Genetic, Behavioral and Environmental Determinants of Male Longevity in *Caenorhabditis elegans*

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ABSTRACT

Males of the nematode *Caenorhabditis elegans* are shorter lived than hermaphrodites when maintained in single-sex groups. We observed that groups of young males form clumps and that solitary males live longer, indicating that male-male interactions reduce life span. By contrast, grouped or isolated hermaphrodites exhibited the same longevity. In one wild isolate of *C. elegans*, AB2, there was evidence of copulation between males. Nine uncoordinated (*unc*) mutations were used to block clumping behavior. These mutations had little effect on hermaphrodite life span in most cases, yet many increased male longevity even beyond that of solitary wild-type males. In one case, the neuronal function mutant *unc-64(e246)*, hermaphrodite life span was also increased by up to 60%. The longevity of *unc-4(e120)*, *unc-13(e51)*, and *unc-32(e189)* males exceeded that of hermaphrodites by 70–120%. This difference appears to reflect a difference in sex-specific life span potential revealed in the absence of male behavior that is detrimental to survival. The greater longevity of males appears not to be affected by *daf-2*, but is influenced by *daf-16*. In the absence of male-male interactions, median (but not maximum) male life span was variable. This variability was reduced when dead bacteria were used as food. Maintenance on dead bacteria extended both male and hermaphrodite longevity.

THE role of genes in determining the rate of aging, and the primary mechanisms underlying the aging process, are currently the subject of intense investigation. The nematode *Caenorhabditis elegans* is proving to be a useful model organism to address these questions, thanks to the identification of several classes of mutation that extend adult longevity (reviewed in Kenyon 1997). A hitherto little-studied aspect of the biology of aging in *C. elegans* is the effect of gender on life span.

In animal species where sex differences in life span have been observed, it is the male that is typically shorter lived (Comfort 1979; Smith 1989). For example, in 1990 in the United States, the mean life expectancy of men at birth was 6.8 years less than that of women (Metropolitan Life 1992). Likewise, *C. elegans* males (XO) have been observed to be shorter lived than hermaphrodites (XX), whether maintained in liquid culture (Johnson and Wood 1982; Johnson and Hutchinson 1993) or on agar plates (Gems and Riddle 1996). (The *C. elegans* hermaphrodite is essentially a female whose germline produces and stores sperm prior to oogenesis.)

It is unclear whether the observed shorter life span of males of many species is the result of fundamental differences between the sexes in the underlying aging process, or of factors relatively distal to aging, such as behavior. Sex-specific life spans in the medfly *Ceratitis capitata* have been described as resulting from an underlying "constitutional" longevity minus the deleterious effects of reproductive biology, *e.g.*, progeny production and effects of reproductive hormones; and sex-related behavior, *e.g.*, seeking and competing for mates, or territorial defense (Carey *et al.* 1995). In *C. elegans*, mating with hermaphrodites slightly increases male life span relative to that of grouped, unmated males (Gems and Riddle 1996). Mating reduces hermaphrodite life span by up to half (Gems and Riddle 1996). Reproduction by self-fertilization does not appear to affect life span (Friedman and Johnson 1988; Kenyon *et al.* 1993), although the potential hermaphrodite life span is reduced by the presence of the germline (Hsin and Kenyon 1999).

In this study we investigate the sex-specific constitutional longevity of *C. elegans* by excluding key elements of its reproductive biology and behavior. The results reveal that aspects of male behavior, including homosexual interactions between males, greatly reduce life span. If these behaviors are blocked, the potential longevity of males exceeds that of hermaphrodites by a factor of 1.7–2.2. This sexual dimorphism in longevity appears not to be mediated by *daf-2*, a gene known to regulate larval development and adult life span. However, it is mediated by *daf-16*, which acts downstream of *daf-2*.

