# GENETIC VARIANTS AND MUTATIONS OF *CAENORHABDITIS ELEGANS* PROVIDE TOOLS FOR DISSECTING THE AGING PROCESSES

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## ABSTRACT

*Caenorhabditis elegans* is a short-lived species that has been widely used in the genetic dissection of development. This species is becoming important in the genetic analysis of aging because strains with mean life spans more than 70% longer than wild type have been identified both through the use of recombinant inbred lines and by the induction of single-gene mutants. Its unique hermaphroditic mode of reproduction leads to a lack of inbreeding depression and simplifies genetic analyses of quantitative traits such as length of life or behavior. Aging in this organism is composed of at least three independent processes: that specifying length of life, that specifying reproductive senescence, and that specifying senescence of the general motor system. These data suggest that aging is not a unitary process but that many different processes or independent components may be involved in various aspects of aging. Most importantly, an apparent single-gene mutation has been mapped to the middle of linkage group II; this mutation lengthens mean and maximum life span 60-110% and also decreases fertility about five-fold.

## INTRODUCTION AND BACKGROUND

*Caenorhabditis elegans* is a self-fertilizing hermaphroditic species of nematode that can be grown in petri plates on a simple diet of Escherichia coli. As such it has been widely used as a model experimental organism in many different areas of biology but especially in analyses of development, muscle physiology, and behavior. The almost invariant cell lineage of *C. elegans* has facilitated the complete cell lineage description of the 959 somatic cells of the hermaphrodite (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston

**Table 1.** Advantages of *C. elegans* for the Genetic Analysis of Aging.

| |
|---|
| -Small (1.2 mm) |
| - Rapid life cycle ( < 3 days) |
| - Short life span (20 days) |
| - Self-fertilizing hermaphrodite |
| - easy isolation of recessive mutants |
| - lack of inbreeding depression |
| - Spontaneous males (obligate outcrossers) |
| - Dauer larvae ( > 90 day survival) |
| - Cryogenic preservation of strains |
| - no loss of mutants |
| - no accumulation of modifiers or suppressors |
| - all stocks in same genetic background |
| - Genetic transformation |
| - Transposon-mediated mutagenesis |
| - Physical map 98% complete in a series of cosmid arrays |
| - Many laboratories: many types of mutants (developmental, behavioral, Ts, amber suppressible, lethals, *etc.*) |

*et al.*, 1983). This analysis is aided by the small size of the animal (1.2 mm length), its 2-day period of development, and its optical transparency, which makes the analysis of cell lineage feasible.

## Genetics: Classical, Quantitative, and Molecular

Sophisticated tools that include simple techniques for classical and molecular genetic analysis have been developed. The recent beginnings of quantitative and population genetic analyses prove that unique applications are available in these areas as well.

The isolation of mutants is straightforward in *C. elegans*, and a large number of mutants have been identified after treatment with ethyl methanesulphonate or a wide variety of other mutagens (Brenner, 1974; Herman and Horvitz, 1980; Herman, 1988). Recently, molecular genetic analysis of this species has progressed rapidly. Many putative transposable elements have been identified in *C. elegans* (Emmons, 1988). Two of these have been shown to transpose at measurable frequencies in strains that have mobilized the Tc1 transposable element. Such "transposon-tagged" loci have facilitated the cloning of genes identified only by mutations (Herman, 1988). Efficient techniques for genetic transformation (Fire, 1986) and the availability of overlapping arrays of cosmids that cover as much as 95% of the genome (Coulson *et al.*, 1986, 1988), when combined with the long-lived genetic variants, offer possibilities for the analysis of aging unrivaled by any other species.

**Table 2.** Comparisons of Genetic Approaches.

| | Induced Mutants | Selective Breeding | Molecular Approaches |
|---|---|---|---|
| Type of Phenotype | Qualitative | Quantitative | Molecular |
| Origin | Mutant Induction | Existing Variation | Molecular Construct /Transgenic Animals |
| Genes Involve | Usually Single Gene | Usually Polygenic | Single or Multiple Genes |
| Level at Which Understanding is Obtained | Organismic | Population | Molecular |
| Gene Localization Strategy | Classical Mapping | Interval Mapping | Physical Mapping |
| Method of Assessing Gene Number | Complementation | Numerical Estimates | DNA Sequence |

Several additional advantages in the use of *C. elegans* for genetic analyses of aging and senescence are listed in Table 1. The life style of *C. elegans* is such that during the fourth larval stages the worm produces sperm which are then stored in the spermatheca throughout the adult period when the worm produces only oocytes; both spermatocytes and oocytes result from regular meiotic divisions (Brenner, 1974). Two principal advantages, easy identification of recessive mutants and a lack of inbreeding depression, result from the fact that *C. elegans* is a self-fertilizing hermaphrodite. As a consequence, recessive mutations can be isolated with relative ease, and this has been quite important in the identification of long-lived mutants (Klass, 1983) and in their subsequent analysis (Friedman and Johnson; 1988a,b).

## Classical, Quantitative, and Molecular Approaches to Aging

Table 2 compares the mutational, selective breeding, and molecular genetic approaches in their ability to estimate genetic contributions, localize genetic effects to one region of the genome, and determine the physiological or molecular basis of phenotypes in any genetic system. Most of these approaches have already been applied to answering the question: What specifies length of life in *C. elegans*?

The approach of choice for the dissection of any biological process is the identification of single-gene mutations that alter the process of interest (Botstein and Mauer, 1982). Since there are relatively straightforward ways to clone a gene in *C. elegans* once mutants in that gene are available, the

mutational approach leads directly to the molecular identification of the gene and the subsequent illumination of the molecular details of the process of interest. Moreover, the mutational approach has significant advantages over both selective breeding and molecular approaches. Unlike selective breeding (Rose, 1984; Luckinbill *et al.*, 1984), mutational analysis is not limited to allelic variants already present in the population, and unlike molecular approaches through transgenic stock construction there is no bias as to which genes are relevant for analysis nor limitations based on the availability of cloned genes. In studies on aging in metazoans, the mutational approach has proven effective only in *C. elegans*. [Two exceptionally long-lived (44 and 48 months) individual C57BL6/NIA mice were identified in colonies sponsored by the National Institute on Aging but post-mortem confirmation of age was not possible (Sprott, personal communication)]. Short-lived mutants have been identified in other species, but these mutants are likely to carry mutations in genes not specifically involved in aging. Problems of inbreeding depression that effectively prevent a simple assessment of underlying genotype based on the phenotype of one or a few related individuals may have prevented the identification of long-lived mutants in other genetic systems.

The selective breeding approach has been applied in Drosophila. Although the response usually results from the action of multiple genes and is limited to existing allelic variants, selective breeding has been effective in producing strains with increased maximum life span (Rose, 1984; Luckinbill *et al.*, 1984; see also Hutchinson and Rose, and Rose this volume). In contrast, mutations can be induced in any gene; the identification of these mutants is limited only by the time and ingenuity of the investigator.

A traditional, but non-essential, difference between these approaches has been the fact that quantitative phenotypes (characters that must be measured to assess genotype) have usually been studied using selective breeding, whereas qualitative traits (those that can be distinguished quickly by just looking at the wild type and mutant) are more amenable to mutant and molecular analyses. This distinction is, however, arbitrary in that any qualitative trait is potentially a quantitative trait if the distinction is small enough that measurements must be made or if a population must be monitored to assess genotype. Moreover, major effects of single-gene mutations that affect quantitative traits are well known. Thus, although the methodology and nomenclature are different (see Table 2), there is a surprisingly large amount of overlap between the information obtained by one technique and by an alternative approach.

## GENETIC CHARACTERIZATION OF LIFE SPAN

### Lack of Heterosis

Heterosis, or hybrid vigor, is defined as an improved performance of the $F_1$ hybrid in comparison with its two inbred parents. More precisely, heterosis is observed whenever the value of the $F_1$ hybrid is significantly different from that expected of the midparent as predicted by a model of simple additive genetic factors (Falconer, 1981). Although common in hybrid progeny resulting from crosses between inbred parents, the cause of heterosis is not completely understood and at least three major models have been proposed for the genetic basis of heterosis (Mitton and Grant, 1984).

