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Age-Specific Demographic Profiles of Longevity Mutants in *Caenorhabditis elegans* Show Segmental Effects

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Demographic profiles of several single-gene longevity mutants of the nematode *Caenorhabditis elegans* reveal segmental (age-specific) effects on mortality. The mortality profiles of wild-type worms were examined across multiple replicate cultures containing 100,000 or more nematodes and found to be quite replicable, although clear environmental effects are routinely found. The combined profile of wild type was compared with those of three long-lived mutants to determine how age-specific mortality is altered by mutations in *age-1*, *clk-1*, or *spe-26.* In all four genotypes, death rates fit a two-stage Gompertz model better than a one-stage Gompertz; that is, mortality levels off at later ages. The largest genetic effect on mortality was that of an *age-1* mutation, which lowered mortality more than fivefold at most later ages. In contrast, a *spe-26* mutant had a tenfold lower mortality until approximately 2 weeks of age but ultimately achieved a higher mortality, whereas *clk-1* mutants show slightly higher mortality than wild type during the fertile period, early in life, but ultimately level off at lower mortality. Each mutant thus has a distinctive profile of age-specific mortalities that could suggest the time of action of each gene.

THE United States is undergoing a demographic revolution. Current population estimates show a larger percentage of elderly individuals (over the age of 65) now than has ever before existed in the history of the country—12.5% now versus 8.1% in 1950 and 4.1% in 1900. Many gerontologists and demographers believe that mortality at advanced ages cannot be substantially reduced (1–5). The canonical view has been that death rates at advanced ages in developed countries are close to limits that can only be relaxed by fundamental breakthroughs in slowing the process of aging. Recent studies in model systems such as *Caenorhabditis elegans*, a nematode worm, have shown that mortality at advanced ages can be dramatically reduced by simple genetic interventions. These findings offer significant challenges for the view that mortality at old age is intractable and suggest that the fraction of the elderly population could increase even more dramatically than projected.

The traditional view in biological demography is that a single, universal process of aging produces an exponential increase in mortality with age—the so-called Gompertz law (6)—and, to cite a 1991 report in *Scientific American*, that "like a clock" every individual "is constructed to run a certain time." The emerging evidence from our current projects and other research suggests a new paradigm of aging that recognizes a rich variety of diverse and often quite variable aging processes that can be influenced by both genetic and environmental changes. The research presented within focuses on the genetic changes in age-specific mortality of long-lived mutants in the nematode *C. elegans.*

C. elegans has been extensively used to identify single-gene "Age" mutants that have an increased life span (7–11). Mortality increases with chronological age in wild-type (N2) *C. elegans*, just as in most, if not all, species (12), and the rate at which mortality changes is under broad genetic control (13,14). The mortality doubling time (MDT) of normal, wild-type worms is approximately 3 to 5 days at 20°C (14–16). The MDT of strains carrying mutations in the best studied gerontogene, *age-1*, is approximately twofold longer (15). The increased longevity of the Age mutants stems from any one of several types of mutational alterations. These longevity mutants fit into one of six categories (10,17): polygenic (RI), dauer type (Daf), slow growth (Clk), fertility deficient (Spe), overexpression (Old), and slow eating (Eat). A total of 39 mutations (both published and unpublished) that extend longevity of one or both sexes in *C. elegans* has been reported (9), with more than a dozen still remaining to be published.

A major limitation on the trajectory of age-specific mortality is the lack of analysis of large populations of nematodes. With only two exceptions (16,18), longevity and survival of long-lived variants have been studied only in small populations. The current study is designed to extend and deepen our understanding of the kinetic changes in mortality underlying the increased life expectancy of specific Age mutants. The focus of this paper is on detailed demographic profiles of a representative mutant of the Daf, Clk, and Spe gerontogene classes, using methodologies involving large mass cultures of 100,000 nematodes or more.

Materials and Methods

Strain Maintenance

Populations of *C. elegans* exist primarily as hermaphrodites (XX), with males (XO) occurring only rarely in a ratio of approximately 700:1 (19). Hermaphrodites reproduce by self-fertilization, producing sperm during their fourth larval stage (L4) and then oocytes for the remainder of their 5- to 8-day reproductive period. Each wild-type hermaphrodite produces approximately 250 to 300 offspring, peaking at 5 days of age at 20°C (20).

Strains were propagated at 20°C on nematode growth media (NGM) plates with *Escherichia coli* strain OP50 as a food source, as previously described (13). Liquid transfers of animals were performed in S basal media (13,21). Strains used in this study were TJ1060 [*spe-9(hc88) I; fer-15(b26) II*], TJ1062 [*spe-9(hc88) I; fer-15(b26) age-1(hx546) II*], both constructed by Tom Fabian (22); TJ1067 [*spe-9(hc88) I; fer-15(b26) II; clk-1(e2519) III*]; and BA821 [*spe-26(hc138) IV*]. Most strains carried two fertility mutants [*fer-15(b26)* and *spe-9(hc88)*] that together completely eliminate the production of progeny (22); because of difficulty in strain construction, *spe-26* mutants were studied without any additional sterile genes added to the background. Life spans of all of these strains have been validated by comparisons with the single gerontogene mutants alone (22); however, studies showing that different backgrounds of N2 can affect longevity (23) continue to plague the observations on BA821. Strains were constructed by using standard methods (21). Genotype was confirmed by back crossing to both parental single-mutant strains and by showing failure to complement.

