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Chapter 10

Biodemography of Aging and Age-Specific Mortality in *Drosophila melanogaster*

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I. Introduction

For the last 15 years there has been a high level of interest in combining the methods of biology and demography to investigate aging in experimental populations. The hybrid field of biodemography addresses a wide range of questions about aging organisms and aging populations, and also attempts to provide insights into human aging (Wachter & Finch, 1997). A handful of issues have preoccupied the nascent field: To what extent are the genetic phenomena that influence life histories age-specific in their effects? How malleable are the patterns of survival and death among the oldest organisms? Why do populations often exhibit mortality plateaus? How have observed survival patterns evolved under the influence of mutation and natural selection? To what extent do survival patterns in populations reflect underlying changes in individual organisms? All of these questions are challenging, and none fully answered yet. Addressing them requires a set of analytical techniques that are commonplace to demographers but foreign to most biologists. Here we review some basic analytic methods from demography and lay out essential biological methods and questions, hoping to introduce both biologists and demographers to the hybrid field.

The integration of genetic and demographic methods requires an experimental system that is genetically defined and amenable to large-scale population studies. The fruit fly *Drosophila melanogaster* is an obvious candidate, being one of the premiere experimental systems for basic research in genetics. The genome is completely sequenced, and the flies can be reared in large numbers (tens of thousands of organisms). The nematode *Caenorhabditis elegans* and some yeast species also have those desirable characteristics, but other standard experimental systems do not. The genetics of house mice (*Mus musculus*) is an important and growing area of research, but large-scale population studies with rodents are impractical. Demography of medflies

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(*Cerititus capitata*) and several parasitic wasp species has been investigated in large experimental populations (Carey, 2003), but those systems are genetically undefined. An interesting feature of *Drosophila* as an experimental model is the similarity of its mortality kinetics to that of humans, first noted by Raymond Pearl (1922). Both species have a relatively short period of high initial mortality, followed by a relatively long period of mortality increase, and then deceleration at advanced ages (although the period of mortality deceleration and mortality plateau in *Drosophila* is longer than in humans).

Here we concentrate on *D. melanogaster*, a holometabolous insect. Larvae hatch from eggs about 24 hours after laying, feed voraciously for a week, and then pupate. Adults emerge from the pupal case after a few days of metamorphosis and are sexually mature within 24 hours. In the wild, *D. melanogaster* adults probably live one to two weeks. In laboratory culture, flies are normally maintained on a two-week generation schedule but can live much longer as adults. In a typical outbred population, adults survive 30 to 50 days on average, depending on temperature and other environmental conditions. Inbreeding and increased temperature reduce mean adult life spans, while artificial selection for increased life span is capable of doubling it. Maximum adult life spans observed in large experiments typically exceed 100 days. There is no precise definition of young, middle-aged, or old adult flies. At two weeks after emergence, metabolic rate and gene expression reach low levels characteristic of remaining adult life (Tahoe *et al.*, 2004; Van Voorhies *et al.*, 2003, 2004). For females, old age in flies is probably best understood as the age after egg laying has ceased, usually 40 to 60 days after emergence, depending on genotype and environmental conditions.

A. Collection of Survival Data

Survival experiments with laboratory populations of *Drosophila* are typically longitudinal, large scale, and complete. That is, age-synchronized cohorts consisting of thousands or tens of thousands of experimental animals are established with newly emerged adults and are observed over time. As the cohorts age, dead animals are removed, counted, and recorded on a daily basis. Observations continue until the last fly dies, typically around 100 days after emergence (depending on genotype and sample size; see below). Experimental populations are maintained under controlled environmental conditions, including temperature, light cycle, and humidity. Initial population density is also controlled, at least approximately; in smaller experiments, exact numbers of flies are counted, whereas in larger experiments, density is approximated by volume or weight of anesthetized flies (one large female weighs ~ 1mg., whereas males are typically ~30 percent smaller). Experimental populations are often housed in cages of one to several liters in volume, each holding up to a thousand individuals, but half-pint milk bottles and finger-sized glass vials are sometimes used. There is always fresh fly food in the containers, which serves as both an oviposition medium and a source of nutrition for adults and larvae. Frequent replacement of the medium and changing cages prevents unwanted recruitment of new adults into experimental populations.

Populations used for survival studies typically consist of males and females in approximately equal proportions when experiments are initially set up, but because of differential survival, the sex ratio changes over time. In mixed-sex population cages, females actively reproduce and generally exhibit shorter average life

spans than males (Curtsinger & Khazaeli, 2002; Curtsinger *et al.*, 1998; Fukui *et al.*, 1993, 1995, 1996; Khazaeli & Curtsinger, 2000; Khazaeli *et al.*, 1997; Pletcher, 1996; Resler *et al.*, 1998;). Because flies reach sexual maturity soon after emergence, mating behavior begins almost immediately in mixed-sex populations. It is possible to study the survival characteristics of unmated flies in single-sex populations by anesthetizing newly emerged adults and then sorting the sexes under a dissecting microscope when cohorts are initially established (Miyo & Charlesworth, 2004; Semenchenko *et al.*, 2004).

There is significant uncontrolled environmental variation that affects death rates in experimental populations of *Drosophila*. The magnitude of the variation is perhaps underappreciated. For instance, it is not unusual to see four- or five-fold variation in individual life spans among flies of the same genotype sharing the same food and population cage. This is not a peculiarity of fly life spans; biologists have long recognized that quantitative traits vary between organisms, even if they are genetically identical and reared under carefully controlled conditions (for a review, see Finch & Kirkwood, 2000; Gavrilov & Gavrilova, 1991). Because of this irreducible variation, which is not well understood, survival experiments should be highly replicated, in some cases involving hundreds of populations. Ideally, data from genotypes or treatments that are to be contrasted are collected simultaneously in order to avoid confounding uncontrolled environmental variations with treatment or genotype effects.

B. Data Analysis: Mean Life Span and Survivorship

The central problem in survival analysis is to summarize and interpret large amounts of information hidden in the survival data. Raw data consist of estimated ages at death. Mean life span, the arithmetic average survival time, has intuitive appeal as a descriptor of survival ability, but the information contained in that summary statistic is limited. The most critical limitation in the present context is that the mean gives little information about the age-specificity of survival patterns. Two cohorts could have very similar means but experience vastly different life histories. For instance, if one population suffers mortality only at middle age, whereas a second experiences mortality equally and exclusively at early and late ages, mean life spans in the two populations will be similar. Maximum observed life span is also frequently reported but is similarly uninformative about age-specific events.

The central conceptual tool for organizing and analyzing age-specific aspects of survival data in experimental populations of *Drosophila* and other species (indeed, other objects) is the cohort life table. It is interesting that *Drosophila* was the second species, after humans, for which such demographic life tables were constructed (Pearl & Parker, 1921). The essential features of the life table are that age classes are defined by sampling intervals, and for each age class (life table row) specific variables (life table columns) are estimated. The first variable is the fraction of the total population dying while in age class x, denoted d_x. The distribution of d_x, a typical example of which is shown in Figure 10.1a, is approximately bell-shaped but not symmetrical, in contrast to the normal curve. The long right-skewed tail represents the oldest survivors of the cohort and is observed even in genetically homogeneous populations. The second variable, survivorship, is represented as l_x and is defined as the probability of survival from the beginning of the experiment until the beginning age interval x. That probability is estimated by the proportion of the initial cohort that remains alive at age x. Survival curves, which show plots of l_x versus x, start at

100 percent and decline to zero at the age when the last animal in the cohort dies (see Figure 10.1b). Survival curves have built-in smoothing because they are nonincreasing (the proportion of the initial cohort remaining alive at age x can only be the same or lower at age $x + \Delta x$). For this reason, even relatively small cohorts produce smoothly declining survivorship curves. Life-table values of l_x and d_x are related as follows: $l^{x+\Delta x} = l_x - d_x$, where Δx is the length of the sampling (age) interval, typically equal to one day for fly experiments. It is important to emphasize that both l_x and d_x are cumulative indicators that depend on preceding death rates. Events early in the life history, such as a temporary epizootic, can affect survivorship and the fraction dying in later age classes, even in old age. In this sense, l_x and d_x reflect the survival history of the cohort up to and including age x.

C. Data Analysis: Probability of Death and Mortality Rate

Unlike survivorship and fraction dying, which have "memory," some other life-table variables are noncumulative and better suited to detecting age-specific effects. Age-specific probability of death (q_x) is defined as the conditional probability of dying in the interval Δx for individuals that survive to the beginning of interval x. It is estimated as the number of deaths that occur in age class x, divided by the number of individuals entering class x. An example of age-specific probability of death is shown in Figure 10.1c. Note that in this particular example, the age-specific probability of death grows monotonically with age up to an advanced age and then levels off, a phenomenon discussed in detail later.

Although probability of death is useful and intuitive, it has limitations. The main problem is that the value of q_x depends on the length of the age interval Δx for which it is calculated, which hampers both analyses and interpretation. For example, one-day probabilities of death may follow the Gompertz law of mortality, but probabilities of death calculated for other age intervals with the same data may not (Gavrilov & Gavrilova, 1991; le Bras, 1976). A meaningful descriptor of the dynamics of survival should not depend on the arbitrary choice of age intervals. Another problem is that, by definition, q_x is bounded by unity, which makes it difficult to scale the variable for studies of mortality at advanced ages.

A more useful indicator of mortality is the instantaneous mortality rate, or hazard rate, μ_x, which is defined as follows:

$$\mu_x = -\frac{dN_x}{N_x dx}$$

where N_x is the number alive at age x. The hazard rate does not depend on the length of the age interval; it reflects instantaneous risk of death. It has no upper bound and has the dimension of a rate (time^{-1}). One of the first empirical estimates of hazard rate μ_x was proposed by Sacher (1956):

$$\mu_x = \frac{1}{\Delta^x}\left(ln\, l_{x-\frac{\Delta^x}{2}} - ln\, l_{x+\frac{\Delta^x}{2}}\right)$$

$$= \frac{1}{2\Delta^x} ln \frac{l_{x-\Delta^x}}{l_{x+\Delta^x}}$$

This estimate is unbiased for slow changes in hazard rate (Sacher, 1966). A simplified version of the Sacher estimate (for small age intervals equal to unity) is often used in biological studies of mortality: $\mu_x = -ln(1-q_x)$ (see Carey, 2003) and assumes constant hazard rate in the age interval.