There is little heterosis effect for life history traits in *C. elegans*. For example, the $F_1$ hybrid of two different wild-type strains (Bristol and Bergerac) that have significant genetic differences between them (Emmons, 1988) has a life expectancy not significantly different from either parent (Figure 1, Johnson and Wood, 1982). This result has been extended to other wild strains (Johnson, unpublished). This lack of heterosis is also seen in other life-history traits of the hermaphrodite, such as fecundity, rate of development, and fertility. Because *C. elegans* reproduces by self-fertilization in the wild, these populations are largely homozygous. Homozygotes cannot carry recessive alleles with significant deleterious effects; when a new deleterious allele enters a population by mutation, it is rapidly eliminated by selection against the recessive allele due to its expression in the homozygote. Thus, the unusual lack of heterosis effects and inbreeding depression may result from the self-fertilizing lifestyle of this organism.

For the genetic analysis of life history and other quantitative traits, the advantages of working with an organism that has little apparent overdominance is extremely significant. This is seen especially in the genetic analysis of single-gene mutants where the ability to measure life history traits in homozygous populations has simplified the mapping of a major gene that affects length of life (Friedman and Johnson; 1988a; see also Figure 10 and below).

### Males, Dauers, and Cryogenic Preservation of Stocks

*C. elegans* spontaneously produces XO males by nondisjunction of the X-chromosome at frequencies of about 1 in 700 (Hodgkin *et al.*, 1979). These males are obligate outcrossers and thus can be used to construct new strains and to perform other crosses. Interestingly, males of the N2 wild-type strain

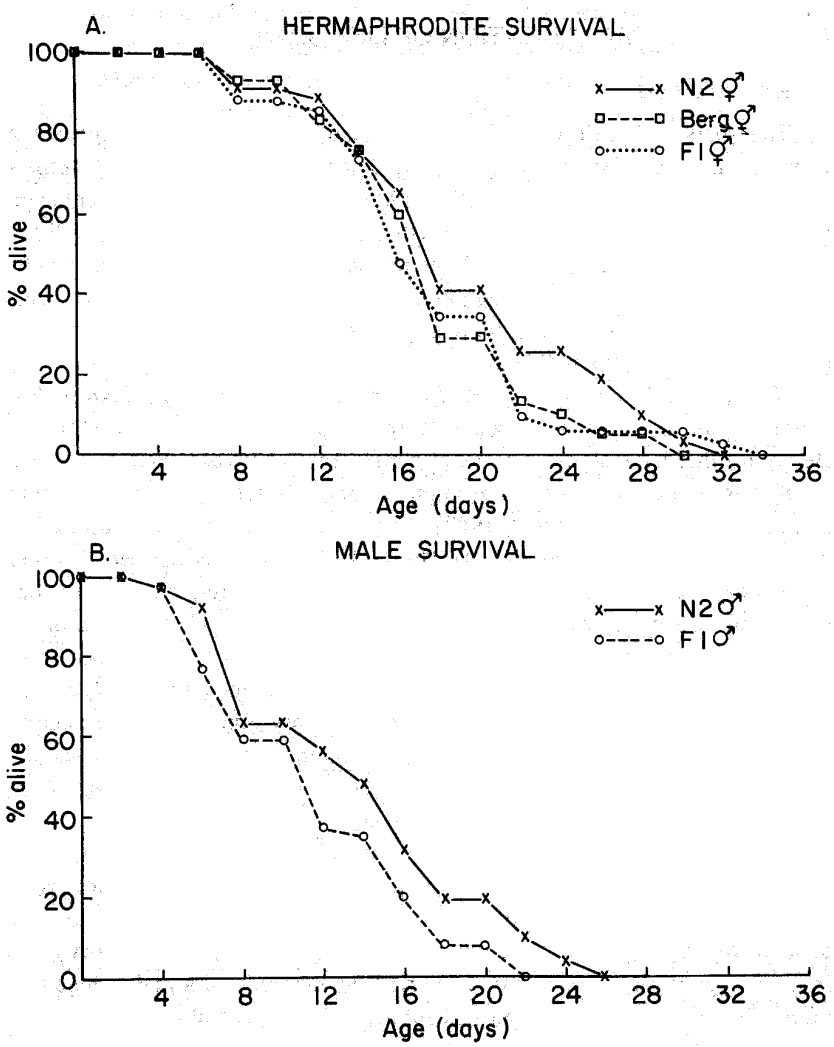


**Figure 1.** Survival curves for parental and $F_1$ hybrid populations. (A) Hermaphrodites. (B) Males. Mean ± SEM life spans for the Bristol, Bergerac, and $F_1$ hybrid hermaphrodites were 18.2 ± 1.1, 16.6 ± 0.8, and 16.6 ± 1.1 days, respectively. Mean life spans for the Bristol and $F_1$ hybrid males were 12.9 ± 0.8 and 10.7 ± 0.7 days, respectively. Comparisons of the hermaphrodite means by the Gehan and Log-rank tests indicated no significant differences in life span among the three populations ($P > 0.35$ in all pairwise tests). Similar comparisons of the male means also showed no significant differences ($P = 0.08$ and 0.10 from the Gehan and Log-rank tests, respectively). However, all pairwise comparisons between a male and a hermaphrodite population showed a significant differences ($P < 0.02$).

are shorter lived than the wild type (Johnson and Wood, 1982), although this is not true of all wild strains (Johnson, 1984).

Dauers are an alternative developmental stage to the normal third larva. They are resistant to desiccation and other environmental insults that would kill a normal worm (Riddle, 1988) and presumably evolved to survive harsh conditions. Dauers can be induced by starvation and survive up to 3 months with no apparent loss of remaining life span (Klass and Hirsh, 1976) while retaining normal or near normal levels of fertility. The dauer can be used in selection paradigms where genetically identical siblings of possibly long-lived mutants are kept in an immature stage until the long-lived cultures are identified, at which point the new mutations can be recovered by inducing the dauer to complete development (Klass, 1983).

A final, major advantage is that *C. elegans* strains can be preserved indefinitely by freezing in liquid $N_2$. This means that novel mutants are easy to maintain and even a small nematode lab can have several hundred mutants on hand. Most important for aging research is the fact that the strains do not accumulate new suppressor and modifier mutations as do Drosophila and mice, which must be maintained by continual passages. Such modifier mutations can significantly affect length of life and can lead to wide variation in quantitative aspects of supposedly identical strains (for examples of these types of problems in Drosophila, see Baker *et al.*, 1985). Thus, for example, almost all *C. elegans* laboratories work with the N2 wild type as a control, ensuring a common genetic background, identical with that of every other lab.

## Characterization of Length of Life

Interestingly, several different wild-type strains (Figure 2 and Johnson, 1984) have fairly similar life expectancies, maximum life spans, and survival curves, consistent with a functional significance for length of life or a tight relationship between length of life and some other life history traits. A number of different types of mutants have been identified in *C. elegans*. These include morphological variants such as short, squat "dumpies" (Dpy), longer than normal (Lon), etc.; behavioral variants such as uncoordinated (Unc), worms that roll instead of swimming smoothly (Rol), etc.; and more unusual mutants such as temperature-sensitive lethals which can affect almost any stage of life and transformer stocks that cause stocks of one sexual genotype to masquerade as the other sex. In a preliminary survey seeking strains that had normal life expectancy - and could therefore be used to map variants with longer life - we discovered that many strains (30%) have life expectancies not significantly different from wild type (Figure 3).