Mass Cultures

Generation of large numbers of age-synchronous young adults.—E. coli strain OP50 was used as a food source. Each subculture was initiated by transferring agar plugs containing dauer larvae from a small NGM plate stock to three to five large (10 cm) NGM plates spread with OP50. The worms were then washed off the plates with S basal and transferred to 2% peptone plates (same as NGM but with 2% peptone) spread with RW2 (a wild-type strain of *E. coli*.)

Eggs were isolated by treatment with alkaline hypochlorite (24). Eggs were inoculated into sterile 10-cm glass Petri dishes at 0.1-ml egg pellet per dish, and S basal was added to a total volume of 10 ml. Eggs were allowed to hatch at 20°C over the next 24 hours in the absence of *E. coli* and cholesterol. First stage larvae (L1's) were then inoculated onto 2% peptone plates and allowed to develop at 25.5°C for 48 hours. More details can be found in Fabian and Johnson (22).

Maintenance of Age-Synchronous Cultures of Adults

Young adults were harvested by washing them off the plates and were inoculated at a concentration of 100 worms/ml into 2-l Erlenmeyer flasks containing 1000 ml of survival medium, which was *E. coli* (1×10^9/ml) and cholesterol (10 μg/ml) in S basal. Liquid cultures were maintained at 25.5°C and 250 rpm in a rotary air shaker (Series 25D Incubator Shaker, New Brunswick Scientific, Edison, NJ). At 2-day intervals, approximately one half of the medium was removed and replaced with fresh medium by allowing the worms to settle out before the medium was removed.

Assessment of Age Trajectory of Survival

Every-other-day subsampling was used to assess mortality. A subsample of approximately 2000 nematodes (20 ml) was withdrawn from the mass culture every other day and all dead worms in this sample were discarded (Figure 1). The mortality of this subsample was then assessed over the next 24-hour period, after which the subsample was discarded and a fresh subsample taken. Our assessment of death is based on the following: lack of spontaneous movement, loss of ability to respond to touch, visible tissue degeneration, and lack of osmotic turgor. The criteria used for distinguishing live and dead worms were developed 20 years ago (13) and are the same in standard assays and mass cultures.

Estimates of the number of worms in the mass culture were obtained from the daily counting of the 20-ml sample. This represents approximately 2% of the mass culture at early ages and a larger fraction at later ages, because the

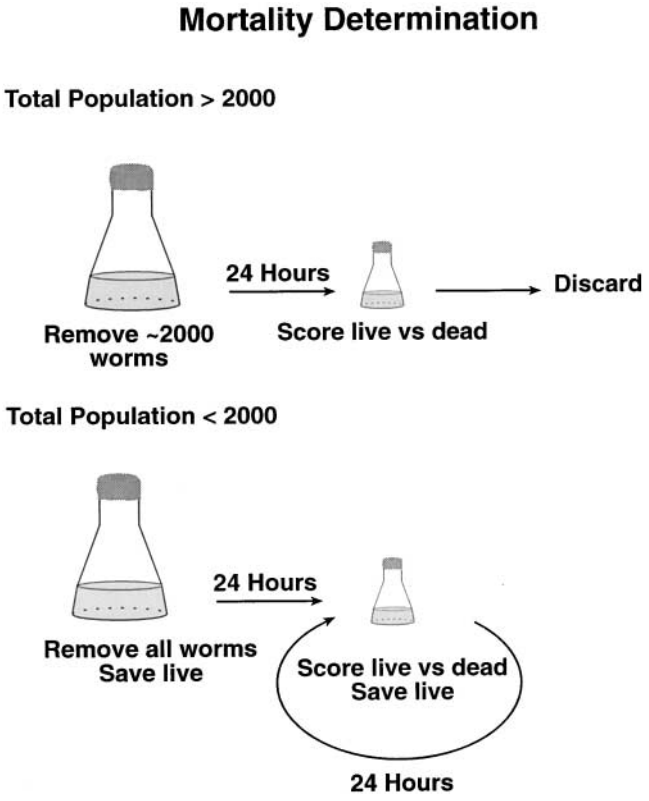


Figure 1. Procedures for measuring survival in mass cultures of *C. elegans.* Approximately 100,000 young adult worms are suspended in S basal at 100 worms/ml. After 24 hours, approximately 2000 worms are withdrawn and live worms are saved and resuspended in a small flask at 100 worms/ml. After 24 hours, these worms are counted for living and dead and then discarded. When the total living population is estimated to be less than 2000, all worms are sorted into living and dead and the living resuspended in a culture where they remain, being counted daily, until all are dead.

concentration of worms is maintained constant at 100 worms/ml by adjusting the culture volume. When 2000 worms, more or less, were alive, we harvested and counted the entire population, retaining the alive worms for subsequent daily assessments of mortality. Small worms (anything < 3/4 adult size), males, and accidentally killed animals are scored as "lost" and removed from the population. This "lost" category was usually quite small in number.

