

A Mutation in the *age-1* Gene in *Caenorhabditis elegans* Lengthens Life and Reduces Hermaphrodite Fertility

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ABSTRACT

age-1(hx546) is a recessive mutant allele in *Caenorhabditis elegans* that results in an increase in mean life span averaging 40% and in maximal life span averaging 60% at 20°; at 25° *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). 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°. Using two novel strategies for following the segregation of *age-1*, we present 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 and are tightly linked to *fer-15*, a locus on linkage group II. *age-1(hx546)* does not affect the timing of larval molts, the length of embryogenesis, food uptake, movement, or behavior in any way tested. Although *age-1(hx546)* lowers hermaphrodite self-fertility, it does not markedly affect the length of the reproductive period with all the increase in life expectancy due to an increase in the length of postreproductive life. In so far as we are aware, this mutant in *age-1* is the only instance of a well-characterized genetic locus in which the mutant form results in lengthened life. It is likely that the action of *age-1* in lengthening life results not from eliminating a programmed aging function but rather from reduced hermaphrodite self-fertility or from some other unknown metabolic or physiologic alteration.

HOW genes are involved in specifying the limited life span of metazoans is a major unsolved problem in biology. Although some have argued that genes may not be involved at all, no informed critic can seriously doubt the contribution of the genotype in specifying species-specific life span and other attributes of aging (RUSSELL 1976; LINTS 1978; ROTHSTEIN 1983, 1985, 1987). Although there are many theories suggesting molecular and physiologic processes that may be responsible for this limitation (ADELMAN and ROTH 1982; FINCH and SCHNEIDER 1985), there are few systems in which predictions of these theories can be directly tested with regard to aging and senescence. A problem peculiar to research in aging has been the lack of good experimental systems with which to manipulate the rates of aging. For example, dietary restriction is the only technique currently available that has been reliably shown to lengthen maximum life span (MASORO 1985; WEINDRUCH 1984).

Mutants with altered rates of aging would be quite useful but criteria that can be used to identify such mutants are unclear. The set of genes most directly involved in the specification of life span might be

identified by screening for mutants that have increased life expectancy, especially those with increased maximum life span. By identifying mutants that extend life span we can identify those genes and processes that limit the normal life span. HAYFLICK (1985) has said of this approach "... only by increasing life span or maximum age of death of members of a species will important insight be made into the aging process...."

Mutants that alter the length of life and presumably the rate of aging have been sought in a variety of metazoan species. For example, GOULD and CLARK (1977) and LEFFELAAR and GRIGLIATTI (1984) have isolated adult-onset-lethal mutations in *Drosophila melanogaster*; LEFFELAAR and GRIGLIATTI (1984) have also identified one mutant which they interpret as showing accelerated senescence. In humans several clinical syndromes have been described as examples of accelerated senescence (for reviews see GOLDSTEIN 1971; MARTIN 1978). These human syndromes accelerate only some of the normal characteristics of aging, and it is now widely believed that they only mimic normal aging processes (MARTIN 1978). In contrast, mutants that extend the maximum length of life must have altered those rate-determining processes that limit the life of the wild-type organism.

KLASS (1983) reported the isolation of eight long-lived mutant strains of *Caenorhabditis elegans*. Exami-

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This paper is dedicated to the memory of Dr. Laurence Sandler, a gifted teacher and geneticist, deceased February 7, 1987, at his home in Seattle, Washington.

nation of these strains led KLASS to conclude that each strain had a visible defect that led to life span prolongation. The extended life of two strains resulted from the fact that they formed dauer larvae constitutively; one strain was chemotaxis defective; the remaining five strains (MK7, MK31, MK248, MK542 and MK546) showed decreased food ingestion which led KLASS to conclude that life extension in these five strains resulted from food restriction. KLASS concluded that life-extension loci are rare and that there are no loci that display only specific alterations in life span without affecting other characteristics as well. The observation that these mutants showed lower rates of food ingestion were not replicable and may have resulted from an unlinked mutation in *unc-31* (JOHNSON 1986; FRIEDMAN and JOHNSON 1988) although the elucidation of the mutant defects remains to be determined.

These strains are of interest because the mutations resulting in increased life expectancy (which we will refer to as the Age phenotype) must have somehow altered those molecular and physiologic processes that normally limit life span. The very existence of loci that, when mutated, cause life extension suggests that the gene thus identified produces a product that is responsible in some way (either directly or indirectly) for limiting life span in the wild-type nematode. Genetic and molecular characterization of such mutations will thus lead to an understanding of the mechanisms that normally limit life span in *C. elegans*.

This paper shows that the Age phenotype is recessive and that original mutant stocks also display changes in other life history traits, such as hermaphrodite self-fertility. The life-extension characteristic (Age) segregates as a single gene that we call *age-1*. We also find that Age cosegregates with a fourfold reduction in hermaphrodite self-fertility; *age-1* is linked to *fer-15* and maps to a well studied region of linkage group II.

MATERIALS AND METHODS

Strains and media: The wild-type strain of *C. elegans*, N2 (var. Bristol), and the DH26 strain which carries the temperature sensitive spermatogenesis mutant *fer-15(b26ts)* II, were obtained from D. HIRSH; a phenotypically identical DH26 strain was also obtained from M. KLASS as were MK7, MK31, MK542 and MK546. These latter four strains are long-lived mutants that were isolated by KLASS (1983) following EMS mutagenesis of DH26 and have the following genotypes (FRIEDMAN and JOHNSON 1988): MK7 [*age-?*; *fer-15(b26ts)* II]; MK31 [*age-1(hx31)* *fer-15(b26ts)* II; *unc-31(z3)* IV]; MK542 [*age-1(hx542)* *fer-15(b26ts)* II; *unc-31(z2)* IV]; and MK546 [*age-1(hx546)* *fer-15(b26ts)* II; *unc-31(z1)* IV]. These four strains were all derived in the same mutant hunt using protocols that failed to insure independence of mutations (M. R. KLASS, personal communication); so the different allele designations refer only to the strain of origin and cannot ensure independence without additional external verification. Several other long-lived mutants of KLASS

(1983) have been lost; these included MK248 and MK508. BA15 (*fer-15(hc15)* II) was obtained from S. L'HERNAULT. TJ401 [*age-1(hx546)* *fer-15(b26ts)* II] is an *age-1 fer-15(b26ts)* II, *unc-31*⁺ strain reisolated from a cross between N2 males and MK546 hermaphrodites (Figure 4A). CB169 carries *unc-31(e169)* IV.

The following mutant alleles were used in mapping; they have been described by BRENNER (1974) and were obtained from the Caenorhabditis Genetics Center:

Linkage group (LG) I: *dpy-5(e61)*

LG II: *dpy-10(e128)*

LG III: *dpy1(e1)*

LG IV: *dpy-9(e12)*, *dpy-13(e184)*, *unc-17(e245)*, *unc-24(e138)*, *unc-28(e15)*, *unc-31(e169)*, *unc-33(e204)*

LG V: *dpy-11(e224)*

LG X: *dpy-6(e14)*

Escherichia coli, OP50, was used as the food source. Stock maintenance, general techniques for handling *C. elegans*, S salts, and prespotted NGM (nematode growth media) have been described (BRENNER 1974; JOHNSON 1984). All studies were performed at 20° unless otherwise indicated.