The Cutler-Ederer (1958) estimate (also called the actuarial hazard rate) is based on the assumption that deaths are

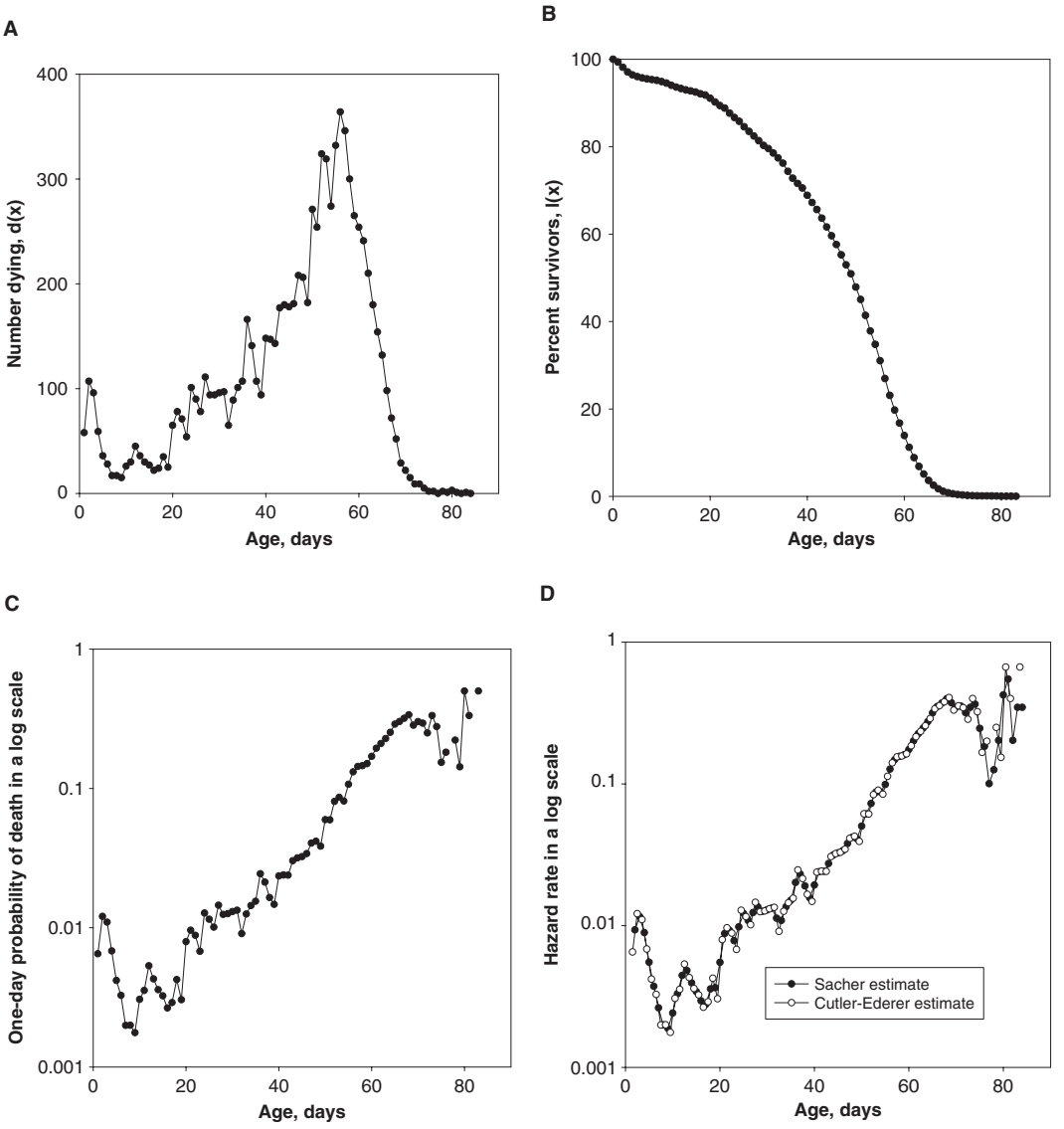

Figure 10.1 Life-table variables as a function of adult age, estimated for experimental population of 8,926 *D. melanogaster* males. (a) Number dying d_x; (b) survivorship l_x; (c) age-specific probability of death q_x; (d) age-specific mortality (hazard) rate μ_x. The subscript "x" indicates adult age in days since eclosion. (Unpublished data of Khazaeli, Gavrilova, Gavrilov, & Curtsinger)

uniformly distributed in the age interval and that all cases of withdrawal (censoring) occur in the middle of the age interval:

$$\mu_{x+\frac{\Delta^x}{2}} = \frac{d_x}{\Delta^x\left[l_x - \frac{c_x}{2} - \frac{d_x}{2}\right]}$$

Here, c_x is number of censored individuals during the age interval (for example, number of flies accidentally escaping the cage during food replacement). The hazard rate is measured at the midpoint of the age interval. Gehan and Siddiqui (1973) used Monte Carlo simulation to show that for samples less than 1,000,

the Sacher method may produce biased results compared to the Cutler-Ederer method, whereas for larger samples, the Sacher estimate is more accurate. The advantage of the Cutler-Ederer estimate is its availability in standard statistical packages (such as SAS and *Stata*), which compute actuarial life tables. Despite the apparent differences between Cutler-Ederer and Sacher estimates, the methods produce very similar results for real data (see Figure 10.1d). Note that the mortality curve, depicting μ_x as a function of x, describes survival events in true age-specific fashion. It clearly illustrates the rate of actuarial senescence, usually defined as the slope of the mortality curve, and is particularly useful for examining details of death rates among the oldest survivors of a cohort. In contrast, the details of shape in a survivorship curve as it approaches the x-axis are generally indistinct (but see Pearl & Parker's method, described below).

The differences between survivorship and mortality are fundamental. The former depends on all previous cohort history, whereas the latter reflects risk specific solely to the age group under study. This distinction has often been misunderstood or overlooked by biologists. Rose's (1991) influential text on evolutionary biology of aging contains dozens of figures, extensive discussion of age-specific life-history phenomena, and not a single depiction of a mortality curve, either experimental or theoretical. Similarly, Kirkwood's (1999) general text on causes of aging gives considerable notice to age-specific phenomena but employs survivorship rather than mortality throughout. In an otherwise excellent paper on chromosomal mapping of genes that influence mean life spans in *Drosophila*, Nuzhdin and colleagues (1997) test an evolutionary model of senescence by examining age-specific variance in l_x, when the issue is clearly variance in μ_x.

Perhaps the most common misunderstanding among biologists about survivorship and mortality is the widespread assumption that rates of senescence can be easily seen in the slopes of survivorship curves. The apparent or actuarial rate of senescence, defined as the rate at which risk of death increases with age, is precisely reflected in the slope of the mortality curve: a steep slope indicates rapid actuarial senescence, a shallow slope indicates negligible senescence, and a zero slope indicates no senescence. Of course, the slope of the survivorship curve bears a mathematical relationship to the slope of the corresponding mortality curve, but not one that is easily grasped by visual inspection. The problem is that even populations that experience no apparent senescence (constant probability of death at all ages) will exhibit exponentially declining survivorship with increasing age. Thus, information about the rate of senescence is present in a survivorship curve only as a deviation from the exponential, a quantitative measure that is not well suited to casual inspection. Pearl and Parker (1924) addressed this problem by examining survivorship in semi-logarithmic plots. This approach may be useful in defining periods of mortality leveling-off (mortality plateaus): survivorship curves in semi-logarithmic scale should be linear if mortality is constant. Economos (1979, 1980) used this method for demonstrating non-Gompertzian mortality kinetics at advanced ages, but the technique has not been widely used in recent years.

There are probably several reasons that biologists in some fields have not, until recently, adequately appreciated the information that can be gained by estimating mortality rates. Survivorship curves have intrinsic smoothing, as mentioned above, whereas mortality curves tend to be jumpy. For a single data set plotted both ways, the mortality estimation makes the data look noisy, whereas

the survivorship curve gives an appearance of orderly behavior. Accurate estimation of age-specific mortality rates requires larger sample sizes than those needed for estimating means or survivorship but provides extra sensitivity in studies of short-term response to phenomena such as heat shock (Khazaeli *et al.*, 1997) and dietary restriction (Mair *et al.*, 2003; Pletcher, 2002). The sample-size requirement is especially critical for the oldest ages; large initial cohort sizes are required in order to have adequate numbers of animals alive for estimation of death rates at the older ages.

In the 1920s, Raymond Pearl, an early advocate of biostatistics and experimental investigation of populations, published a series of papers on *Drosophila* life spans that employed relatively large sample sizes. For instance, Pearl and Parker (1924) collected survival data on about 4,000 flies from two strains. Since the 1950s, radiobiologists have routinely employed large sample sizes to estimate mortality rates in survival studies with experimental organisms. However, in spite of those pioneering efforts, up until around 1990, it was standard practice among experimental gerontologists, evolutionary biologists, and geneticists to employ small populations in studies of *Drosophila* survival, typically on the order of 50 to 100 animals per experimental treatment or genotype. Such sample sizes sufficed to give reasonably accurate estimates of mean life spans and aesthetically pleasing survivorship curves but provided virtually no information about death rates in old age.

Sample size requirements will depend on the specific question being asked. For accurate estimates of hazard rates, it is necessary to have some events (deaths) in each age interval. At younger ages, when mortality rates are low, it would be desirable to have at least one death in each observation interval. In small samples there might be no deaths during some intervals, in which case intervals will have to be combined and the accuracy of hazard rate estimation will decline. Thus, the minimum sample size of experimental populations for hazard rate studies may be estimated on the basis of expected risk of death during younger ages, when mortality is low.

For example, if the expected risk of death is 1 per 1,000 during a one-day period, then the sample size should be at least 1,000. If mortality at younger ages is higher, then smaller sample sizes will suffice. This rule of thumb does not apply to studies of mortality deceleration and leveling-off. This phenomenon happens later in life, after a significant part of population has died and the remaining number of animals is a small fraction of the initial cohort. The empirical rule here may be to have at least 50 animals alive at the age when mortality deceleration starts so that hazard rate estimations would not be distorted by small numbers of deaths. If one is interested in short-term effects of caloric restriction or other interventions on mortality kinetics at middle ages close to the modal life span, then much smaller sample sizes may be sufficient because numbers of organisms at risk and numbers of deaths will be substantial.

D. Smoothing and Model Fitting

Two approaches are commonly used to describe trends in the (often noisy) data on age-specific mortality. One approach is to apply a non-parametric smoothing procedure. For data organized in the form of a life table, smoothing can be accomplished by widening the age intervals. If times to death for each individual in the sample are known with reasonable accuracy, and/or small sample size does not allow construction of a conventional life table, then the method of hazard rate smoothing using kernel functions may be more appropriate (Ramalu-Hansen,

1983). The latter method is more computationally complex, although special routines are available now in SAS and *Stata*. Applying methods of non-parametric smoothing decreases statistical noise and facilitates visual inspection of mortality plots but does not allow quantitative analysis of life-span data.

The second major approach for summarizing and simplifying mortality estimates is parametric model fitting, which allows researchers to describe the observed mortality kinetics using a small number of parameters of a specified mortality model. Although there are many possible models in the literature, three are widely used by biologists. The venerable model of Gompertz (1825) specifies exponentially increasing hazard rate with increasing age:

$$\mu_x = Ae^{Bx}$$

where A is initial mortality rate, e is the base of the natural logarithms, and B, the slope parameter, controls the rate at which mortality increases with age. Estimates of A in laboratory populations of *D. melanogaster* are typically in the range 0.005 to 0.010 per day, whereas B often lies in the range 0.04 to 0.10 per day (Fukui *et al*, 1993). The Gompertz model produces a straight line in semi-log plots of hazard rate versus age, with the y-intercept estimating the initial mortality rate and the slope estimating the rate of senescence. The aging rate is sometimes summarized by the mortality rate doubling time (MRDT), defined as $\ln(2)/B$. However, this measure has limited applicability to *Drosophila* because of non-Gompertzian mortality dynamics at advanced ages; in particular, as B approaches zero in old age, the MRDT approaches infinity.