Statistical Analysis

Data were collected by hand and transferred to Dr. Deqing Wu at the Max Planck Institute in Rostock, Germany, for analysis. Some analysis was also performed at our site.

The direct calculation of the conditional probability of dying (q_x) was calculated as

$$q_x = \frac{N_x - N_{x+1}}{N_x}$$

where N is the number of worms alive on day x.

Smoothing with locally weighted least squares was carried out as follows (25,26). Given scatter plot data, $(X_j, Y_j)_{j=1,\ldots,n}$ and associated weights W_j, the basic assumptions are as follows: (i) $E(Y_j|X_j = x) = f(x)$ with a smooth function f, and (ii) $\mathrm{var}(Y_j|X_j = x)$ is finite. Then a consistent curve estimate of $f(x)$ using locally weighted squares is:

$$S[x,b,(X_j,Y_j,W_j)_{j=1,\ldots,n}] =$$

$$\underset{a_0}{\operatorname{argmin}}\ \min_{a_1}\left(\sum_{j=1}^{n} K\left(\frac{x-X_j}{b}\right)\{Y_j - [a_0 + a_1(X_j - x)]\}^2 W_j\right).$$

Here j is day, b is the bandwidth of the smoother, and K is a nonnegative kernel function. This estimate fits local linear lines by weighted least squares to the data falling into a window $(x - b, x + b)$ around the point x where the estimate is desired. We chose the kernel function

$$K(x) = (1 - x^2),\ |x| \le 1,\ K(x) = 0,\ |x| > 1$$

and we chose $X_j = j$ (in days); Y_j is the force of mortality at day j, and W_j is the number of animals at risk at the beginning of day j. The bandwidth b is 5 days.

For the two-stage Gompertz model, we used the equations

$$q(x) = \frac{D(x)}{N(x)},$$

$$\mu(x) = -\ln[1 - q(x)]$$

where $q(x)$ is the probability of death at day x, $D(x)$ is the number of deaths at day x, $N(x)$ is the number of animals at risk at day x, and $\mu(x)$ is the force of mortality at day x.

The form of the two-stage Gompertz model (27) is

$$\mu(x) = ae^{bx},\ x \le c$$

$$\mu(x) = ae^{bc + \beta(x-c)},\ x > c$$

where x denotes day (age), a is the initial death rate, b is the rate of mortality increase before age c, β is the rate thereafter, and c is the breakpoint. We use maximum likelihood methods to estimate the values of the parameters of the model. To test whether the slope parameters b and β are significantly different, a Gompertz curve with $b = \beta$ was also fitted to the data, and then a likelihood ratio test was used.

The logistical regression used the formula

$$\mu(x) = \frac{ae^{bx}}{1 + (ae^{bx}/c)}$$

where a is the initial death rate, b is the rate of mortality increase at early age, and c is the ceiling of mortality. We use the maximum likelihood method to estimate the values of parameters of the model. To test whether ceiling c exists, a Gompertz model with $C \to \infty$ was also fitted to the data and then a likelihood test was used.

To test whether the daily mortality between two groups is significantly different, we use the normally distributed test statistic $Z_{q(x)}$ (28):

$$Z_{q(x)} = \frac{|q_{i(x)} - q_{j(x)}| - 1/2[1/N_{i(x)} + 1/N_{j(x)}]}{\{\mathrm{var}[q_{i(x)}] + \mathrm{var}[q_{j(x)}]\}^{1/2}}$$

where i and j are groups, x is day (age), $q_{i(x)}$ is the probability of death at day x for group i, $q_{j(x)}$ is the probability of death at day x for group j, and $N_{i(x)}$ and $N_{j(x)}$ are the number of animals at risk at the beginning of day x for group i and group j, respectively. The form of the variance of $q_{i(x)}$ is

$$\mathrm{var}[q_{i(x)}] = q_{i(x)}[1 - q_{i(x)}]/N_{i(x)}.$$

Significance tests of mortality in total between two groups were performed by using the k-sample test (29), which can be described as follows: suppose that, in the pooled sample, failures occur at times $t_1 < t_2 < \ldots < t_N$. Let n_{ij} be the number of individuals in sample j still at risk just prior to t_i, and let d_{ij} be the number of failures in sample j at time t_i. Let the corresponding numbers in the pooled sample be n_{i+} and d_{i+}, respectively ($n_{i+} = \Sigma_j n_{ij}$, and $d_{i+} = \Sigma_j d_{ij}$). Under the hypothesis of no difference between samples, the conditional expected value of d_{ij}, given n_{ij}, can be estimated as $E_{ij} = n_{ij}(d_{i+}/n_{i+})$. Let $E_{+j} = \Sigma_i E_{ij}$. Then the test statistic is