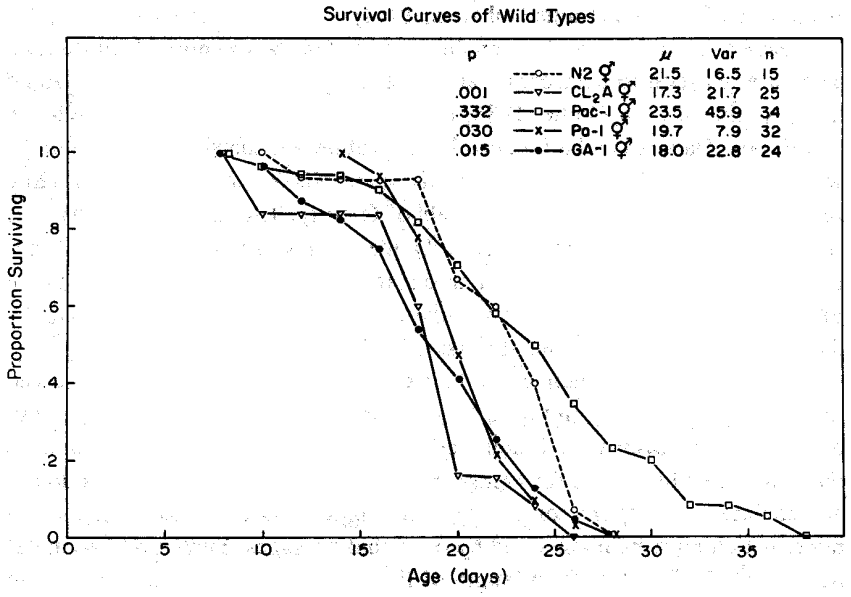


**Figure 2.** Survival curves of several wild-type strains of *C. elegans*.

We will describe two complementary genetic approaches used in our laboratory over the last seven years for the generation and analysis of long-lived lines of the nematode. The first approach, that of generating recombinant inbred lines (RIs) between two common laboratory strains of *C. elegans* (Johnson and Wood, 1982; Johnson, 1987), as well as the more recent analysis of mutants (Friedman and Johnson, 1988 a,b), have each resulted in strains with life expectancy more than 60% longer than that of the parental strains. The details of these studies will be reviewed herein.

## RECOMBINANT INBRED LINES

### Creation of Long-lived RIs

Two laboratory wild-type strains of *C. elegans*, the Bristol (N2) and the Bergerac BO, were crossed (Figure 4). $F_1$ hermaphrodites were allowed to self-fertilize and the $F_2$ hermaphroditic progeny were cloned and independently inbred through 19 subsequent rounds of self-fertilization. Within two months, recombinant inbred lines (Bailey, 1981), inbred to less than one part in $10^6$ (roughly equivalent to the rate of spontaneous mutation in *C. elegans*, Moerman *et al.*, 1986), were established.

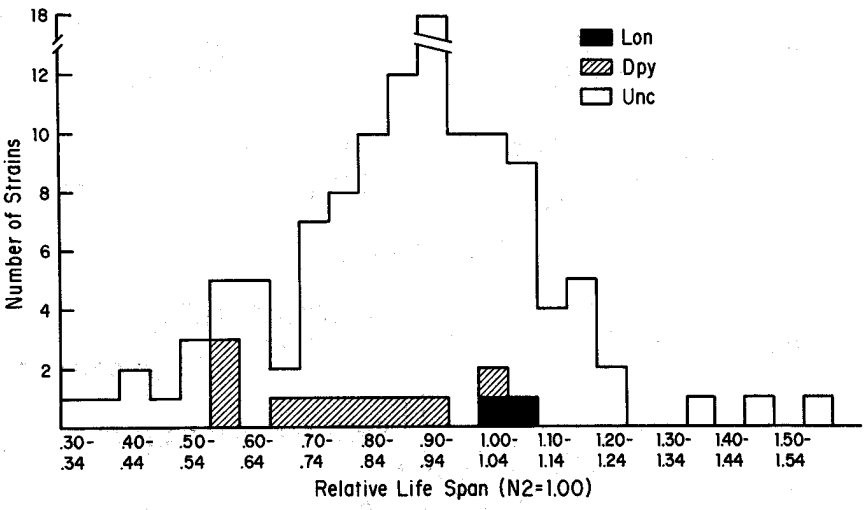


**Figure 3.** Distribution of life expectancies for 114 morphological and behavioral mutants: *Unc*, uncoordinated behavior; *Lon*, longer; *Dpy*, dumpy. For more information on strains see Brenner (1974).

These RI lines have been assayed several times for length of life. In typical experiments mean life spans of these lines vary three-fold, ranging from 13.8 days to 37.9 days (Figure 5A). More importantly, maximum life spans of the RI lines are also altered; maximum life spans both shorter (17 days) and up to 63% longer (63 days) than N2 (40 days) are observed among the RI lines. As expected, there are strong positive correlations between mean life span and the 90th percentile, the 95th percentile, or maximum life span (Figure 5B). These strong positive correlations are not trivial, because a higher mean life span can result either from decreased early life mortality or from increased maximum life span. In these lines, longer life results from an increase in life expectancy at all chronological ages.

The shape of the survival curve was examined in detail in three selected RI lines and in the two parental genotypes (Figure 6). The exact shape of the survival curves varies slightly between lines, but all are rectangular.

We undertook a more detailed analysis of the kinetics of mortality in the parent stocks and in five selected RI lines to see if mortality increases exponentially with chronological age as modeled by the Gompertz equation.

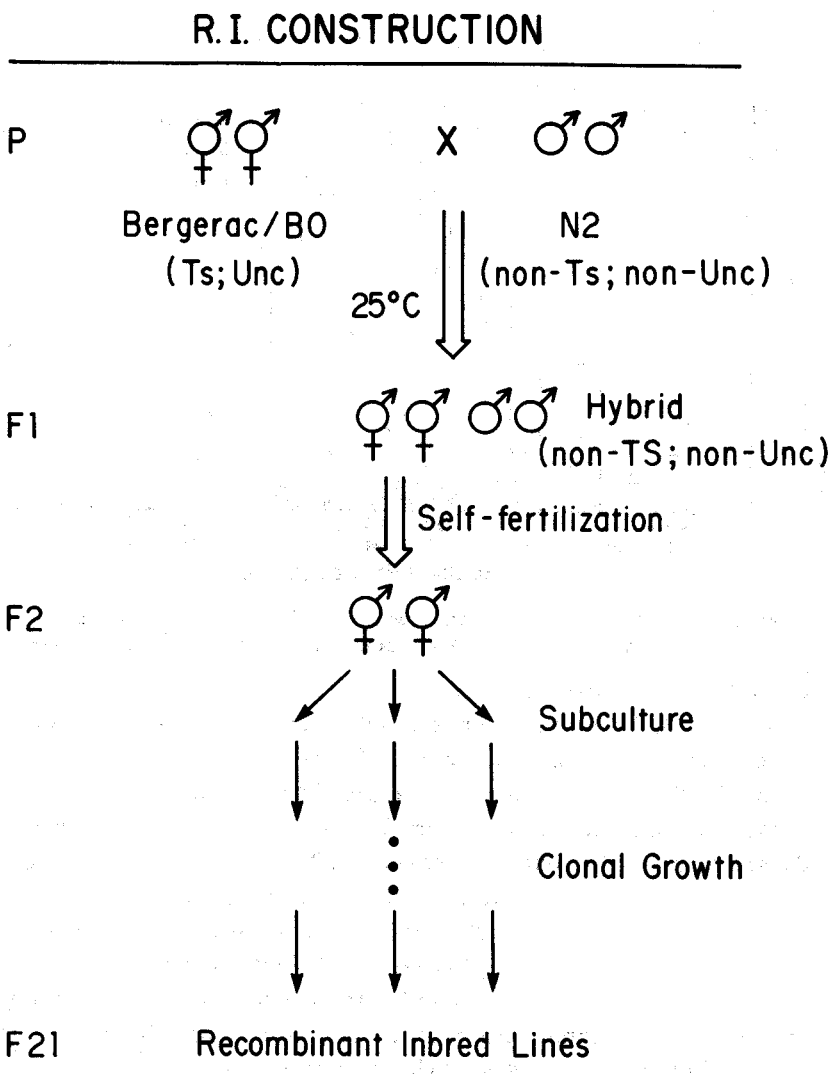


**Figure 4.** Scheme for constructing recombinant inbred lines in *C. elegans*. Two common laboratory wild types, N2 (Bristol) and Bergerac BO, were crossed. $F_1$ cross progeny were distinguished from self-progeny of the parental Bergerac hermaphrodites by the non-Ts, non-Unc phenotypes of the $F_1$'s. Individual fourth larval stage $F_1$ hermaphrodites were isolated to individual small petri plates. Subsequent generations were produced by self-fertilization. Fourth larval stage hermaphrodites were transferred to fresh NGM plates at each generation. This inbreeding procedure was continued for 21 generations (from Johnson *et al.*, 1988).