Age-synchronous cultures: Cultures were produced as described by JOHNSON and WOOD (1982). Briefly, four to ten young adult hermaphrodites were placed onto a fresh NGM plate for a period of egg deposition that varied depending on the number of progeny needed for each assay; this interval was usually between 4 and 8 hr and never more than 24 hr. Alternatively, eggs were isolated from gravid adults using hypochlorite as described by EMMONS and YESNER (1984), except that we used S salts and placed the eggs on spotted NGM plates prior to hatch.

Crossing techniques: All crosses were done on NGM plates preseeded with a spot of *E. coli* OP50. Typical crosses consisted of ten males placed together with four fourth-larval-stage hermaphrodites. After 24 hr, adults were transferred to fresh NGM plates until sterile or no longer needed. When crosses involved *fer-15*, eggs which were laid by four to six fecund *fer-15* hermaphrodites at 20° were allowed to develop at 25° producing self-sterile hermaphrodite progeny which were then mated at 20°. This procedure was used in the backcrosses to ensure that only cross progeny were analyzed. Self-sterility was monitored by placing identically treated *fer-15* control hermaphrodites at 20° on fresh NGM at the same time the crosses were performed. In crosses where the hermaphrodite parent had a visible phenotype (Unc or Dpy), cross progeny were identified as being phenotypically wild type. Male ratios were determined as an additional check for crossing; if the F₁ did not contain approximately 50% male progeny, the data were suspect. The Dpy or Unc males used in these experiments are almost sterile (HODGKIN 1983); therefore, crosses between stocks both of which carry such genes were performed using heterozygous males.

Comparison of parent and progeny life spans: Individual hermaphrodites were cultured in 0.6 ml of survival medium in 24-well microtiter plates and were transferred daily. Progeny were collected from each hermaphrodite on the first day of reproduction and were transferred to small NGM plates to develop. At 3 days of age populations of these progeny were transferred to survival medium following standard protocols and followed until dead.

Generation of *age-1* reisolates: MK546 [*age-1(hx546)* *fer-15(b26ts)* II; *unc-31(z1)* IV] or MK542 [*age-1(hx542)* *fer-15(b26ts)* II; *unc-31(z2)* IV] hermaphrodites were hatched and grown to the fourth larval stage at 25° which results in self-sterility from the action of *fer-15(b26ts)*. Several self-sterile fourth larval stage hermaphrodites were then crossed

with ten or more N2 males at 20°. The F₁ were picked and allowed to self-fertilize at 20°; the F₂ were cloned, and individually inbred by self-fertilization (Figure 4A) for five generations in the first experiment and for 10 to 15 generations in others resulting in 96% to more than 99.9% inbreeding, respectively. Due to the length of time between stock construction and testing, many of the reisolates in the first experiment were lost prior to testing for both life span and hermaphrodite fertility so only data from fully characterized isolates are included here; this problem was avoided in the other crosses presented in this paper.

Measurement of life span: Life span was scored on synchronous populations as described before (JOHNSON and WOOD 1982) except that some assays of life span were conducted at 25°. Briefly, the assays involve the suspension of 25 or 50 age-synchronous worms per genotype at 3 days of age in 4 ml of S Basal plus cholesterol (BRENNER 1974) plus 10⁹/ml of *E. coli*. Worms were transferred daily for the first 6 days and thrice weekly thereafter until sterile at which point transfers were performed weekly. All studies were performed at 20° unless otherwise indicated. At the time of transfer and at least three times a week the number of worms that were living, dead, nonresponsive, bags, killed, or lost were recorded. Worms recorded as dead had died of natural causes and were nonmotile, failed to respond to touch, showed visible degeneration of tissue due to bacterial invasion, and had no osmotic turgor when cut (JOHNSON and WOOD 1982).

Life expectancy is defined as mean life span at birth; maximum life span is used here to indicate the longest-lived individual observed; this is a poor statistical variable but has theoretical significance in aging research (SACHER 1977).

Measurement of self-fertility: Fourth larval stage hermaphrodites were serially transferred to fresh, spotted NGM plates at 12- to 48-hr intervals, until sterile. One to two days after eggs were laid, the hatched progeny were counted as they were removed with a Pasteur pipette attached to a vacuum line. Each data point represents the mean number of progeny \pm standard error of the mean of three to five worms except in the few cases noted otherwise.

Time of intermolt lethargis and average time of reproductive maturity: Unhatched eggs were obtained via hypochlorite treatment (EMMONS and YESNER 1984) and were allowed to hatch in S Basal. Newly hatched worms immediately arrest development in the absence of food but recover normal rates of development almost immediately upon return to food (JOHNSON *et al.* 1984). Approximately 100 arrested larvae were aliquoted onto prespotted NGM plates at 8-hr intervals, initiating three parallel synchronous populations separated by 8 hr in their stage of development. All three parallel populations were maintained at 20° and were assayed simultaneously thus allowing us to follow 24 hr of development in a convenient 8-hr assay period and providing us with internal controls so that asynchrony of one population, with respect to the others, could be immediately detected. As a control to ensure that worms were arrested in early first larval stage (length ~0.20 mm) (BYERLY, CASSADA and RUSSELL 1976), ten nematodes from each population were heat killed (10 min at 60°) and measured with an ocular micrometer.

Individual nematodes temporarily cease pharyngeal pumping during the lethargis associated with every larval molt; this lack of pumping serves as a convenient assay for determining the precise time of molt (CASSADA and RUSSELL 1975). Fifty worms were scored for pharyngeal pumping using a Nikon Microphot compound microscope equipped with Nomarski differential interference contrast optics. The

time at which the fewest worms were pumping was taken as the midpoint of each larval molt.

The time of reproductive maturity was determined in the same assays. A worm was considered mature if any fertilized eggs were found in the uterus. Approximately 50 worms were scored and the time when 50% contained eggs was obtained by interpolation.

Mapping the Unc phenotype: There were obvious behavioral changes in three of the Age mutants: MK31, MK542 and MK546. The mutants were sluggish in their normal movements but moved well after touch or when starved. The gene responsible for this phenotype was assigned to linkage group IV by genetic mapping with Dpy mutants on each linkage group; complementation tests between the mutants and *unc-31(e169)* confirmed this map position and showed that all three stocks carry alleles of *unc-31*. MK542 and MK546 were noticeably more uncoordinated than MK31.

