

A Demographic Analysis of the Fitness Cost of Extended Longevity in *Caenorhabditis elegans*

Jianjun Chen,¹ Damla Senturk,² Jane-Ling Wang,³ Hans-Georg Müller,³ James R. Carey,^{4,5}
Hal Caswell,⁶ and Edward P. Caswell-Chen¹

¹Department of Nematology, ³Division of Statistics, and ⁴Department of Entomology, University of California, Davis.

²Department of Statistics, Pennsylvania State University, University Park.

⁵Center for the Economics and Demography of Aging, University of California, Berkeley.

⁶Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

We monitored survival and reproduction of 1000 individuals of *Caenorhabditis elegans* wild type (N2) and 800 individuals of *clk-1* and *daf-2*, and used biodemographic analysis to address fitness as the integrative consequence of the entire age-specific schedules of survival and reproduction. Relative to N2, the mutants *clk-1* and *daf-2* extended average life span by 27% and 111%, respectively, but reduced net reproductive rate by 44% and 18%. The net result of differences in survival and fertility was a significant differential in fitness, with both *clk-1* ($\lambda = 2.74$) and *daf-2* ($\lambda = 3.78$) at a disadvantage relative to N2 ($\lambda = 3.85$). Demographic life table response experiment (LTRE) analysis revealed that the fitness differentials were due to negative effects in mutants on reproduction in the first 6–7 days of life. Fitness costs in *clk-1* and *daf-2* of *C. elegans* are consistent with the theory of antagonistic pleiotropy for the evolution of senescence.

THE nematode *Caenorhabditis elegans* has become a widely used model organism for studies of aging and biodemography (1–10). Its developmental biology and genetics are being intensively studied. There exist many genetically characterized longevity mutants of *C. elegans*, and their study is an important and growing part of gerontology (10–12). There has been a huge amount of work undertaken on longevity genes relative to longevity extension and other traits including aspects of reproduction, competitive ability, and survival relative to environment and stresses (3,4,9,10,13–18).

Here we focus on two of these longevity mutants that are particularly well characterized and understood: *clk-1* and *daf-2*. The *clk-1* gene codes an enzyme required for coenzyme Q synthesis, and mutations in *clk-1* influence metabolic activity and lead to reduced respiration, slowed developmental and physiological processes, and extended longevity that may be due in part to reduced production of reactive oxygen species (5,7,9,12,19–21). The gene *daf-2* codes for an insulin/insulin-like growth factor type I (IGF-I) receptor involved in an insulin-like signaling cascade, and mutants are temperature-sensitive dauer-constitutive with extended longevity (1,3,7,10). Insulin/IGF-I signaling is part of a signaling cascade that influences life span; this signaling pathway has been reviewed by Kenyon (10).

In this article, we extend the analysis of *clk-1* and *daf-2*; our focus is on the demographic differences among genotypes and their fitness consequences (22–26). Studies of the evolution, as opposed to the mechanisms, of aging require estimates of the fitness consequences, but they have seldom been estimated for *C. elegans*. This is an important and potentially confusing point; fitness is an integrative consequence of the entire age-specific (or, more generally, stage-

specific) schedules of survival and reproduction. Comparisons of survival alone, or of fertility alone, do not reveal fitness differences. Nor do comparisons of summary indices of survival and fertility (e.g., median longevity, total brood size, average reproductive output, generation time). It might appear that the fitness effects of longevity mutants have been documented, but much of the research has addressed effects of mutations on fitness components, not on fitness itself. For example, measurements of realized population growth [e.g., (14,16)] allow the population itself to integrate survival and fertility, and thus do provide an index of fitness. They have the drawback, however, of providing no information on the causation of the putative fitness differences revealed (i.e., are differences due to differences in survival, or fertility, in what proportions, at what ages?).

Biodemographic studies of aging must address the evolution of life span, which requires estimates of fitness. Senescence (the increase of mortality rate with age) has long been a particularly difficult evolutionary problem (22,27,28). One explanation views the evolution of senescence as resulting from an indirect effect of selection for genes with favorable effects on fitness at early ages but negative effects at later ages—an explanation termed “antagonistic pleiotropy” (22,29). Studies of mortality in general, and senescence in particular, must include complete measures of fitness, including survival, fertility, and the timing of events in the life cycle, as only then will the pleiotropic effects on fitness of longevity mutants be revealed.

Especially when dealing with longevity as a trait, analysis of fitness is rendered more powerful by the use of large cohorts, because such cohorts provide sufficient numbers for the actuarial properties of the cohort to be measured, including those of the oldest individuals (30).

Such large-cohort studies exist for the Mediterranean fruit fly *Ceratitis capitata* (26), *Drosophila melanogaster* (31), and *C. elegans* (9). However, most studies of the life span of *C. elegans* have used relatively small cohorts (32,33).

Here we subject a large cohort data set to demographic analysis, and report on deleterious fitness consequences of extended life span in *C. elegans* longevity mutants *clk-1* and *daf-2*. Our goals are to: 1) analyze the relationship of reproduction and longevity, 2) quantify the fitness of each strain, 3) document the demographic bases of fitness differences in terms of tradeoffs between survival and reproduction, and 4) explore the relationships between life span and age-specific fertility at the individual level. We do this using a combination of survival analyses, event history diagrams, matrix population models, and life table response experiment (LTRE) analyses. Our results provide, for the first time, a quantitative analysis of the fitness tradeoffs associated with longevity mutations in *C. elegans*.

METHODS

Genotypes

Strains used in the study were: 1) N2, wild type, 2) MQ 130, *clk-1*(qm30) III, and 3) DR1572, *daf-2*(e1368) III (a class 1 allele of *daf-2*). The wild-type (N2 var Bristol; DR subclone of CB original, Tc1 pattern I), *clk-1*, and *daf-2* worms were obtained from the *Caenorhabditis* Genetic Center at the University of Minnesota, St. Paul in October 2000. All experimental cohorts were two generations removed from a frozen culture maintained at -80°C (34).