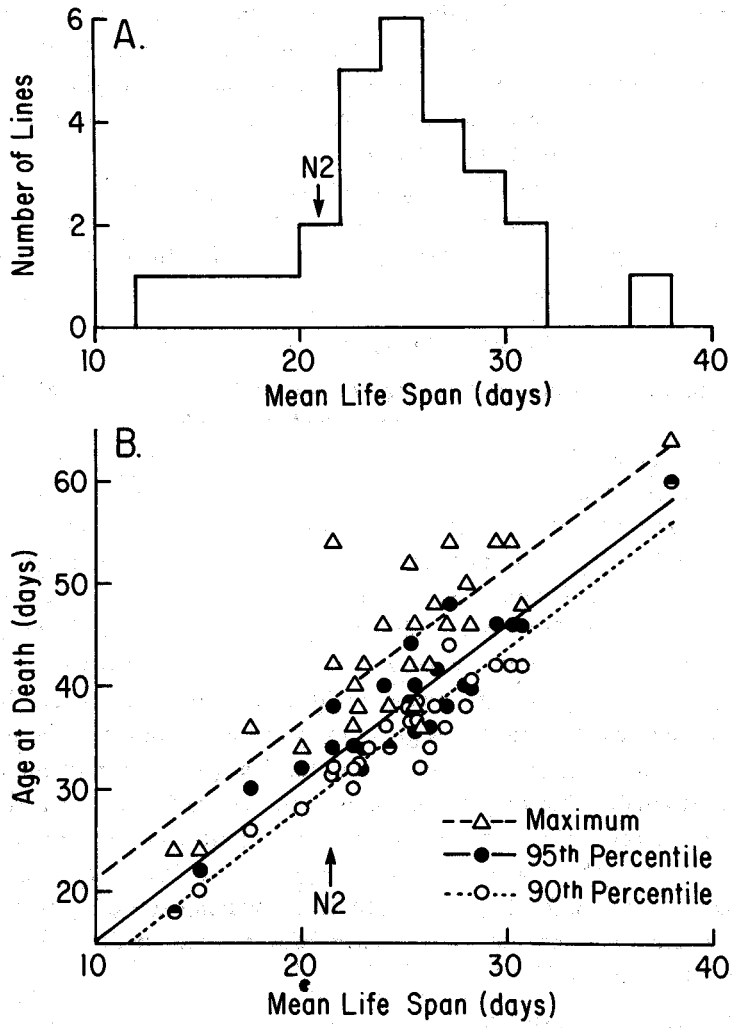


**Figure 5.** Life spans of hermaphrodites from RI lines. (A) Mean life spans of 27 RI lines. Data are the average of two survival experiments, each containing 50 nematodes. The entire experiment involved the assay of 2950 nematodes; 2206 died of natural causes. (B) Regression of mean life span (same nematodes described in Figure 4A) on either maximum life span, the 95th percentile of life span, or the 90th percentile of life span. Mean life span is highly correlated ($P<.001$) with maximum life span ($r = 0.83$), the 95th percentile of life span ($r = 0.93$), and the 90th percentile of life span ($r = 0.96$) (from Johnson, 1987).

In both wild type and RI strains, the age-dependent component increases exponentially with increasing chronological age. This is most clearly seen by plotting mortality rate against chronological age on a semilogarithmic scale (Figure 6 B, D). Because increased mean life span could result from lower basal mortality rate or from a slower rate of increase in mortality with chronological age, we asked how these components varied in the RI lines. The age-dependent component varies between lines and explains most of the variance in length of life (Johnson, 1987). No significant change in the age-independent component was observed.

## Coinheritance of Fertility and Life Span

Based on the findings with *age-1* and on theoretical models for the evolution of senescence we have also looked at the inheritance of fertility in these recombinant inbred lines (Foltz and Johnson, unpublished). A significant genetic component for life expectancy was observed in each of five trials, consistent with earlier observations (Johnson and Wood, 1982; Johnson, 1986). Hermaphrodite fertility also showed significant heritability in each of three trials. A significant positive phenotypic and genetic covariance for life span and fertility was also observed in two of three experiments (Table 3). Age-specific fertility was positively correlated with fertility on consecutive days but was negatively correlated with fertility on more distant days.

Three to five independently segregating genes are estimated to be specifying these traits within the RI lines; two of three single-gene markers used to generate strain distribution patterns for these lines were found to be associated with one or more loci which had a statistically significant effect on life span and/or fertility. There was also evidence for a significant environmental component affecting fertility and length of life, which leads us to be cautious about generalizing the observed positive covariances to the environment encountered in the wild.

## Dissecting the Aging Process

The length of developmental periods and the length of the reproductive period are unrelated to increased life span in these lines or in *age-1* mutants (Johnson, 1987; Friedman and Johnson; 1988a). Lengthened life is due entirely to an increase in post-reproductive life span. Development, reproduction, and life span are each under independent genetic control. General motor activity decays linearly with chronological age in all RI genotypes examined (Johnson, 1987). The decay in general motor activity is both correlated with and a predictor of mean and maximum life span, suggesting that both share at least one common rate-determining component. These observations can be

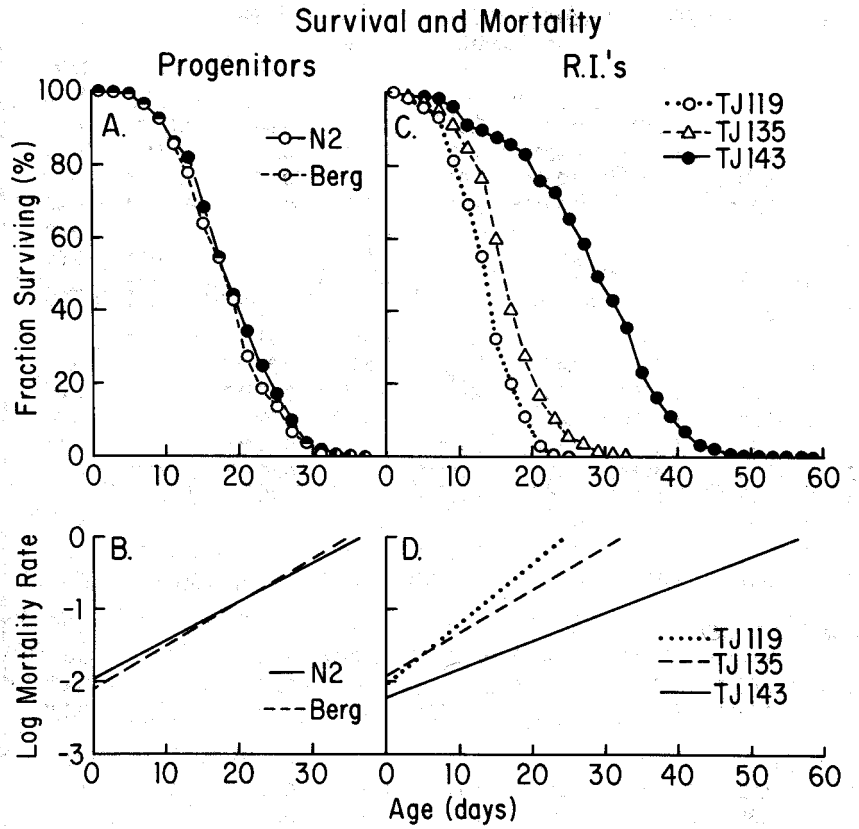


**Figure 6.** Survival data (A and C) and mortality rates (B and D) for the parental strains and three RI lines. Panels A and B show survival data obtained as described in Figure 5 except that each curve is the result of assays on 400 nematodes, 200 in each of two experiments, and that survival assays were performed every 12 hours. Subgroups of the same genotype were tested for consistency. One sample of 50 hermaphrodites (TJ143) showed significant differences in survival from the other three TJ143 subgroups and was excluded from this analysis; however, inclusion of that sample does not noticeably affect the results. Panels B and D are plots of age-specific mortality rates versus chronological age. Age-specific mortality rates were calculated for each 2-day period throughout life using SPSS subprogram, Survival. Lines are weighted regression estimates (SPSS). (A and B) Survival curves and mortality data for the parental stocks, N2 and Bergerac BO. (C and D) Survival curves and mortality data for TJ119, TJ135, and TJ143. TJ143 is one of the longest-lived stocks generated (from Johnson, 1987).