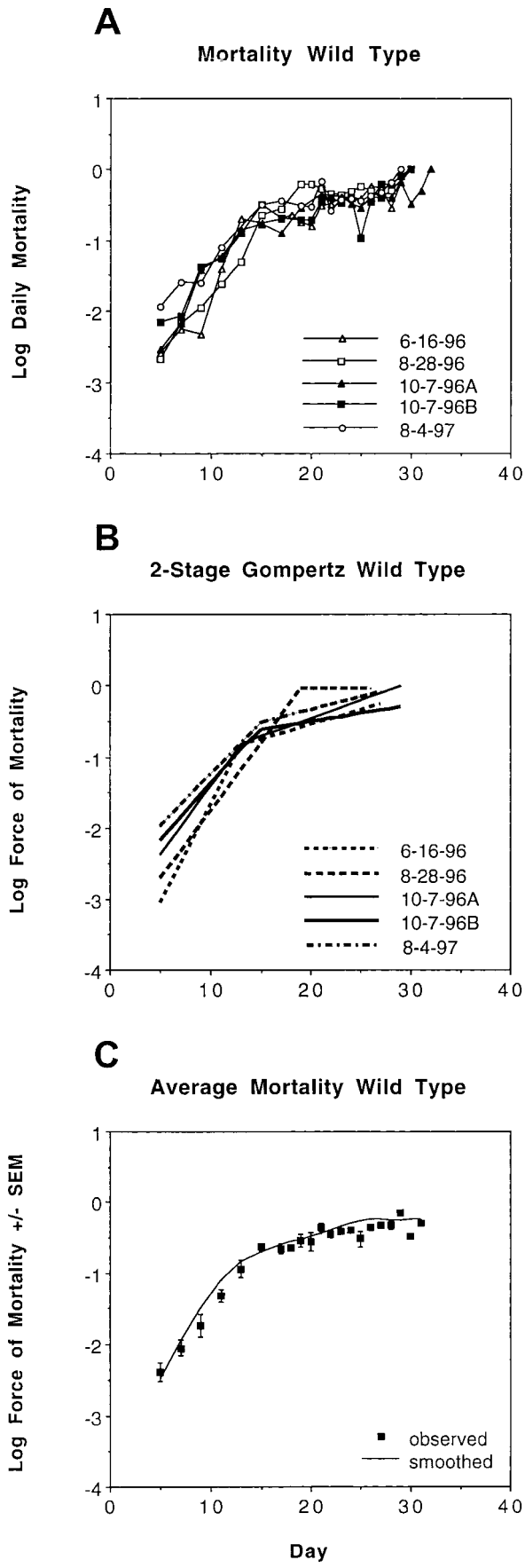
$$\sum_j (E_{+j} - d_{+j})^2 / E_{+j}$$

which is treated as a chi square with $(k - 1)$ degrees of freedom, with k being the number of samples involved.

RESULTS

Mortality in Mass Culture of the Wild Type

The assessment of mortality in large populations of *C. elegans* has proven difficult to achieve. Three problems make it quite difficult to follow populations larger than a few thousand by using a daily census procedure: the large



number of progeny produced by each hermaphrodite worm (~300), the rapid loss of carcasses after death, presumably as a result of rapid digestion by the *E. coli* in which these animals are raised, and, most importantly, the effort involved in daily censuses of large populations. To solve the problem of large numbers of progeny, we incorporated strains that were made completely sterile by using two mutations (*fer-15* and *spe-9*) blocking sperm development (22). Such strains contain two fertility mutants that completely sterilize the worms but do not cause any alteration in life span (22). (Note that no single temperature-sensitive mutation that we tested was completely effective at eliminating progeny production; absolute sterility is essential to ensure that the longest-lived individuals in a mass culture are not offspring of the original cohort.) The problem of loss of dead worm carcasses was addressed in preliminary experiments in which worms were killed and then followed under conditions similar to those used for the mass culture where little loss of carcasses was observed after 2 days and probably up to 1 week. The problem of counting 100,000 or more worms per day was solved by using a sampling strategy such that we counted a smaller number of worms each day and ascertained mortality in this subpopulation, as in Brooks and colleagues (16). A subsample of approximately 2000 nematodes (20 ml) was withdrawn from the mass culture every other day. All dead worms in this sample were discarded (Figure 1) and the mortality of this subsample assessed over the next 24-hour period, after which the subsample was discarded. When only approximately 2000 worms, more or less, were left alive, we harvested and counted the entire population.

