Rates of Mortality in Populations of *Caenorhabditis elegans*

A. Brooks *et al.* (*1*) report that mortality increased exponentially for a cohort of 180,000 nematodes of the species *Caenorhabditis elegans* of the single genotype TJ1060 [*spe-9*(*hc88*) *fer-15*(*b26*)]. A closer look at the data (Fig. 1) reveals that from days 5 through 8 mortality increased at a rate of 0.58 ± 0.0004, which is more than twice the rate thereafter (0.21 ± 0.02). Such a "biphasic pattern," with death rates increasing rapidly at younger ages and more slowly at older ages, was also found in a genetically heterogeneous population of 79 recombinant-inbred (RI) nematode strains (*1*). The deceleration of mortality for the heterogeneous population is of high statistical significance ($P < 0.001$) even when the population is divided into quartiles on the basis of life expectancy. Gompertz' law (*2*), which states that death rates increase exponentially at the same rate at all ages older than some initial age (such as the age of sexual maturity), does *not* hold for either the isogenetic or heterogeneous nematode populations.

The genotype TJ1060 used in the isogenic experiment suffered higher mortality and a more rapid rate of mortality increase with age (Fig. 1) than the genetically heterogeneous RI population [table 2 in (*1*)]. This resulted from growth at a higher temperature (25.5°C as opposed to 20°C) and at higher food concentrations (on agar plates rather than liquid survival medium); these environmental conditions affect life expectancy (*3*). The different life chances of the two populations make the finding that mortality decelerates in both populations even more significant.

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REFERENCES AND NOTES

1. A. Brooks, G. J. Lithgow, T. E. Johnson, *Science* **263**, 668 (1994).
2. B. Gompertz, *Philos. Trans. R. Soc. London Ser. A* **57**, 289 (1825).
3. M. R. Klass, *Mech. Ageing Dev.* **6**, 413 (1977).
4. J. W. Curtsinger, H. H. Fukui, D. R. Townsend, J. W. Vaupel, *Science* **258**, 461 (1992); H. H. Fukui, L. Xiu, J. W. Curtsinger, *Exp. Gerontol.* **28**, 585 (1993).
5. Gompertz formulated his law in terms of what he called the intensity of mortality (*2*) and what is also known as the force of mortality or hazard of death, generally denoted by μ. The central death rate m provides a convenient empirical estimate of the intensity of mortality. The probability of death per unit time q is smaller than the intensity of mortality and is not a useful approximation when mortality is high. It is clear that q cannot be described by Gompertz' formula, because a Gompertz curve rises exponentially from its initial value toward infinity as age increases, but the probability q is bounded by 1. This is apparent in figure 1 of (*1*); note that some of the values of m in Fig. 1 exceed 1.
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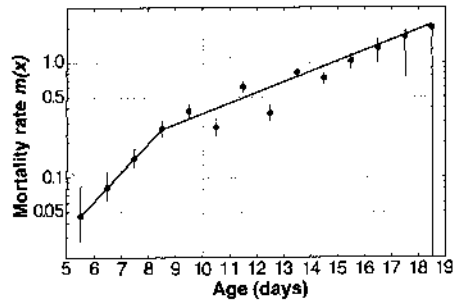

Fig. 1. Age-specific central death rates (•) and estimated trajectory of mortality for TJ1060 nematodes. The vertical bars at each point indicate the range of death rates that, with probability exceeding 1%, might have produced a death count at least as extreme as that observed. For days 5 through 13, the observed death rates are based on samples of the population that were subcultured (*1*). Because there are no significant differences between age-specific death rates in the subcultures, available data were combined for each day of observation. The central death rate at age x is given by

$$m(x) = [N(x) - N(x + 1)]/\{[N(x) + N(x + 1)]/2\}$$

where N is the number of surviving individuals. The trajectories were estimated by maximum likelihood methods using a two-stage Gompertz curve (*4*) of the form