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MATERIALS AND METHODS

Media and strains: Animals were maintained on NG agar plates (60-mm diameter) with *Escherichia coli* OP50 as a food source (Brenner 1974). AB2, CB4855, PA2, and RC301 are *C. elegans* wild isolates in which males deposit copulatory plugs upon hermaphrodites (Hodgkin and Doniach 1997). The wild-type Bristol (N2) strain was taken from the Riddle laboratory strain collection. The male stock DRM, from which the current Caenorhabditis Genetics Center N2 male stock is derived, was used. Some strains were first backcrossed three times to the DRM strain to reduce the potential effect of variation in the genetic backgrounds on life span. In this way GA2 [*unc-13(e51) I*] was derived from CB51 [*unc-13(e51)*], GA12 [*unc-4(e120) II*] from CB120 [*unc-4(e120)*], GA16 [*unc-32(e189) III*] from DR129 [*daf-2(e1370*ts*) unc-32(e189)*], and GA14 [*fog-2(q71) V*] from JK574 [*fog-2(q71)*]. GA27 [*fer-15(b26) II*; *unc-64(e246) III*] was derived from DR1620 and DH26 [*fer-15(b26)*]. Other mutants used were LG I, *daf-16(m26),* CB193 [*unc-29(e193)*], DR49 [*unc-35(e259)*]; LG III, *daf-2(m120*ts*), daf-2(m577*ts*), tra-1(e1099), dpy-18(e1096),* DR1620 [*unc-64(e246)*]; LG IV, CB138 [*unc-24(e138)*]; LG V, DR96 [*unc-76(e911)*]; and LG X, *lon-2(e678), unc-10(e102),* CB78 [*unc-6(e78)*]. Abbreviations for phenotypes are as follows: Age, extension of adult life span; Daf-c, constitutive dauer larva formation; Daf-d, unable to form dauer larvae; Fog, feminization of germline; Lon, long body; ME, mating efficiency; and Unc, uncoordinated.

Strain construction and preparation of males: The backcrossed *fog-2(q71)* strain was prepared as follows. *fog-2* females were mated with N2 (DRM) males, and F_1 *fog-2(q71)*/+ males and hermaphrodites were crossed. Approximately 25% of the resulting F_2 animals were expected to be *fog-2* homozygotes. From the F_2, late fourth larval stage (L4) hermaphrodites or females were picked individually and incubated overnight at 25°. *fog-2* homozygotes, identified by the absence of eggs laid, were crossed with single F_2 males. L4 hermaphrodite (or female) progeny from these crosses were examined for self-progeny, the absence of which indicated that both parents were *fog-2* homozygotes.

Unc males were prepared by crossing N2 (DRM) males with Unc hermaphrodites. F_1 *unc*/+ males were crossed with Unc hermaphrodites, and Unc males were picked from among the resulting progeny. *unc-13; fog-2* males and females were prepared as follows. *fog-2* males were crossed with *unc-13* hermaphrodites, and then heterozygous F_2 males and hermaphrodites were crossed. From the resulting progeny Unc L4 hermaphrodites and females were isolated and incubated for 24 hr, and Unc Fog females were identified by the absence of eggs laid. These were crossed with *fog-2* males, giving rise to *unc-13*/+; *fog-2* males and females. These were crossed, and *unc-13; fog-2* males and females were picked from among the resulting progeny. *daf-2* males were obtained from male stocks raised at 15°, prepared using spontaneously formed males.

daf-16(m26) I; unc-4(e120) II and *daf-16(m26) I; unc-32(e189) III* strains were constructed by crossing *unc*/+ males with *daf-16* hermaphrodites. Single Unc L4 hermaphrodites from the F_2 were selfed to form starved populations, and *daf-16*; *unc* strains were identified by their failure to form dauer larvae. *daf-16*; *unc* males were prepared from these strains using *daf-16*; *unc*(+) males and backcrossing as described above for *daf-16*(+); *unc* males.

Culture of animals on UV-killed bacteria: Agar plates were spread with a suspension of *E. coli* strain OP50 and incubated for 6–8 hr at 37°, or overnight at 20°. Plates were then irradiated for 4 min at 100 mJ/cm^2 in a Spectrolinker XL-100 (Spectronics Corp., Westbury, NY), or a UV Stratalinker (Stratagene, La Jolla, CA), containing bulbs producing 254-nm radiation. The efficacy of the killing protocol was established as follows. UV-irradiated plates were incubated overnight at 37°. A sterile wire loop was then drawn across the entire surface of the lawn and used to inoculate a fresh plate. Absence of bacterial colonies after 24 hr at 37° indicated effective killing. On occasion, plates became contaminated with live *E. coli*, which was easily recognizable. Such plates were excluded from further study. To culture *C. elegans* on UV-killed bacteria, *E. coli*-free eggs were prepared using alkaline hypochlorite (Sulston and Hodgkin 1988).