Statistical techniques: Statistical analyses of survival populations were conducted as described (JOHNSON and WOOD 1982). All comparisons of survival were carried out using the generalized Kruskal-Wallis test (BRESLOW 1970) as implemented in the Statistical Package for the Social Sciences (SPSS) survival procedure (HULL and NIE 1979); the log-rank test (PETO and PETO 1972) has also been used to confirm statistics obtained by the generalized Kruskal-Wallis in several instances. Descriptive statistics were obtained using the SPSS condcriptive procedure (NIE *et al.* 1975). Life spans are reported as mean \pm standard error of the mean (SEM). Student's *t*-tests were employed to compare means of different genotypes (SOKAL and ROHLF 1981).

RESULTS

Mutants live longer than wild type: KLASS (1983) reported that several mutants were longer-lived when grown on a bacterial lawn on solid NGM. Our assay conditions are liquid S Basal at 10⁹ bacteria per ml, so we first determined that mutants were longer-lived under our assay conditions. In the first experiment (Figure 1A), the life expectancies of three Age mutants (MK7, MK31 and MK546) were significantly greater than N2 ($P < 0.01$). Mean life spans were extended 30–50% in this experiment, and maximum life spans were extended 10–60%.

MK7, MK31 and MK546 were retested for survival and compared with DH26, the strain in which the mutants were isolated (Figure 1B). DH26 has a life span which is indistinguishable from N2 at all temperatures (data within KLASS 1977; JOHNSON 1984; JOHNSON and McCAFFREY 1985). Again, all of the mutant stocks were significantly longer-lived ($P < 0.001$). Mean life spans were extended 50–80% over that of DH26, while maximum life spans were increased 60–150%. Another mutant also described by KLASS, MK542, was not assayed at this time but was later shown to have mean and maximum life spans comparable to MK546.

Since the three Age mutants, MK31, MK542 and MK546, contain mutations leading to longer life (*age-1*), are mutant at the *unc-31* locus (FRIEDMAN and JOHNSON 1988), and were isolated in the same mutant

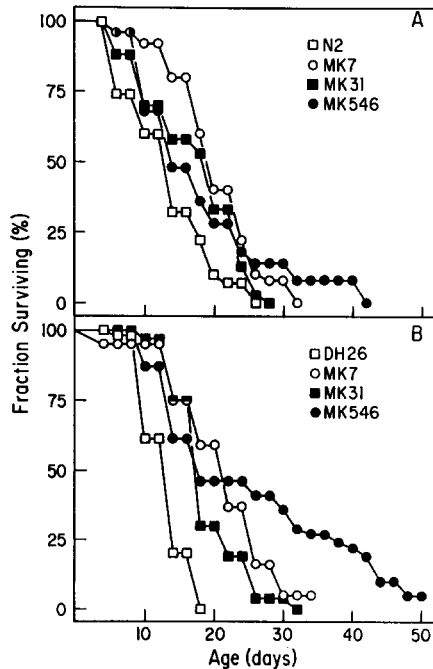


FIGURE 1.—Survival curves of MK7, MK31 and MK546 are compared to N2 or DH26; all survivals were at 25°. Mean life spans \pm SEM and probability of significance of survival in comparison to wild type are (A) N2, 13.7 ± 1.0 days; MK7, 20.9 ± 0.9 days ($P < 0.001$); MK31, 17.5 ± 1.0 days ($P < 0.01$); MK546, 18.4 ± 1.3 days ($P < 0.01$); (B) DH26, 13.1 ± 0.5 ; MK7, 20.7 ± 1.6 ($P < 0.001$); MK31, 19.2 ± 0.9 ($P < 0.001$); MK546, 23.8 ± 2.0 ($P < 0.001$). For MK7 and MK546 in experiment B, the last surviving individual(s) were lost before death.

hunt in such a way that their independence cannot be assured (KLASS 1983 and personal communication), it is possible that these Age mutant strains contain identical *age-1* alleles. We therefore chose to concentrate on the genetic analysis of MK546, one of the longest-lived strains.

Many measurements of the life span of MK546 have been made throughout these studies. A comprehensive summary (Table 1) shows that MK546 [*age-1(hx546) fer-15(b26ts) II; unc-31(z1) IV*] averages a 40% increase in life expectancy and a 60% increase in maximum life span at 20° and a 65% increase in life expectancy and a 110% increase in maximum life span at 25°. TJ401 [*age-1(hx546) fer-15(b26ts) II; unc-31⁺ IV*], was derived from a backcross of MK546 to N2, (Figure 4A) and shows a 65% increase in life expectancy and averages a 50–55% increase in maximum life span at both temperatures (Table 1). Moreover, males carrying the *age-1(hx546)* allele are also longer-lived than wild type showing a 30–50% increase in mean and maximum life span (Table 1). Considerable variation is seen between replicates especially at 25° (Table 1); this results from as yet uncontrolled environmental variance between experiments (JOHNSON and WOOD 1982) and necessitates the inclusion of a reference strain with wild-type life

span, either N2 or DH26 [*fer-15(b26ts)*], in all experiments.

Self-fertility is decreased in the Age strains: Incidental observations had suggested to us that the Age mutant strains had a significant reduction in fitness as compared to wild type. Indeed, quantitative analyses of the number of progeny produced by the Age mutants showed that MK31, MK542 and MK546 produce significantly fewer progeny ($P < 0.001$; FRIEDMAN and JOHNSON 1988). MK546 displayed a dramatic decrease in hermaphrodite self-fertility to levels only 10–20% that of wild type or DH26 (Table 2). TJ401 also displayed a similar decrease to levels only 20% that of wild type. This decreased reproductive fitness of *age-1(hx546)* may explain the skewed observations in favor of the wild-type *age-1⁺* allele in preliminary studies on the segregation of the Age phenotype (JOHNSON 1986).

Inheritance of mortality kinetics, stability of Age phenotype: In initial observations, MK546 seemed to exhibit biphasic mortality in which some individuals began dying relatively early in life while siblings lived several times longer (Figure 2A). Such mortality kinetics presumably reflect the action of the *age-1(hx546)* mutant allele but could result from the presence of two genetically distinct subpopulations in the MK546 strain. To distinguish between these hypotheses, the life spans of individual hermaphrodites were compared with the life expectancy of their respective progeny. If MK546 were composed of two or more genetically distinct subpopulations, we would expect a positive correlation between the life expectancy of progeny and the life span of their respective parent. When we compared progeny whose parents died early in life, in mid life, or late in life, we found no significant difference between the respective survival curves (Figure 2B; $P = 0.39$). Nor did we see any significant correlation between life spans of individual parents and the life expectancy of their respective progeny (Figure 2C; $r = -0.12$; not significant).

Segregation of Age in backcross populations: We wished to determine how many mutational events specified the differences between N2 and MK546 in length of life. Since it is impossible to follow the segregation of a gene that affects life span such as *age-1* in individual F₂ progeny, we thought it judicious to perform a preliminary experiment by measuring survival on populations of MK546, N2, F₁ cross progeny, and progeny from successive backcrosses of the F₁ to MK546 (Figure 3; B₁ and B_{1,1}). The life expectancy of the F₁ (*age-1(hx546)/age-1⁺*) was not significantly different from N2 ($P = 0.41$; Figure 3C and Table 3) showing that *age-1(hx546)* is a recessive mutant allele. F₁ males mated with MK546 yielded backcross progeny (B₁), 50% of which are expected to be *age-1(hx546)* homozygotes and thus long-lived (Figure 3, A and B).