$$\mu(x) = a \exp(bx)\, I(x \leq c) + a \exp[bc + \beta(x - c)]\, I(x > c)$$

where x denotes age, a is the initial death rate, b is the rate of mortality increase before age c, β is the rate thereafter, I is an indicator that equals one if the condition is true and zero otherwise, and $\mu(x)$ is the hazard of death at age x (*5*). The estimated values of the parameters of the model are: $a = 0.0019 \pm 0.000003$, $b = 0.58 \pm 0.0004$, $\beta = 0.21 \pm 0.02$, and $c = 8.5 \pm 0.4$. To test whether the slope parameters b and β are significantly different, a Gompertz curve with $b = \beta$ was also fit to the data. A likelihood ratio test indicated that the two-stage Gompertz curve fits significantly better, $P = 0.0000008$. The breakpoint of the fitted curve, c, is estimated in the model: it falls in the middle of day 8. The central death rate $m(x)$ is related to the daily probability of death $q(x)$ by $m(x) = 1/\{[1/q(x)] - 0.5\}$. Because $N(18) = 1$ and $N(19) = 0$, it follows that $m(18) = 2$ and $q(18) = 1$. The long vertical bar indicates that values of $m(18)$ corresponding to values of $q(18) \geq 0.01$ might have produced, with probability ≥0.01, the final death on day 18.

Mortality Rates and Immigration in Laboratory Populations of *Drosophila*

Brooks *et al.* (*1*) suggest that the slowing of age-specific mortality rates observed in earlier studies of *Drosophila* cohorts at older ages (*2*) may have resulted from contamination by recruitment of progeny. However, in these earlier experiments (*2*) (i) only male flies were studied and no sexing errors were detected during daily observations and (ii) vials were changed once a week, which is too fast to allow hatching, larval development, pupation, and emergence of new adults.

For all but about 20 min per day experimental flies were kept in an air-tight incubator with no other stocks; there were no other *Drosophila* cultures in the third-floor laboratory where survivorship was studied, and the nearest foreign *Drosophila* cultures were in a basement laboratory. Dead flies were counted daily, but once a week live flies were counted, which allowed a direct assessment of immigration rates. In two vials (out of thousands) a discrepancy was found that suggested immigration, which was attributed to miscounting (live flies can become trapped between the glass and the vial plug and miss being counted). These two vials were excluded from the analysis. Even if there had been a few immigrants, it would not have affected the conclusions (*2*), because in the largest experiment a zero acceleration of mortality rate was observed among the last 1906 deaths in a cohort of 5751 males from a single inbred line. Finally, the observation of slowing mortality rates in genetically homogeneous cohorts of *Drosophila* has been replicated and extended to both sexes and more genotypes (*3*).

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REFERENCES

1. A. Brooks, G. J. Lithgow, T. E. Johnson, *Science* **263**, 668 (1994).
2. J. W. Curtsinger, H. H. Fukui, D. R. Townsend, J. W. Vaupel, *ibid.* **258**, 461 (1992).
3. H. H. Fukui, L. Xiu, J. W. Curtsinger, *Exp. Gerontol.* **28**, 585 (1993).

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One problem with the analysis of Brooks *et al.* (*1*) occurs because a substantial fraction of the observed lifetimes turn out to be right censored, and those censored observations are discarded in the statistical analysis (*2*). This problem may lead to biases in statistical estimators like mean lifetimes and makes tests invalid (*3*).

Although this censoring problem does not affect the analysis of one large cohort of 180,000 individuals (*1*), there is a lack of fit of the proposed Gompertz model (*4*) with data from this cohort. This problem becomes evident when one uses a nonparametric locally weighted least squares method (*5*), which takes the unequal variance feature into account (*6*). This nonparametric curve estimation procedure lets the data "speak for themselves" without imposing any restrictive model assumptions—in contrast to Gompertz model or other parametric fits. Such a method is particularly suitable to assess the goodness-of-fit of parametric models. The nonparametric fit (*5*) for double log-transformed mortality (*6*) deviates from the linear fit corresponding to the Gompertz model and shows deceleration patterns of mortality as compared with the Gompertz model at ages between 8 and 10 days. This deceleration is followed by a slight acceleration, which leads to the impression of a "shoulder" on the curve. We noted this phenomenon of a shoulder previously (*7*) in the survival of medflies.