We have collected at least partial data from seven distinct cohorts on ~700,000 wild-type worms of the strain TJ1060 (22). Five such experiments have been successfully carried to completion out of a total of seven attempted (Figure 2). In the wild-type TJ1060, the mortality profiles were remarkably homogenous between replicates (Figure 2A) and showed two phases: an exponential and a plateau. To further estimate replicability, we split a population and assessed mortality independently, but simultaneously, in both subpopulations (Figure 2A, 10-7-96A and 10-7-96B). There was complete homogeneity in the daily force of mortality, with these replicates showing no significant difference. Although some daily variation in force of mortality is observable, this was not statistically significant. Note especially that variability of small peaks is not reliable or reproducible. Two-stage Gompertz modeling (Table 1), which provides a much better fit than does a one-stage Gompertz model, is used throughout. In the five different wild-type populations, variation in estimates of the Gompertz parameters is small for b but much larger for β (Table 1). The slopes of the first stage of the Gompertz plots ranged between 0.334 and

Figure 2. Daily mortality of five replicates of wild-type nematodes. **A**, Age-specific mortality from five separate populations of the wild-type strain TJ1060 (see Table 1) that were studied over a period of 2 years. All populations, except the two from 10-7-96, were set up at different times. **B**, Two-stage Gompertz for each of the experiments shown in **A**. **C**, Average force of mortality for the five populations shown in **A**, as well as a smoothed curve.

Table 1. Best-Fit Parameters From the Gompertz Two-Stage Model*

Mutant & Strain	Experiment (date)	Initial Mortality (Day 0) (Prob./day × 10^4)	Slope		Inflection Point		Big Count		Life Span	
			Stage 1 (*b*)	Stage 2 (Beta)	Age (d)	Observed Mortality	Day	No. Alive	Mean	Maximum
"wild type"	6/16/96	0.270	0.638	0.094	13.5	0.224	16	15,768	16.624	30
TJ1060	8/28/96	1.77	0.439	0	19.5	0.960	18	3,426	16.864	30
	10/7/96A	3.70	0.444	0.115	13.64	0.147	22	360	16.438	32
	10/7/96B	8.96	0.369	0.052	15.1	0.380	18	2,779	15.768	30
	8/4/97	17.32	0.334	0.081	15.5	0.384	18	7,654	15.229	29
	Summary	3.14	0.442	0.097	14.67	0.195			16.385	
age-1	4/27/97**	13.80	0.000	0.14	NR	NR	40	1,343	28.180	50
TJ1062	9/19/97	1.17	0.338	0.025	22.99	0.342	40	67	22.422	51
	12/5/97	0.32	0.609	0.084	11.5	0.0500	38	2,633	23.176	55
	Summary	1.00	0.463	0.095	11.5	0.0316			24.184	
clk-1	4/18/98	0.00	1.503	0	9.8	0.124	27	819	14.539	38
TJ1067	11/24/98	3.29	0.319	0	24.8	0.789	27	336	20.366	33
	1/11/99	0.03	0.514	0.039	22.46	0.331	29	3,687	22.188	40
	Summary	31.0	0.261	0.04	15.5	0.245			16.884	
spe-26	3/17/99	0.95	0.365	0.036	24.79	0.545	30	65	21.871	33
BA821	5/2/99	0.66	0.431	0.169	21.47	0.801	23	432	18.395	28
	Summary	0.45	0.435	0.038	22.59	0.555			19.587	

*All "ages" use day of egg lay as the zero point in life-span measurements.

**This population is best fit by the one-stage Gompertz; thus, no inflection point is seen; NR = Not Relevant.

0.638 and varied only approximately 25%. Stage 2 slopes varied from 0 to 0.115. Thus, the genetic specification of longevity more rigidly determines early mortality than the later "plateau" stage. The age at which the inflection point occurred differed only slightly between experiments on TJ1060 (Table 1), varying from 13.5 to 19.5 days of age with only 2.43 days as a standard error. The mortality level at which the inflection occurred was more variable, but the mass summary statistic suggests that the inflection occurred at 19.5% mortality. Thus, the family of five best-fit curves for the two-stage Gompertz model for the wild-type strain TJ1060 (Figure 2B) are remarkably consistent among themselves. We saw considerable variation among replicates in the number of worms alive when the second stage of the Gompertz was reached (Table 1). This variation was not explained by any easily assessed parameter. Because of the environmental variation between genetically identical replicates, multiple replicates of each mutant strain provide a better estimate of the genetic effects by allowing multiple sampling of these inconsistent environmental variations across replicates.

Mortality of Age Mutants

Several experiments have been completed on large populations of *age-1*, *clk-1*, and *spe-26* mutants (Table 1). Less consistency in daily mortality among the replicates of the different genotypes is apparent. We again found that a two-stage Gompertz led to a better fit of all the data sets except for one of TJ1062, which fit a one-stage model best (Table 1). Comparisons of daily mortality between genotypes showed that the *age-1* (TJ1062) mutant resulted in lower mortality at all ages (Figures 3A and 3C). In contrast, mutations in *clk-1* (TJ1067) resulted in little change in mortality for the 11 days of life, but much lower mortality subsequently. The *spe-26* mutant (BA821) had the lowest mortality before 14 days of age but showed much higher forces of mortality after 3 weeks, higher even than the wild-type strain, TJ1060, which carries the two fertility mutants.