A second widely used model is the logistic, which is motivated by the possibility that individuals in the same population can have different frailties (age-dependent chances of death). Differences in frailty might be innate and fixed throughout life, or modified over the life history. Strehler and Mildvan (1960) showed that when there is such heterogeneity, the observed population mortality pattern deviates from the underlying mortality for individuals. Following Beard (1963), the observed mortality in the population is

$$\mu_x = Ae^{Bx}/[1 + \sigma^2 \Lambda(x)],$$

where A and B are as defined in the Gompertz model, σ^2 is the variance for frailty in the population, and $\Lambda(x) = (A/B)(e^{Bx} - 1)$. Note that when $\sigma^2 = 0$, there is no heterogeneity in the population, and the logistic reduces to the Gompertz model. However, if $\sigma^2 > 0$, then the logistic curve increases exponentially at early ages and plateaus at more advanced ages (as x becomes large, u_x approaches B/σ^2). Yashin and colleagues (1994) showed that this model applies under two biologically different circumstances: when individuals possess a fixed frailty from birth that differs from that of other individuals, and when all individuals start life with identical frailties but then randomly acquire differences in frailty during adulthood.

A third model used by biologists is also motivated by the observation that mortality data often exhibit plateaus at older ages. This approach involves fitting two curves to the mortality data. Curtsinger and colleagues (1992) proposed a two-stage Gompertz model, in which a Gompertz curve is fit to the data at young ages up to some breakpoint age, and then a second Gompertz curve with shallower slope is fit to the older ages. This model includes five parameters: two intercept and two slope parameters for two Gompertz curves, and a fifth parameter for the breakpoint. Zelterman and Curtsinger (1994, 1995) applied the method to fly data, and Vaupel and colleagues (1994) used it for nematodes.

Drapeau and colleagues (2000) employed a similar method, except older ages were fit to a linear rather than exponential curve. It should be noted that mortality trajectories following the Weibull (power) law of mortality may resemble a two-stage Gompertz model in semi-log coordinates (see Chapter 1 in this volume).

The two major methods of parameter estimation for nonlinear models are maximum likelihood and nonlinear least squares. The maximum likelihood approach is based on maximizing the likelihood function, or the probability of obtaining a particular set of data given the chosen probability model. Maximum likelihood methods provide unbiased and efficient parameter estimates for large data sets (though the estimates may be heavily biased for small samples). Another advantage is that maximum likelihood generates theoretically more accurate confidence bounds for parameter estimates. An important property of maximum likelihood for survival data is that censored observations can be readily introduced (see Filliben, 2004). The limitation of this method is the need for specifying the maximum likelihood equations for each particular function not implemented in the standard statistical software packages, which often is not trivial. Standard statistical packages provide maximum likelihood estimates for a limited number of models. For example, the *Stata* package has a procedure for maximum likelihood estimation of Gompertz and logistic models. Maximum likelihood estimation of Gompertz, Gompertz-Makeham, logistic, and logistic-Makeham models is implemented in *WinModest,* a program written and distributed by S. Pletcher (Baylor College of Medicine, Houston) specifically for calculating basic statistics, fitting mortality models to survival data, and partitioning mean longevity differences between populations (Pletcher & Curtsinger, 2000a).

The nonlinear least squares method provides an alternative to maximum likelihood. This method is implemented in most statistical software packages and allows researchers to fit a large variety of nonlinear models. The limitation of this method is its theoretically less desirable optimality properties compared to the maximum likelihood, and less applicability to censored data. Both methods are sensitive to the choice of initial parameter estimates and outliers.

There is a tradeoff between flexibility and convenience of the nonlinear least squares method and the accuracy of the maximum likelihood approach. In practice, the theoretical considerations mentioned above are apparently not crucial, and the two approaches generate similar results. For example, Gehan and Siddiqui (1973) conducted a simulation study of fitting Gompertz and some other hazard models to survival data. The authors concluded that the least squares estimates are nearly as efficient as maximum likelihood when sample size is 50 or more. They also found that the weighted least squares approach, which accounts for systematic decrease of the sample size with age, generated more efficient but less accurate parameter estimates compared to the nonweighted method. Thus, maximum likelihood is a preferred method in those cases where the statistical software is readily available or the optimization procedure can be easily implemented. Otherwise, the nonlinear least squares may be a reasonable choice.

It is important to recognize the limitations and pitfalls of model fitting. The main problem is uneven statistical power. At young ages, there are relatively few deaths; at the oldest ages, death rates are high, but there are relatively few organisms. At middle ages, there are large numbers of both organisms at risk and deaths, and so statistical power for estimation of mortality rates is

concentrated in those middle age classes. Consequently, model fitting to the entire life history can give very accurate descriptions of the dynamics of middle age and can be systematically biased at early and late ages.

II. Experimental Evidence for Age-Specific Effects

If new mutations and genetic variants segregating in populations modify chances of survival by a constant factor at all ages (a situation known among demographers as "proportional hazards"), then there is no true age specificity; all is known from observations at a single age. However, if genes alter survival characteristics specifically at certain prescribed ages or stages of the life cycle, with no effect or very different effect at other ages, then the situation is more complex, and much more interesting. The evolutionary theory for the evolution of senescence requires age-specificity of genetic effects (Charlesworth, 1980; Curtsinger, 2001; Hamilton, 1966; Medawar, 1952; Williams, 1957). As we discuss below, evolutionary models currently under investigation are sensitive to the precise degree of age specificity. Proving the existence of such age-specific genetic variation is difficult, especially at the older ages, but mounting evidence suggests that there may be a substantial degree of age specificity of genetic effects in *Drosophila*. In the following sections, we describe several different types of experimental evidence that address that issue.

A. P-Element Tagging

P-elements are naturally occurring transposable genetic elements (transposons) specific to *Drosophila*. Their ability to insert into random chromosomal locations throughout the genome makes them useful tools for genetic research, because they potentially disrupt gene expression or function at the insertion site. Screening of P-element inserts led to the discovery of life-extending "methuselah" (*mth*) and "I'm not dead yet" (*Indy*) single-gene mutations (Lin *et al.*, 1998; Rogina *et al.*, 2000). Clark & Guadalupe (1995) used P-element insertion lines to investigate the genetic basis of senescence and found that otherwise genetically identical lines differed in survivorship and mean life span under the influence of P-induced insertions. The authors claimed that some of the P-element insertions led to reduced post-reproductive survival without affecting early life history, and that P-element inserts altered the ages at which mortality curves leveled off, though few demographic details were given.

B. Mutation Accumulation Experiments

The term *mutation accumulation* refers to both a theory of the evolution of senescence (Medawar, 1952) and an experimental design pioneered in *Drosophila* (Mukai, 1964). It is the latter sense of the term that concerns us for the moment, although the former will be relevant later. The goal of a mutation accumulation experiment is to measure the rate at which new genetic variation spontaneously arises in a population, and to measure the phenotypic effects of those new mutations. General features of mutation accumulation experiments using *Drosophila* are as follows: starting with a single highly inbred line of flies, multiple sub-lines are established and maintained separately in small populations for dozens or even hundreds of generations. Spontaneous germline mutations occur independently in the various sub-lines, causing them to diverge both genetically and phenotypically. The sub-lines are kept at small census numbers so that new mutations have a reasonable chance to increase to fixation within

each particular line by random genetic drift. The rate at which sub-lines diverge phenotypically provides an estimate of the rate of input of new genetic variation affecting the particular trait assayed.

The first mutation accumulation study of age-specific mortality was executed by Pletcher and colleagues (1998), who established 29 sub-lines of *D. melanogaster* from a single highly inbred progenitor pair. Sub-lines were maintained for 19 generations, and then survival data were collected on approximately 100,000 flies. Mutational effects were detected by comparing age-specific mortality rates in each sub-line with that of the progenitor stock, which was maintained in nonmutating condition by cryopreservation. Significant mutational variance for age-specific mortality was detected, but only for flies aged less than 30 days post-emergence. Most of the new mutations were highly age-specific, each affecting survival rates over a well-defined age window of one or two weeks. Mutations that affected mortality at all ages were also detected, but their contribution to overall mutational variance was small. The conclusion from this study is that most new mutations have age-specific effects, but the failure to detect mutational variance at very old ages is difficult to interpret. It is unclear whether the failure to detect late-acting mutations is due to smaller sample sizes and loss of statistical power, to inherently lower mutation rates for alleles that specifically affect old age survival, or a combination of those factors.

Pletcher and colleagues (1999) continued the mutation accumulation experiment, assaying mortality rates at 47 generations of divergence, and also jointly analyzing data at three time points (10, 19, and 47 generations). These assays involved approximately a quarter of a million flies. Further evidence for highly age-specific mutation was found, and once again there was evidence for higher levels of mutation affecting early survival than late survival. Surprisingly, there appeared to be no upward or downward bias of mutational effects on mortality rates (mutations increasing mortality are as frequent as mutations decreasing mortality), contradicting the usual assumption that almost all mutations are deleterious to carriers. One possible explanation of this paradox may be related to elimination of many deleterious mutations through selective deaths at early larval stages of *Drosophila* development.

Mack and colleagues (2000) and Yampolsky and colleagues (2001) used a different experimental design, the "middle class neighborhood" method, to accumulate mutations affecting mortality, fecundity, and male mating ability on a genetically heterogeneous background of recently collected flies. They found clear evidence of age-specific effects of new mutations after 20 generations of mutation accumulation, including many effects limited to middle and advanced ages. This result contrasts with that of Pletcher and colleagues (1998, 1999), who found mostly early age effects. In both studies, the degree of age specificity declined in later generations of the experiment.

Martorell and colleagues (1998) executed a large mutation accumulation experiment to study life history in *D. melanogaster*, maintaining 94 sublines for 80 generations. They found evidence for small mutational effects on mean life span, but because mortality rates were not assayed, the experiment provides no information about age specificity of genetic effects. If Pletcher and colleagues (1999) are correct about mutations decreasing mortality as often as they increase it, then Martorell and colleagues (1998) might have underestimated the rate of mutations that modify mean life spans. Similar remarks apply to studies of life span and related characters in flies exposed to mutagenic chemicals (Keightley & Ohnishi, 1998). Mutation

accumulation experiments on life-history traits have also been executed using the nematode *C. elegans* (Keightley *et al*., 2000).

C. Neurogenetics and Gene Expression

Adult *Drosophila* are entirely post-mitotic organisms; that is, all cell division is completed when the animal metamorphoses from larval to adult stage. This contrasts sharply with other organisms, such as humans, in which cell division continues throughout the adult life span. It has been suggested that the lack of cell division in adult flies precludes late-onset genetic effects in *Drosophila*. However, recent evidence from several areas of biology that are not normally part of the discourse of demography suggests otherwise.

Neurodegenerative diseases in human, including Alzheimer's, Huntington, and Parkinson's disease, are characterized by late onset of pathology. Because *Drosophila* and humans share many functionally and structurally related genes, it is possible to model some of the human neurodegenerative pathologies by creating lines of flies that carry foreign or artificially modified genes (Driscoll & Gerstbrein, 2003; Fortini & Bonini, 2000; Mutsuddi & Nambu, 1998). Feany and Bender (2000) constructed transgenic flies carrying normal or mutant forms of the human gene for α-synuclein, a candidate cause of Parkinson's disease. All transgenics exhibited normal neural morphology and geotactic behavior as young adult flies, but beginning at 25 days after eclosion, mutant transgenics developed Parkinson-like neural morphology and a dramatic loss of locomotor ability, whereas nonmutant transgenics escaped the morphological and behavioral manifestations of disease. Of course, the primary importance of such research is its potential application to treating human disease, but the α-synuclein case and others like it also demonstrate that genetic variation can produce specific late-onset phenotypes in adult *Drosophila*. Evidently, lack of cell division in adults does not preclude age-specific effects in older flies.