Experiments

Experiments were based on cohorts followed until the death of the last individual. To initiate cohorts, frozen stock was placed on nematode growth medium (NGM) seeded with *Escherichia coli* strain OP-50 (35) at 20°C . Four days later, the eggs laid on the plate were transferred onto new NGM with OP-50. In 3 days, these eggs developed into mature hermaphrodites laying eggs. First-stage juveniles, newly hatched from the eggs, were used to initiate cohorts. Cohorts were followed 200 worms at a time, and all experiments were conducted in the same laboratory using the same equipment under the same conditions, with the same personnel, to provide consistency.

Worms were transferred individually onto 60 mm \times 15 mm NGM plates seeded with 1-day-old OP-50 and then maintained in the dark at 20°C in a constant temperature incubator. Worm survival was monitored daily. Survival was determined by observing worms for movement. If no movement was observed for 5–10 seconds, the plate was gently tapped to elicit movement; absent motion, the worm was gently touched near the head with a small piece of agar and then a nematode pick (8). Worms that failed to move were considered dead.

During the time that a worm was laying eggs it was transferred each day to new NGM. To avoid mechanical damage, a small block of agar was cut from beneath the worm and transferred, with the worm, to new medium. After the worm had crawled off of the agar block, the block was

removed from the plate. Each day, individual worm survival was assessed, and progeny were counted as juveniles emerging from eggs (1 day after eggs were laid) (8). Because facultative vivipary is a life-history trait in *C. elegans* (36,37), the few adults that died because of the internal hatch of eggs were included in this study. Experiments were initiated with 200 individual worms, with new experiments started at 2-week intervals to yield a total of 2600 individual worms. The experimental cohorts included wild-type (1000 individual worms total) and two longevity mutant strains (800 individual worms each).

Demographic Analysis

Standard life table parameters were calculated as described by Carey (24,26). Age-specific survivorship l_x was calculated as the proportion of individuals surviving to age x . The expectation of life (e_0 ; the average days remaining to an individual at birth) is defined as:

$$e_0 = \int_0^{\infty} l_x dx$$

In practice, we calculated it from the fundamental matrix [(38), eq. 3.5]. The force of mortality at age x was calculated as:

$$\mu_x = -\ln\left(\frac{l_{x+1}}{l_x}\right)$$

The maternity function m_x was measured as the mean number of juvenile progeny produced per worm per day at age x . The survival and reproduction history of each individual was depicted using a color-coded event history chart (39).

The cohort generation time is the mean age of the parents of the offspring produced by a cohort over its lifetime. It is defined as:

$$G = \frac{\int_0^{\infty} x l_x m_x dx}{\int_0^{\infty} l_x m_x dx};$$

in practice, we computed it from the fundamental matrix (38).

For analysis of population growth and fitness, the survival and maternity data were combined to construct an age-classified matrix population model [birth-flow, projection interval of 1 day; see (25)]

$$\mathbf{n}(t+1) = \mathbf{A}\mathbf{n}(t)$$

where \mathbf{n} is a vector giving the abundance of the age classes, and \mathbf{A} is a population projection matrix which contains age-specific survival probabilities P_i on the subdiagonal and age-specific fertilities F_i in the first row. Such a population will eventually grow exponentially at a rate λ given by the dominant eigenvalue of \mathbf{A} . This rate is a measure of fitness that integrates survival, reproduction, and the effects of the timing of reproduction; it can be interpreted as either a measure of mean fitness (23) or as the invasion exponent (40). We also calculated the net reproductive rate R_0 (the average number of offspring produced by an individual over its lifetime) and the sensitivity of population growth rate to changes in age-specific survival and fertility.

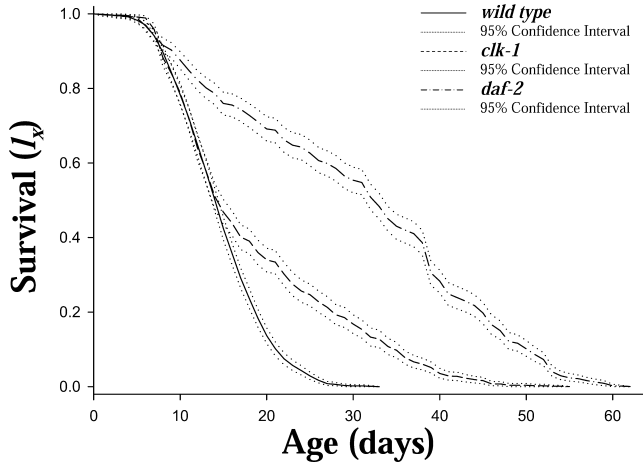


Figure 1. Cohort survival (I_x) of *Caenorhabditis elegans* (strains N2, *clk-1*, and *daf-2*) maintained as individuals on nematode growth medium and OP-50 at 20°C ($n = 1000, 800, \text{ and } 800$ for N2, *clk-1*, and *daf-2*, respectively) with survival and reproduction monitored daily (bootstrap 95% confidence intervals shown).

To determine the sources of the differences in fitness among genotypes, we performed an LTRE analysis [(25) Section 10.1 (41)]. Let \mathbf{A}_{N2} , \mathbf{A}_{clk-1} , and \mathbf{A}_{daf-2} be the projection matrices for the three genotypes. Using \mathbf{A}_{N2} as a reference, the fitness difference between wild type (N2) and the strain of interest (here *clk-1*) can be written as follows:

$$\lambda_{clk-1} - \lambda_{N2} \approx \sum_i (P_i^{(clk-1)} - P_i^{(N2)}) \frac{\partial \lambda}{\partial P_i} \Big|_{(A_{clk-1} + A_{N2})/2} + \sum_i (F_i^{(clk-1)} - F_i^{(N2)}) \frac{\partial \lambda}{\partial F_i} \Big|_{(A_{clk-1} + A_{N2})/2}$$

where the superscripts denote genotypes. The terms in the first summation are the contributions to the fitness effect of differences in age-specific survival. The terms in the second summation are the contributions of differences in age-specific fertility. The partial derivatives are the sensitivities

of λ to age-specific survival and fertility, and are calculated from \mathbf{A} following Caswell [(25), Section 9.1].