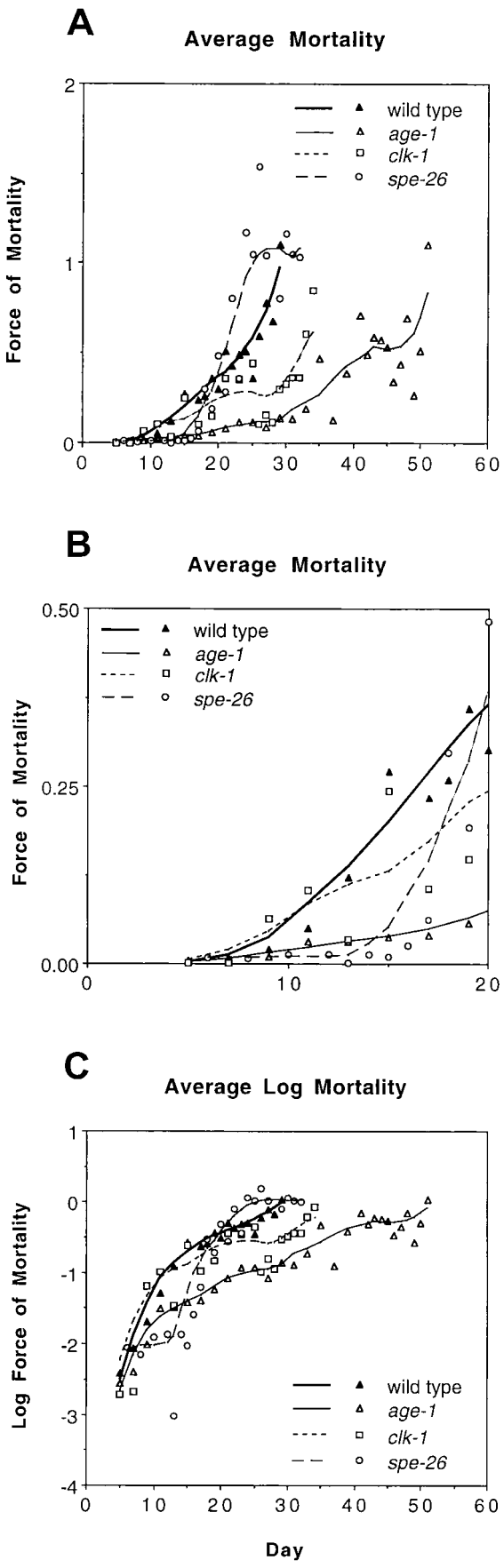
Interesting differences among genotypes are apparent (Table 1). The *age-1* mutant has a lower mortality than wild type at essentially all ages. This observation suggests continual action of the normal AGE-1 protein to increase mortality rate throughout life as compared with the mutant. The *age-1* mutant departs from wild type dramatically by 13 days of age, leveling off at a mortality that is almost tenfold lower than that of the wild-type strain. The inflection point is at 3% daily mortality in the *age-1* mutant, as compared with daily mortality of 20% in the wild type at the inflection point, which occurred at 11 days in *age-1* mutants, as compared with 15 days for wild type. The rates of mortality increase were well fit by a two-stage Gompertz analysis (Table 1) and were very similar in both stages for the *age-1* mutant and for the wild-type strain.

In contrast, *clk-1* started at a tenfold higher initial mortality but then had a twofold slower rate of increase in mortality in both stages of the two-stage Gompertz (Table 1). For *clk-1*, the age at which mortality levels off as well as the level of mortality at the inflection point are approximately the same as wild type. *clk-1* shows decreased mortality only late in life (Figure 3).

The *spe-26* mutant strain seems to start at a sevenfold lower mortality than the wild type and to then show a wild-type rate of mortality increase until approximately 1 week later in life than wild type (Table 1, Figure 3). Then, at ~3 weeks of age, mortality tends to level off but at a higher mortality than that of wild type. Thus *spe-26* mutants show lower mortality only early in life.

DISCUSSION

We have discovered several things both about normal mortality and about how this mortality is altered by each mutant. First, mortality is quite sensitive to environmental



variance. In the best-studied case (wild-type TJ1060), we had five completed replicates. Mortality profiles of each of these were distinct by the k-sample test (30), with one exception: the two samples taken at the same time from a split culture were not different, showing that the differences between replicates are likely a result of environmental differences prior to the establishment of the culture or to differences between cultures (such as food heterogeneity, etc.) and do not result from assessment variation. We know that temperature, food concentration (20,31), and age of the food (live *E. coli*; 32) affect survival. It is not unreasonable to ascribe much of the variation between replicates done at different times to these sources. Variation in life expectancy of replicate wild-type (N2) cultures has been repeatedly observed (33,34), necessitating the inclusion of controls within each assessment of life span (13). In these assessments, it was not possible to maintain multiple populations simultaneously; so, we rely instead on the pooling of multiple replicates prior to comparing mortality kinetics.

We observed that mortality tends to level off (always increasing slightly with increasing age) at a high level in all strains and experiments. This is consistent with many experiments (see 18 for a review) in a variety of species, showing a similar tendency to level off or even decrease. Thus, mortality does not increase indefinitely but levels off at a rate that is under genetic control, as shown by the results of mutants described within. Mortality kinetics are well described by a two-stage Gompertz model.

