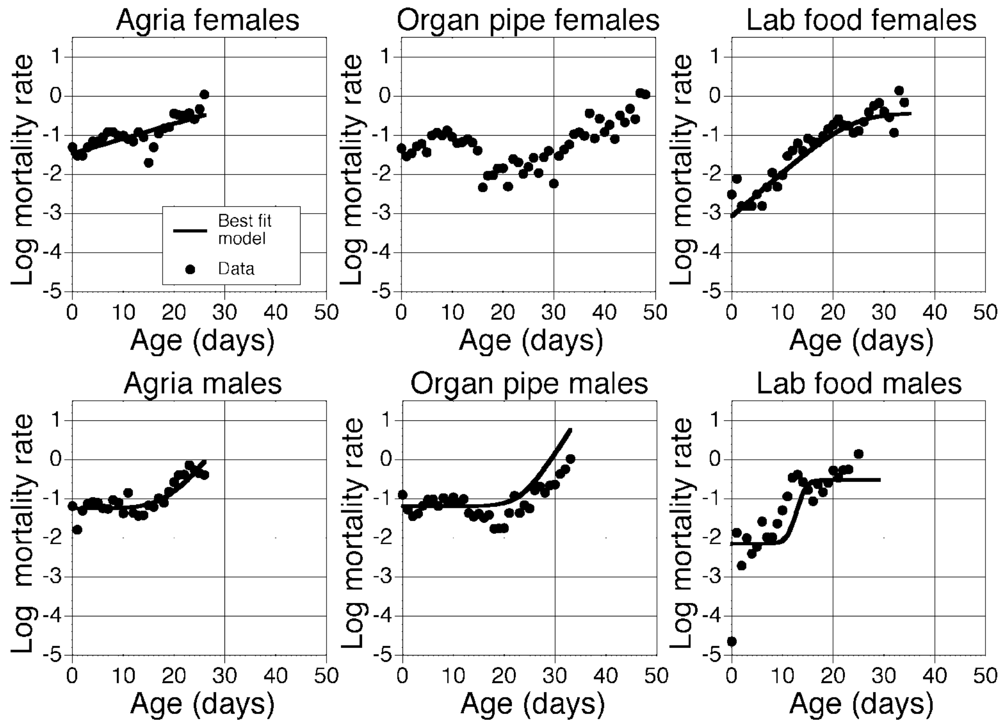

Fig. 3. Log of mortality rate versus time for adult flies feeding on agria, organ pipe cactus, and lab food. Four different models were fitted to mortality rates (Gompertz, Gompertz-Makeham, Logistic, and Logistic-Makeham) based on parameters estimated by maximum likelihood procedures. Some data points at older ages were not plotted since log(μ) was equal to log(0), which is undefined. However, since maximum likelihood was used, parameter estimation and therefore best fit line shape was not affected (Pletcher, 1999a; Promislow *et al.*, 1999).

Estimates of mortality using flies grown on standard lab food were far different from those experiencing fermenting cactus tissues and low concentrations of atmospheric volatiles, conditions designed to be similar to those found in nature. Although these cactus tissues contain carbohydrates, they are stored as complex triterpene glycosides and are unavailable to the flies (Fogleman and Danielson, 2001). Once fermented, these cactus substrates are carbohydrate-poor/volatile-rich compared with lab food, which may mimic low carbohydrate dietary restriction (Mair *et al.*, 2005). Even though flies grown on lab food showed the highest mean longevities, the effects of free carbohydrate restriction may influence maximum life span (Pletcher *et al.*, 2002; Mair *et al.*, 2005), as suggested by the organ pipe females that showed a maximum life span of 50 days (Fig. 2). Mortality rates were lowest at intermediate ages for organ pipe-reared females, a new pattern that does not fit any of the four models (see below) or the common view that either a Gompertz/Gompertz Makeham or Logistic/Logistic Makeham can always be used to explain patterns of mortality. This also suggests that non-Gompertz models may have explained increases/decreases in mortality rate (see Fig. 3, organ pipe females). Thus, our results may belong to a reduced group of studies where curves of contrasting shape (i.e. not described by a single group of models) have been found, or results like ours are quite common, but have been neglected.