TABLE 1
Life spans of *age-1(hx546)* and wild-type strains

		Strain and genotype														
		N2 <i>age-1⁺ fer-15⁺</i>			DH26 <i>age-1⁺ fer-15(b26)</i>			MK546 <i>age-1(hx546) fer-15(b26) unc-31(z1)</i>				TJ401 <i>age-1(hx546) fer-15(b26)</i>				
		Life span (days)			Life span (days)			Life span (days)				Life span (days)				
Date ^a	Sex	Temp	Mean ± SEM	Max	N	Mean ± SEM	Max	N	Mean ± SEM	Max	N	P ^b	Mean ± SEM	Max	N	P ^b
3/5/84	Herm	20	23.2 ± 1.2	38	40				34.5 ± 4.2	57	33	0.02	32.6 ± 4.2	47	8	0.03
7/23/84	Herm	20	23.6 ± 1.4	41	29				26.8 ± 2.8	65	21	0.18	37.1 ± 2.3	58	37	<0.001
	Male	20	20.6 ± 1.1	29	21								26.1 ± 0.8	35	46	0.001
8/17/84	Herm	20	20.5 ± 1.6	33	19				28.0 ± 4.1	48	21	0.25				
2/11/85	Herm	20	21.7 ± 1.9	37	12				32.4 ± 8.9	58	5	0.07				
5/24/85	Herm	20	24.1 ± 1.0	35	43				39.1 ± 4.0	68	12	0.001	39.5 ± 1.9	61	34	<0.001
2/5/83	Herm	25	13.7 ± 1.0	27	42				18.4 ± 1.3	42	49	0.01				
4/19/83	Herm	25				13.1 ± 0.5	19	46	23.8 ± 2.0	48	43	<0.001				
4/10/85	Herm	25	16.3 ± 0.7	17	9				36.9 ± 4.2	56	14	0.002	25.1 ± 2.2	39	16	0.03
2/10/86	Herm	25				17.6 ± 0.7	29	24	27.5 ± 2.4	49	25	0.01	27.9 ± 1.7	43	25	<0.001
	Male	25				5.1 ± 0.3	6	13					10.0 ± 0.5	18	24	<0.001
11/8/86	Herm	25				16.3 ± 0.9	23	42	19.7 ± 1.4	36	23	0.06	24.7 ± 1.1	43	44	<0.001
	Male	25				8.8 ± 0.5	14	46					12.2 ± 0.5	21	47	<0.001

^a Date on which cohort was born.

^b Probability that differences are by chance when compared with N2 or DH26 using the modified Kruskal-Wallis procedure.

TABLE 2
Hermaphrodite self-fertilities

		Strain and genotype							
		N2 wild type		DH26 <i>fer-15(b26) age-1⁺</i>		MK546 <i>fer-15(b26) age-1(hx546) unc-31(z1)</i>		TJ401 <i>fer-15(b26) age-1(hx546)</i>	
Date ^a		Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N
7/27/84		224.3 ± 55.7	3			23.7 ± 5.2	3	53.7 ± 9.2	3
8/10/84		341.5 ± 9.4	3			18.0 ± 0.8	3	80.5 ± 24.1	3
2/14/85		254.3 ± 11.0	3			48.0 ± 17.5	3		
6/20/85		103.5 ± 15.9	3					81.3 ± 10.3	3
								68.3 ± 2.7	3
1/23/86		226.4 ± 12.7	5	185.5 ± 26.1	4	31.8 ± 4.2	4	73.2 ± 4.3	5
2/11/86				234.4 ± 19.7	5			60.2 ± 8.6	5
3/26/86		253.4 ± 15.6	5	258.3 ± 16.1	4	32	1		
10/26/86		302.6 ± 7.3	10	317.4 ± 14.9	7	30.9 ± 3.8	10	71.9 ± 3.9	10

^a Date on which experimental cohorts were born.

B₁ progeny display a survival curve in which about 1/2 of the progeny had survival kinetics similar to N2 and nearly 1/2 lived longer, similar to MK546 (Figure 3C). Another backcross of the B₁ males to MK546 yielded progeny (B_{1,1}) which displayed a survival curve where about 1/4 of the progeny had wild-type life spans and 3/4 lived longer, with life spans similar to MK546 (Figure 3C). These data are consistent with the segregation of a single gene that specifies the increased life span of MK546 but are difficult to interpret precisely.

The life expectancy (mean life span) of these populations shows the same trend as do survival curves (Table 3). The F₁ displays a life expectancy not significantly different from N2, and much shorter than MK546, as expected if MK546 carries a mutation

recessive to wild type. The B₁ displays a mean life span that is consistent with it being 1/2 Age and 1/2 non-Age, and the B_{1,1} displays a mean life span that is consistent with it being 3/4 Age and 1/4 non-Age. Since maximum life span is a characteristic of an individual, we anticipate maximum life spans of the B₁ and B_{1,1} to be comparable to the MK546 parent; the B₁ have a lower maximum life span than might be expected but the B_{1,1} have a maximum life span identical to MK546 (Figure 3C, Table 3). As also expected, the coefficient of variation is highest in the B₁ and slightly smaller (not significant) in the B_{1,1}. These data are consistent with the interpretation that the major increase in length of life results from a mutational event in a single nuclear gene.

Segregation of Age in inbred reisolates: Based

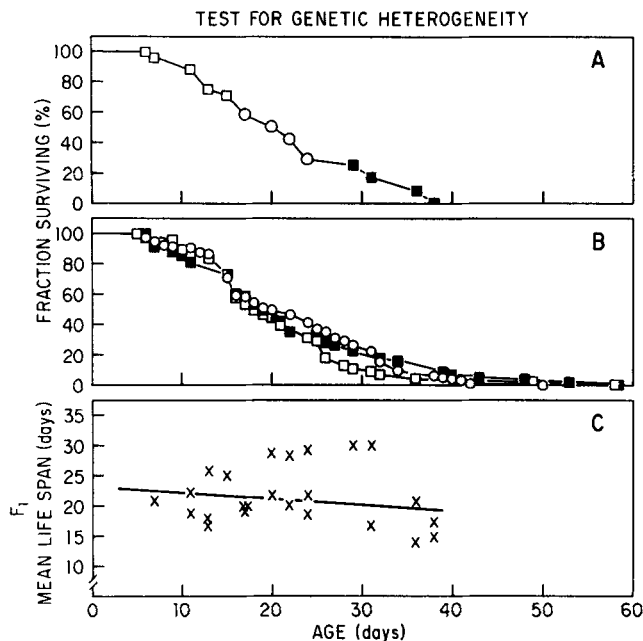


FIGURE 2.—Comparison of parental and progeny life spans. (A) Survival curve of MK546 ($N = 24$). (B) Survival curves of progeny were obtained by combining survival curves of progeny whose parents died during the early (\square — \square), median (\circ — \circ), or late (\blacksquare — \blacksquare) periods of life as shown in (A), ($P = 0.39$). (C) Linear regression between individual parental life spans and mean life spans of their respective progeny ($r = -0.12$, not significant).