Statistical Analysis

Confidence intervals were computed on all estimated quantities using bootstrap resampling methods (42), following [(25), Section 12.1]. Each individual, with its age at death and its history of reproduction, was treated as a unit. Bootstrap data sets were created by randomly sampling 1000 individuals (for N2) or 800 individuals (for *clk-1* and *daf-2*), with replacement, from the real data sets. Bootstrap estimates of all demographic parameters were created by applying to the bootstrap data set the same algorithm used for the real data. The 95% confidence intervals were computed using the percentile method [because all quantities were nearly median-unbiased, no bias correction was applied; cf. (42)].

Significance tests were carried out using nonparametric randomization tests (25,43). Comparisons of N2 with *clk-1* and *daf-2* were conducted for survival (I_x), reproduction (m_x), age at death (d_x), mortality (μ_x), lambda (λ), life expectancy (e_x), and generation time. Test statistics, measuring the differences between strains, were defined for each estimated quantity, as follows.

1. For all scalar measures (life expectancy, λ , generation time), the absolute value of the difference between strains.
2. Survivorship. Let \mathbf{l} be the vector of age-specific survivorship. The test statistic was the ∞ -norm of the difference between the two functions,

$$\|\mathbf{l}_1 - \mathbf{l}_2\|_{\infty} = \max_x |l_1(x) - l_2(x)|.$$

This is equivalent to the test statistic used for the 2-sample Kolmogorov–Smirnov test of the difference between two cumulative probability distributions.

3. Age at death. Let \mathbf{d} be the vector giving the probability of death at each age. The test statistic was the 1-norm of the difference between the two distributions,

Table 1. Demographic Parameter in a Large Cohort of Individually Maintained *Caenorhabditis elegans* (N2, *clk-1*, and *daf-2*)

Parameter	N2	<i>clk-1</i>	<i>daf-2</i>
Fitness* (95% CI) [†]	3.85 (3.83, 3.87)	2.74 (2.73, 2.76)	3.78 (3.76, 3.80)
Net reproductive rate (R_0) (95% CI) [†]	285.6 (281.8, 289.5)	160.8 (158.0, 163.6)	233.5 (230.3, 237.0)
Life expectancy (e_0) (95% CI) [†]	14.33 (14.02, 14.62)	18.25 (17.57, 18.93)	30.26 (29.22, 31.33)
Generation time (d)	3.85 (3.84, 3.87)	4.42 (4.40, 4.44)	3.73 (3.71, 3.75)
Preproductive life span (d)	3.01 ± 0.01 [‡]	3.76 ± 0.02	3.01 ± 0.01
Change relative to N2		+24.9%	+0.0%
Reproductive life span (d)	6.04 ± 0.05	5.15 ± 0.07	6.43 ± 0.09
Change relative to N2		-14.7%	+6.5%
Worms with interrupted reproductive period [§]	14%	26%	40%
Preproductive and reproductive life span (d)	9.05 ± 0.05	8.91 ± 0.07	9.44 ± 0.09
Change relative to N2		-1.5%	+4.3%
Postreproductive life span (d)	5.77 ± 0.16	9.83 ± 0.37	21.3 ± 0.53
Change relative to N2		+70.4%	+269%

Notes: *Fitness calculated as λ , the dominant eigenvalue of the population projection matrix \mathbf{A} , determined from cohorts of 1000, 800, and 800 individual worms (N2, *clk-1*, and *daf-2*, respectively).

[†]95% confidence intervals (CI) were calculated from 2000 bootstrap samples.

[‡]Days ± standard error.

[§]Worms in which egg-laying was not continuous after initiation.