Table 3. Some recent ageing studies where mortality rates were estimated in different populations or species exposed to various experimental conditions such as temperature or diet

Organism	Type of comparison where mortality was measured	Model used	Reference
Drosophila melanogaster	Alternative diets	L, G, LM	Min and Tatar (2006)
	Exercise (flight activity)/control	G	Magwere *et al.* (2006)[a]
	Alternative diets	G	Magwere *et al.* (2004)[a]
	Five genotypes ± *Wolbachia* infection	G, GM	Fry and Rand (2002)
	Five genotypes ± *Wolbachia* infection	G, GM, L, LM	Fry *et al.* (2004)
	Effects of mutations and virgin and mated flies	G	Yampolsky *et al.* (2001)
	Alternative diets	G, L	Bross *et al.* (2005)[a]
	Reproducing and non-reproducing males	L	Miyo and Charlesworth (2004)[a]
	10 genetically distinct strains	LM	Johnson *et al.* (2006)
	Several levels of inbreeding	G	Swindell and Bouzat (2006)[a]
	Ablation of neurosecretory cells in the brain vs. controls	G	Broughton *et al.* (2005)[a]
	Individuals with different gene × genetic background combinations	G	Spencer and Promislow (2005)[a]
	Laboratory-adapted vs. artificially selected lines	G	Linnen *et al.* (2001)[a]
	Long-lived mutants vs. wild-type flies	G, GM	Marden *et al.* (2003)
	Mutants affecting the insulin/IGF signal pathway	GM	Clancy *et al.* (2001)
	Several genotypes and genetic variance	GM	Snoke and Promislow (2003)
	Parental age in outbred and inbred strains	G	Priest *et al.* (2002)[a]

Drosophila spp.	Species comparisons	GM	Promislow and Haselkorn (2002)
D. buzzatii and *D. koepferae*	Temperature and populations from different elevations	G	Sambucetti *et al.* (2005)[a]
Apis melifera	High and low pollen hoarding lines	L, GM	Rueppell *et al.* (2005)
Daphnia spp.	Multi-environments defined by food and temperature	G	Dudycha (2003)[a]
Mus musculus	Strain derived from the wild	G	Klebanov *et al.* (2001)
	A variety of experimental interventions. Data from other papers	G	de Magalhães *et al.* (2005)[a]
Phaseolus vulgaris	Strain under alternative selective regimens	G	Maklakov *et al.* (2005)
Callosobruchus maculatus and *Stator limbatus*	Sex	L	Fox *et al.* (2003)
Callosobruchus maculatus	Between populations	L	Fox *et al.* (2004)
Pararge aegeria	Sex and population	L	Gotthard *et al.* (2000)
Brachionus calyciflorus and *Synchaeta pectinata*	Differences between species	G	Kirk (2001)[a]
Caenorhabditis elegans	Transgenic individuals with increased glutathione transferase expression	G	Ayyadevara *et al.* (2005)[a]

Note: Only studies showing survival distribution and parameter estimation, derived from theoretical models, were included. In all studies, treatments were compared with the model specified. More than one model indicates alternative treatments/manipulations were described by different models. G = Gompertz, GM = Gompertz-Makeham, L = Logistic, LM = Logistic-Makeham.

[a] Studies where a particular model is assumed or it was not specified if alternative models were tested previous to analyses.

The availability of free carbohydrates has been shown to affect adult longevity of cactophilic *Drosophila* (Brazner *et al.*, 1984; Kircher and Al-Azawi, 1985). If *Drosophila* larvae and the yeasts/bacteria responsible for fermentation compete for free sugars (Brazner *et al.*, 1984), then carbohydrate restriction models of longevity may apply to *D. mojavensis* and help explain the lower mean longevities in cactus-reared flies than in flies grown on lab food. Also, fermentation rates are slower in organ pipe than in agria cactus (Etges, 1989b), which should further decrease the availability of free sugars for adult flies. If fermentation by-products from the interaction between cactus and yeasts increased life span in organ pipe-reared flies (Batterham *et al.*, 1982; Brazner *et al.*, 1984; Ganter *et al.*, 1989), then this system may offer a unique opportunity to study the interplay between dietary restriction due to low levels of free carbohydrates and their effects on different measures of ageing like longevity, rates of mortality, and maximum life span.