upon the results of these preliminary experiments we decided to pursue a novel protocol in which we followed the segregation of length of life in inbred populations derived from backcrosses of MK546 to N2. The assessment of genotype relies on the measurement of length of life, which is obtained from a quantitative analysis that must be performed on a population of individuals. It is most helpful if each population is composed of genotypically identical individuals. Moreover, *age-1(hx546)/age-1⁺* heterozygotes would be scored as wild type but would segregate long-lived progeny. To confirm the results of backcrosses and to minimize problems resulting from nonuniform populations, we followed the scheme described in MATERIALS AND METHODS and shown in Figure 4A. Briefly, instead of scoring phenotype in the F_2 generation, F_2 were first inbred by self-fertilization before measuring life span. This results in stocks that are homozygous and therefore unlikely to segregate new phenotypes which facilitates the use of these stocks in further genetic studies.

When life span was determined on these reisolates, two distinct groups were detectable with respect to length of life. One class (*fer-15 age-1*) were long-lived, having mean life spans more than 120% that of wild type. The second class (*fer-15⁺ age-1⁺*) had mean life spans that were between 80% and 120% that of wild type (Figure 4, B and C, and JOHNSON 1986). The ratios of normal to long-lived were 10:3 in experiment

1 (Figure 4B) and 12:7 in experiment 2 (Figure 4C); both of these segregation ratios are consistent with the single gene model ($\chi^2 = 3.84$, $P = 0.05$, in A and $\chi^2 = 1.37$, not significant, in B); but also fit a two-gene model wherein two independently segregating loci must both be mutant to observe long life ($\chi^2 = 0.18$ for B and 1.72 for C, both not significant). In similar crosses with a second long-lived strain MK542 [*age-1(hx542) fer-15(b26ts) II; unc-31(z2) IV*] similar segregation of two distinct populations were observed (Figure 4D); the segregation ratios from this latter cross are inconsistent with a two-gene model ($\chi^2 = 10.23$, $P < 0.005$) but fit well the predictions of the one-gene model ($\chi^2 = 0.12$, not significant). Moreover, with one possible exception (Figure 4B) all long-lived, low-fertility strains were also *fer-15*, showing that the *age-1* locus is tightly linked to *fer-15* and maps to the middle of linkage group II.

Length of development is normal in the Age mutants: Longer life could result from extending a process functioning only in development, only post-developmentally, or during both periods. Prolonging the period of development either as dauer larva (KLASS and HIRSH 1976) or in other larval stages (JOHNSON *et al.* 1984) has been shown to result in an increase in mean life span of 1 day for each day development is prolonged. If longer life were either due entirely to extensions in development or to extensions affecting both development and adult life span, the long-lived stocks would have a longer developmental period. However, the length of embryonic development is unchanged as are the lengths of each larval period and the time of reproductive maturity (Figure 5A). As mentioned in MATERIALS AND METHODS and by KLASS (1983), altered behavior in the original Age mutant strains (MK31, MK542 and MK546) was quantitatively discernible and results from mutational event(s) in the *unc-31* locus on linkage group IV. We have not been able to repeat KLASS's observations that rates of food uptake are decreased in the mutant strains (JOHNSON 1986 and D. B. FRIEDMAN and T. E. JOHNSON, in preparation); indeed, preliminary findings (M. CRUZEN and T. E. JOHNSON, unpublished data) suggest that the *age-1* mutants may have a reduced metabolic rate.

Length of the fecund period in *age-1* hermaphrodites is the same as in *age-1⁺* populations. Although fewer viable eggs are laid in any given period, the total length of the fertile period is the same for *age-1* stocks as it is for wild type (Figure 5B). There are several statistically significant differences in relative age-specific fertility. For example, MK546 and TJ401 produced about 80% of their total progeny in the first 30 hr of hermaphrodite fertility while N2 and DH26 produced only about 60%. In both *age-1⁺* and *age-*

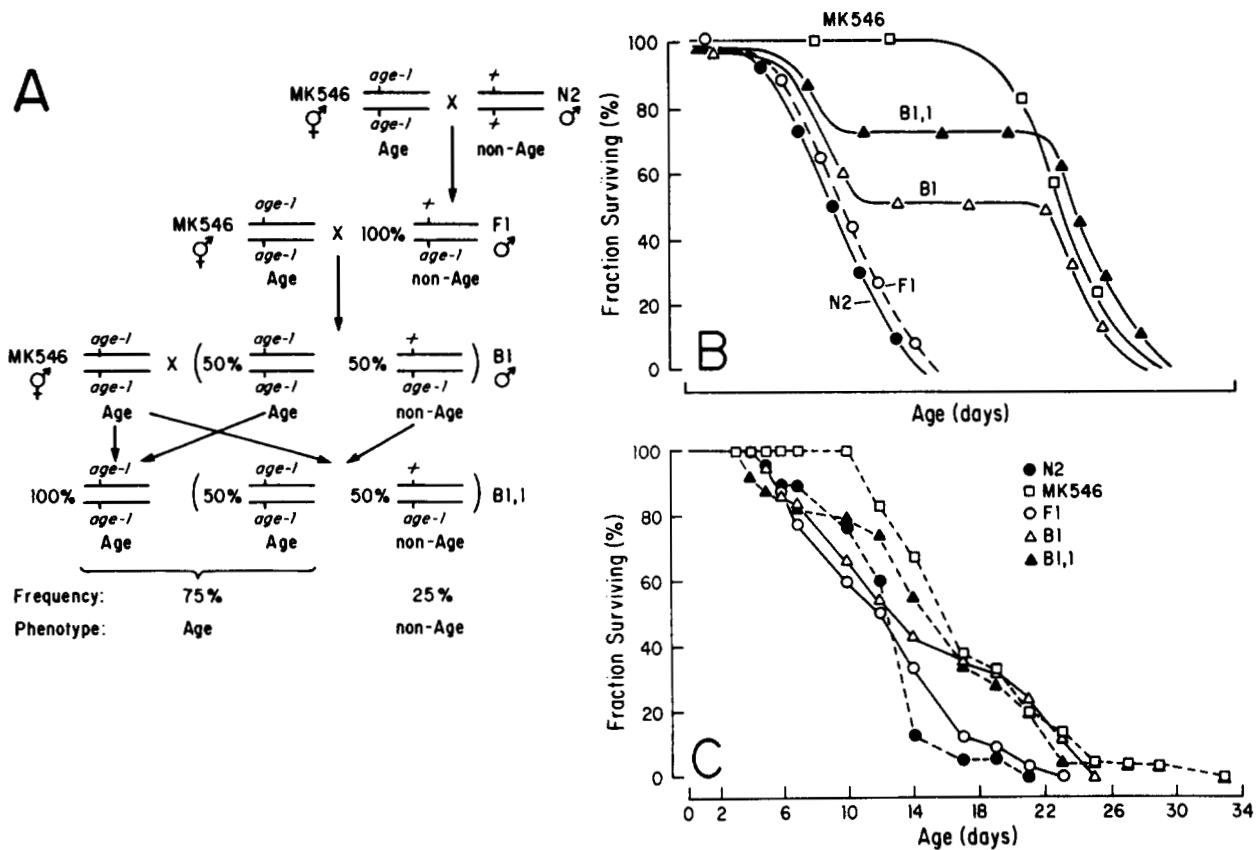


FIGURE 3.—Scheme for following the segregation of a life span determining gene in genetically heterogeneous populations. (A) MK546 hermaphrodites were backcrossed to N2 males to measure life span on the F₁. F₁ males were backcrossed to MK546 hermaphrodites to obtain a B₁ generation which were similarly 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.