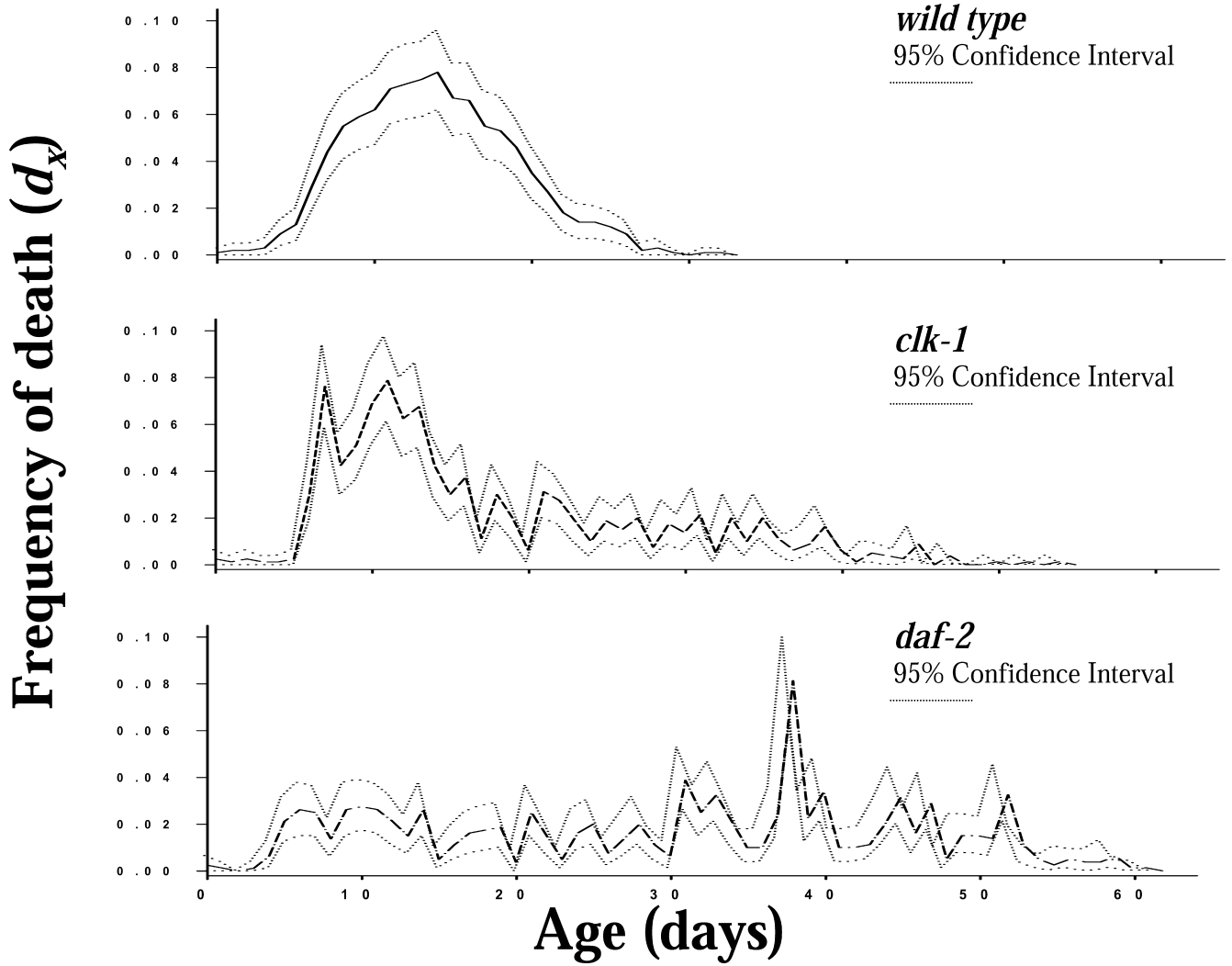


Figure 2. Frequency of death (d_x) for cohorts of *Caenorhabditis elegans* (strains N2, *clk-1*, and *daf-2*) individuals maintained on nematode growth medium and OP-50 at 20°C ($n = 1000, 800,$ and 800 for N2, *clk-1*, and *daf-2*, respectively) with survival and reproduction monitored daily (bootstrap 95% confidence intervals shown).

$$\|\mathbf{d}_1 - \mathbf{d}_2\|_1 = \sum_x |d_1(x) - d_2(x)|;$$

this is a standard measure of the difference between two probability distributions.

4. Fertility and the force of mortality. Let \mathbf{m} be the vector giving age-specific fertility. The test statistic was the 2-norm of the difference between the two vectors,

$$\|\mathbf{m}_1 - \mathbf{m}_2\|_2 = \sqrt{\sum_x (m_1(x) - m_2(x))^2},$$

which is appropriate as these are simply non-negative vectors. This test statistic was also used for mortality (μ_x).

To obtain the distribution of the test statistics under the null hypothesis, individuals (with their complete record of reproduction and age at death) were randomly permuted between treatments, maintaining sample sizes. The permuted data were then subjected to the same analyses as the original data, and the relevant test statistic calculated for each of 2000 permuted data sets. The statistical significance of the observed test statistic is the proportion of the permutation statistics greater than or equal to the observed value.

RESULTS

Survival (l_x), reproduction (m_x), age at death (d_x), mortality (μ_x), lambda (λ), life expectancy (e_x), and generation time were significantly different ($p \leq .0005$) between N2 and *clk-1* and between N2 and *daf-2* ($p = .002$ for the comparison of μ_x between N2 and *clk-1*).

Survival

The *clk-1* and *daf-2* mutants both increased survival relative to N2 (Figure 1). Life expectancies at birth (e_0) for N2, *clk-1*, and *daf-2* were 14.3, 18.3, and 30.3 days, respectively (Table 1). The distribution of age at death (d_x) (Figure 2) is concentrated between 5 and 20 days for N2, between 5 and 15 days with a long tail extending to 50 days in *clk-1*, and nearly uniformly distributed between 5 and 60 days, with fluctuation, in *daf-2*. The age patterns of the mortality ($\ln \mu_x$ Figure 3) were different among the strains. N2 exhibited a generally increasing mortality, with the slope changing at about Day 8, with a decreasing slope until approximately Day 23, whereas *clk-1* and *daf-2* showed an

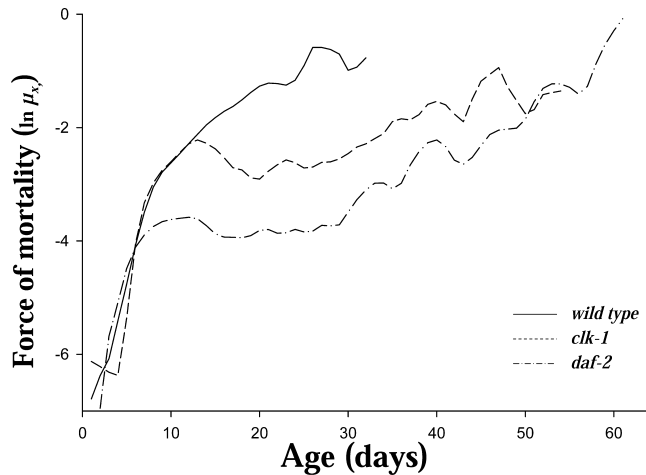


Figure 3. Force of mortality (μ_x) for cohorts of *Caenorhabditis elegans* (strains N2, *clk-1*, and *daf-2*) individuals maintained on nematode growth medium and OP-50 at 20°C ($n = 1000, 800,$ and 800 for N2, *clk-1*, and *daf-2*, respectively) with survival and reproduction monitored daily. The smoothed mortality rate curves were obtained using a locally weighted regression (LOESS) procedure.

increase until Day 6 to Day 8, followed by a decline and a period of essentially no age-related increase in mortality until approximately Day 30 followed by increasing, fluctuating mortality (Figure 3).

The period of most intense reproduction was between Days 3 and 6 in N2 and *daf-2* and Days 4 and 6 for *clk-1*, with the reproductive period essentially ended by about Day 9 for all three strains (Figures 4 and 5, and Table 1). About 10% of *daf-2* worms had died by this time, compared to about 16% for the N2 and *clk-1* (Table 2). Mortality was relatively low during the prereproductive period, with only 4.0%, 3.8%, and 6.4% of N2, *clk-1*, and *daf-2* individuals, respectively, dying prior to reproduction.