There is also evidence for age-specific genetic effects in modern studies of gene expression. It used to be widely assumed that the regulation of gene expression, which is capable of transforming single cells into highly differentiated and spatially structured mature organisms, becomes chaotic in old age. This view is now rejected, in part because of evidence from *Drosophila* (Helfand & Rogina, 2000, 2003; Rogina & Helfand, 1995; Rogina *et al*., 1998). Regulation of gene expression throughout the adult life span, including old age, sets the stage for age-specific genetic effects. DNA microarrays are powerful tools for the study of genome-wide patterns of gene expression in *Drosophila* and other organisms. Microarrays have been used to detect genes that vary in expression levels over the lifetimes of flies, and to detect genome-wide transcriptional responses to experimental treatments that modify life spans (McCarroll *et al*., 2004; Pletcher *et al*., 2002). Results from microarray studies bolster the view that gene expression is regulated throughout the adult life span and is therefore likely to be subject to genetic modification. Tahoe and colleagues (2005) demonstrated that age-specific patterns of gene expression differ between lines of *Drosophila* with very different mean life spans, and in some cases, including the genes encoding anti-microbial peptides, the line differences are manifest only in old age. Such observations do not prove that there are genetic differences between lines that alter survival specifically at advanced ages, but the observation of late-onset transcriptional differences does render the existence of such effects more likely. As more longitudinal studies of genome-wide transcription levels are

published in the next few years, we can expect a more complete picture of genome function and its variability throughout the adult life span.

D. Mortality QTLs

Quantitative trait locus (QTL) mapping is a set of procedures for identifying approximate chromosomal locations of segregating genes that influence polygenic traits (Mackay, 2001, 2002; see Chapter 8, this volume). QTLs affecting mean life span in *Drosophila* have been identified in a number of studies (Curtsinger *et al.,* 1998; De Luca *et al*., 2003; Forbes *et al*., 2004; Leips & Mackay, 2000, 2002; Luckinbill & Golenberg, 2002; Khazaeli *et al*., 2005; Nuzhdin *et al*., 2005; Nuzhdin *et al.,* 1997; Pasyukova *et al*., 2000; Resler *et al.,* 1998; Valenzuela *et al*., 2004; Vieira *et al*., 2000).

In principle, it is possible to apply the methods of QTL mapping to localize genes that affect age-specific mortality rates rather than just mean life spans. However, the requirements are stringent: not only is there the prerequisite for large sample sizes, as in any estimation of mortality rates, but it is also necessary that the populations be genetically highly defined and contain a high density of genetic markers for QTL localization. To date this has been accomplished in only two cases. Curtsinger and Khazaeli (2002) identified QTLs that affect age-specific mortality rates in recombinant inbred populations of *D. melanogaster*, finding evidence for several genetically variable chromosomal regions that influence survival in age-specific fashion. The authors also developed a graphical method for presenting age-specific QTL results, as follows. QTL mapping results are typically presented in two-dimensional graphs: the abscissa represents chromosomal position, measured in units of recombination from the left telomere, while the ordinate represents a statistical measure, likelihood or LOD score indicating the probability that a QTL is present at a particular chromosomal position. A typical QTL map has peaks and valleys; genes affecting the quantitative trait are most likely to be located in chromosomal regions under the peaks, provided that the peaks exceed some likelihood threshold. Curtsinger and Khazaeli (2002) extended the usual analysis by mapping QTLs that affect mortality in each week of adult life and then adding a third dimension to the QTL map, indicating age. An example of a three-dimensional QTL map is shown in Figure 10.2. There is a QTL that affects age-specific mortality near the left end of chromosome III; the QTL has significant effects on

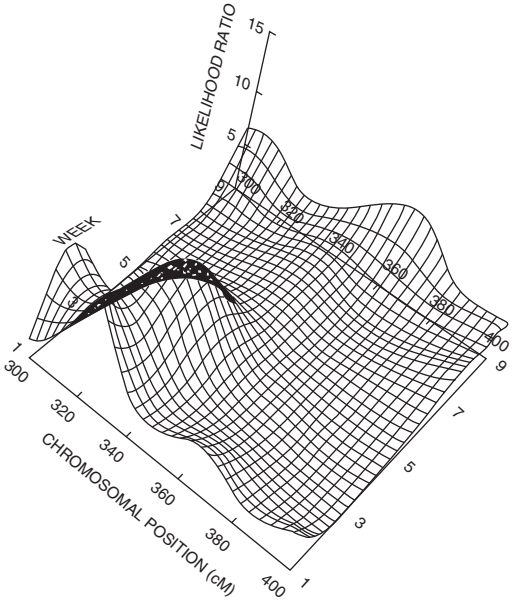

Figure 10.2 Three-dimensional QTL map of age-specific mortality rates for experimental populations of male *D. melanogaster* (Curtsinger & Khazaeli, 2002). The figure shows the chromosomal location and ontogenic timing of effects of quantitative trait loci that influence weekly mortality rates throughout the adult life span. The peak near the left telomere of chromosome III indicates genetic effects on mortality rates primarily in early adult life, with no evidence for significant effects late in adult life.

mortality in the first few weeks of adult life but has no effect on survival at later ages.

One other study of age-specific mortality rates using QTL mapping methods is that of Nuzhdin and colleagues (2005). QTLs affecting weekly mortality rates in both sexes were mapped in 144 recombinant inbred lines. Twenty-five statistically significant QTLs were found; most had positively correlated effects on mortality at several different ages, but in two cases the correlations were negative. Overall, the results suggest that the standing genetic variation in survival consists of a mixture of transient deleterious mutations that tend to increase mortality at younger ages, and a few mutations with opposing age-specific effects that are maintained by balancing selection. The latter are potentially examples of antagonistic pleiotropy, although finer genetic resolution will be required to rule out the competing linkage hypothesis.

III. Leveling-Off of Mortality Rates

In many biological species, including *Drosophila* and humans, death rates increase exponentially with age for much of the life span. However, at extreme old ages, a "mortality deceleration" occurs—the pace of mortality growth decelerates from an expected exponential curve. Sometimes this mortality deceleration progresses to the extent that mortality "leveling-off" is observed, leading to a "mortality plateau." Thus, at extreme old ages, a paradoxical situation is observed when one of the major manifestations of aging—increasing death rate—apparently fades away or even disappears.

The phenomenon of mortality deceleration has been known for a long time, although its mechanisms were not intensively studied prior to the 1990s. The first person who noticed that the Gompertz curve is not applicable to extreme old ages was Benjamin Gompertz himself (Gompertz, 1825, 1872; see review by Olshansky, 1998). In 1867, William Makeham noted that for humans "the rapidity of the increase in the death rate decelerated beyond age 75" (p. 346). In 1919, Brownlee wondered whether it is "possible that a kind of Indian summer occurs after the age of 85 years is passed, and that conditions improve as regards length of life" (p. 385). Perks (1932) observed that "the graduated curve [of mortality] starts to decline in the neighborhood of age 84" (p. 15). Greenwood and Irwin (1939) confirmed that "the increase of mortality rate with age advances at a slackening rate, that nearly all, perhaps all, methods of graduation of the type of Gompertz's formula overstate senile mortality" (p. 14). They also suggested "the possibility that with advancing age the rate of mortality asymptotes to a finite value" (p. 14), and made the first estimates for the asymptotic value of human mortality plateau (expressed in one-year probability of death, q_x). According to their estimates of human mortality plateaus, "the limiting values of q_x are 0.439 for women and 0.544 for men" (Greenwood & Irwin, 1939, p. 21). In 1960, *Science* published an article on a "General theory of mortality and aging" that listed some "essential observations which must be taken into account in any general theory of mortality." (Strehler & Mildvan, 1960, p. 14). The first of these essential observations was the Gompertz law of mortality, while the second essential observation stated that "the Gomperzian period is followed by a gradual reduction in their rate of increase of the mortality" (Strehler & Mildvan, 1960, p.14). This observation of mortality deceleration was confirmed for several species, including *Drosophila* and *C. elegans* (Economos, 1979). The author

concluded "that after a certain species-characteristic age, force of mortality and probability of death cease to increase exponentially with age . . . and remain constant at a high level on the average for the remainder of the life span." (p. 74). The author called these findings "a non-Gompertzian paradigm for mortality kinetics" (Economos, 1979, p. 74). A year later, the same author analyzed data for thoroughbred horses (mares), Dall mountain sheep, houseflies, and some other species and came to a conclusion that "Gompertz's law is only an approximation, not valid over a certain terminal part of the lifespan, during which force of mortality levels off." (Economos, 1980, p. 317). These findings failed, however, to receive attention, and the topic stagnated.

A. Recent Studies of Mortality Plateaus

Prior to 1990, the most popular explanation of mortality plateaus was based on the idea of initial population heterogeneity, suggested by British actuary Robert Eric Beard (1911–1983). Beard developed a mathematical model in which individuals were assumed to have exponential increase in their risk of death as they age, but their initial risks differed from individual to individual and followed a gamma distribution (Beard, 1959, 1963, 1971). This model produces a logistic function for mortality kinetics that is very close to the exponential function at younger ages, but then mortality rates decelerate and reach a plateau in old age. This compositional interpretation of mortality plateaus explained them as an artifact of mixture, perhaps reducing their intrinsic interest to biologists.

The situation changed in 1991, when it was found that the general theory of systems failure (known as reliability theory) predicts an inevitable mortality leveling-off as a result of redundancy exhaustion, even for initially identical individuals (Gavrilov & Gavrilova, 1991). Thus, a testable prediction from this theory was that mortality deceleration should be observed even for genetically identical individuals kept in strictly controlled laboratory conditions. Shortly thereafter, Carey and colleagues (1992) and Curtsinger and colleagues (1992) published back-to-back papers in *Science* demonstrating mortality plateaus in laboratory populations of medflies and *Drosophila*, respectively. The medfly study employed genetically heterogeneous populations, whereas the companion study in *Drosophila* used highly inbred lines that were essentially devoid of within-line genetic heterogeneity.

The medfly and *Drosophila* experimental papers generated a flurry of criticisms and responses (Carey *et al.*, 1993; Curtsinger *et al.*, 1994; Gavrilov & Gavrilova, 1993; Graves & Mueller, 1993, 1994; Kowald & Kirkwood, 1993; Nusbaum *et al.*, 1993; Robine & Ritchie, 1993; Vaupel & Carey, 1993). Within a few years, even the most ardent critics were convinced that mortality plateaus were real phenomena and not merely artifacts of contamination or declining density in population cages (Khazaeli *et al.*, 1995a, 1996). Mortality plateaus were subsequently documented on very large scales in a variety of experimental species, including yeast, nematodes, *Drosophila*, medflies, parasitic wasps, and humans (see Vaupel *et al.*, 1998, for a review).

Typical characteristics of a mortality plateau in *Drosophila* are shown in Figure 10.3 (from Pletcher & Curtsinger, 1998). In this sample of 122,000 males, age-specific mortality increases in approximately exponential fashion from emergence until 60 days. After 60 days, when 5 percent of the original cohort remains alive, mortality decelerates and remains fairly constant until 80 days of age. Thus,

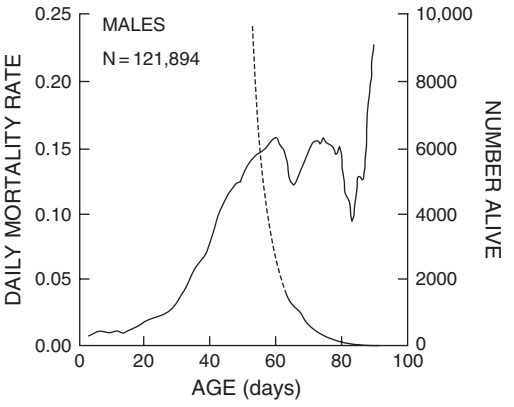

Figure 10.3 Age-specific mortality rate and survivorship in a large cohort of male *D. melanogaster* (Pletcher & Curtsinger, 1998). Age-specific mortality increases approximately exponentially until 60 days post-eclosion, and then reaches a plateau on days 60 to 80.

for a period of 20 days, or about 20 percent of the maximum life span in this particular experiment, there is no trend toward increasing mortality with increasing age. After 80 days the mortality curve shoots up, as the last few survivors die. The latter behavior is of no particular significance, and is best understood as an artifact of finite sample size, occurring when fewer than 10 flies remain alive.