**Table 3.** Correlations[a] Among Age-Specific Self-Fertility, Total Self-Fertility, and Life Span

| | | | Age-Specific Self-Fertility | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Age: 3 | | 4 | 5 | 6 | 7 | >7[b] | Total | Life |
| Trial | | | | | (days) | | | Fertility | Span |
| 1 | 3 | 1 | 0.55*** | 0.07 | 0.02 | 12 | 0.06 | 0.74*** | 0.11 |
| 2 | 3 | 1 | 0.60*** | 0.43** | -0.30** | -0.19** | -0.19* | 0.54*** | 0.15 |
| 3 | 3 | 1 | 0.57*** | 0.24** | -0.03 | -0.18** | -0.17* | 0.69*** | -0.13 |
| 1 | 4 | 0.54* | 1 | 0.48*** | 0.15 | 0.15 | -0.02 | 0.83*** | 0.40*** |
| 2 | 4 | 0.69** | 1 | 0.41*** | -0.50*** | -0.44*** | -0.29*** | 0.47*** | 0.08 |
| 3 | 4 | 0.64** | 1 | 0.65*** | 0.36*** | 0.07 | -0.07 | 0.88*** | 0.08 |
| 1 | 5 | 0.07 | 0.06 | 1 | 0.47*** | 0.32*** | 0.16* | 0.64*** | 0.24*** |
| 2 | 5 | 0.48* | 0.54* | 1 | 0.18* | 0.11 | 0.02 | 0.84*** | 0.11 |
| 3 | 5 | 0.38 | 0.79** | 1 | 0.68*** | 0.44*** | 0.21* | 0.84*** | 0.32*** |
| 1 | 6 | 0.02 | 0.13 | 0.45* | 1 | 0.54*** | 0.30*** | 47*** | 0.08 |
| 2 | 6 | 0.37 | -0.63** | 0.14 | 1 | 0.74*** | 0.42*** | 0.39*** | 0.06 |
| 3 | 6 | 0.20 | 0.61** | 0.85*** | 1 | 0.56*** | 0.32*** | 0.60*** | 0.28* |
| 1 | 7 | 0.11 | 0.13 | 0.31 | 0.53* | 1 | 0.41*** | 0.42*** | 0.10 |
| 2 | 7 | -0.23 | -0.62** | 0.13 | 0.96*** | 1 | 0.67*** | 0.38*** | 0.09 |
| 3 | 7 | -0.18 | 0.07 | 0.47* | 0.77*** | 1 | 0.67*** | 0.34*** | 0.14 |
| 1 | >7 | -0.06 | -0.04 | 0.16 | 0.29*** | 0.40*** | 1 | 0.14 | -0.02 |
| 2 | >7 | -0.28 | -0.63* | 0 | 0.92*** | 0.94*** | 1 | 0.24** | 0.03 |
| 3 | >7 | -0.29 | -0.19 | 0.21 | 0.57*** | 0.93*** | 1 | 0.17* | 0.01 |
| Total Self-Fertility | | | | | | | | | |
| 1 | | 0.73*** | 0.81*** | 0.62* | 0.47 | 0.42 | 0.13 | 1 | 0.30* |
| 2 | | 0.60** | 0.48* | 0.91* | 0.31 | 0.34 | 0.24 | 1 | 0.17 |
| 3 | | 0.69** | 0.91* | 0.90* | 0.80*** | 0.36 | 0.18 | 1 | 0.14* |
| Life Span | | | | | | | | | |
| 1 | | 0.08 | 0.37 | 0.20 | 0.06 | 0.08 | -0.03 | 0.25 | 1 |
| 2 | | 0.25 | 0.17 | 0.30 | 0.07 | 0.05 | 0.09 | 0.32 | 1 |
| 3 | | -0.16 | 0.27 | 0.60** | 0.43 | 0.32 | 0.21 | 0.34 | 1 |

a* Phenotypic correlations above diagonal; genetic, below diagonal; analysis of three trials.

b* Last progeny: Day 10 in trial 1; Day 13, trial 2; Day 14, trial 3. Significance: $P \leq 0.05$ (*); $P \leq 0.01$ (**); $P \leq 0.001$ (***); 2-tailed test.

combined with earlier studies suggesting a model in which development is completed before the onset of aging (Johnson *et al.*, 1984) to give a comprehensive summary of the independent processes involved in the specification of length of life (Figure 7).

## INDUCED MUTANTS

*age-1(hx546)* is a recessive mutant allele in *Caenorhabditis elegans* that results in an average 40% increase in life expectancy and an average 60% increase in maximal life span at 20° C (Figure 8; Table 4; and Friedman and

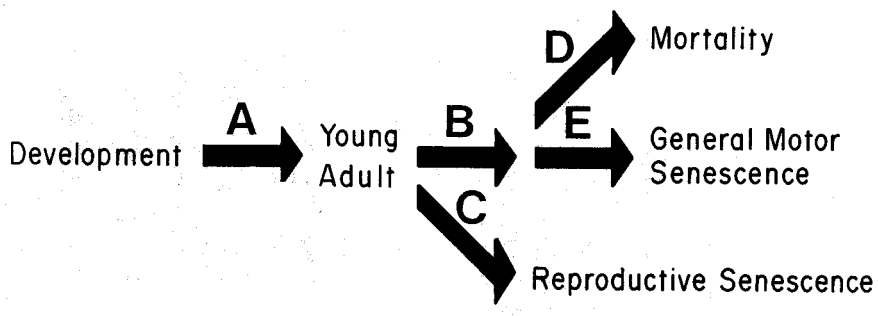


**Figure 7.** Diagram describing the order of dependency of events in senescence of *C. elegans*. Arrows indicate dependency relationships (from Johnson, 1987).

Johnson, 1988a,b); at 25°C, *age-1(hx546)* averages a 65% increase in mean life span (25.3 days *vs.* 15.0 days) and a 110% increase in maximum life span (46.2 days *vs.* 22.0 days for wild-type hermaphrodites; Friedman and Johnson, 1988a). Mutant males also show extended life spans. *age-1(hx546)* is associated with a 75% decrease in hermaphrodite self-fertility as compared to the *age-1*$^{+}$ allele at 20°.

## Physiological Characteristics of Long-lived Strains: Food Uptake

Klass (1983) reported that eight long-lived strains isolated in his screens had behavioral or developmental alterations that led to longer life and implied that the uncoordinated phenotype (Unc) might lead to the observed reduction in food uptake and thereby lead to longer life. In pilot studies on the segregation of life expectancy it became clear that the Unc character segregated independently of any locus or loci that specified long life (Johnson, 1986); the Unc mutation was subsequently shown to map to linkage group V and to be an allele of *unc-31* (Friedman and Johnson; 1988b). It was also clear that the long-lived mutant strains did not ingest less food than did wild type (Figure 8 C, E and Johnson, 1986). Pharyngeal pump rates were determined for age-synchronous cultures at the first larval stage (immediately after refeeding cultures starved at the time of hatching from the egg), second larval stage (20 hours after refeeding), and fourth larval stage (40 hours after refeeding), and on young adult (50 hours after refeeding) hermaphrodites of N2, DH26, MK7,