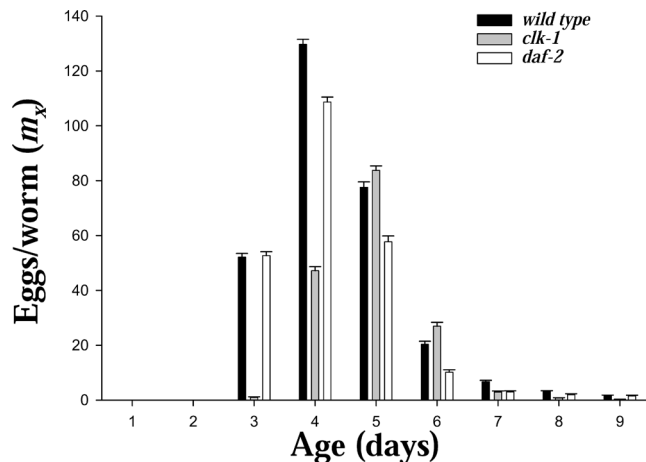


Figure 4. Age-specific reproduction (m_x) from cohorts of *Caenorhabditis elegans* (strains N2, *clk-1*, and *daf-2*) maintained as individuals on nematode growth medium and OP-50 at 20°C ($n = 1000, 800,$ and 800 for N2, *clk-1*, and *daf-2*, respectively) with survival and reproduction monitored daily (bootstrap 95% confidence intervals shown).

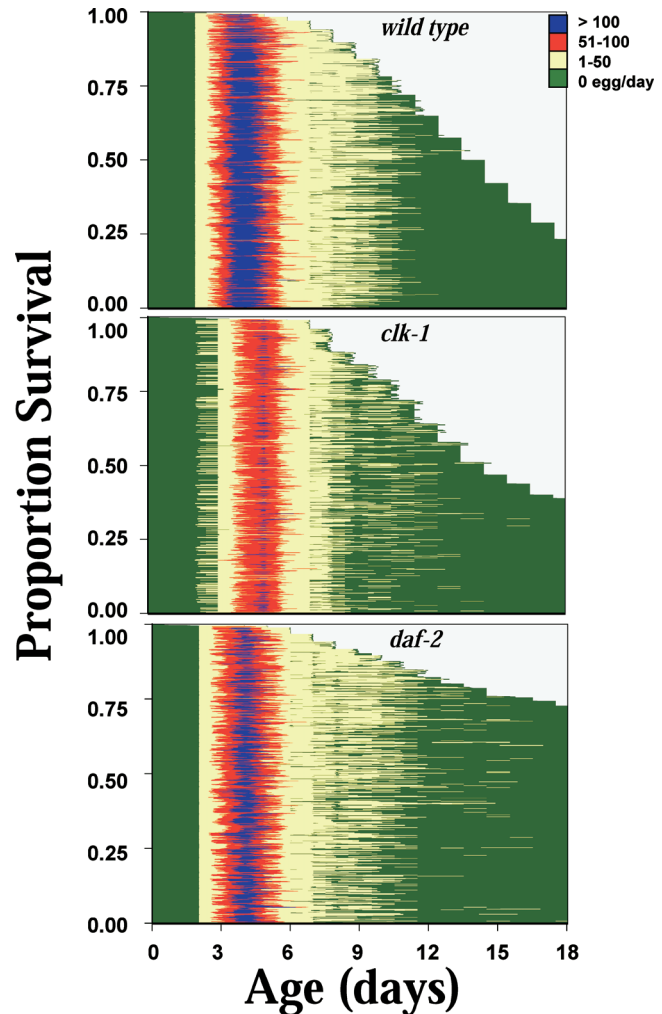


Figure 5. Event history diagrams showing daily survival versus age for *Caenorhabditis elegans* cohorts, with individual reproduction at each age indicated by color. Individuals of *C. elegans* (strains N2, *clk-1*, and *daf-2*) were maintained separately on nematode growth medium and OP-50 at 20°C ($n = 1000, 800,$ and 800 for N2, *clk-1*, and *daf-2*, respectively) with survival and reproduction monitored daily.

Differences in survival after most reproduction was completed (i.e., after age 9) accounted for the differences in total life span among strains (Table 1). The expectation of life at Day 9 for N2, *clk-1*, and *daf-2* worms was 6.7, 11.3, and 23.8 days, respectively. Between Days 10 and 18, both longevity mutants exhibited reduced average daily mortality (0.08 for *clk-1* and 0.02 for *daf-2*; 0.15 for N2). By Day 18, when the last *daf-2* worm had finished reproduction, about 76% of N2 worms had died, compared to 61% for *clk-1*, and about 27% for *daf-2* (Table 2). Life expectancy on Day 18 was 3.3, 11.2, and 19.6 days for N2, *clk-1*, and *daf-2*, respectively. The remaining life expectancy on Day 33, when the last N2 worm died, was 5.6 days for *clk-1* and 10.4 days for *daf-2*.

Reproduction

Individual life span and lifetime reproduction were not correlated (Table 3 and Figure 6), and lifetime egg

Table 2. Demographic Parameters for Cohorts of Individual *Caenorhabditis elegans* (N2, *clk-1*, and *daf-2*) Over Three Successive Age Intervals

Demographic Trait	Age Interval (d)								
	0–9			10–18			18– ω^*		
	N2	<i>clk-1</i>	<i>daf-2</i>	N2	<i>clk-1</i>	<i>daf-2</i>	N2	<i>clk-1</i>	<i>daf-2</i>
% Worms dying in interval	15.8	16.0	9.75	60.6	45.0	17.5	23.6	39.0	72.8
Average daily mortality	0.026	0.025	0.014	0.148	0.083	0.023	0.365	0.159	0.143
Life expectancy at end of interval [†]	6.68	11.3	23.8	3.32	11.2	19.6	2.25	8.04	13.9
No. of eggs per hermaphrodite	292.6	167.4	237.3	0.59	0.54	1.37	0	0	0

Notes: The mean prereproductive and reproductive life span is approximately 9 days for all three genotypes ($n = 1000, 800,$ and 800 for N2, *clk-1*, and *daf-2*, respectively). Day 18 is the last day for egg production among 2600 worms in three strains.