We also reanalyzed the survival data from the other 79 cohorts (*1*) that consisted of a single genotype. These data are subject to heavy censoring, and we first constructed appropriate lifetables, from which estimates of the log mortality rate were obtained with the locally weighted least squares method (*6*). A covariate of interest identified by Brooks *et al.* is the mean lifetime associated with each strain. We recalculated mean lifetimes to accommodate censoring, then divided the 79 strains into four groups corresponding to quartiles of associated mean lifetimes. The rates were well synchronized within each quartile group (*6*). Moreover, every single strain showed marked deceleration of transformed mortality. In view of these observations it is likely that deceleration of mortality of cohorts of genetically heterogeneous individuals is not caused by genetic heterogeneity per se.

We observe that deceleration becomes more pronounced as mean lifetime increases. The longer a genetically homogeneous strain lives on the average, the more the curve of transformed mortality will bend (Fig. 1). We find, upon fixing age and varying mean lifetime, that log mortality declines almost monotonously as mean lifetime increases. Conversely, fixing mean lifetime and varying age shows that concavity and deceleration of log mortality for higher age become more pronounced for increasing mean lifetime. These changes in the shape of the curve allow an assessment of mortality factors associated with higher mean lifetime. The major factor appears to be the intensity of a sharp localized deceleration of log mortality occurring around day 10 (*5*, *8*). The higher mean lifetimes seem to be associated with increased intensity of deceleration, as well as a lower rate of the initial transformed mortality.

Our conclusion from this reanalysis of the data of Brooks *et al.* is that deceleration of transformed mortality rates appears to be a universal feature of strains of *C. elegans*. The Gompertz model cannot adequately serve as a life model for nematodes. We found evidence that shape and slope (deceleration) of the curve of mortality is associated with the mean lifetime of the corresponding strain, and that deceleration increases with increasing mean lifetime.

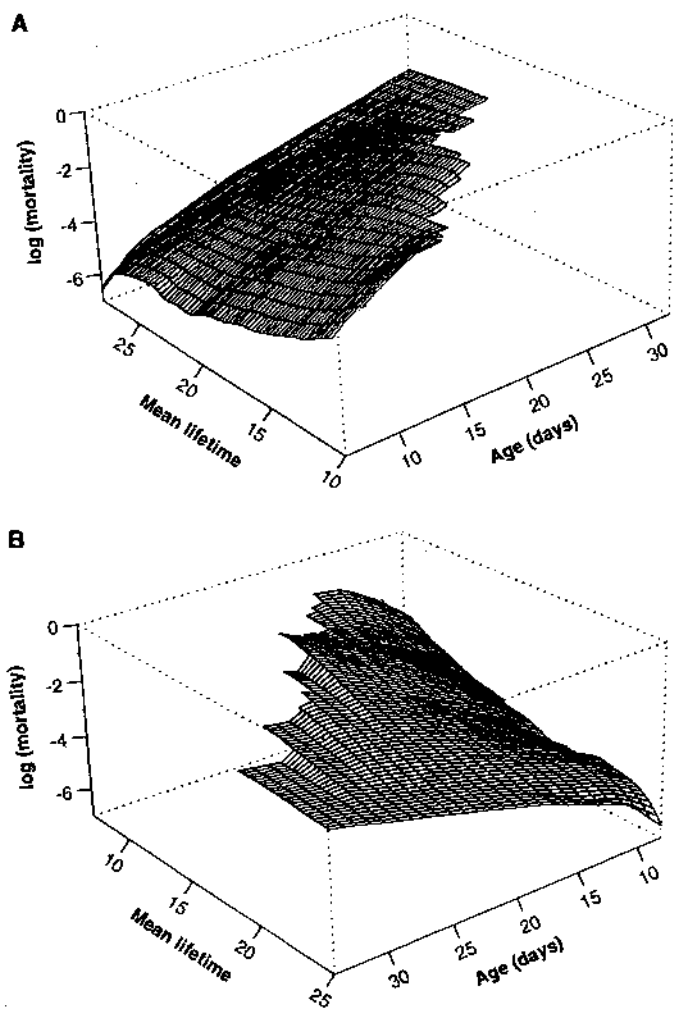

Fig. 1. Nonparametric estimate of the double log–transformed mortality surface as a function of age (in days) and mean lifetime (in days) with the use of two-dimensional locally weighted least squares (*6*). This surface estimate is calculated from the lifetime data of 2755 nematodes in 79 cohorts, including 1625 uncensored and 1130 censored lifetimes. (**A**) The perspective with origin of mean lifetime and age in front. (**B**) The perspective with the largest values of mean lifetime and age in front.