TABLE 3
Mean life spans of successive backcrosses to MK546

Generation	Life span (days)				Probability ^c	
	Observed ^a			Expected ^b mean	N2	MK546
	Mean ± SEM	Max	N			
N2	12.6 ± 0.6	21	40			
MK546	17.2 ± 1.1	32	23		0.002	
F ₁	11.9 ± 0.9	23	30	12.6	0.407	0.002
B ₁	13.9 ± 1.2	25	33	14.9	0.706	0.142
B _{1,1}	15.3 ± 1.6	32	22	16.0	0.181	0.598

^a Life spans shown were calculated from the survival data shown in Figure 3C.

^b Calculations of expected means were based on the model presented in Figure 3A.

^c Probability that differences are by chance when compared with N2 or MK546 using the modified Kruskal-Wallis procedure.

l(hx546) strains a few percent of the progeny are produced at seven days of age and later.

DISCUSSION

Mutants have extended maximum life span: We have verified the observations of KLASS (1983) that four EMS-induced mutants isolated after brute force

screens for extended life do indeed have significant extensions in both mean (40–65%) and maximum (60–110%) life span (Figure 1, Table 1). We also extended these observations to males (TJ401, Table 1) carrying the *age-1(hx546)* mutant which also showed extensions in mean and maximum life span of 30–60%, comparable to that of hermaphrodites (Table 1). The increase in life span is thus observed in a variety of environments (liquid as in this paper or solid medium as in KLASS 1983) and maintenance temperatures (20° or 25°). Long life results from an increase in the probability of survival at all chronological ages including an increase in maximum life span.

MK546 [*age-1(hx546) fer-15(b26ts) II; unc-31(z1) IV*] displays mortality kinetics that seem initially to be somewhat dissimilar to that of DH26 (Figure 2, A and B) in that there appeared to be two phases of mortality. This difference in mortality was not heritable, although there is considerable variation among individuals in time of death all progeny have the same probability of survival (Figure 2, B and C).

Development is normal in the long-lived mutants: No significant change in the length of the embryonic period, in the lengths of any larval period, or in the

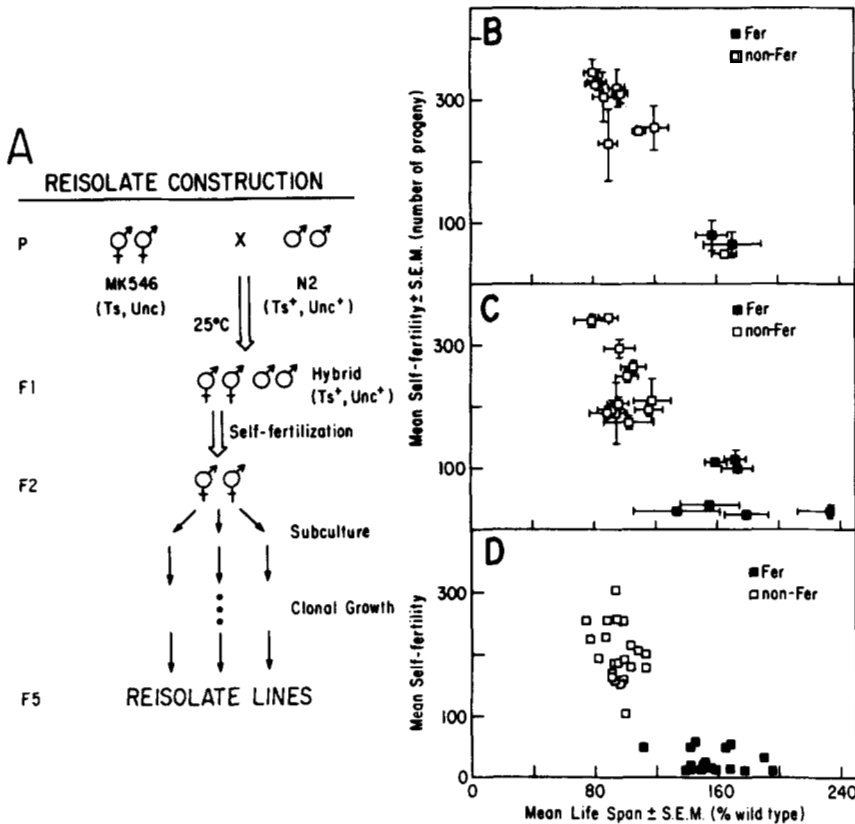


FIGURE 4.—(A) Method for constructing homozygous populations from crosses between N2 and MK546. (B and C) Life expectancy at 20° of reisolates from the cross of MK546 [*age-1(hx546) fer-15(b26ts) II; unc-31(z1) IV*] to N2 is plotted relative to hermaphrodite self-fertility. (B) F₅ reisolates from experiment 1, and (C) F₁₅ reisolates from experiment 2. (D) Life expectancy at 25° of F₁₀ 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 4D but ranged from 5 to 15% of the mean life span while self-fertility is the average of three to five hermaphrodites whose progeny were counted collectively rather than individually.

average time of reproductive maturity could be detected (Figure 5A). Slight changes in the age of reproductive maturity were observed as reported by KLASS (1983) but are not significant, given that these changes are minor and do not cosegregate with *age-1*.

All four long-lived strains had a significant reduction in hermaphrodite self-fertility (Table 2). This reduction ranged from only about 30% for MK7, the mutant with the shortest life span, to almost 80% for the *age-1* mutants: MK31, MK542 and MK546. Despite decreased fertility, there was no change in the length of the reproductive period as a result of the *age-1(hx546)* mutation (Figure 5B). Like wild-type hermaphrodites which can produce more than 500 progeny when continuously mated with young wild-type males (WARD and CARRELL 1979), *age-1* hermaphrodites can also be mated, and produce more progeny after mating (N. L. FOLTZ and T. E. JOHNSON, unpublished observations). This observation may suggest that low hermaphrodite self-fertility results from a lack of viable sperm. However, direct measurement suggests that *age-1* hermaphrodites have near normal numbers of sperm.