Life-span analysis: Life spans were assayed as previously described (Gems and Riddle 1996). All measurements were made at 20° unless otherwise stated, with the zero time point as the late fourth larval stage. During the egg-laying period hermaphrodites were transferred daily to new plates. Males and older hermaphrodites were transferred to freshly streaked plates at approximately weekly intervals. When measuring male life span we found that a proportion of animals crawled up the wall of the petri plate and died from desiccation. This was a particular problem with solitary wild-type males and also both sexes of *unc-4* animals, perhaps due in the latter case to their inability to back. Such deaths were excluded from life-span analysis. Life-span measurements were initially carried out at the University of Missouri-Columbia and subsequently at University College London. For unknown reasons, at the latter site a higher proportion of males were lost due to wall climbing. Despite this, no consistent differences were observed in life-span measurements performed in Missouri and London.

In the demographic analysis, core mortality rate, $m(x)$, was calculated as $m(x) = [N(x) - N(x + 1)]/\{[N(x) + N(x + 1)]/2\}$, where x = age, in days, and $N(x)$ = number of animals alive on day x. Statistical analysis of mortality data and fitting to the Weibull equation were performed using the program JMP version 3.2.1 (SAS Institute).

RESULTS

Differences in age-related mortality between grouped males and hermaphrodites: The effect of gender on aging in *C. elegans* was examined using animals maintained on agar plates (Brenner 1974) in groups of 25–60. A long tail on the survival curve, representing a small minority of long-lived survivors, was seen in male but not hermaphrodite populations (Figure 1A). In hermaphrodites, age-specific mortality increased exponentially during most of the senescent phase, as previously observed in liquid culture (Johnson 1990). In males, daily mortality at first increased more rapidly with age than in hermaphrodites, reaching a peak on days 10–11 of up to 49%, after which it declined (Figure 1B). Thus, male life expectancy actually increased with age from days 11–13. We sought to understand the reason for the different pattern of mortality in males and hermaphrodites.

Male-male interactions: Single-sex groups of males, but not hermaphrodites, were observed to congregate into clumps of animals attempting to mate with one another (Figure 2A). Since mating shortens hermaphrodite life span (Gems and Riddle 1996), we examined the effect of population density on longevity to test whether male clumping might have a similar effect. Male life span proved to be inversely related to population density, whereas hermaphrodite life span was not

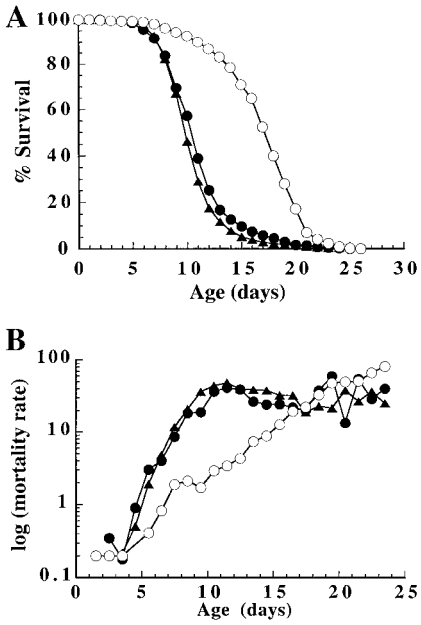

Figure 1.—Gender and adult life span in grouped wild-type animals at 20°. (A) Survival; (B) age-specific mortality. Open circles, hermaphrodites; summed data from 14 trials, 507 animals, of which 476 died apparently of old age; starting population per trial, 25–60 animals, mean 36 ± 15 (SE) animals. Median life span, 17.1 ± 1.1 days (mean from 13 trials, life span measured from late in the final larval stage); maximum life span, 22.1 ± 1.5 days (mean from 11 trials). Solid circles, males; summed data from 18 trials, 549 animals, of which 482 died apparently of old age; starting population per trial, 25–53 animals, mean 30 ± 10 animals. Median life span, 10.4 ± 0.7 days; maximum life span, 17.9 ± 2.4 days (mean from 18 trials). In both cases data are from trials performed over a 10-month period. Solid triangles, males; summed data from 32 simultaneous trials, 1811 animals, of which 1689 died apparently of old age; starting population per trial, 43–68 animals, mean 57 ± 4 animals. Median life span, 9.8 ± 0.6 days; maximum life span, 19.5 ± 3.7 days (mean from 31 trials). In each case only deaths resulting from old age were included in the analysis.