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REFERENCES AND NOTES

1. A. Brooks, G. J. Lithgow, T. E. Johnson, *Science* **263**, 668 (1994).
2. The right censoring occurs as only senescent deaths were counted as observed deaths. In this approach, all individuals with deaths due to other specific causes, individuals which are lost for follow-up and those which were observed to become pregnant give rise to right censored observations which correspond to the last time at which such individuals were still observed to be at risk.
3. T. R. Fleming and D. P. Harrington, *Counting Processes and Survival Analysis* (Wiley, New York, 1991).
4. B. Gompertz, *Philos. Trans. R. Soc. London Ser. A* **57**, 289 (1825).
5. W. S. Cleveland, *J. Am. Stat. Assoc.* **74**, 829 (1979); H. G. Müller, *ibid.* **82**, 231 (1987); J. Fan, *ibid.* **87**, 998 (1993).
6. J.-L. Wang *et al.*, unpublished paper.
7. J. R. Carey, P. Liedo, D. Orozco, J. W. Vaupel, *Science* **258**, 457 (1992); J. W. Curtsinger, H. F. Fukui, D. R. Townsend, J. W. Vaupel, *ibid.*, p. 461.
8. Sharp changes in mortality rates are closely connected with the change-point problem [H. G. Müller and J. L. Wang, *Biometrika* **77**, 305 (1990)].

9. We thank A. Brooks, G. J. Lithgow, and T. E. Johnson for providing the data on *C. elegans* and M. Tatar for critical review of our comment. We acknowledge

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Response: The analysis by Vaupel *et al.* allows the extension of our data in ways that we (*1*) did not originally envision and provides a much better fit of the raw data. The slope of the Gompertz curve changes at 8 days of age, but mortality still continues to increase exponentially until the end of life. However, large errors in the estimation of mortality rates late in life, which result from the small number of deaths during this period, prevent us from determining the accuracy of this statement with a high degree of precision.

I find the statements from Curtsinger *et al.* to be convincing, and these precautions seem to adequately rule out the possibility that there was significant contamination by progeny in the aging cohorts displayed in their earlier report (*2*). Nevertheless, any significant amount of progeny contamination can produce a huge artifact when only a small minority of the starting population are being examined as is done in examining the oldest old. Great care must be taken to avoid it.

The analyses conducted by Wang *et al.* purport to show (i) that a "nonparametric locally weighted least squares method" provides a better fit (*3*) and detects a decrease in mortality rates at "between 8 and 10 days" and (ii) that each of the 79 genotypes analyzed in our report (*1*) themselves appear to be composed of two slopes. We completely concur on the first aspect of their analysis (*4*). However, we are uncertain that the deviations from the exponential curve are biologically significant. It remains to be demonstrated that the details of these deviations from the Gompertz will be replicated in further analyses. It is our opinion that experimental replication, not extensive mathematical analyses, should be the critical basis for testing theories in this area.

We disagree on the second aspect of the comment by Wang *et al.* The analyses have a high inherent inaccuracy because the analysis is based on total cohorts of 30 animals or less. Many of the individual estimates of age-specific rates of mortality are based on none or only a single death in a given time period. However, too few details are presented to allow us to determine the accuracy of the methodology. We do agree that there was censoring of the data [this was stated in the original report (*1*)] and that this censoring has resulted in apparently higher mortality rates early in life. In analyzing this data, Wang *et al.* suggest that the four quartiles behave synchronously; because the division into quartiles was based on mean life span (*1*), it is not unexpected that there is a fairly uniform distribution of mean life span in each quartile. However, the mean life spans of these RIs are distributed essentially normally (*5*, *6*) and all RIs are derived from crosses between the Bristol and Bergerac wild-type strains of *C. elegans* (*5*, *6*, *7*).