The turnaround in views about applicability of the Gompertz model, which had been revered for well over a century, raises an obvious question: Why was Gompertz widely accepted until recently, and even raised to the stature of "Gompertz' law" despite various exceptions being pointed out? In addition to science's predilection for simple laws of nature, the likely explanation is that most survival experiments prior to the 1990s had been too small to detect plateaus. Mortality plateaus are late-life phenomena. Small experiments fail to detect them because there are few survivors to the age at which mortality rates begin to level off. It is also possible that biologists' habit of examining survivorship curves rather than mortality rates contributed to ignorance about plateaus; it is difficult to see a plateau in the tail of a survivorship curve, even if sample sizes are relatively large.

B. Explaining Mortality Plateaus

Although the existence of mortality plateaus is now universally accepted, explaining why plateaus exist is controversial. It is convenient to define two general, non-exclusive classes of explanations: population heterogeneity and individual aging. Heterogeneity refers to the idea that individuals in a cohort differ in frailty, which is most conveniently parameterized as a multiplicative factor of the Gompertz hazard model. The hazard rate of an individual of age x and frailty Z is

$$\mu_{x,z} = ZAe^{Bx},$$

where Z is a gamma-distributed random variable with mean 1 and variance σ^2. Under those circumstances, the mean age-specific mortality in the population is given by the logistic equation. Individual differences in frailty can be genetic or environmental in origin and tend to produce mortality deceleration. This occurs because weaker organisms die first, leaving preferentially more robust members of the population alive for later survival measurements. The process of sorting weaker and stronger individuals by death within a generation is often referred to as "demographic selection," the first part of the term being necessary to distinguish it from selection of the Darwinian sort.

Frailty may be fixed at birth, or acquired and modified through life experience, as mentioned above. For instance, for the fixed frailty situation, we might imagine that a population of flies contains different genotypes, each with its characteristic hazard rate. Or, in a genetically homogeneous population such as an inbred line or F_1 cross between two inbred lines, differences

in frailty between organisms could arise from micro-environmental effects, such as slight uncontrolled spatial variation in temperature or quality of food experienced at pupation sites. In either case, the essential feature of the fixed frailty models is that the organisms carry a certain frailty factor Z with them throughout their lives. In contrast, flies could acquire different frailty factors during their adult lifetimes as a result of exposure to infectious organisms, or differential rates of reproduction. In either case, the logistic model predicts the expected population mortality dynamics (Yashin *et al.,* 1994), and the magnitude of population variance for frailty has a strong influence on mortality dynamics.

Gavrilov and Gavrilova (1991, 2001; see Chapter 1, this volume) developed several classes of aging models based on reliability theory. Interestingly, all these models predict a mortality deceleration, no matter what assumptions are made regarding initial population heterogeneity or its complete initial homogeneity. Moreover, these reliability models of aging produce mortality plateaus as inevitable outcome for any values of considered parameters. The only constraint is that the elementary steps of the multistage destruction process of a system should occur by chance only, independent of age. The models also predict that an initially homogeneous population will become highly heterogeneous for risk of death over time (acquired heterogeneity).

Another class of explanations for mortality plateaus depends not on differences between individuals, but on changes within individuals as they age. If the hazard rates for individual organisms decelerate at older ages, then so, too, will the observed population mortality. One can imagine various biological reasons that individual hazard rates might decelerate. Older flies might incur less physiological and metabolic cost from mating behavior and reproduction, or lower activity levels in old age might entail less exposure to infectious agents and less generation of harmful oxygen radicals. For humans, a similar hypothesis was proposed by Greenwood and Irwin (1939), who suggested that lower-than-expected mortality of centenarians could be explained by their less risky behavior.

There is a growing body of evolutionary theory that addresses ultimate causes of mortality plateaus. The basic problem to be solved by theoreticians is that evolutionary models of age-specific mortality tend to generate very high mortality rates ("walls of death") at post-reproductive ages (Charlesworth, 1980; Curtsinger, 1995a,b; Partridge & Barton, 1993; Pletcher & Curtsinger, 1998). Imagine a population in which there is initially no senescence—that is, the hazard rate is the same for all age classes. Over time, new mutations occur, some of which have age-specific effects on survival. Many of the new mutations are deleterious at all ages and are quickly eliminated from the population by natural selection. Some mutations, presumably very few, improve survival of carriers at early ages, are positively selected, and increase in frequency in the population; this causes an evolutionary lowering of the population mortality curve at juvenile and reproductive ages. Some mutations increase or decrease mortality specifically at post-reproductive ages, but because post-reproductive survival is irrelevant to Darwinian fitness, natural selection does not discriminate. The net result is that there is no evolutionary force "pushing down" on the late-life part of the mortality curve. If the majority of mutations that affect old-age survival cause a deterioration of vitality, then post-reproductive survival will erode under mutation pressure, with nothing to stop it from eventually producing a wall of death. This scenario presumes the existence of exclusively late-acting mutations, as originally postulated by Medawar (1952), and is known as the mutation accumulation model of the

evolution of senescence. The central problem for evolutionists trying to understand mortality trajectories is to discover some means of counteracting the tendency of recurrent mutation to drive post-reproductive hazard rate to infinity.

One possibility, not widely considered, is that mutations that affect only the old might improve survival as often as they erode it. This might seem at first glance to be nonbiological, violating the widely held view that the vast majority of mutations are deleterious to their carriers. However, reasonable scenarios can be imagined; for instance, a mutation that reduces mobility in old age might increase survival by causing carriers to generate fewer damaging oxygen radicals. There is some suggestion in the results of mutation accumulation experiments described above that mutations increase survival as often as they decrease it, but it must be admitted that the distribution of mutational effects for old-age-specific mutations is not known in detail.

Abrams and Ludwig (1995) addressed the mortality plateau problem in an evolutionary context by analyzing an optimality model in which organisms are presumed to allocate resources to either somatic repair or reproduction. The optimal allocation was presumed to be that which maximizes lifetime reproductive output. Abrams and Ludwig (1995) found that an optimal allocation involves declining investment in repair with increasing age, which, the authors suggest, could lead to late-life mortality plateaus. However, Charlesworth and Partridge (1997) re-examined the optimality model and found that the death rate tends to infinity with increasing age. We also note that the optimality approach does not specifically incorporate deleterious mutations with age-specific effects, an important omission.

Mueller and Rose (1996) used numerical simulations to study the evolution of mortality under antagonistic pleiotropy—that is, the assumption that mutations have negatively correlated effects on survival at young and old ages. They argued that such models easily explain mortality plateaus, but their results have been widely criticized. Mueller and Rose (1996) assumed that every mutation increases survival in one randomly chosen age class, and reduces it in another; there are no unconditionally deleterious mutations in the model. Charlesworth and Partridge (1997) noted that the Mueller-Rose model was not iterated to equilibrium, and suggested that late-life survival rates would approach zero in this model as more evolutionary time elapsed. In general, the evolutionary equilibrium state is difficult to define in numerical simulations of finite populations. Pletcher and Curtsinger (1998) argued that the Mueller-Rose model includes a strange feature that biases the results: there is an assumption that when the population mortality rate is low, new mutations tend to increase mortality, but when the mortality rate is high, new mutations tend to make it decrease. The net effect is that mortality rates are forced toward an intermediate value. Pletcher and Curtsinger (1998) showed that removing that assumption leads to a late-life wall of mortality. The most telling critique is by Wachter (1999), who obtained analytical results for a generalized class of Mueller-Rose–type models and concluded that mortality plateaus cannot be accounted for by their equilibrium behavior. Wachter (1999) states unequivocally that the Mueller-Rose model fails in this respect. Thus, it seems likely that the simulation of Mueller and Rose (1996) produced transient mortality plateaus that were erroneously interpreted as equilibrium evolutionary states.

Given strong criticisms of the Mueller-Rose simulation model and analytical invalidation of its results, it is surprising that Drapeau and colleagues (2000), Rose and Mueller (2000), and Rose and colleagues (2002) have continued to promote it. All three of those papers failed to

cite the analytical results of Wachter (1999). Mueller and colleagues (2003) address the various criticisms, including Wachter's (1999) analytical results, but the responses are unconvincing (de Grey, 2003a, 2004; Service, 2004). Technical details aside, the broader point is that Rose, Mueller, and their associates endorse individual aging over population heterogeneity as a general explanation for mortality plateaus, a position that could ultimately prove to be correct. They refer to their argument as "the evolutionary theory" (Rose & Mueller, 2000, p. 1,660), implying that heterogeneity explanations are "un-evolutionary" or "anti-evolutionary." The nomenclature is unfortunate. Phenotypic variability between organisms, including genetically identical ones, is an essential feature of quantitative genetic variability and micro-evolutionary change (Falconer & Mackay, 1996). Labeling the argument "evolutionary" is just a rhetorical device, with few constraints on its use: Graves and Mueller (1993, 1994; see also Curtsinger, 1995a,b) raised the "evolutionary" flag when they argued *against the existence* of mortality plateaus in *Drosophila*, a stance that was eventually abandoned.

Pletcher and Curtsinger (1998) presented simulation results for the evolution of mortality plateaus, focusing on positive pleiotropy, in which mutations exert positively correlated effects on mortality rates at different ages. In these simulations, positive pleiotropy seemed to produce mortality plateaus, but, as in any simulation of finite populations, the definition of stable evolutionary state is difficult, and the outcomes were probably transient. Charlesworth (2001) used analytical techniques to study a similar situation by assuming that all deleterious mutations have deleterious effects at reproductive ages. This assumption prevents mutation frequencies from exploding at older ages and, thus, preserves mortality plateaus.

Service (2000a) simulated mortality dynamics under the assumption of population heterogeneity in individual age-specific risk of death. Heterogeneity was modeled by assigning each individual a unique Gompertz mortality function, with means and variances of Gompertz parameters based on the published literature for *Drosophila*. He found that the heterogeneity generated by variation in Gompertz parameters was sufficient to explain late-life mortality plateaus and could also account for late-life declines in genetic variance of mortality rates. Similar conclusions were reported by Pletcher and Curtsinger (2000b).

The reliability models of multistage destruction (Gavrilov & Gavrilova, 1991, 2001) were recently reformulated in mathematical terms of a stochastic Markov process (Steinsaltz & Evans, 2004). The authors define a Markov mortality model as a stochastic process, which is "killed" at random stopping times according to the behavior of a Markov process. A general feature of such multistage models is that they usually produce mortality plateaus, as it was demonstrated earlier with a more simple approach (Gavrilov & Gavrilova, 1991, 2001). As Steinsaltz and Evans (2004) put it, "the mortality rate stops increasing [with increasing age], not because we have selected out an exceptional subset of the population, but because the condition of the survivors is reflective of their being survivors, even though they started out the same as everyone else." Thus, the Markov mortality models explain mortality plateaus by a type of heterogeneity in acquired frailty because the underlying assumptions are similar to the earlier reliability models.