Generalizing our attempts to understand how extrinsic mortality and mortality deceleration influence the shape of mortality curves requires comparisons with other such multi-environment studies. Including alternate hypotheses to the Gompertz model should help to better describe mortality rates under different conditions or when mortality curves are compared (Table 3). Fitting predetermined models without assessing alternate hypotheses is not uncommon in mortality studies (Table 3) (Kirk, 2001; Magwere *et al.*, 2004; Broughton *et al.*, 2005; Sambucetti *et al.*, 2005): this has been pointed out previously (Wilson, 1994; Pletcher, 1999a). For example, our preliminary attempts to fit a Gompertz function resulted in estimates of 0.032 for baseline mortality and an exponential increase in mortality of 0.0869 (Table 4) in males reared on agria cactus. However, the Gompertz-Makeham model best described mortality rates of these individuals with a low but constant mortality during the first 16 days (0.00009) and then an increase (0.35272) until the end of the experiment. In the beetle *Callosobruchus maculatus*, deceleration rates have been shown to differ between the sexes (Fox *et al.*, 2003). Population and sex differences in life span were also partially explained by differences in this parameter in the butterfly *Pararge aegeria* (Gotthard *et al.*, 2000). Like mortality deceleration, age-independent mortality has been considered to be responsible for shaping mortality curves. Mortality rates under this model (Gompertz-Makeham) are relatively constant early in life and then increase exponentially. Such mortality trajectories have been shown in some species of *Drosophila* (Clancy *et al.*, 2001; Promislow and Haselkorn, 2002; Tu *et al.*, 2002) and bees (Rueppell *et al.*, 2005). Additionally, a combination of deceleration rate and age-independent mortality (Logistic-Makeham model) can also be a part of models describing mortality (Fry *et al.*, 2004).

Table 4. Maximum likelihood estimates of baseline mortality (a) and rate of ageing (b), and 95% confidence intervals (in parentheses) assuming all six mortalities are best described by the Gompertz model

Group	a	b
Agria females	0.035 (0.030–0.041)	0.088 (0.077–0.100)
Agria males	0.032 (0.027–0.100)	0.087 (0.076–0.038)
Organ pipe females	0.044 (0.038–0.050)	0.018 (0.013–0.026)
Organ pipe males	0.053 (0.046–0.061)	0.030 (0.023–0.041)
Lab food females	0.003 (0.002–0.004)	0.170 (0.159–0.181)
Lab food males	0.005 (0.004–0.007)	0.198 (0.185–0.213)

There have been few studies (Pletcher *et al.*, 2000) in which different models fit alternative treatments within the same experiment. This suggests that differences among treatments have often been assessed using the simplest model (i.e. Gompertz) because it fits most data (de Magalhães *et al.*, 2005) or treatment effects are best described by different parameter values under a single model (Table 3).

Disparate patterns of mortality rates between cactus- and lab food-reared *D. mojavensis* invoke two important considerations in ageing studies. First, organisms exposed to conditions similar to those experienced in nature may not always show the same patterns of mortality and longevity as organisms grown on artificial laboratory conditions. This is not surprising. Second, ecological determinants of ageing may have a significant impact in the expression of mortality rates, which calls for more careful interpretations of ageing studies when model organisms are used (e.g. *D. melanogaster*). Thus, broadening the spectrum of species used in ageing studies will promote an integrative understanding of genetic and environmental factors influencing ageing.

ACKNOWLEDGEMENTS

We thank S. Tuljapurkar and the Biodemography group for useful discussion. S. Pletcher helped in implementing *Winmodest*, and C. Fox provided useful editorial input. This research was funded by the Center on the Economics and Demography of Aging (CEDA) at UC Berkeley and NSF grant DEB-02111025 to W.J.E.