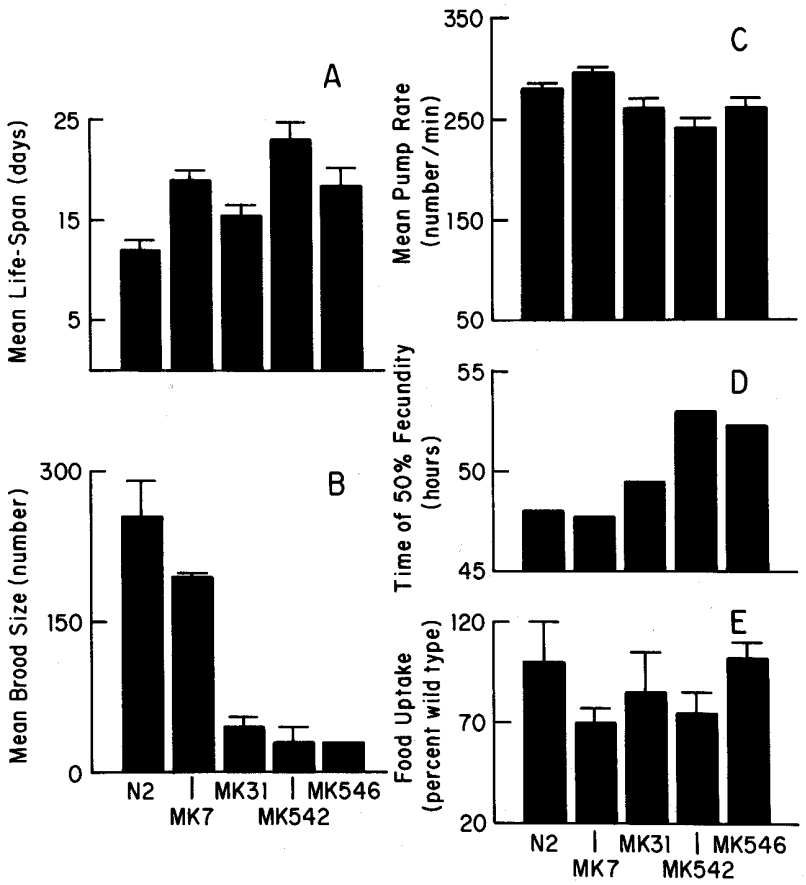


**Figure 8.** Physiological characteristics of four long-lived mutant strains and wild type, N2: (A) mean life spans; (B) average number of progeny; (C) pharyngeal pump rates; (D) length of time to 50% of population being fertile; (E) relative amount of radiolabelled *Escherichia coli* ingested. Procedural details can be found in Friedman and Johnson (1988a,b) and in Johnson and McCaffrey (1986).

MK31, MK542, and MK546. First stage larvae had lower pharyngeal pump rates than other larval stages. Pharyngeal pump rates (an indirect assessment of rate of food ingestion) and rates of radiolabelled food uptake of the long-lived stocks were comparable to the wild-type controls (Table 5).

**Table 4.** Average Life of Age Mutants[a].

| Strain | Number of Repeats | Average Life Expectancy ± SD | Average Maximum Life ± SD |
|---|---|---|---|
| N2 | 2 | 15.0 ± 1.3 | 22.0 ± 5.0 |
| DH26 | 3 | 15.7 ± 1.3 | 23.7 ± 2.9 |
| MK7 | 3 | 19.2 ± 1.6 | 29.3 ± 2.3 |
| MK31 | 4 | 18.4 ± 0.3 | 30.3 ± 1.1 |
| MK542 | 2 | 28.1 ± 6.3 | 46.5 ± 7.5 |
| MK546 | 5 | 25.3 ± 3.4 | 46.2 ± 3.4 |

[a]. Data from Friedman and Johnson (1988b).

**Table 5.** Pharyngeal Pump Rates and Food Uptake

| Strain | Geno-type | Mean Pump Rate ± SEM[a] (pumps/min) | | | | Mean Food Uptake[b] (cpm) | | |
|---|---|---|---|---|---|---|---|---|
| | | First stage lar-vae | Second stage lar-vae | Fourth stage lar-vae | Young Adult | Experi-ment 1 (liquid) | Experiment 2 (agar) | (liquid) |
| N2 | *age*$^+$ | 130 ± 12 | 225 ± 4 | 279 ± 4 | 281 ± 6 | 2648 ± 508 | — | — |
| DH26 | *age*$^+$ | 107 ± 2 | 198 ±14 | 207 ± 25 | 295 ± 9 | — | 1799 ± 64 | 1372 ± 335 |
| MK7 | *age-?* | 110 ± 16 | 220 ± 20 | 259 ± 7 | 293 ± 6 | 1860 ± 187 | 1770 ± 163 | 660 ± 295 |
| MK31 | *age-1 (hx31)* | 163 ± 4 | 216 ± 10 | 256 ± 7 | 260 ± 9 | 2273 ± 636 | 7240 ± 90 | 1712 ± 45 |
| MK542 | *age-1 (hx542)* | 140 ± 3 | 174 ± 20 | 224 ±22 | 241 æ 13 | 1949 ± 333 | 2065 ± 10 | 2200 ± 628 |
| MK546 | *age-1 (hx546)* | 127 ± 16 | 232 ± 7 | *234* ±12 | 262 ± 13 | 2696 ± 257 | 3257 ± 238 | 1595 ± 8 |

[a] Hatched larvae were fed and assayed at the following times: 0-1 hours for first stage larvae, 20- 21 hours for second stage larvae, 39-40 hours for fourth stage larvae, and 49- 50 hours for young adult worms. Data are from 20-second assays on 5 individuals.
[b] 1000 worms were assayed except for experiment 2, liquid media, where 500 worms were used; assays are described in Johnson and McCaffrey (1986).

Further longitudinal studies of individual animals, as well as longitudinal studies on mass cultures (Johnson and Conley, unpublished), showed that the relationship between food uptake and chronological age is complex, with the mutants ingesting greater than normal amounts of food early in life and lesser amounts later. Thus, two apparently contradictory observations (Klass, 1983; Johnson, 1986), which had been obtained at different chronological ages, are not necessarily conflicting.

In an attempt to resolve this problem, we pursued another approach to answering the question of whether self-imposed food restriction leads to the longer life of *age-1*. Mean life spans of wild-type and long-lived mutants were determined in solutions of *E. coli* at concentrations ranging from $10^8$ to $10^{10}$ cells per ml. In these experiments we took advantage of observations by Nicholas *et al.* (1973), Schiemer *et al.* (1980), and Schiemer (1982) that food ingestion rates in other nematodes are proportional to bacterial concentration over concentrations from $2 \times 10^8$ to $5 \times 10^{10}$ cells per ml.

We reasoned that if the physiological processes affected by food restriction are independent of the process(es) affected in the long-lived mutants, then an additional component of life span might be added to the already longer life span of the mutants by food restriction. Alternatively, if the long life of the mutant strains is due entirely to food restriction, we would expect that no additional life extension due to food restriction would be observed in the mutants and that maximal life span would be observed at bacterial concentrations higher than those that maximize mean life span of DH26.

DH26 exhibited a bell-shaped response to varying bacterial concentrations, with a maximal mean life span at $10^9$ cells per ml (our standard survival conditions; Figure 9). With only one exception (MK7 at $3 \times 10^9$ bacteria per ml), each of the three mutant strains tested lived significantly longer than DH26 at comparable concentrations ($P < 0.001$). Maximal mean life span of MK546 was at $3 \times 10^8$ whereas MK31 and MK542 were maximal at $10^8$ bacteria per ml. This result suggests that whatever processes are acting to increase life span in the mutant strains, they function independently of, that is to say in addition to, the life-extension effects of food restriction.

## Mapping of the *age-1* Locus

Using two novel strategies for following the segregation of *age-1*, we obtained evidence that longer life results from a mutation in a single gene that increases the probability of survival at all chronological ages. The long-life and reduced-fertility phenotypes cosegregate in backcrosses to N2 (Figure 10). Surprisingly, both are tightly linked to *fer-15*, a locus on linkage group II; our current model suggests that *age-1* may be a further mutation in *fer-15* or tightly linked to it, and we are attempting to clone this region using overlapping deficiencies (Sigurdson *et al.*, 1984) and congenic strains (Link and Johnson, unpublished). *age-1(hx546)* does not affect the timing of larval molts, the length of embryogenesis, food uptake, movement, or behavior in any way tested.