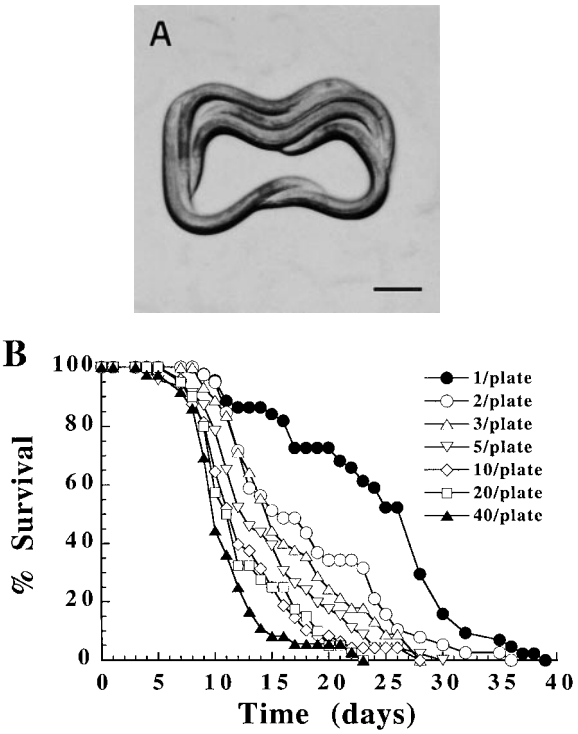

Figure 2.—Male clumping and its effect on survival. (A) Five wild-type *C. elegans* males exhibit clumping behavior. Bar, 0.2 mm. (B) Effect of population density on male survival. Initial sample size, 40–50.

density dependent (Figure 2B; Table 1). Male longevity, particularly when expressed as median life span, was reduced by the presence of even one other male.

Given that very few solitary males died before day 13, we conclude that most of the 80% of the grouped-male populations that died in that interval (Figure 1A) must have died as the result of male-male interactions. The lethal effect of male-male interactions was not immediate, since homosexual clumps dispersed after days 2–5 of adulthood (mean age when last clump observed, 2.9 ± 0.5 days; 9 trials), although infrequent male-male interactions may have occurred after day 5. After day 13 the surviving 20% of the grouped males exhibited an approximately constant mortality rate (Figure 1B). Given that senescing populations are characterized by an increase in mortality rate with increasing age, this 20% may have died from delayed effects of earlier male-male interactions, rather than senescence.

In the absence of male-male effects, median and maximum male life spans exceeded those of the hermaphrodite by ~20 and 70%, respectively. The maximum life span of solitary males was significantly greater than that of hermaphrodites ($P < 0.001$, Student's *t*-test), but median life span was not, due in large part to the relatively high variance in the median life span of solitary males. Greater variation in mean (but not maximum) life span in males than in females has also been reported in Drosophila (Lints *et al.* 1983).

Male life span is less variable on UV-killed bacteria: One factor contributing to the variation in male life span between trials was the age of the live bacterial lawn. Longevity was reduced when solitary males were maintained throughout their lives on the same bacterial lawn. Median and maximum life spans were 12.0 ± 0.2 (SE) and 16.0 ± 1.0 days, respectively (two trials, 61 deaths scored), compared to 18.9 ± 1.5 days and 35.8 ± 1.6 days, respectively, where animals were frequently transferred (every 1–4 days) to plates with freshly prepared bacterial lawns (6 trials, 188 deaths scored). Median and maximum life spans of solitary males were also more variable than those of grouped males or of hermaphrodites, even where frequent transfers were carried out. Varying the frequency of transfer had little effect on hermaphrodite life span where live *E. coli* was used, or male life span where UV-killed *E. coli* was used (data not shown).