REFERENCES

Ayyadevara, S., Engle, M.R., Singh, S.P., Dandapat, A., Lichti, C.F., Bene, H. *et al.* 2005. Lifespan and stress resistance of *Caenorhabditis elegans* are increased by expression of glutathione transferases capable of metabolizing the lipid peroxidation product 4-hydroxynonenal. *Aging Cell*, **4**: 257–271.

Batterham, P., Starmer, W.T. and Sullivan, D.T. 1982. Biochemical genetics of the alcohol longevity response of *Drosophila mojavensis*. In *Ecological Genetics and Evolution: The Cactus–Yeast–Drosophila Model System* (J.S.F. Barker and W.T. Starmer, eds.), pp. 307–321. Sydney, NSW: Academic Press.

Brazner, J.C. and Etges, W.J. 1993. Pre-mating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. II. Effects of larval substrates on time to copulation, mate choice, and mating propensity. *Evol. Ecol.*, **7**: 605–624.

Brazner, J., Aberdeen, V. and Starmer, W.T. 1984. Host-plant shifts and adult survival in the cactus breeding *Drosophila mojavensis. Ecol. Entomol.*, **9**: 375–381.

Bross, T.G., Rogina, B. and Helfand, S.L. 2005. Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. *Aging Cell*, **4**: 309–317.

Broughton, S.J., Piper, M.D.W., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y. *et al.* 2005. Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc. Natl. Acad. Sci. USA*, **102**: 3105–3110.

Carey, J.R., Liedo, P., Orozco, D. and Vaupel, J.W. 1992. Slowing of mortality rates at older ages in large medfly cohorts. *Science*, **258**: 457–461.

Chapman, T. and Partridge, L. 1996. Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. *Proc. R. Soc. Lond. B*, **263**: 755.

Clancy, D.J., Gems, D., Harshman, L.G., 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.

Comfort, A. 1964. *Ageing: The Biology of Senescence*. New York: Holt, Rinehart & Winston.

Curtsinger, J.W., Fukui, H.H., Townsend, D.R. and Vaupel, J.W. 1992. Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster. Science*, **258**: 461–463.

de Magalhães, J.P., Cabral, J.A.S. and de Magalhães, D. 2005. The influence of genes on the aging process of mice: a statistical assessment of the genetics of aging. *Genetics*, **169**: 265–274.

Dudycha, J.L. 2003. A multi-environment comparison of senescence between sister species of *Daphnia. Oecologia*, **135**: 555–563.

Elandt-Johnson, R. and Johnson, N.L. 1980. *Survival Models and Data Analysis*. New York: Wiley.

Etges, W.J. 1989a. Divergence in cactophilic *Drosophila*: the evolutionary significance of adult ethanol metabolism. *Evolution*, **43**: 1316–1319.

Etges, W.J. 1989b. Evolution of developmental homeostasis in *Drosophila mojavensis. Evol. Ecol.*, **3**: 189–201.

Etges, W.J. 1993. Genetics of host-cactus response and life-history evolution among ancestral and derived populations of cactophilic *Drosophila mojavensis. Evolution*, **47**: 750–767.

Etges, W.J. 1998. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. IV. Correlated responses in behavioral isolation to artificial selection on a life history trait. *Am. Nat.*, **152**: 129–144.

Etges, W.J. and Heed, W.B. 1987. Sensitivity to larval density in populations of *Drosophila mojavensis*: influences of host plant variation on components of fitness. *Oecologia*, **71**: 375–381.

Etges, W.J. and Heed, W.B. 1992. Remating effects on the genetic structure of female life histories in populations of *Drosophila mojavensis. Heredity*, **68**: 515–528.

Etges, W.J. and Klassen, C.S. 1989. Influences of atmospheric ethanol on adult *Drosophila mojavensis*: altered metabolic rates and increases in fitness among populations. *Physiol. Zool.*, **62**: 170–193.

Filchak, K.E., Etges, W.J., Besansky, N.J. and Feder, J.L. 2005. Ecological genetics of host use in Diptera. In *The Evolutionary Biology of Flies* (D.K. Yeates and B.M. Wiegman, eds.), pp. 340–370. New York: Columbia University Press.