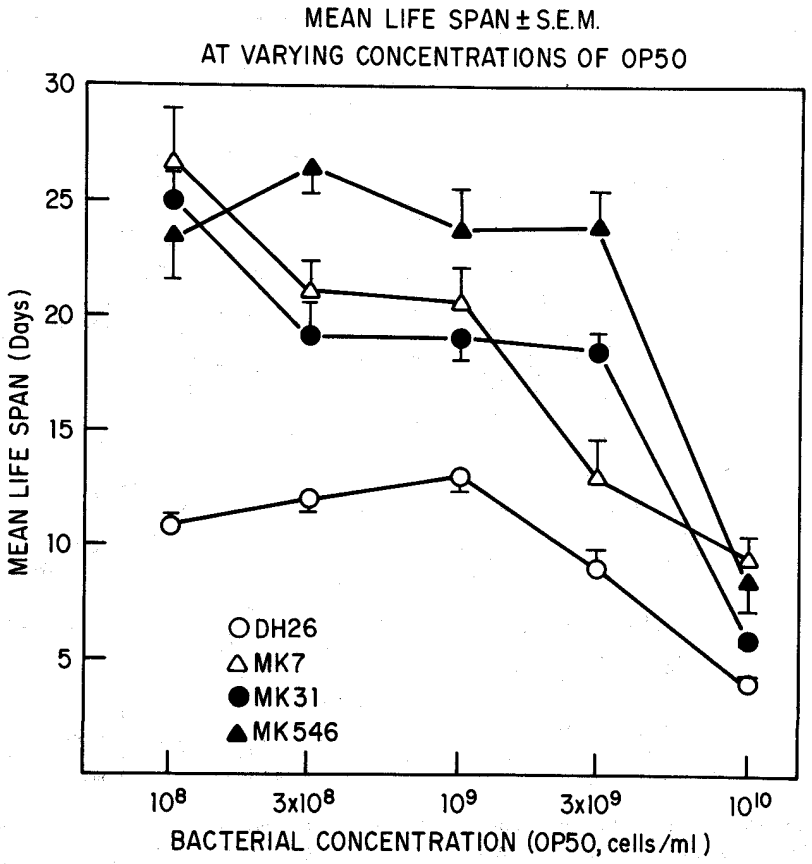


**Figure 9.** Survival populations of MK7, MK31, MK546, and DH26 at different concentrations of OP50. Mean life spans ± SEM were calculated from populations of 50 worms. Survivals of all mutants are significantly different from DH26 at the same concentration ($P < 0.001$) with the exception of MK31 at $3 \times 10^9$ bacteria per ml ($P = 0.308$).

In performing these mapping experiments we took advantage of the self-fertilizing nature of *C. elegans* and its lack of inbreeding depression in that we were able to establish homozygous populations for subsequent quantitative genetic analyses and that the lack of inbreeding depression simplifies the inference of genotype based on length of life of each resultant isolate. In contrast, sexually reproducing organisms face problems resulting from the segregation of alleles after crossing, as well as from inbreeding depression. These complexities are graphically illustrated by examining survival curves

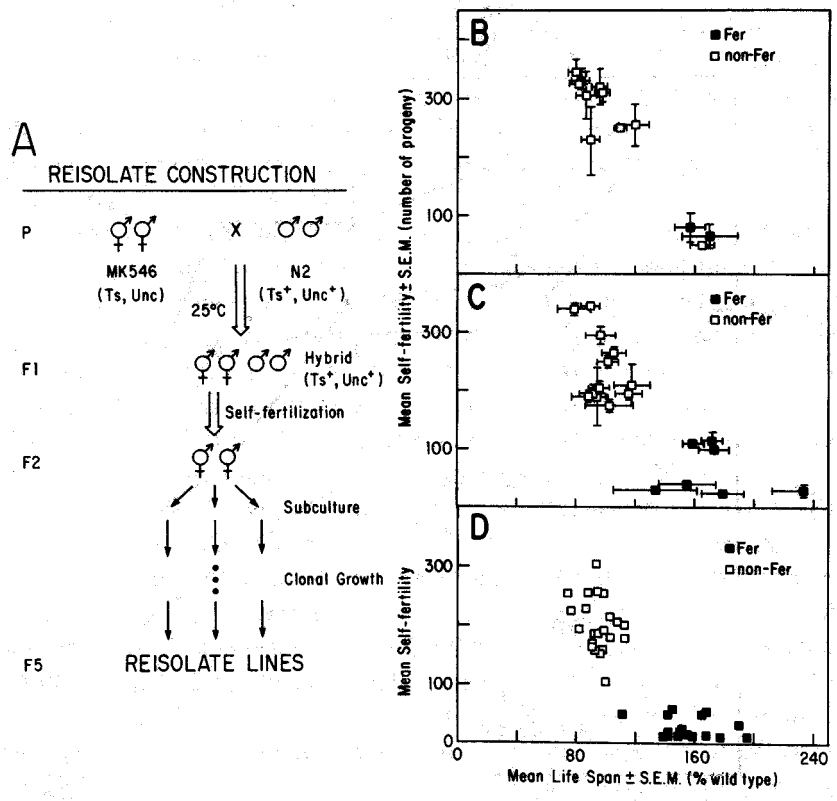


**Figure 10.** (A) Method for constructing homozygous populations from crosses between N2 and MK546. (B and C) Life expectancy at 20° of reisolates from the cross of MK546 [*age- 1(hx546) fer-15(b26ts)* II; *unc-31(z1)* IV] to N2 is plotted relative to hermaphrodite self-fertility; (B) $F_5$ reisolates from experiment 1, and (C) $F_{15}$ reisolates from experiment 2. (D) Life expectancy at 25° of $F_{10}$ reisolates from crosses of MK542 [*age-1(hx542) fer-15(b26ts)* II; *unc-31(z2)* IV] to N2. Fer (■) and non-Fer (□) stocks are indicated; because of the large number of points, standard errors are not shown in Figure 10D, but ranged from 5% to 15% of the mean life span, while self-fertility is the average of 3 to 5 hermaphrodites whose progeny were counted collectively rather than individually (from Friedman and Johnson, 1988a).

shown in Figure 11; the inference that a single gene specifies length of life would be quite difficult to make from these data alone.

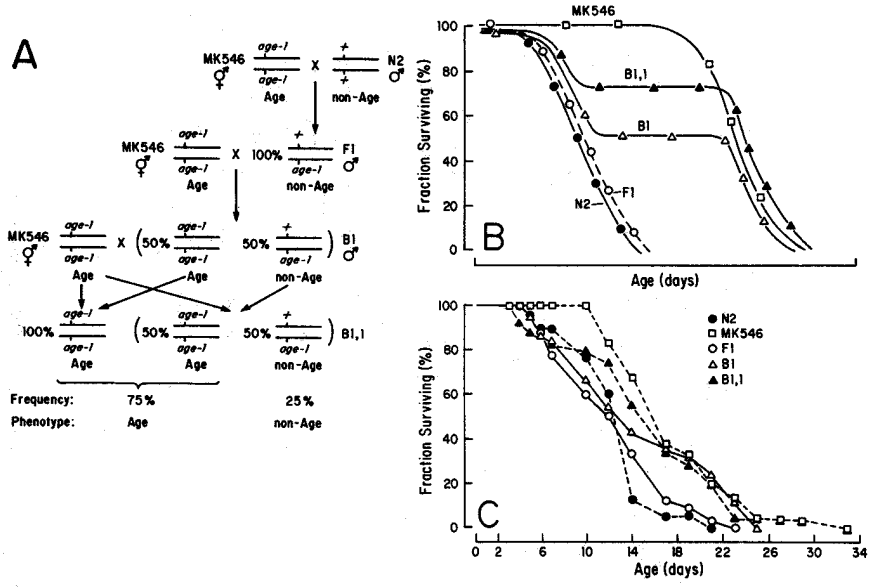


**Figure 11.** Scheme for following the segregation of a life-span determining gene in genetically heterogeneous populations. (A) MK546 hermaphrodites were backcrossed to N2 males to measure life span in the $F_1$. $F_1$ males were backcrossed to MK546 hermaphrodites to obtain a $B_1$ generation which were similarly backcrossed to obtain the $B_{1,1}$ generation. Also shown in this figure are the proportions of Age and non-Age progeny expected in each generation. (B) Idealized survival curves expected at each generation, assuming single gene segregation for *age-1*. (C) Actual survival curves obtained from the crossing scheme described in A (from Friedman and Johnson, 1988a).

## Fine Structure Mapping of *age-1*

Using both three-point crosses and deficiency analysis we have further localized *age-1* with respect to outside markers. In recombinants selected between *dpy-10* and *unc-4*, the lower fertility characteristic associated with *age-1* is shown to cosegregate with *fer-15* (Figure 12; Fitzpatrick and Johnson, unpublished). An alternate approach, deficiency mapping, uses a series of deficiencies covering much of the region between *dpy-10* and *unc-4* (Sigurdson *et al.*, 1984) and assigns *age-1* to a region between the nominal breakpoints of mnDf91 and mnDf92 which puts it into the region containing *fer-15* and *emb-27* (Figure 13; Shoemaker and Johnson, unpublished).