In evaluating the various theories, it is important to remember that the fact that a particular mathematical model or simulation can fit or "predict" an experimental outcome is not proof that the assumptions of the model are correct. For

example, the venerable Hardy-Weinberg model of population genetics predicts certain genotypic frequencies, but observation of those frequencies in real populations does not validate the underlying assumptions of the model (random mating, absence of natural selection, etc.). Theory guides our thinking, but critical tests must come from well-designed experiments, efforts at which are described in the next section.

C. Testing the Theories

Designing critical experiments to address the causes of mortality plateaus has proven to be exceptionally difficult; in fact, all experimental tests in this area are flawed in one way or another. Thus, no final answers can be given at present, but it is instructive to review the relevant experiments and consider the pitfalls.

The first experiment specifically designed to test heterogeneity theory used lethal stress to manipulate the magnitude of population heterogeneity (Khazaeli *et al.*, 1995b) and was inspired by demographic studies of human populations after a catastrophe (Vaupel *et al.*, 1987). Using a single highly inbred line of flies, multiple age-synchronized cohorts were established. In control populations, flies were maintained under the usual conditions, whereas in experimental populations, flies were subjected to 24 hours of desiccation at a young age. About 20 percent of the flies died during and immediately after the desiccation stress. Post-stress mortality rates are informative about population heterogeneity; in particular, in the absence of population heterogeneity, post-stress mortality in experimental and control populations is expected to be identical. However, if there is significant population heterogeneity at the time of the stress, then post-stress mortality in the experimental populations is expected to drop below that of the control populations, at least temporarily, because the more frail individuals will have been eliminated. The latter pattern was observed, and was interpreted by Khazaeli and colleagues (1995b) as evidence for significant levels of heterogeneity. However, the authors retracted that result when it was realized that there was a flaw in the interpretation (Curtsinger & Khazaeli, 1997). The problem is that exposure to an external stress does more than kill the more frail flies; it also induces a stress response in the survivors. This phenomenon, known as hormesis, is well documented in a variety of species and involves a rapid genomic response to severe stress. The stress response is an interesting phenomenon, but it creates difficult problems in the interpretation of the stress experiment. In particular, the post-stress decline in mortality among experimentals compared to controls could be due to reduced heterogeneity through elimination of weaker flies, hormesis induced among survivors, or both factors. The experimental design of Khazaeli and colleagues (1995b) does not permit separation of the heterogeneity and hormetic effects, and so the result is inconclusive regarding heterogeneity. Recently, the stress experiment was redesigned to correct the confounding flaw, and data have been collected in the Curtsinger lab on 100,000 male flies of one inbred genotype. Five intensities of stress were applied, including one sufficient to induce a stress response but not severe enough to cause immediate deaths. The mild stress will allow estimation of the hormesis effect independent of the heterogeneity effect, unconfounding the variables. Data analysis by the authors of this chapter and Dr. A. Khazaeli is underway.

A different and more benign experimental design was used by Khazaeli and colleagues (1998), who attempted to manipulate population heterogeneity by fractionating genetically homogeneous populations. Working with two

highly inbred lines, experimental populations were subjected to the most stringent environmental controls possible, far beyond what is normally employed in fly husbandry. Eggs were collected over a seven-hour period, instead of the usual 24 hours. First instar larvae were collected from that sample for only three hours, and emerging adults were collected in three-hour windows. The result of all this careful timing of development is that within a cohort, adult flies experienced larval and pupal environmental conditions that are as similar as possible. The question then is whether the environmentally "homogeneous" populations exhibit mortality plateaus to a lesser extent than normal environmentally "heterogeneous" control populations. Khazaeli and colleagues (1998) found that 93 percent of experimental populations and 100 percent of control populations exhibited statistically significant mortality deceleration late in life. The authors concluded that reducing environmental heterogeneity during larval and pupal stages has negligible effect on adult mortality trajectories. Drapeau and colleagues (2000, p. 72) overstated this experimental result when they wrote that "Khazaeli *et al.* (1998) found no evidence to support the hypothesis that environmental heterogeneity among individual flies is a primary factor in determining late-life mortality rates." The experiment actually gives information only about larval and pupal stages, and is in the strictest sense relevant only to the "fixed-heterogeneity" model. The results are not informative about heterogeneity acquired in adulthood, which may be substantial. Perhaps a broader lesson from this study is that there is a substantial and intrinsic environmental heterogeneity in experimental populations that cannot be removed experimentally, even by Herculean efforts.

The most widely discussed experimental test of heterogeneity theory is that of Drapeau and colleagues (2000), who argued that there is a close connection between frailty and sensitivity to environmental stresses in experimental populations of *Drosophila*. They further suggested that, according to heterogeneity theory, populations differing in tolerance to stress should have different late-life mortality characteristics, though the nature of the expected differences was not spelled out. They compared mortality trajectories in fly populations that had been selected for resistance to starvation with those of unselected controls. No statistically significant differences were found, which the authors interpreted as evidence against the heterogeneity theory. Service (2000b) questioned the assertion that the populations are expected to differ in late-life mortality, noting that for the logistic model the plateau occurs at B/σ^2. Consequently, populations could differ in the intercept parameter A and have the same levels of late-life mortality. As noted by Mueller and colleagues (2000) in their response to Service (2000b), the force of this criticism is blunted by the generally accepted theoretical observation that large and biologically unrealistic amounts of variation in the intercept parameter would be required to produce mortality plateaus, if that were all that varied between individuals. Service (2000b) also noted that if σ^2 is lower in the selected population, then it is expected to have higher mortality rate than controls (when all other parameters are fixed), especially at early ages, as observed. Service concludes that the results of Drapeau and colleagues (2000) are entirely consistent with the predictions of the heterogeneity model. de Grey (2003b) criticized the use of maximum likelihood methods by Drapeau and colleagues (2000) and argued that heterogeneous Gompertz parameters could explain the experimental results. Steinsaltz (2005) reanalyzed the experimental results of Drapeau and colleagues (2000) and questioned the claim that

there is no difference in late-life mortality schedules between populations. The original claim was based on comparisons of means averaged over populations. Steinsaltz (2005) noted that the data are bimodal, and means are therefore misleading. He reanalyzed the data and found that populations were actually quite different, the mortality plateau being lower in the selected populations. He concluded that the experimental results lend mild support to the heterogeneity theory, although the expected differences in timing of the plateau were not observed. In sum, the critiques of Drapeau and colleagues (2000) are varied and instructive, and illustrate some of the difficulties of the experimental task and complexities of the analysis.

Rose and colleagues (2002) studied mortality trajectories in populations of *Drosophila* that had been artificially selected for long life and compared them to unselected control populations. Mortality trajectories had previously been studied in the same populations by Service and colleagues (1998), who invoked a heterogeneity explanation. Rose and colleagues (2002) showed that control populations consistently exhibited earlier onset of mortality plateaus than selected populations. This result was interpreted as being consistent with an "evolutionary" (i.e., individual aging) model. The result is suggestive, but not critical; it is not clear that the observations are inconsistent with predictions of any particular heterogeneity model. In general, we consider it very unlikely that critical tests of heterogeneity and individual aging models can be executed with outbred experimental populations. The problem is that the variance parameter plays a central role in the predictions of heterogeneity models but is generally unknown in either relative or absolute terms for outbred, genetically uncharacterized populations. It is widely assumed that selected populations are less heterogeneous than unselected controls because some genotypes have been eliminated by selection. However, several factors could cause selected populations to be *more* heterogeneous, both in genetic and environmental variance. If the selection response entails an increase in frequencies of initially rare alleles, genetic variance is expected to increase under selection, a prediction that has been verified experimentally (Curtsinger & Ming, 1997). This counterintuitive result occurs because the contribution to total genetic variance by any particular locus depends on $2pq$, where p and q are allelic frequencies (Falconer & Mackay, 1996); rare and common alleles contribute little to population genetic variance, but alleles at intermediate frequencies potentially contribute much. The same effect occurs if new mutations increase to appreciable frequencies during the selection process. Another factor that complicates matters is genetic homeostasis. It is well known that homozygous genotypes generally exhibit greater environmental variance than heterozygotes (see review by Phelan & Austad, 1994). If selection and/or inbreeding increase homozygosity in selected populations, then the environmental component of variance is expected to increase. In short, there are too many unknown variables in genetically uncharacterized outbred populations to allow critical tests of predictions of heterogeneity models. A better experimental design is that of Miyo and Charlesworth (2004), who studied mortality rates in hybrid progeny of crosses between inbred lines of *Drosophila*. In such populations, all individuals are genetically alike, except for recent mutations, and heterozygous at loci that differ between parental lines. Miyo and Charlesworth (2004) found that populations of both mated and unmated hybrid males exhibited mortality plateaus, and suggested that their results were consistent with underlying heterogeneity of mortality rates.

In the final analysis, evaluating the various heterogeneity models is a purely quantitative question. No reasonable person would deny that there is some heterogeneity for frailty within populations, even genetically homogeneous ones; the question is whether there is sufficient heterogeneity to produce late-life mortality plateaus. We are optimistic that large-scale, multilevel stress experiments and other designs using genetically defined populations will provide the relevant estimates. On the other hand, if the individual aging theory is correct, then there must be some important biological processes that differ between organisms at pre- and post-plateau ages and account for the change in mortality trajectory.

IV. Conclusions

The integration of biology and demography proceeded sporadically for most of the 20th century. Pearl, Sacher, Strehler, and others showed the way toward integration of the fields, but their efforts were not always widely appreciated. Now we are in a period of widespread dissemination of demographic techniques among experimental biologists. The new field of biodemography is flourishing and has rich conceptual bases to draw on in demography, evolutionary biology, reliability theory, and even theoretical physics (Pletcher & Neuhauser, 2000). Its first major conceptual challenge is to explain mortality plateaus. We are optimistic that consensus will emerge in this area as experimental designs and methods of data analysis become more sophisticated. Other important challenges include defining the nature of age-specific genetic variation and explaining the high degree of environmental variation in demographic parameters.

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References

Abrams, P. A., & Ludwig, D. (1995). Optimality theory, Gompertz' law, and the disposable soma theory of senescence. *Evolution*, 49, 1056–1066.

Beard, R. E. (1959). Note on some mathematical mortality models. In G. E. W. Wolstenholme & M. O'Connor (Eds.), *The lifespan of animals.* Boston: Little, Brown.

Beard, R. E. (1963). A theory of mortality based on actuarial, biological, and medical considerations. *Proceedings of the International Population Conference, New York*, 1, 611–625.

Beard, R. E. (1971). Some aspects of theories of mortality, cause of death analysis, forecasting and stochastic processes. In W. Brass (Ed.), *Biological aspects of demography* (pp. 57–68). London: Taylor & Francis.

Brownlee, J. (1919). Notes on the biology of a life-table. *Journal of the Royal Statistical Society*, 82, 34–77.

Carey, J. R. (2003). *Longevity: the biology and demography of life span.* Princeton, NJ: Princeton University Press.

Carey, J. R., Curtsinger, J. W., & Vaupel, J. W. (1993). Response to letters. *Science*, 260, 1567–1569.

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. (1980). *Evolution in age-structured populations*. New York: Cambridge University Press.

Charlesworth, B. (2001). Patterns of age-specific means and genetic variances of mortality rates predicted by mutation-accumulation theory of ageing. *Journal of Theoretical Biology*, 210, 47–65.