Longevity is specified by a single recessive mutation called *age-1*: Three lines of evidence suggest that the 30–70% increase in life expectancy characteristic of MK546 results from a single recessive mutational event. First, in backcrosses to wild type the Age characteristic behaves as a single autosomal recessive mu-

tation that increases life expectancy. Crosses between N2 and MK546 yield F₁ progeny whose length of survival is not significantly different from wild type (Figure 3, Table 3). The B₁ generation (backcross of F₁ to MK546) is composed of two distinct genotypes: about ½ are *age-1/age-1*⁺ heterozygotes and die with wild-type kinetics and about ½ are *age-1/age-1* homozygotes and are longer-lived; similarly, in the B_{1,1} generation about ¼ are short-lived (*age-1/age-1*⁺) and ¾ are long-lived (homozygous for *age-1*).

The second line of evidence is derived from the analysis of homozygous stocks derived from backcrosses of MK546 to N2. Backcrosses to wild type are necessary to separate mutations that may have been coincided by the typical mutational scheme used in *C. elegans* (BRENNER 1974) but were not performed by KLASS (1983). We used the first backcross as an opportune time for the analysis of the segregation of *age-1*. To facilitate this analysis, we followed a novel procedure in which F₂ individuals were inbred for several generations by self-fertilization yielding almost homozygous populations (Figure 4A). After such an inbreeding regimen, single gene mutants are expected to segregate 1:1 as in a haploid, rather than 3:1 as typical of the F₂ in a normal diploid cross. The 1:1 segregation of Age:non-Age lines is seen in the data shown in Figure 4, B–D, which best fit a model where Age is specified by a single recessive gene. The deficit of *age-1* progeny (Figure 4, B and C) may have been

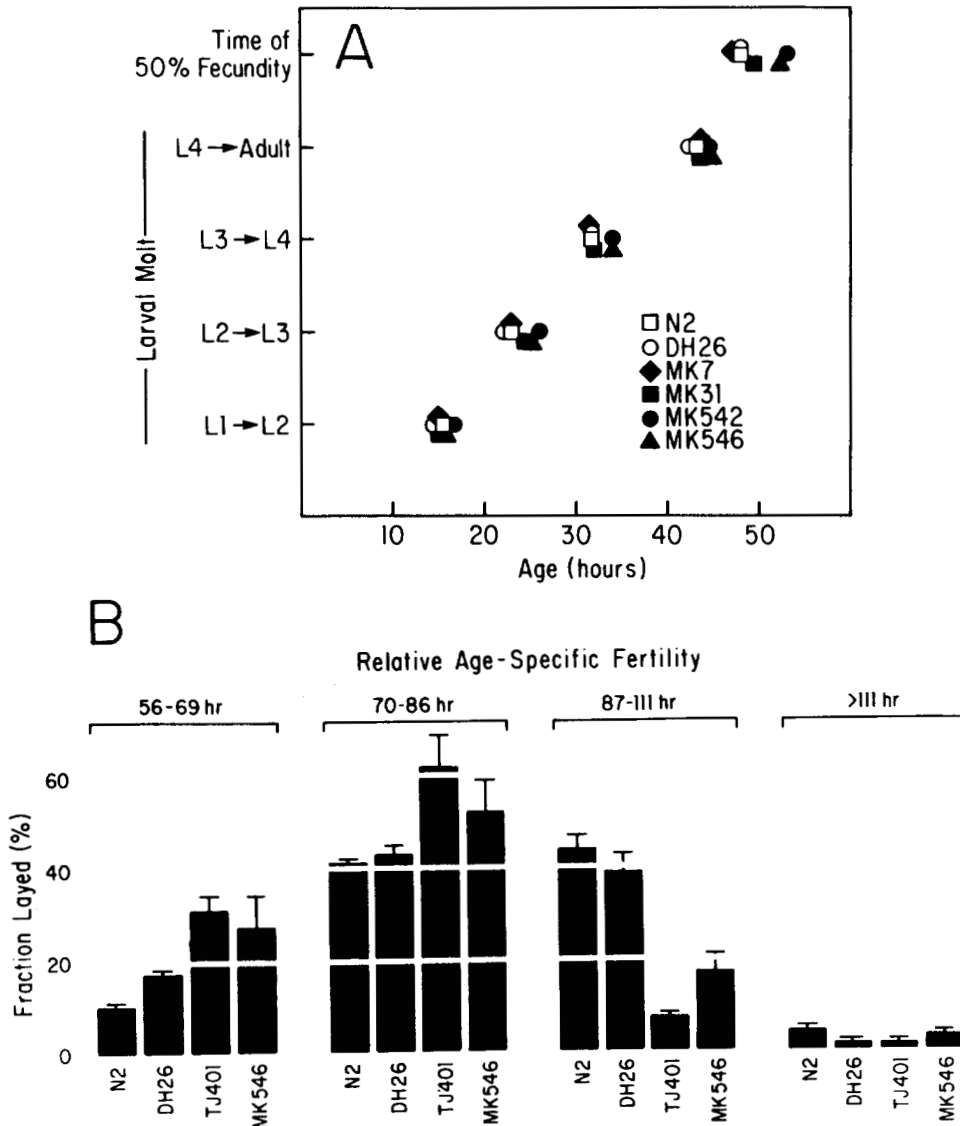


FIGURE 5.—(A) Time of intermolt lethargis and time of reproductive maturity for N2, DH26, MK7, MK31, MK542 and MK546. Points represent the time of minimal proportion of animals displaying pharyngeal pumping (CASSADA and RUSSELL 1975) and the time at which 50% of hermaphrodites carried visible eggs. Two of the three synchronous cultures were slightly asynchronous at the L3-L4 larval molt. (B) Relative age-specific fertilities of wild-type and *age-1* strains. Relative fertility (fraction of total progeny produced) was obtained from measurements on progeny produced during four consecutive periods of the fertile portion of adult life, 56 hr after hatch to sterility. Total hermaphrodite self-fertilities (mean \pm SEM) were as follows: N2: 302.6 ± 7.3 ($N = 10$); DH26: 317.4 ± 14.9 ($N = 7$); TJ401: 71.9 ± 3.9 ($N = 9$); MK546: 30.9 ± 3.8 ($N = 10$).

due to the fivefold reduction in self-fertility associated with *age-1* which makes *age-1*⁺ individuals reproductively more fit resulting in their preferential selection during the generation of inbred populations.

The most compelling line of evidence that the Age phenotype results from a single mutation is the cosegregation of *age-1* with *fer-15* (Figure 4B). All Age isolates, with one possible exception that could not be confirmed, were also *fer-15(b26ts)*. The lack of independent assortment was highly significant (Figure 4, B-D, $P \ll 0.001$). It was not expected, *a priori*, that *age-1* should map near to *fer-15*, a temperature-sensitive mutant used by KLASS (1983) to facilitate screens for long-lived mutants by making the strains self-sterile at 25°. We have no explanation for this fortuitous association but continue to explore alternatives other than the simplest conclusion which has been presented here. Assay of *age-1(hx546)* opposite small deficiencies in this region of linkage group II (SIGURD-

SON, SPANIER and HERMAN 1984) and four-point crosses using *dpy-10* and *unc-4* as flanking markers, confirms the tight linkage between the low self-fertility phenotype in *age-1* and *fer-15* (P. A. FITZPATRICK, J. E. SHOEMAKER, D. B. FRIEDMAN and T. E. JOHNSON, unpublished data).