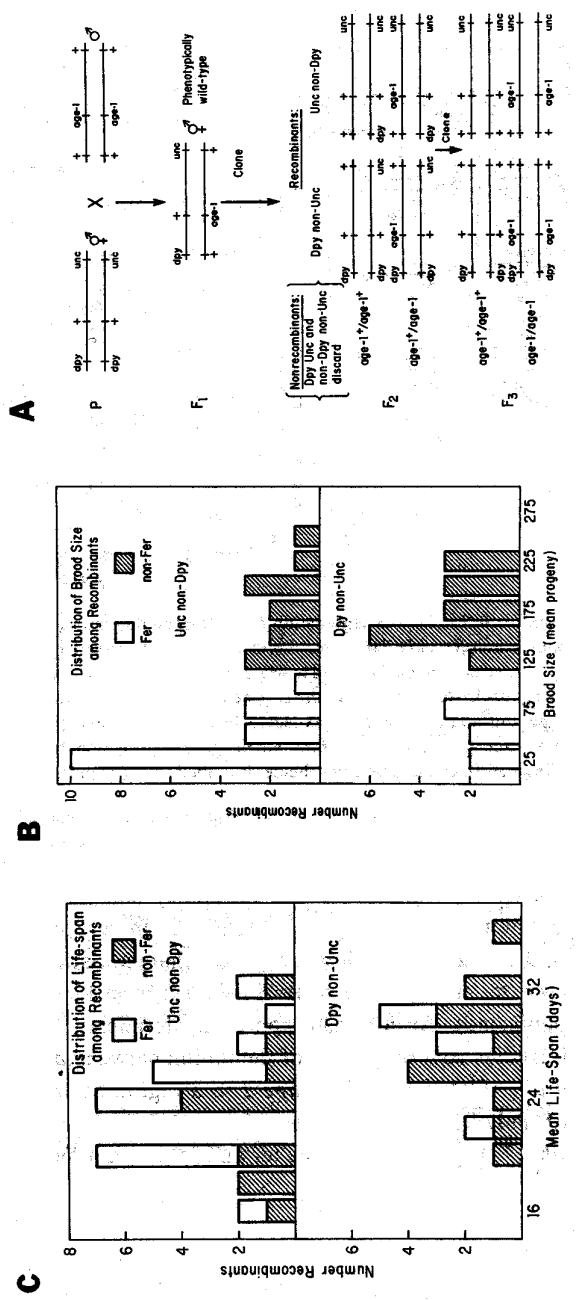


**Figure 12.** Scheme for performing three-point crosses (A) and results for fertility (B) and for life span (C).

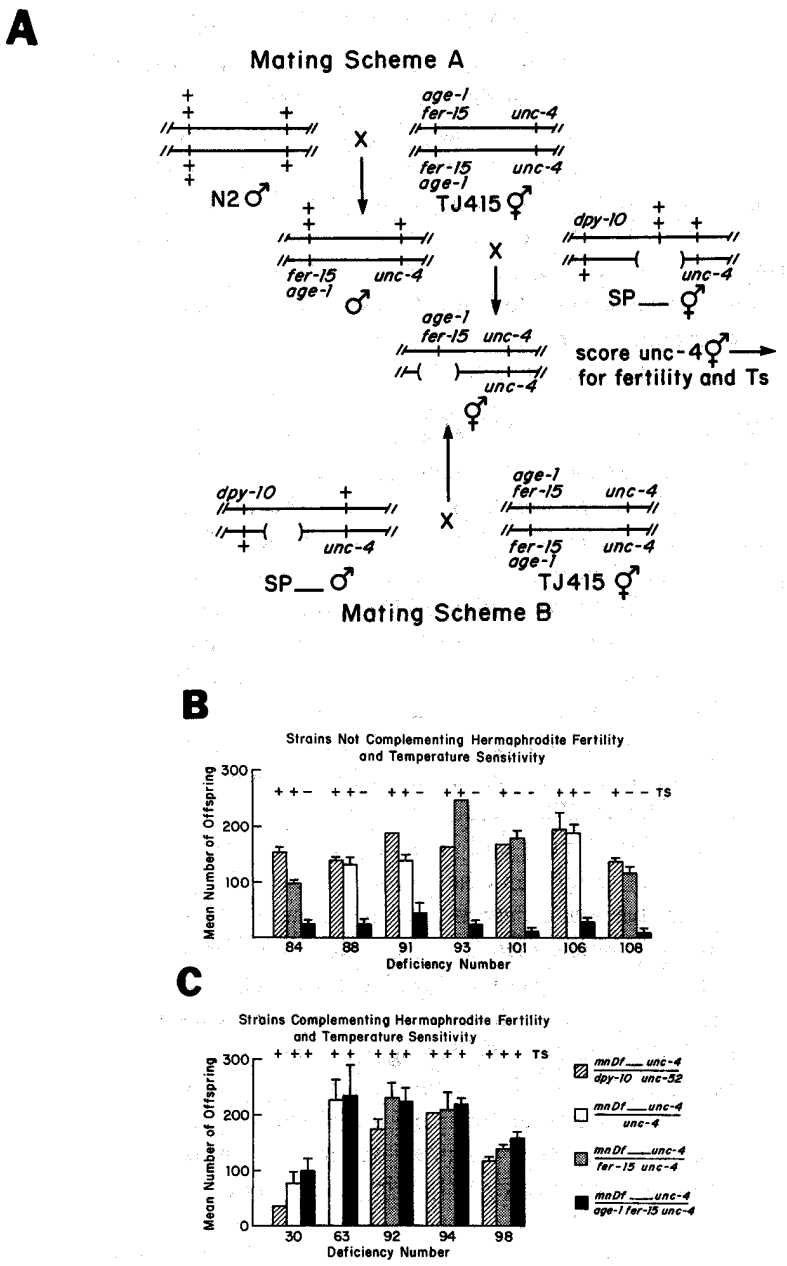


**Figure 13.** Scheme for performing deficiency analyses on fertility (A) and results (B).

### Effects on Other Physiological and Developmental Traits

Although *age-1(hx546)* lowers hermaphrodite self-fertility, it does not markedly affect the length of the reproductive period. All of the increase in life expectancy is due to an increase in the length of postreproductive life. Small changes in rate of movement at different chronological ages are seen when *age-1* and wild type are compared; similarly, changes in age-specific levels of lysosomal enzymes can be detected (Conley and Johnson, unpublished; Russell and Seppa, this volume). However, lipofuscin levels do not appear to be altered (Le Noir and Johnson, unpublished), suggesting that *age-1* changes one of the aspects of aging in *C. elegans* - that limiting length of life - without dramatically affecting other physiological systems.

## SUMMARY AND PERSPECTIVES

So far as we are aware, this mutation in *age-1* is the only instance of a well-characterized genetic locus in which the mutant form results in lengthened life. Consistent with the recessive nature of *age-1(hx546)* is the interpretation that longer life results from the elimination of normal gene function; deficiency analysis (Figure 13) suggests that *age-1(hx546)* is a null allele. Thus life span is shortened by the normal action of a gene whose function may be primarily involved in increasing reproduction. The *age-1* mutation dramatically displays a trade-off wherein reproductive effort is increased by the normal allele at a "cost" of loss of 1/2 of postreproductive life as is predicted by the Antagonistic Pleiotropy model for the evolution of senescence (Williams, 1957; Rose, 1985).

The molecular cloning and characterization of this locus is likely to provide significant insights into heretofore theoretical arguments concerning the evolutionary basis of senescence. Some of these arguments are described elsewhere in this volume (see Charlesworth, Rose, Kirkwood, Hutchinson, this volume; Partridge and Harvey, 1988) and may help to distinguish between rival theories. In light of such evolutionary arguments we favor a model in which the action of *age-1* in lengthening life results *not* from elimination of a programmed aging function but rather from the lack of the detrimental action of a gene whose primary function is involved in hermaphrodite reproduction. The existence of such genes offers an optimistic view about our ability to intervene in the aging processes to mimic the action of mutant alleles such as *age-1*.

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## DISCUSSION

1. Asked where the variability comes from that makes the survival curve less than perfectly square Johnson pointed out that the environment affects a quantitative trait like aging, especially over time, so that all individuals do not die at the same time. This is also true in inbred mice.

2. An unanswered question was why do the worms die? Do they run out of something? This seems unlikely, as they are independent organisms. However, adults have no replicating cells except for reproductive cells. DNA repair should be no problem, as the worms can survive 100,000 R of irradiation.

3. Questioned whether *age-1* and *fer-15* may be alleles at the same locus, Johnson answered that he was testing this possibility with sequencing.