The principal problem with the analyses put forth by Wang *et al.* is twofold. First, the small population size results in considerable inaccuracy in the estimates of mortality rates and the rates estimated could be off by several orders of magnitude. Second, Wang *et al.* found absolute mortality rates early in life to range from 10^{-8} to 10^{-6}, which can be compared with our estimates of 10^{-3} or less (*1*, *5*, *8*) in populations of about 200 worms. Error estimates in the latter two studies (*5*, *8*) were obtained directly by analysis of the four component populations, each of 50 worms, and direct estimation of error. The standard error of the mortality rate at 3 days of age in these estimates (*8*) was 15 to 40% of the mean. Also, the log mortality rate at 3 days of age in two different estimates for the wild type (N2) were −2.09 (*5*) and −2.70 (*8*), which suggests considerable variation between experiments. In contrast, Wang *et al.* suggest that less than one worm out of 100 million is dying per day early in life; surely an estimate such as this cannot be made on a cohort of population size 30.

Before the methods proposed by Wang *et al.* are accepted for the analysis of mortality in small cohorts, they should show that their procedure allows the accurate reconstruction of the original mortality rates with the use of small, simulated data sets. One could simulate a population of organisms dying with exponential kinetics to see if their analytic method would generate an exponential model after sampling populations of size 30. Indeed, one of the principal arguments put forth in an earlier study "is that it may not be possible to determine the mortality pattern of a species from data on 100 or even fewer individuals . . ." (*3*, p. 460).

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REFERENCES

1. A. Brooks, G. J. Lithgow, T. E. Johnson, *Science* **263**, 668 (1994).
2. J. W. Curtsinger, H. H. Fukui, D. R. Townsend, J. W. Vaupel, *ibid.* **258**, 461 (1992).
3. J. R. Carey, P. Liedo, D. Orozco, J. W. Vaupel, *ibid.*, p. 457.
4. J. W. Vaupel, T. E. Johnson, G. J. Lithgow, *ibid.* **266**, 826 (1994).
5. T. E. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3777 (1987).
6. A. Brooks, thesis, University of Colorado, (1993).
7. ——— and T. E. Johnson, *Heredity* **67**, 19 (1991).
8. T. E. Johnson, *Science* **249**, 908 (1990).

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Identification of Calcium Channels That Control Neurosecretion

The report by David B. Wheeler *et al.* (*1*) addresses the important question of which Ca^{2+} channel types control synaptic transmission in the mammalian central nervous system. Wheeler *et al.* studied glutamatergic transmission between Schaffer collateral fibers and CA1 pyramidal neurons in the rat hippocampus and used synthetic toxins that target high voltage–activated Ca^{2+} channels in an effort to identify which types trigger glutamate release at this synapse. Wheeler *et al.* argue for the primary involvement of a novel class of Ca^{2+} channel, which they have labeled "Q." In pharmacological experiments such as these, three criteria should be met before conclusions can be drawn with confidence: (i) the concentration of antagonists at the synaptic site must be known, (ii) estimates of potency must be made at or near equilibrium for antagonist binding, and (iii) the antagonists employed should be specific. These fundamental criteria have not been consistently met in the report by Wheeler *et al.*

Wheeler *et al.*, measuring the field excitatory postsynaptic potential (fEPSP), demonstrate a slow onset for action of ω-agatoxin IVA [IVA, a P channel antagonist (*2*)]: 30 nM toxin produced no effect in 20 min and 200 nM produced inhibition at a rate of about 1% per minute. They use this to argue for the relative inefficacy of IVA on Schaffer collateral Ca^{2+} channels (and for the lack of P channels in Schaffer collateral nerve terminals). Our recent results suggest that the rate of onset for toxin action in a tissue slice is largely a function of the rate of toxin delivery to the synaptic region rather than the on-rate for toxin binding. The application times that Wheeler *et al.* used in their experiments with 30 nM IVA, therefore, are insufficient to achieve a steady-state concentration in the synaptic region. With the use of whole cell recording from superficial CA1 neurons in the slice, we found that 100 to 200 nM IVA