Increased life expectancy and lower hermaphrodite self-fertility may be pleiotropic effects of *age-1(hx546)*: All long-lived (Age) isolates have decreased hermaphrodite self-fertility and all non-Age isolates have normal hermaphrodite self-fertility, a highly significant deviation from the expectation of independent assortment ($P \ll 0.001$). Even if we alter the cutoff points and score as Age those reisolates with life expectancies intermediate to N2 and the two *age-1* strains, MK546 and MK542 (Figure 4, B-D), we still observe significant deviations from that expected for independent assortment ($P \ll 0.001$). The simplest model is that *age-1* is pleiotropic and results in both

lower fertility and longer life. The fact that we have not been able to separate *age-1* from *fer-15* leads us to be cautious about this interpretation but neither *fer-15* nor other loci that affect sperm formation prolong life (Table 1 and T. E. JOHNSON, unpublished data).

Increased life expectancy is not associated with changes in development: There are three alternative ways in which *age-1* might extend life. (1) *age-1* is a gene that is expressed only during the adult phase of life: life-extension occurs solely as a result of affecting some adult-stage function that causes mortality and *age-1* has no role in altering the timing of developmental events. (2) *age-1* functions in both development and in the adult phase: life-extension results from coordinate extensions of both development and the adult phases of life. (3) *age-1* functions during development only: extended life expectancy results solely from extension in the time of development as is observed when development is prolonged by environmental manipulations that either produce dauer larvae (KLASS and HIRSH 1976) or arrest development by starvation (JOHNSON *et al.* 1984).

age-1 does not function to alter the growth rate or timing of developmental events. There is no evidence to suggest that *age-1* results from mutations in a "clock" as has been hypothesized to account for programmed aging (HAYFLICK 1987; JOHNSON 1987). Indeed, except for the effect in lengthening life there is no evidence that *age-1* has any effect on a hypothetical "rate of aging." For example, *age-1* does not play a role in determining the length of the fecund period of life. The life-extension characteristic of *age-1* presumably results from the elimination or reduction of some process that shortens the life of the wild-type nematode. Moreover, *age-1* functions to prolong the life of the male, although to a somewhat lesser extent (Table 1).

No other traits are altered by *age-1(hx546)*: KLASS (1983) attributed the long life of MK546 to a decreased rate of food uptake presumably resulting from the obvious uncoordinated phenotype of MK546 but these observations have not been replicable in our hands (JOHNSON 1986 and D. B. FRIEDMAN and T. E. JOHNSON, unpublished data). Although the original mutant strains were uncoordinated and male-sterile, *age-1* reisolates can have normal behavior and chemotaxis and are male-fertile, although the male-fertility of *age-1* strains may be reduced (T. E. JOHNSON, unpublished data). We ascribe the male-sterile phenotype of MK546, at least in part, to the *unc-31(z1)* allele, since *unc-31* reduces male-fertility to less than 1% that of wild type (HODGKIN 1983).

Self-fertilizing hermaphrodites have significant advantages in the analysis of quantitative traits: The assessment of genotype for quantitative traits is simplified if all members of the population are genetically

identical. Problems in genotype assessment of heterogeneous populations can be seen graphically in Figure 3C where *age-1* is still segregating in the B₁ and B_{1,1} leading to hybrid survival curves (see also GOULD and CLARK 1977). We have circumvented this problem by generating homogeneous populations using a novel inbreeding protocol that simplifies interpretations by producing a population of identical individuals. A second major advantage of this procedure is that it leads to genetically stable populations which simplifies both confirmation of genotype and the analysis of multiple phenotypic traits on the same genotype; these lines are also useful in further genetic analyses. There are two reasons for the success of this procedure in *C. elegans*: the rapidity with which inbreeding can be achieved in a self-fertilizing hermaphrodite (FALCONER 1981) and the lack of inbreeding depression for life-history traits in *C. elegans* (JOHNSON and WOOD 1982; T. E. JOHNSON, unpublished data) which simplifies the inference of genotype from phenotypic measurements.

Significance of these findings for the study of aging: Among the several evolutionary theories proposed to explain the phenomenon of senescence, two alternative models stand out: adaptive and nonadaptive. It has been popular to hypothesize the existence of genes with time-keeping functions that may play a role in programmed death or senescence and may have adaptive significance in the evolution of aging and senescence but there are good critiques that forcefully argue against such adaptive theories (for a review of such theories and criticisms see CHARLESWORTH 1980 or KIRKWOOD 1985). In contrast, MEDAWAR (1952) and HAMILTON (1966) hypothesized that senescence is nonadaptive and might evolve because of lower selection against deleterious genes that function late in life. An alternative nonadaptive theory is that of negative pleiotropy in which genes that were selectively advantageous early in life but harmful later would be selected for (MEDAWAR 1952; WILLIAMS 1957); such pleiotropic relationships between, for example, increased life expectancy and reduced reproductive fitness could explain the evolution of senescence (CHARLESWORTH 1980; ROSE 1985).

Both ROSE (1984) and LUCKINBILL *et al.* (1984) demonstrated negative pleiotropy in fitness trade-offs between life span and early fertility in *Drosophila* that had been selected for increased late-life reproductive fitness. Negative pleiotropy is consistent with our current model of *age-1*. The *age-1*⁺ allele has presumably been selected because it increases reproductive fitness fourfold, while the decrease in life expectancy is a pleiotropic side effect of this gene. This lowered expectancy of life results from the action of *age-1*⁺ but is itself without evolutionary consequence because the loss of life is entirely postreproductive.

age-1 should not be interpreted as an example of a genetic locus whose primary function is to keep time so as to cause programmed aging or senescence: Indeed, such loci are unlikely to exist in iteroparous organisms because of a lack of selective pressure for the evolution of such loci. It seems much more likely that the normal *age-1* gene product has some physiologic role, perhaps in sperm formation and/or intermediary metabolism, that leads to an increased rate of mortality.

The simplest model consistent with our results is that *age-1(hx546)* is a recessive mutant on linkage group II which causes a five-fold decrease in hermaphrodite fertility and a 1.4–1.7-fold increase in life expectancy and maximum life span as compared to the *age-1⁺* allele. The *age-1(hx546)* mutation prolongs the life of males (Table 1) and so must play some role in male physiology or metabolism as well. Many mutations increase the rate with which an organism ages or appears to age but the *age-1* locus is the only identified and well-characterized example of a gene whose *normal* function results in the acceleration of the time of onset and/or rate of progression of an aging process.

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