Finch, C.E. 1990. *Longevity, Senescence and the Genome*. Chicago, IL: University of Chicago Press.

Fogleman, J.C. and Abril, J.R. 1990. Ecological and evolutionary importance of host plant chemistry. In *Ecological and Evolutionary Genetics of Drosophila* (J.S.F. Barker, W.T. Starmer and R.J. MacIntyre, eds.), pp. 121–141. New York: Plenum.

Fogleman, J.C. and Danielson, P.B. 2001. Chemical interactions in the cactus–microorganism–*Drosophila* model system of the Sonoran desert. *Am. Zool.*, **41**: 877–889.

Fogleman, J.C. and Heed, W.B. 1989. Columnar cacti and desert *Drosophila*: the chemistry of host plant specificity. In *Special Biotic Relationships of the Southwest* (J.O. Schmidt, ed.), pp. 1–24. Albuquerque, NM: University of New Mexico Press.

Fogleman, J.C. and Starmer, W.T. 1985. Analysis of community structure of yeasts associated with the decaying stems of cactus. III. *Stenocereus thurberi. Microb. Ecol.*, **11**: 165–173.

Fox, C.W., Dublin, L. and Pollitt, S.J. 2003. Gender differences in lifespan and mortality rates in two seed beetle species. *Funct. Ecol.*, **17**: 619–626.

Fox, C.W., Bush, M.L., Roff, D.A. and Wallin, W.G. 2004. Evolutionary genetics of lifespan and mortality rates in two populations of the seed beetle, *Callosobruchus maculatus. Heredity*, **92**: 170–181.

Fry, A.J. and Rand, D.M. 2002. *Wolbachia* interactions that determine *Drosophila melanogaster* survival. *Evolution*, **56**: 1976–1981.

Fry, A.J., Palmer, M.R. and Rand, D.M. 2004. Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster. Heredity*, **93**: 379–389.

Fukui, H.H., Xiu, L. and Curtsinger, J.W. 1993. Slowing of age-specific mortality rates in *Drosophila melanogaster. Exp. Gerontol.*, **28**: 585–599.

Ganter, P.F., Peris, F. and Starmer, W.T. 1989. Adult life span of cactophilic *Drosophila*: interactions among volatiles and yeasts. *Am. Mid. Nat.*, **121**: 331–340.

Gasser, M., Kaiser, M., Berrigan, D. and Stearns, S.C. 2000. Life-history correlates of evolution under high and low adult mortality. *Evolution*, **54**: 1260–1272.

Gompertz, B. 1825. On the nature of the function expressive of the law of human mortality and on a new mode of determining life contingencies. *Phil. Trans. R. Soc. Lond.*, **115**: 513–585.

Gotthard, K., Nylin, S. and Wiklund, C. 2000. Mating opportunity and the evolution of sex-specific mortality rates in a butterfly. *Oecologia*, **122**: 36–43.

Harshman, L.G. 2003. Life span extension of *Drosophila melanogaster*: genetic and population studies. In *Life Span: Evolutionary, Ecological and Demographic Perspectives* (J. Carey and S. Tuljapurkar, eds.), pp. 99–126. New York: Life Span Population Council.

Heed, W.B. and Mangan, R.L. 1986. Community ecology of the Sonoran desert *Drosophila*. In *The Genetics and Biology of Drosophila* (M. Ashburner, H.L. Carson and J.N. Thompson, eds.), pp. 311–345. New York: Academic Press.

Hughes, K.A., Alipaz, J.A., Drnevich, J.M. and Reynolds, R.M. 2002. A test of evolutionary theories of aging. *Proc. Natl. Acad. Sci. USA*, **99**: 14286–14291.

Johnson, T.K., McKechnie, S.W. and Clancy, D.J. 2006. Water balance and cation levels in *Drosophila*: can early physiological decline predict aging and longevity? *J. Gerontol. A: Biol. Sci. Med. Sci.*, **61**: 146–152.

Kircher, H.W. and Al-Azawi, B. 1985. Longevity of seven species of cactophilic *Drosophila* and *Drosophila melanogaster* on carbohydrates. *J. Insect Physiol.*, **31**: 165–169.