There are six or more classes and a total of 39 reported genes in *C. elegans* in which mutations result in longer adult life expectancy and maximum life span (9). We have examined representative mutants from three of these classes: *age-1*, representing the dauer-constitutive class, *clk-1*, representing the clock class of mutants, and *spe-26*, representing the fertility-defective class of mutants. Each of these three classes (and the individual mutant representing each class) is thought to represent a quite distinct mechanism for life prolongation. Our demographical results bear out this prediction in that each mutant alters the demographic profile in a characteristic fashion. The first class consists of those loci where mutants have a dauer-constitutive (Daf) phenotype (10,11). In addition to the *age-1* mutant, described within, mutants in *daf-2* result in a more than twofold extension of life expectancy in the adult phase and *daf-2* interacts with *daf-12* to cause an almost fourfold increase in life expectancy. Finally, *pdk-1* mutants show a life-extension phenotype. All of the effects (dauer formation, life extension, and stress resistance) of *age-1* and *daf-2* are suppressed by mutant alleles of *daf-16*, a gene necessary for dauer formation. So far we have only assessed mortality in mass cultures of one representative of this class of genes, *age-1*, and that mutation lowers mortality at all ages.

Figure 3. Summary of mortality profiles. **A**, Force of mortality for wild type (TJ1060), *age-1* (TJ1062), *clk-1* (TJ1067), and *spe-26* (BA821) mutants, plotted on an arithmetic scale. **B**, Enlargement of mortality data for days 1–20 from Figure 3**A**, showing both actual data points and smoothed curve. **C**, Force of mortality for wild type, *age-1*, *clk-1*, and *spe-26* mutants, plotted on a semilog scale.

A second class (Fertility deficient, or Fer) contains only two genes: *spe-26* and *spe-10*. Two of the six mutant alleles of *spe-26*, a gene specifying proper segregation of cellular components affecting sperm activation, were reported (35) to result in life extensions of approximately 80% for the hermaphrodite and the mated male, although the details are contentious (36). Results presented here show that life extensions fall short of that previously reported for *spe-26*. The *spe-10* mutant is also long lived, perhaps more long lived than *spe-26* (37).

The third class studied is the "clock" class (Clk; 9–11), where two of four alleles of *clk-1*, both of which have altered cell cycle and developmental timing, also have increased life expectancy. Other genes in this class (*clk-2*, *clk-3*, and *gro-1*) all have modest (typically 20–30%) extensions of life span, but only for some alleles. Other classes are the Eat (*eat-1*, *-2*, *-3*, *-6*, *-13*, *-18*, and *unc-26*) that slow the consumption of food and also result in life extension (9–11), presumably through a self-imposed, dietary restriction; hypermorphic (Old for overexpression longevity determinants) (38), a new class of gerontogene that increases life span when overexpressed; and polygenic mutants, in which life prolongation results from the combined action of many genes (quantitative trait loci; 14,39–41).

We assessed detailed mortality kinetics in representative mutants from each of the first three classes of mutants listed above. As researchers found previously in insects (42,43), we observed a deflection of the Gompertz curve at late ages. This is more pronounced than that previously reported in *C. elegans* (27). We confirmed that all three mutants (*age-1*, *clk-1*, and *spe-26*) are longer lived. Maximum life spans of all three were also greater than that of the wild-type TJ1060, where the last living worm typically died at approximately day 30. In contrast, the last living *age-1* mutant worm died at 50 to 55 days. For *clk-1* mutants the last worm died at approximately day 33 to 40 in most experiments, and for *spe-26* only approximately a 3-day life extension was observed (last dead at day 28 or 33). This modest increase in life expectancy of *spe-26* is consistent with that previously reported (35,36).

Each mutant had a distinct, statistically different pattern of mortality that accounted for its extended longevity. By far, the most impressive were the *age-1* mutants, which showed extremely significant, lower mortality throughout life (almost tenfold at some ages). In contrast, *clk-1* and *spe-26* mutants showed age-specific (but highly significant statistically) alterations in mortality that we refer to as "segmental." The *spe-26* mortality was reduced only early in life, prior to day 18, whereas *clk-1* had consistently lower mortality only after approximately 20 days. It could be that the life extension of *spe-26* results from the elimination of some cause of death related to reproduction, which occurs only early in life and has been shown to cause increased mortality in *Drosophila* (44), or that uncontrolled background effects (see 23) played a role. However, these assessments were made in a sterile background, so it cannot be that reproduction per se underlies the difference in survival of *spe-26*. In contrast, *clk-1*, which may have reduced metabolic rates (45,46), only lowers mortality later in life. For *clk-1*, early mortality is actually higher than in the wild type. Because large numbers of animals at risk are necessary to accurately estimate such low mortality rates early in life and because we have only assessed three representative mutant alleles in these large culture conditions, it is not clear whether the generalizations distinguishing each mutant can be extended to include other members of the same class of mutant.