Charlesworth, B., & Partridge, L. (1997). Ageing: leveling of the grim reaper. *Current Biology*, 7, R440–R442.

Clark, A. G., & Guadalupe, R. N. (1995). Probing the evolution of senescence in *Drosophila melanogaster* with P-element tagging. *Genetica*, 96, 225–234.

Curtsinger, J. W. (1995a). Density and age-specific mortality. *Genetica*, 96, 179–82.

Curtsinger, J. W. (1995b). Density, mortality, and the narrow view. *Genetica,* 96, 187–89.
Curtsinger, J. W. (2001). Genetic theories of senescence. *International encyclopedia of social and behavioral sciences.* Oxford: Pergamon Press.
Curtsinger, J. W., & Khazaeli, A. (1997). A reconsideration of stress experiments and population heterogeneity. *Experimental Gerontology*, 32, 727–729.
Curtsinger, J. W., & Khazaeli, A. A. (2002). Lifespan, QTLs, age-specificity, and pleiotropy in *Drosophila*. *Mechanisms of Ageing and Development*, 123, 81–93.
Curtsinger, J. W., Fukui, H. H., Resler, A. S., Kelly, K., & Khazaeli, A. A. (1998). Genetic analysis of extended lifespan in *Drosophila melanogaster*. I. RAPD screen for genetic divergence between selected and control lines. *Genetica,* 104, 21–32.
Curtsinger, J. W., Fukui, H., Townsend, D., & Vaupel, J. W. (1992). Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*. *Science*, 258, 461–463.
Curtsinger, J. W., & Ming, R. (1997). Non-linear selection response in Drosophila: A strategy for testing the rare-alleles model of quantitative genetic variability. *Genetica*, 99, 59–56.
Curtsinger, J. W., Service, P., & Prout, T. (1994). Antagonistic pleiotropy, reversal of dominance, and genetic polymorphism. *American Naturalist,* 144, 210–228.
Cutler, S. J. , & Ederer, F. (1958). Maximum utilization of the life table methodology in analyzing survival. *Journal of Chronic Disease*, 8, 699–712.
de Grey, A. D. (2003a). Overzealous maximum-likelihood fitting falsely convicts the slope heterogeneity hypothesis. *Experimental Gerontology*, 38, 921–923.
de Grey, A. D. (2003b). Critique of the demographic evidence for "late-life non-senescence." *Mathematical Society Transactions*, 31, 452–454.
de Grey, A. D. (2004). Reply to Mueller's and Rose's letter to the editor: models on trial: falsifying overstated claims of generality does not falsify correctly-stated ones. *Experimental Gerontology*, 39, 453.
De Luca, M., Roshina, N. V., Geiger-Thornsberry, G. L., Lyman, R. F., Pasyukova, E. G., & Mackay, T. F. C. (2003). Dopa-decarboxylase affects variation in *Drosophila* longevity. *Nature Genetics*, 34, 429–433.
Drapeau, M. D., Gass, E. K., Simison, M. D., Mueller, L. D., & Rose, M. R. (2000). Testing the heterogeneity theory of late-life mortality plateaus by using cohorts of *Drosophila melanogaster*. *Experimental Gerontology*, 35, 71–84.
Driscoll, M., & Gerstbrein, B. (2003). Dying for a cause: invertebrate genetics takes on human neurodegeneration. *Nature Reviews Genetics*, 4, 181–194.
Economos, A. C. (1979). A non-Gompertzian paradigm for mortality kinetics of metazoan animals and failure kinetics of manufactured products. *AGE*, 2, 74–76.
Economos, A. C. (1980). Kinetics of metazoan mortality. *Journal of Social and Biological Structures*, 3, 317–329.
Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Harlow, UK: Longman Science and Tech. Feany, M. B., & Bender, W. W. (2000). A *Drosophila* model of Parkinson's disease. *Nature*, 404, 394–398.
Filliben, J. J. (2004). Exploratory data analysis. In *NIST/SEMATECH e-Handbook of Statistical Methods*. Available at http://www.itl.nist.gov/div898/handbook.
Finch, C. E., & Kirkwood, T. B. L. (2000). *Chance, development, and aging*. New York: Oxford University Press.
Forbes, S. N., Valenzuela, R. K., Keim, P., & Service, P. M. (2004). Quantitative trait loci affecting life span in replicated populations of *Drosophila melanogaster*. I. Composite interval mapping. *Genetics*, 168, 301–311.
Fortini, M. E., & Bonini, N. M. (2000). Modeling human neurodegenerative diseases in *Drosophila*. *Trends in Genetics*, 16, 161–167.
Fukui, H. H., Ackert, L., & Curtsinger, J. W. (1996). Deceleration of age-specific mortality rates in chromosomal homozygotes and heterozygotes of *Drosophila melanogaster*. *Experimental Gerontology*, 31, 517–531.
Fukui, H., Pletcher, S., & Curtsinger, J. W. (1995). Selection for increased longevity in *Drosophila melanogaster*: A response to Baret and Lints. *Gerontology*, 41, 65–68.
Fukui, H, Xiu, L., & Curtsinger, J. W. (1993). Slowing of age-specific mortality rates in

Drosophila melanogaster. *Experimental Gerontology*, 28, 585–599.
Gavrilov, L. A., & Gavrilova, N. S. (1991). *The biology of lifespan: a quantitative approach*. New York: Harwood Academic Publishers.
Gavrilov, L. A., & Gavrilova, N. S. (1993). Fruit fly aging and mortality. *Science*, 260, 1565.
Gavrilov, L. A., & Gavrilova, N. S. (2001). The reliability theory of aging and longevity. *Journal of Theoretical Biology*, 213, 527–545.
Gehan, E. A., & Siddiqui, M. M. (1973). Simple regression methods for survival time studies. *Journal of the American Statistical Association*, 68, 848–856.
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. *Philosophical Transactions of the Royal Society (London)*, 115, 513–585.
Gompertz, B. (1872). On one uniform law of mortality from birth to extreme old age, and on the law of sickness. *Journal of the Institute of Actuaries*, 16, 329–344.
Graves, J. L., & Mueller, L. D. (1993). Population density effects on longevity. *Genetica*, 91, 99–109.
Graves, J. L., & Mueller, L. D. (1994). Population density effects on longevity revisited: a note in response to 'Density and age-specific mortality' by J. W. Curtsinger. *Genetica*, 96, 183–186.
Greenwood, M., & Irwin, J. O. (1939). The biostatistics of senility. *Human Biology*, 11, 1–23.
Hamilton, W. D. (1966). The molding of senescence by natural selection. *Journal of Theoretical Biology*, 12, 12–45.
Helfand, S. L., & Rogina, B. (2000). Regulation of gene expression during aging. *Results and Problems in Cell Differentiation*, 29, 67–80.
Helfand, S. L., & Rogina, B. (2003). Molecular genetics of aging in the fly: is this the beginning of the end? *Bioessays*, 25, 134–141.
Keightley, P. D., & Ohnishi, O. (1998). EMS-induced polygenic mutation rates for nine quantitative characters in *Drosophila melanogaster*. *Genetics*, 148, 753–766.
Keightley, P. D., Davies, E. K., Peters, A. D., & Shaw, R. G. (2000). Properties of ethylmethane sulfonate-induced mutations affecting life-history traits in *Caenorhabditis elegans* and inferences about bivariate distributions of mutation effects. *Genetics*, 156, 143–154.
Khazaeli, A. A., & Curtsinger, J. W. (2000). Genetic analysis of extended lifespan in *Drosophila melanogaster*. III. On the relationship between artificially selected lines and wild stocks. *Genetica*, 109, 245–253.
Khazaeli, A. A., Pletcher, S. D., & Curtsinger, J. W. (1998). The fractionation experiment: reducing heterogeneity to investigate age-specific mortality in *Drosophila*. *Mechanisms of Ageing and Development*, 105, 301–317.
Khazaeli, A. A., Tatar, M., Pletcher, S., & Curtsinger, J. W. (1997). Heat-induced longevity extension in *Drosophila melanogaster* I. Longevity, mortality, and thermotolerance. *Journal of Gerontology, Biological Sciences*, 52A, B48–B52.
Khazaeli, A. A., Van Voorhies, W., & Curtsinger, J. W. (2005). Longevity and metabolism in *Drosophila melanogaster*: positive pleiotropy between life span and age-specific metabolic rate in populations artificially selected for long life. *Genetics*, 69, 231–242.
Khazaeli, A. A., Xiu, L., & Curtsinger, J. W. (1995a). Effect of adult cohort density on age-specific mortality in *Drosophila melanogaster*. *Journal of Gerontology, Biological Sciences*, 50A, 262–269.
Khazaeli, A. A., Xiu, L. & Curtsinger, J. W. (1995b). Stress experiments as a means of investigating age-specific mortality in *Drosophila melanogaster*. *Experimental Gerontology*, 30, 177–184.
Khazaeli, A. A., Xiu, L., & Curtsinger, J. W. (1996). Effect of density on age-specific mortality in *Drosophila melanogaster*: A density supplementation experiment. *Genetica*, 98, 21–31.
Kirkwood, T. B. L. (1999). *Time of our lives: the science of human ageing*. New York: Oxford University Press.
Kowald, A., & Kirkwood, T. B. L. (1993). Explaining fruit fly longevity. *Science*, 260, 1664–1665.
le Bras, H. (1976). Lois de mortalité et age limité. *Population*, 31, 655–692.
Leips, J., & Mackay, T. F. C. (2000). Quantitative trait loci for lifespan in

Drosophila melanogaster: interactions with genetic background and larval density. *Genetics,* 155, 1773–1788.

Leips, J., & Mackay, T. F. C. (2002). The complex genetic architecture of *Drosophila* life span. *Experimental Aging Research*, 28, 361–390.

Lin, Y. J, Seroude, L., & Benzer, S. (1998). Extended life-span and stress resistance in the *Drosophila* mutant *methuselah. Science*, 282, 943–946.

Luckinbill, L. S., & Golenberg, E. M. (2002). Genes affecting aging: mapping quantitative trait loci in *Drosophila melanogaster* using amplified fragment length polymorphisms (AFLPs). *Genetica*, 114, 147–156.

Mack, P. D., Lester, V. K., & Promislow, D. E. L. (2000). Age-specific effects of novel mutations in *Drosophila melanogaster*. II. Fecundity and male mating ability. *Genetica*, 110, 31–41.

Mackay, T. F. C. (2001). Quantitative trait loci in *Drosophila*. *Nature Reviews*, *Genetics*, 2, 11–20.

Mackay, T. F. C. (2002). The nature of quantitative genetic variation for *Drosophila* longevity. *Mechanisms of Ageing and Development,* 123, 95–104.

Mair, W., Goymer, P., Pletcher, S. D., & Partridge, L. (2003). Demography of dietary restriction and death in *Drosophila. Science*, 301, 1731–1733.

Makeham, W. M. (1867). On the law of mortality. *Journal of the Institute of Actuaries*, 13, 325–358.

Martorell, C., Toro, M. A., & Gallego, C. (1998). Spontaneous mutation for life-history traits in *Drosophila melanogaster*. *Genetica*, 102/103, 315–324.

McCarrol, S. A., Murphy, C. T., Zou, S., Pletcher, S. D., Chin, C. S., Jan, Y. N., Kenyon, C., Bargmann, C. I., & Li, H. (2004). Comparing genomic expression patterns across species identifies shared transcriptional profiles in aging. *Nature Genetics*, 36, 197–204.