Kirk, K.L. 2001. Dietary restriction and aging: comparative tests of evolutionary hypotheses. *J. Gerontol. A: Biol. Sci. Med. Sci.*, **56**: B123–B129.

Klebanov, S., Astle, C.M., Roderick, T.H., Flurkey, K., Archer, J.R., Chen, J. *et al.* 2001. Maximum life spans in mice are extended by wild strain alleles. *Exp. Biol. Med.*, **226**: 854–854.

Leslie, M. 2005. Death in the dirt. *Sci. Aging Knowl. Environ.*, **2005**: nf35 (available at: http://sageke.sciencemag.org/cgi/content/full/2005/2019/nf2035).

Linnen, C., Tatar, M. and Promislow, D.E.L. 2001. Cultural artifacts: a comparison of senescence in natural, laboratory-adapted and artificially selected lines of *Drosophila melanogaster. Evol. Ecol. Res.*, **3**: 877–888.

Magwere, T., Chapman, T. and Partridge, L. 2004. Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster. J. Gerontol. A: Biol. Sci. Med. Sci.*, **59**: 3–9.

Magwere, T., Pamplona, R., Miwa, S., Martinez-Diaz, P., Portero-Otin, M., Brand, M.D. *et al.* 2006. Flight activity, mortality rates, and lipoxidative damage in *Drosophila. J. Gerontol. A: Biol. Sci. Med. Sci.*, **61**: 136–145.

Mair, W., Goymer, P., Pletcher, S.D. and Partridge, L. 2003. Demography of dietary restriction and death in *Drosophila. Science*, **301**: 1731.

Mair, W., Piper, M.D.W. and Partridge, L. 2005. Calories do not explain extension of life span by dietary restriction in *Drosophila. PLoS Biol.*, **3**: e223.

Maklakov, A., Kremer, N. and Arnqvist, G. 2005. Adaptive male effects on female ageing in seed beetles. *Proc. R. Soc. Lond. B*, **272**: 2485–2489.

Marden, J.H., Rogina, B., Montooth, K.L. and Helfand, S.L. 2003. Conditional tradeoffs between aging and organismal performance of Indy long-lived mutant flies. *Proc. Natl. Acad. Sci. USA*, **100**: 3369.

Miller, R.A., Harper, J.M., Dysko, R.C., Durkee, S.J. and Austad, S.N. 2002. Longer life spans and delayed maturation in wild-derived mice. *Exp. Biol. Med.*, **227**: 500–508.

Min, K.-J. and Tatar, M. 2006. *Drosophila* diet restriction in practice: do flies consume fewer nutrients? *Mech. Ageing Dev.*, **127**: 93–96.

Miyo, T. and Charlesworth, B. 2004. Age-specific mortality rates of reproducing and non-reproducing males of *Drosophila melanogaster. Proc. R. Soc. Lond. B*, **271**: 2517–2522.

Mueller, L.D. 1987. Evolution of accelerated senescence in laboratory populations of *Drosophila. Proc. Natl. Acad. Sci. USA*, **84**: 1974–1977.

Partridge, L. 1997. Evolutionary biology and age-related mortality. In *Between Zeus and the Salmon: The Biodemography of Longevity* (K.W. Wachter and C.E. Finch, eds.), pp. 78–95. Washington, DC: National Academy Press.

Pletcher, S.D. 1999a. Model fitting and hypothesis testing for age-specific mortality data. *J. Evol. Biol.*, **12**: 430–439.

Pletcher, S.D. 1999b. *Winmodest*. Rostock, Germany: Max Planck Institute for Demographic Research.

Pletcher, S.D., Khazaeli, A.A. and Curtsinger, J.W. 2000. Why do life spans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters. *J. Gerontol. A: Biol. Sci. Med. Sci.*, **55**: B381–B389.

Pletcher, S.D., Macdonald, S.J., Marguerie, R., Certa, U., Stearns, S.C., Goldstein, D.B. *et al.* 2002. Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster. Curr. Biol.*, **12**: 712–723.