*Day ω is the day the last surviving worm died within each strain.

[†]Life expectancy e_9, e_{18}, e_{27} .

production (mean \pm standard error) was 293 ± 1.6 for N2, 168 ± 1.3 for *clk-1*, and 239 ± 1.7 for *daf-2*. In all the strains, fertility (m_x) was concentrated in a limited reproductive window between Days 3 and 7 (Table 1, Figures 4 and 5). Reproduction was initiated by Day 3 in N2 and *daf-2*, but was delayed until Day 4 in most *clk-1* individuals (Table 1, Figures 4 and 5).

The pattern of reproduction among individuals is shown in the event history graph (Figure 5). The peak of daily egg production occurred at Day 4 in both N2 and *daf-2*, but was delayed to Day 5 in *clk-1* (Figures 4 and 5). Egg-laying continued at a greatly reduced rate after Day 7, and had ceased completely by Day 14 in N2, Day 16 in *clk-1*, and Day 18 in *daf-2*. After egg-laying started, it usually continued without stopping, but interruptions in reproduction were observed in 14%, 26%, and 40% of N2, *clk-1*, and *daf-2* worms, respectively (Table 1 and Figure 5). No individual worms were observed to produce progeny at times later than the average total life span of their strain.

In N2 and *clk-1* worms that lived longer than 18 days, there was no relationship between remaining lifetime and total reproduction. However, in *daf-2* nematodes that lived longer than 18 days, there was a negative relationship between remaining lifetime and egg production during Days 10–18, although only about 0.5% of the reproduction occurred during this time interval.

Fitness

Fitness was highest for N2 ($\lambda = 3.85$), and lower for *clk-1* ($\lambda = 2.74$) and *daf-2* ($\lambda = 3.78$). Net reproductive rates (R_0) were 286, 161, and 233 for N2, *clk-1*, and *daf-2*, respectively.

Table 3. Mean and Coefficient of Variation (CV) for Life History Components of Long-Lived and Wild-Type *Caenorhabditis elegans*

Life History Components	Strains					
	Wild Type		<i>clk-1</i>		<i>daf-2</i>	
	Mean	CV	Mean	CV	Mean	CV
Life span (d)	14.8	34.5	18.7	55.1	30.7	48.9
First day of reproduction	3.01	5.55	3.76	13.4	3.01	6.53
Last day of reproduction	9.05	17.7	8.91	21.2	9.44	25.6
No. of days in reproductive period	6.04	26.7	5.15	36.4	6.43	37.6
Total egg production	293	17.6	168	22.8	239	18.3

LTRE analysis decomposed the fitness differential between the mutant strains and N2 into contributions from differences in age-specific survival probability and fertility (Figures 7 and 8). There were large differences in survival after age 5 (and especially after age 30) between *clk-1*, *daf-2*, and N2, but these survival differences contributed nothing to the fitness differential (Figure 7). There are small positive contributions from very small survival differences in the first 8 days of life, but the confidence intervals on these contributions overlap zero, so the contribution of survival differences to the fitness differential is essentially zero. Fertility differences between the mutants and N2, which were limited to the first 10 days of life, made large contributions to the fitness differentials between the strains (Figure 8).

Summing the contributions over age gives the overall contributions of survival and fertility to the fitness differential. Comparing *clk-1* and N2, the contributions are 1.37×10^{-3} for survival and -1.07 for fertility. Comparing *daf-2* and N2, the contributions are 2.34×10^{-3} for survival and -7.53×10^{-2} for fertility. Thus the contributions of fertility differences to fitness costs were between one and 2 orders of magnitude larger than were the contributions of survival differences.

DISCUSSION

The longevity mutants *clk-1* and *daf-2* reduce age-specific mortality and increase postreproductive survival, relative to wild type (N2) *C. elegans*. These mutations, although exerting a positive effect in later life, carry costs due to effects on other demographic parameters, and hence reduce fitness. These costs may be considered to act as tradeoffs influencing the evolution of life histories; they put the antagonism into the antagonistic pleiotropy theory of senescence. The small increases in early reproduction in the wild type more than make up for its reduced late survival relative to these two mutants.

Our analysis of fitness using an assessment of λ is new. The only reported estimates of population growth rate for *C. elegans* that we are aware of were obtained by measurement of food consumption (13) or from the slope of a linear regression of the log of population size versus time (44), not by demographic calculation. An estimate obtained through regression does not provide insight on how fitness is related to specific differences in survival and fertility. The