Medawar, P. B. (1952). *An unsolved problem of biology*. London: H. K. Lewis.

Miyo, T., & Charlesworth, B. (2004). Age-specific mortality rates of reproducing ad non-reproducing males of *Drosophila melanogaster*. *Proceedings of the Royal Society of London*, *Series B*, 271, 2517–2522.

Mueller, L. D., & Rose, M. R. (1996). Evolutionary theory predicts late-life mortality plateaus. *Proceedings of the National Academy of Sciences of the USA*, 93, 15249–15253.

Mueller, L. D., Drapeau, M. D., Adams, C. S., Hammerle, C. W., Doyal, K. M., Jazayeri, A. J., Ly, T., Beguwala, S. A., Mamidi, A. R., & Rose, M. R. (2003). Statistical tests of demographic heterogeneity theories. *Experimental Gerontology*, 38, 373–386.

Mueller, L. D., Drapeau, M. D., & Rose, M. R. (2000). Stress resistance, heterogeneity, and mortality plateaus: response by the authors. *Experimental Gerontology,* 35, 1089–1091.

Mukai, T. (1964). The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics*, 50, 1–19.

Mutsuddi, M., & Nambu, J. R. (1998). *Drosophila* degenerates for a good cause. *Current Biology*, 8, R809–811.

Nusbaum, T. J., Graves, J. L., Mueller, L. D., & Rose, M. R. (1993). Fruit fly aging and mortality. *Science*, 260, 1567.

Nuzhdin, S. V., Khazaeli, A. A., & Curtsinger, J. W. (2005). Survival analysis of life span QTLs in *Drosophila melanogaster*. *Genetics*, 170, 719–731.

Nuzhdin, S. V., Pasyukova, E. G., Dilda, C., & Mackay, T. F. C. (1997). Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA,* 94**,** 9734–9739.

Olshansky, S. J. (1998). On the biodemography of aging: a review essay. *Population and Development Review*, 24, 381–393.

Partridge, L., & Barton, N. H. (1993). Optimality, mutation, and the evolution of aging. *Nature*, 362, 305–311.

Pasyukova, E. G., Vieira, C., & Mackay, T. F. C. (2000). Deficiency mapping of quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Genetics*, 156, 1129–1146.

Pearl, R. (1922). Experimental studies on the duration of life. VI. A comparison of the laws of mortality in *Drosophila* and in man. *American Naturalist*, 56, 398–405.

Pearl, R., & Parker, S. (1921). Experimental studies on the duration of life. I.

Introductory discussion of the duration of life in *Drosophila*. *American Naturalist*, 55, 481–500.

Pearl, R., & Parker, S. (1924). Experimental studies on the duration of life. IX. New life tables for *Drosophila*. *American Naturalist*, 58, 71–82.

Perks, W. (1932). On some experiments in the graduation of mortality statistics. *Journal of the Institute of Actuaries*, 63, 12–57.

Phelan, J. P., & Austad, S. N. (1994). Selecting animal models of human aging: inbred strains often exhibit less biological uniformity than F1 hybrids. *Journal of Gerontology, Biological Science*, 49, B1–B11.

Pletcher, S. D. (1996). Age-specific mortality costs of exposure to inbred *Drosophila melanogaster* in relation to longevity selection. *Experimental Gerontology*, 31, 605–616.

Pletcher, S. D. (2002). Mitigating the tithonus error: genetic analysis of mortality phenotypes. *Science Aging Knowledge Environment*, pe14.

Pletcher, S. D., & Curtsinger, J. W. (1998). Mortality plateaus and the evolution of senescence: Why are old-age mortality rates so low? *Evolution*, 52, 454–464.

Pletcher, S. D., & Curtsinger, J. W. (2000a). Why do lifespans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters. *Journal of Gerontology, Biological Sciences*, 55, B381–B389.

Pletcher, S. D., & Curtsinger, J. W. (2000b). The influence of environmentally induced heterogeneity on age-specific genetic variance for mortality rates. *Genetical Research, Cambridge*, 75, 321–329.

Pletcher, S. D., Houle, D., & Curtsinger, J. W. (1998). Age-specific properties of spontaneous mutations affecting mortality in *Drosophila melanogaster*. *Genetics*, 148, 287–303.

Pletcher, S. D., Houle, D., & Curtsinger, J. W. (1999). The evolution of age-specific mortality rates in *Drosophila melanogaster*: Genetic divergence among unselected strains. *Genetics*, 153, 813–823.

Pletcher, S. D., Macdonald, S. J., Marguerie, R., Certa, U., Stearns, S. C., Goldstein, D. B., & Partridge, L. (2002). Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster*. *Current Biology*, 12, 712–723.

Pletcher, S. D., & Neuhauser, C. (2000). Biological aging: criteria for modeling and a new mechanistic model. *International Journal of Modern Physics C*, 11, 525–546.

Ramalu-Hansen, H. (1983). Smoothing counting process intensities by means of kernel functions. *Annals of Statistics*, 11, 453–466.

Resler, A. S., Kelly, K., Cantor, G., Khazaeli, A. A., Tatar, M., & Curtsinger, J. W. (1998). Genetic analysis of extended lifespan in *Drosophila melanogaster*. II. Replication of the backcross test and molecular characterization of the N14 locus. *Genetica,* 104, 33–39.

Robine, J. M., & Ritchie, K. (1993). Explaining fruit fly longevity. *Science,* 260, 1665.

Rogina, B., & Helfand, S. L. (1995). Regulation of gene expression is linked to life span in adult *Drosophila*. *Genetics*, 141, 1043–1048.

Rogina, B., Reenan, R. A., Nilsen, S. P., & Helfand, S. L. (2000). Extended life-span conferred by cotransporter gene mutations in *Drosophila*. *Science*, 290, 2137–2140.

Rogina, B., Vaupel, J. W., Partridge, L., & Helfand, S. L. (1998). Regulation of gene expression is preserved in aging *Drosophila melanogaster*. *Current Biology*, 9, 475–478.

Rose, M. R. (1991). *Evolutionary biology of aging*. New York: Oxford University Press.

Rose, M. R., & Mueller, L. D. (2000). Ageing and immortality. *Philosophical Transactions of the Royal Society of London, Series B*, 355, 1637–1662.

Rose, M. R., Drapeau, M. D., Yazdi, P. G., Shah, K. H., Moise, D. B., Thakar, R. R., Rauser, C. L., and Mueller, L. D. (2002). Evolution of late-life mortality in *Drosophila melanogaster. Evolution*, 56, 1982–1991.

Sacher, G. A. (1956). On the statistical nature of mortality, with special reference to chronic radiation mortality. *Radiology*, 67, 250–257.

Sacher, G. A. (1966). The Gompertz transformation in the study of the injury-mortality relationship: Application to late radiation effects and ageing. In P. J. Lindop & G. A. Sacher (Eds.), *Radiation and ageing* (pp. 411–441). London: Taylor and Francis.

Semenchenko, G. V., Khazaeli, A. A., Curtsinger, J. W., & Yashin, A. L. (2004). Stress resistance declines with age: analysis of data from a survival experiment with *Drosophila melanogaster*. *Biogerontology*, 5, 17–30.

Service, P. M. (2000a). Heterogeneity in individual mortality risk and its importance for evolutionary studies of senescence. *American Naturalist*, 156, 1–13.

Service, P. M. (2000b). Stress resistance, heterogeneity, and mortality plateaus: a comment on Drapeau *et al*. *Experimental Gerontology*, 35, 1085–1087.

Service, P. M. (2004). Demographic heterogeneity explains age-specific patterns of genetic variance in mortality rates. *Experimental Gerontology*, 39, 25–30.

Service, P. M., Michieli, C. M., & McGill, K. (1998). Experimental evolution of senescence: An analysis using a "heterogeneity" model. *Evolution,* 52, 1844–1850.

Steinsaltz, D. (2005). Reevaluating a test of the heterogeneity explanation for mortality plateaus. *Experimental Gerontology*, 40, 101–113.

Steinsaltz, D., & Evans, S. N. (2004). Markov mortality models: implications of quasistationarity and varying initial distributions. *Theoretical Population Biology*, 65, 319–337.

Strehler, B. L., & Mildvan, A. S. (1960). General theory of mortality and aging. *Science*, 132, 14–21.

Tahoe, N. M., Lande, J., Khazaeli, A. A., & Curtsinger, J. W. (2005). Genome-wide analysis of age-specific gene expression in populations of *Drosophila melanogaster* artificially selected for long life.

Tahoe, N. M, Mokhtarzhadeh, A., & Curtsinger, J. W. (2004). Age-related RNA decline in adult *Drosophila* melanogaster. *Journal of Gerontology: Biological Sciences*, 59, B896–901.

Valenzuela, R. K., Forbes, S. N., Keim, P., & Service, P. M. (2004). Quantitative trait loci affecting life span in replicated populations of *Drosophila melanogaster*. II. Response to selection. *Genetics*, 168, 313–324.

Van Voorhies, W., Khazaeli, A. A., & Curtsinger, J. W. (2003). Selected contribution: long-lived *Drosophila melanogaster* exhibit normal metabolic rates. *Journal of Applied Physiology*, 95, 2605–2613.

Van Voorhies, W.W., Khazaeli, A. A., & Curtsinger, J. W. (2004). Testing the "rate of living" model: Further evidence that longevity and metabolic rate are not inversely correlated in *Drosophila melanogaster*. *Journal of Applied Physiology*, 97, 1915–1922.

Vaupel, J. W., & Carey, J. R. (1993). Compositional interpretations of medfly mortality. *Science,* 260, 1666–1667.

Vaupel, J. W., Carey, J. R., Christiansen, K., Johnson, T. E., Yashin, A. I., Holm, N. V., Iachine, L. A., Khazaeli, A. A., Liedo, P., Longo, V. D., Yi, Z. Y., Manton, K. G., & Curtsinger, J. W. (1998). Biodemographic trajectories of longevity. *Science*, 280, 855–860.

Vaupel, J. W., Johnson, T. E., & Lithgow, G. J. (1994). Rates of mortality in populations of *Caenorhabditis elegans*. *Science*, 263, 668–671.

Vaupel, J. W., Yashin, A. I., & Manton, K. G. (1987). Debilitation's aftermath: stochastic process models of mortality. *Mathematical Population Studies*, 1, 21–48.

Vieira, C., Pasyukova, E. G., Zeng, S., Hackett, J. B., Lyman, R. F. & Mackay, T. F. C. (2000). Genotype-environment interaction for quantitative trait loci affecting lifespan in *Drosophila melanogaster*. *Genetics*, 154, 213–227.

Wachter, K. W. (1999). Evolutionary demographic models for mortality plateaus. *Proceedings of the National Academy of Sciences of the USA*, 96, 10544–10547.

Wachter, K. W., & Finch, C. E. (Eds.). (1997). *Between Zeus and the salmon*. Washington, DC: National Academy Press.

Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, 11, 398–411.

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

Yashin. A., Vaupel, J. W., & Iachine, I. A. (1994). A duality in aging: the equivalence of mortality models based on radically different concepts. *Mechanisms of Ageing and Development*, 74, 1–14.

Zelterman, D., & Curtsinger, J. W. (1995). Survival curves subjected to occasional insults. *Biometrics*, 51, 1140–1146.

Zelterman, D., Li, C., & Curtsinger, J. W. (1994). Piecewise exponential survival curves. *Mathematical Biosciences*, 120, 233–250.