Priest, N.K., Mackowiak, B. and Promislow, D.E.L. 2002. The role of parental age effect on the evolution of aging. *Evolution*, **56**: 927–935.

Promislow, D.E.L. and Haselkorn, T.S. 2002. Age-specific metabolic rates and mortality rates in the genus *Drosophila. Aging Cell*, **1**: 66–74.

Promislow, D.E.L., Tatar, M., Khazaeli, A.A. and Curtsinger, J.W. 1996. Age-specific patterns and genetic variation in *Drosophila melanogaster:* I. Mortality. *Genetics*, **143**: 839–848.

Promislow, D.E.L., Tatar, M., Pletcher, S.D. and Carey, J.C. 1999. Below threshold mortality: implications for studies in evolution, ecology and demography. *J. Evol. Biol.*, **12**: 314–328.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**: 223–225.

Rose, M.R. 1991. *Evolutionary Biology of Aging*. Oxford: Oxford University Press.

Rose, M. and Charlesworth, B. 1980. A test of evolutionary theories of senescence. *Nature*, **287**: 141–142.

Rueppell, O., Fondrk, M.K. and Page, R.E. 2005. Biodemographic analysis of male honey bee mortality. *Aging Cell*, **4**: 13–19.

Ruiz, A. and Heed, W.B. 1988. Host-plant specificity in the cactophilic *Drosophila mulleri* species complex. *J. Anim. Ecol.*, **57**: 237–249.

Sambucetti, P., Sørensen, J.G., Loeschcke, V. and Norry, F.M. 2005. Variation in senescence and associated traits between sympatric cactophilic sibling species of *Drosophila. Evol. Ecol. Res.*, **7**: 915–930.

SAS Institute. 1989. *SAS/STAT User's Guide*, Version 6, Vol. 2. Cary, NC: SAS Institute Inc.

Service, P.M., Ochoa, R., Valenzuela, R. and Michieli, C.A. 1998. Cohort size and maximum likelihood estimation of mortality parameters. *Exp. Gerontol.*, **33**: 331–342.

Snoke, M.S. and Promislow, D.E.L. 2003. Quantitative genetic tests of recent senescence theory: age-specific mortality and male fertility in *Drosophila melanogaster. Heredity*, **91**: 546–556.

Spencer, C.C. and Promislow, D.E.L. 2005. Age-specific changes in epistatic effects on mortality rate in *Drosophila melanogaster. J. Hered.*, **96**: 513–521.

Starmer, W.T. 1982. Analysis of community structure of yeasts associated with the decaying stems of cactus. I. *Stenocereus gummosus. Microb. Ecol.*, **8**: 71–81.

Starmer, W.T., Heed, W.B. and Rockwood-Sluss, E.S. 1977. Extension of longevity in *Drosophila mojavensis* by environmental ethanol: differences between subraces. *Proc. Natl. Acad. Sci. USA*, **74**: 387–391.

Swindell, W.R. and Bouzat, J.L. 2006. Inbreeding depression and male survivorship in *Drosophila*: implications for senescence theory. *Genetics*, **172**: 317–327.

Tu, M.P., Epstein, D. and Tatar, M. 2002. The demography of slow aging in male and female *Drosophila* mutant for the insulin-receptor substrate homologue *chico. Aging Cell*, **1**: 75–80.

Van Voorhies, W.A., Fuchs, J. and Thomas, S. 2005. The longevity of *Caenorhabditis elegans* in soil. *Biol. Lett.*, **1**: 247–249.

Vaupel, J.W., Carey, J.R., Christensen, K., Johnson, T.E., Yashin, A.I., Holm, N.V. *et al.* 1998. Biodemographic trajectories of longevity. *Science*, **280**: 855–860.

Wilson, D.L. 1994. The analysis of survival (mortality) data: fitting Gompertz, Weibull and logistic functions. *Mech. Ageing Dev.*, **74**: 15–33.

Yampolsky, L.Y., Pearse, L.E. and Promislow, D.E.L. 2001. Age-specific effects of novel mutations in *Drosophila melanogaster*. I. Mortality. *Genetica*, **110**: 11–29.