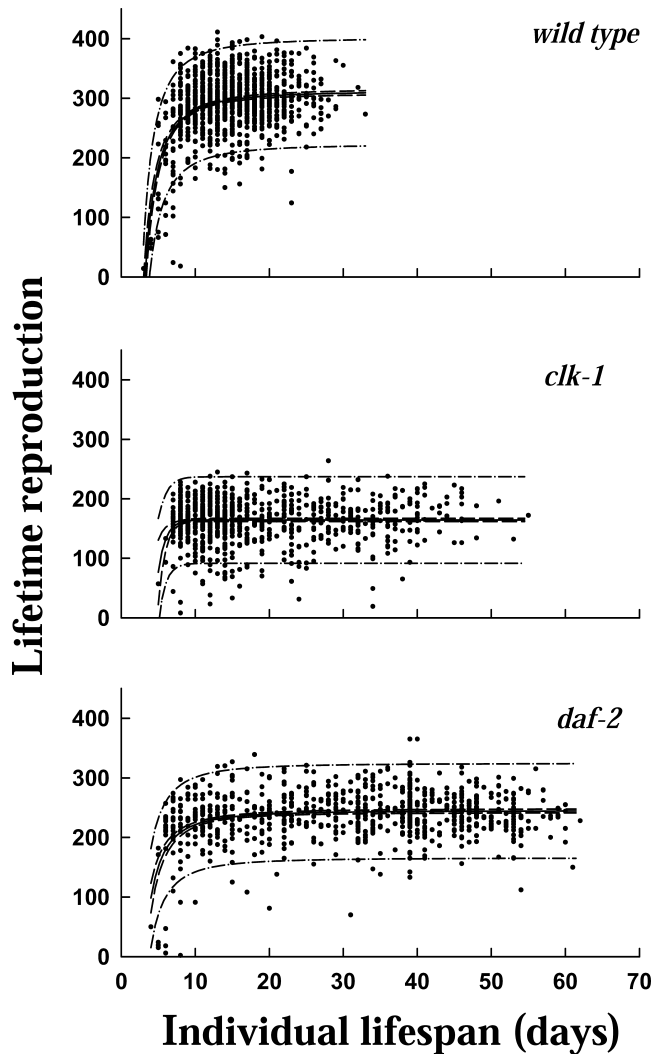


Figure 6. Individual life span (in days) versus lifetime total reproduction for *Caenorhabditis elegans* (strains N2, *clk-1*, and *daf-2*) individuals maintained separately on nematode growth medium and OP-50 at 20°C ($n = 1000$, 800, and 800 for N2, *clk-1*, and *daf-2*, respectively) and survival and reproduction monitored daily. Curves depicted include fit, 95% confidence intervals, and 95% prediction intervals (N2: $y = 312 - 3201/x^2$, Fit Standard Error = 45.4; *clk-1*: $y = 164 - 13045e^{-x}$, Fit Standard Error = 37.1; *daf-2*: $y = 245 - 2346/x^2$, Fit Standard Error = 40.4).

same general limitation applies to the estimates of relative (not absolute) fitness of *C. elegans* strains reported by Walker and colleagues (16) and Jenkins and colleagues (14) that were obtained by following changes in the relative abundance of populations over time. In addition, those estimates appear to have been obtained in a serial transfer environment that could be expected to fundamentally alter the selection regime on longevity mutants. Although such studies provide insights, they are not a substitute for demographic analysis as a method for understanding the survival and fertility components of fitness.

The antagonistic pleiotropy theory of aging suggests that senescence results from genes with positive effects on fitness early in life but negative effects later in life (22,29).

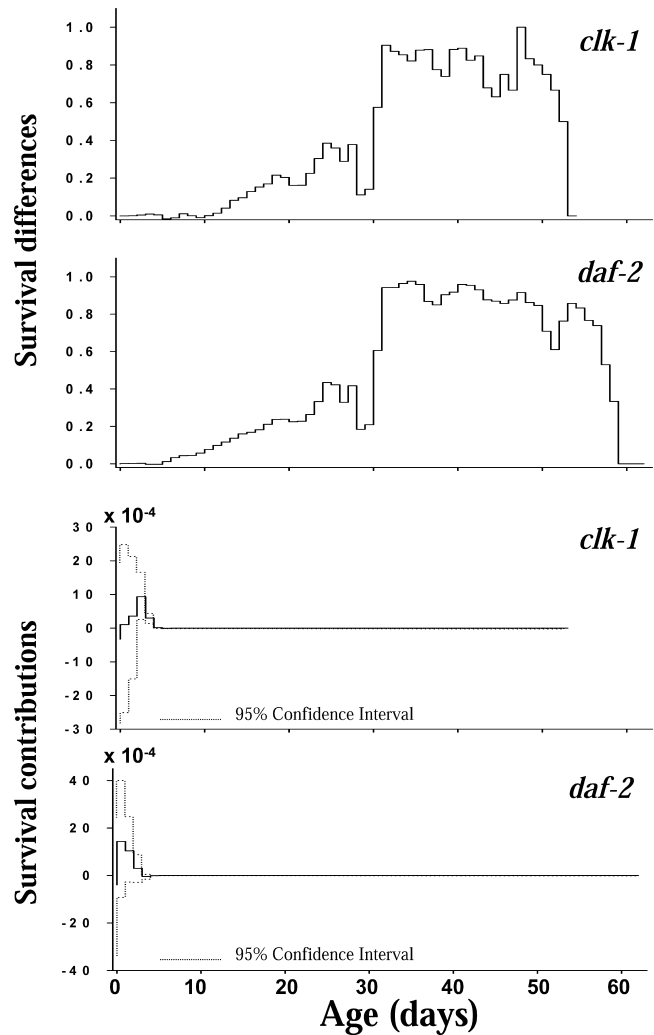


Figure 7. Life table response experiment analysis of the survival differences and survival contributions to fitness in *clk-1* and *daf-2* relative to N2 (bootstrap 95% confidence intervals shown).

Relatively few genes have been demonstrated to have beneficial effects early in life and detrimental effects later in life (45), but the nematode life-span extension mutants *age-1* and *daf-2* have influences on life span and estimated fitness consistent with antagonistic pleiotropy (14,16).

These longevity mutants change the slope of postreproductive age-specific mortality rates. The leveling of mortality after reproduction that was observed in *clk-1* and *daf-2* did not occur as clearly in N2. All three strains exhibited mortality trajectories that differed slightly from the two-stage Gompertz patterns reported by Johnson and colleagues (9), but generally agree with those patterns in having an initial exponential mortality increase followed by a lower rate of increase. It is intriguing to consider *C. elegans* behaviors governed through group interactions (e.g., pheromone influence on dauer formation) relative to the role of postreproductive survival in contributing to the evolution of senescence, given that in social species intergenerational transfers may shape senescence (46).