In summary, different genetic variants, each identified initially as a prolongevity mutant, affect different parts of the nematode life span, segmentally. One possible implication is that differential gene action or the accumulation of toxic gene products or other toxic agents could differentially accumulate among the different types of mutant strains. Clearly *clk-1*, which may have a severe metabolic defect, perhaps involving coenzyme Q biosynthesis (47), is at higher risk early in life, consistent with its delayed development and altered cyclicity (48). However, those worms surviving early mortality are then destined to age slower and show dramatic decreases in mortality approaching that of *age-1*. Because *spe-26* shows lower early mortality, but does not affect late-life mortality, it is tempting to suggest that this segmental pattern has something to do with the fertility defect presumably responsible for the increased longevity of *spe-26* (and perhaps of *spe-10* as well). However, the lack of consistent effects of all *spe-26* alleles on longevity suggest that other factors are at work and the fact that the increased longevity and stress resistance of *spe-26* mutants are suppressed by *daf-16* (49) suggests a direct role in dauer formation. The greatest impact on longevity and mortality is seen in mutants in *age-1*. This is the first gerontogene mutant identified in any species (50,51) and until last year was the only mutant ever identified solely by its longevity phenotype. In the only other published study on mortality in these mutants, Johnson (15) reported a good fit to a one-stage Gompertz model and found a twofold change in the slope but no change in the initial mortality rate. The studies shown here extend those observations and show little effect on initial mortality but reduced increases in mortality with age. Mortality in *age-1* is drastically reduced at all ages after the initial time point, suggesting either continued need for the AGE-1 protein through all of adult life or long-lasting effects of an early-life alteration in AGE-1 activity. Mutants of *age-1* are healthy and move vigorously for much longer than do wild type (52,53), suggesting that it is not just longevity but also health span that is altered.

In considering several potential complications of these assessments, the most important is possible problems from contamination by progeny. Adults were completely sterilized by constructing strains carrying two temperature-sensitive, fertility-defective mutations that together completely block fertility at 25.5°C. In a previous study (22), we found that most single fertility mutants allowed some leakage of small numbers of offspring. For three of the assessments reported here (wild type, *age-1*, and *clk-1*), we were successful using this protocol. For the third mutant (*spe-26*), we relied on only a single mutation and found that *spe-26* mutants produced few progeny under the conditions used in the assessments. Small animals (<3/4 adult size, which represented immature or developmentally abnormal worms) were few, usually 2% or less, and did not increase in num-

ber during the experiment, consistent with a complete block of reproduction.

We have run several controls to ask if this assessment protocol accurately assesses the underlying mortality of the mass culture. One way in which estimates may be biased is if dead worms are lost at higher rates than are the living. We performed defined experiments in which we either killed worms artificially and asked how long we could still recover their carcasses or in which we kept those dying a natural death for several more days, counting them daily. Both studies showed that carcasses survive for several days under these conditions. Alternately, we asked if living worms are more likely to be lost in the flask; again, we found that this was not likely to be true.

Another possible confound is the effect of the day at which assessment protocols were changed (Big Count Day, Table 1). By this day, most of the worms in the culture were dead or had been removed by sampling so that only a completely countable number of worms were still available (Table 1). In the wild-type cultures of TJ1060, this day varied between 16 and 22 days. All three gerontogenes mutants had later Big Count Days: *spe-26* and *clk-1*, approximately 7–8 days later, whereas for *age-1* there was a 16- to 24-day delay in the age at which this assessment occurred. It should also be noted that there is no association between Big Count Day and the inflection point in the two-stage Gompertz model.

It is our hypothesis that the leveling off of the mortality functions in later life observed in previous experiments (18) is driven by acquired heterogeneity, that is, differential mortality between individuals or sub cohorts that was not present in the initial populations. In genetically identical cohorts, we hypothesize that acquired heterogeneity is a major source of variation in subsequent survival (54,55).

In comparing our studies with those of other studies on genetic effects on mortality kinetics, several things are noticeable. First is the major impact of single-gene mutants, especially *age-1*, as compared with relatively minor effects and small heritabilities seen in studies of polygenic variations (53). This also should be compared with fairly small narrow-sense heritabilities in *Drosophila* for longevity (6–9%) versus 19% (narrow sense) to 44% (broad sense) heritability estimates in nematodes (13). The range of differences for age-specific mortality seen in *Drosophila* is not that different from those observed in comparing *age-1* mutants with wild type.

The identification and cloning of genes that play a role in increasing longevity in a variety of species, but especially in *C. elegans*, and the recent demonstration that human genes can play a similar role in model systems (S. Murakami and T.E. Johnson, unpublished data, 1999) clearly demonstrates that longevity is remarkably responsive to genetic interventions. The results presented within show that huge changes in age-specific mortality can be achieved by altering only one gene. In human populations, a drug treatment, instead of a genetic alteration, could be envisioned that would similarly inactivate one protein or gene function. Whether such drug interventions are achievable or desirable is debatable.

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