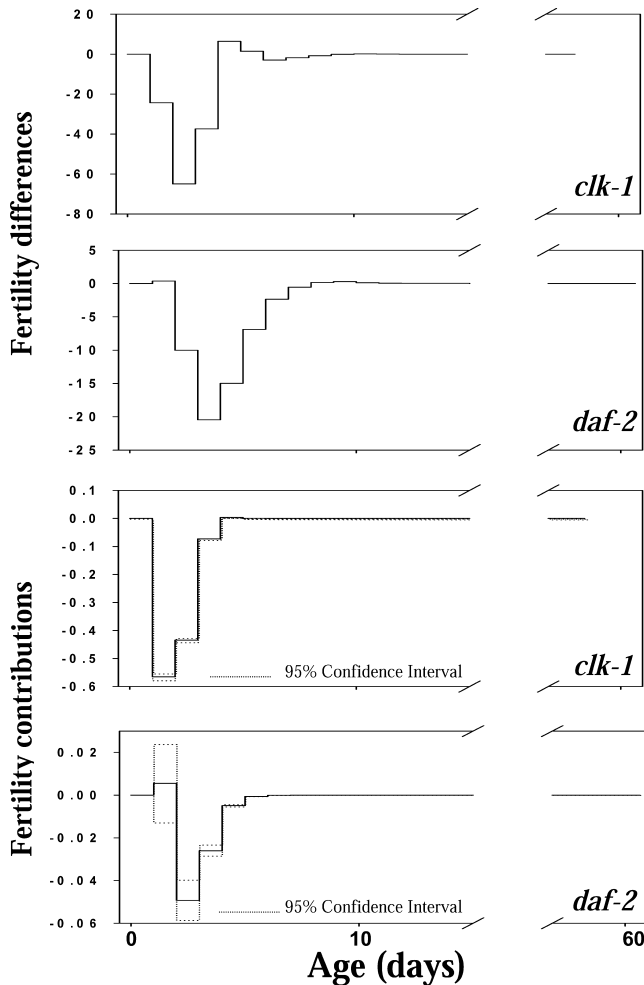


Figure 8. Life table response experiment analysis of the fertility differences and fertility contributions to fitness in *clk-1* and *daf-2* relative to N2 (bootstrap 95% confidence intervals shown).

In our experiments, the *clk-1* and *daf-2* mutants extend average life span relative to the wild type by 27% and 111%, respectively. However, they reduced reproduction in early life, leading to significant fitness costs. The magnitude of these costs can be appreciated by noting that the fitness differentials are sufficient to produce a decline in the frequency, relative to the wild type, of *clk-1* of 29% per day and of *daf-2* of 1.8% per day.

The fitness costs are due to negative effects of the mutations on reproduction in the first 6–7 days of life, as shown by the LTRE analysis. The dramatic improvements in late survival make no contribution to fitness. The positive contributions of increases in early survival are 2 orders of magnitude smaller than the negative contributions of fertility differences during this same period. This is a clear quantitative documentation of the age-specific demographic basis of antagonistic pleiotropic effects on survival and reproduction. Our results are consistent with the quite different study of Hodgkin and Barnes (13), who compared food consumption rates of populations of several strains differing in sperm production, and thus in reproductive rate. They

emphasized the importance of changes in the age at first reproduction; our LTRE analysis quantifies this effect, especially for *clk-1* (see Figure 8). Our results are also consistent with the determination that longevity genes influence relative fitness and survival under stressful environmental conditions or under competition with wild-type worms (14,16).

A key aspect of these effects we report is that, whereas life span is extended by *clk-1* and *daf-2*, the duration of the reproductive window is not. The event history diagram (Figure 6) shows that the beginning and the end of this window are both tightly controlled in N2. In *clk-1* the beginning is delayed by 1 day, but the end is even more tightly controlled. In *daf-2*, both the beginning and end of the reproductive window are very similar to N2, and a linear relationship between life span and postreproductive life span arises from the relatively fixed reproductive schedule.

The developing reproductive system influences life span, and laser ablation of germ line precursor cells, eliminating reproduction, may extend the life span of *C. elegans* (6). Interestingly, both the *clk-1* and *daf-2* mutations dramatically increase the frequency of breaks in individual reproduction. This phenomenon suggests an effect, unknown at this point, on the genetic regulation of reproduction. It is interesting that two different mutations both show this disruption. Because the mutants increased longevity by extending postreproductive survival, there was no direct relationship between lifetime reproductive output and life span. In general, the results appear to represent tradeoffs relative to extended life span—with reduction in total fertility in longevity mutants.

Any estimate of fitness is conditional on the environment in which it is carried out. Our measurements were carried out in controlled laboratory conditions with surplus food. Even under these unstressed conditions, the fitness costs of the longevity mutants were apparent. Stress, for example due to periodic starvation, can exacerbate these effects (14,16); large-cohort demographic data collected under such conditions would permit a detailed analysis of these effects.

The ecology of *C. elegans* is poorly known (47,48). Studies under conditions more ecologically realistic than standard laboratory conditions could provide insights into the selection pressures on life history traits in *C. elegans*. Van Voorhies and colleagues (18), for example, compared survivorship in soil and sand with that on agar for a wild-type strain (*fer-1 wv01*) and a *daf-2* mutant, although reproduction and fitness were not assessed. Survivorship was drastically reduced in soil, more so for the *daf-2* than for the wild type (18). This line of research merits elaboration through experiments that would include monitoring of the introduced bacterial food, given that food concentration can alter life span (49). We anticipate that our N2 1000-worm cohort data will serve as a reference data set for further exploration of *C. elegans* aging in the wild (50).

Our cohorts exhibited considerable interindividual variation in life span. Given the genetic homogeneity of the cohorts and the controlled culture environment, such variation may

reflect the epigenetic stochastic elements described by Finch, Kirkwood, and colleagues (51,52), perhaps including senescent decline at the ultrastructural level and decreased gene regulation in the postreproductive period of life (53). Although discussions of longevity mutants often emphasize the unusually long-lived individuals, not all individuals experience long life. This variation in life span has ramifications relative to possible genetic therapies oriented toward life-span extension, that although life-span extension may be achieved through a given genetic pathway, the maximum possible increases in life span are only realized by a few individuals.

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Address correspondence to Edward P. Caswell-Chen, PhD, Department of Nematology, University of California, One Shields Avenue, Davis, CA 95616. E-mail: epcaswell@ucdavis.edu

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