TABLE 1

Effects of gender and population density on life span of wild-type animals

Sex	Animals per plate	Median life span (days)	Maximum life span (days)	No. of trials[a]
Hermaphrodite	1	17.0 ± 0.7	22.0 ± 0.6	4 (190, 166)
Hermaphrodite	25–26	16.8 ± 0.6	21.7 ± 0.5	6 (153, 149)
Male	1	20.3 ± 2.0	38.0 ± 1.1	4 (240, 201)
Male	2	15.9 ± 0.4*	37.5 ± 2.5	2 (80, 77)
Male	3	14.0 ± 0.3*	28.3 ± 4.5*	3 (131, 116)
Male	5	12.8 ± 0.5*	32.5 ± 4.5	2 (100, 90)
Male	10	11.6 ± 0.4**	24.3 ± 2.7**	3 (140, 133)
Male	20	10.9 ± 0.1**	22.0 ± 0.0***	2 (80, 76)
Male	40	10.0 ± 0.3**	24.0 ± 2.0**	2 (80, 76)

Life span is expressed as the mean of estimates from several trials, with the standard error given. * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$ (Student's *t*-test), where *P* expresses the probability of having the same life span as the solitary males.

[a] In parentheses are the total sample sizes of animals in the starting populations, and of those, the number dying of old age. Those excluded from the initial sample include animals that crawled up the wall of the petri plate and died from desiccation and hermaphrodites that died as the consequence of internal hatching of larvae.

When maintained on UV-killed bacteria, the median life span of solitary males was significantly higher than that of solitary hermaphrodites [$0.001 < P < 0.005$; male life span, 23.8 ± 0.6 days (12 trials, 536 deaths scored); hermaphrodite life span, 19.7 ± 0.6 days (6 trials, 623 deaths scored)]. The maximum life span of males was also significantly greater than that of hermaphrodites ($0.005 < P < 0.01$; 37.0 ± 1.3 days for males; 30.0 ± 2.1 days for hermaphrodites).

Maintenance on UV-killed bacteria resulted in significant extension of median and maximum life spans of hermaphrodites and median life span in males. Median and maximum life spans of hermaphrodites were increased by, on average, 16 and 36%, respectively ($0.01 < P < 0.025$ and $0.001 < P < 0.005$, respectively). Median life span in solitary males was increased by 17% ($0.025 < P < 0.05$). The effect of bacterial killing was even more marked in grouped males, where median life span was increased by 55%, suggesting that male-male interactions render them particularly susceptible to the effects of live *E. coli*.

It is unclear why maintenance on UV-killed bacteria increased nematode life span. Caloric restriction is one possibility. A reduction in caloric intake extends life and reduces reproductive output in many species (Masoro 1995), including *C. elegans* (Klass 1977). While ample UV-killed *E. coli* is available for consumption, it is possible that animals eat less of it. If this were the case, a reduction in fecundity might be expected. Brood sizes of hermaphrodites grown on live or dead *E. coli* were compared and found to be indistinguishable. In two independent trials, hermaphrodites raised on live *E. coli* produced on average 254 ± 18 (SE) and 313 ± 13 progeny per animal (12 animals per trial), whereas on killed *E. coli* they produced 278 ± 11 and 281 ± 10 progeny per animal (12 and 11 animals per trial, respectively). The timing of egg laying was similar in animals raised on live and dead *E. coli*. Furthermore, animals on UV-killed *E. coli* did not have a starved appearance. These observations suggest that caloric restriction is not the cause of the increased life span of *C. elegans* kept on UV-killed *E. coli*.

Evidence for homosexual mating in a wild isolate of *C. elegans*: The behavior of males of one *C. elegans* wild isolate is interesting in the context of the deleterious interactions between males. Males of a number of wild isolates of *C. elegans* deposit a gelatinous-looking mating plug (Barker 1995; Hodgkin and Doniach 1997) over the vulva at the end of copulation (Figure 3A). We observed that grouped males of the AB2 wild isolate of *C. elegans* deposited mating plugs on one another (Figure 3B). In three groups of 40–47 males each, 22–33% of the animals acquired such a mating plug after 3–4 days, invariably on the ventral side of the head. Examination by Nomarski microscopy revealed that the plugs were deposited over the excretory pore. No mating plugs were observed on solitary AB2 males (58 animals scored). By contrast, in three other plug-forming wild isolates, CB4855, PA2, and RC301, such intermale mating plug deposition was not observed.

Although these results suggest that AB2 males deposit mating plugs on each other, it remained possible that they plug their own heads, but only when part of a homosexual clump. To investigate this possibility, single AB2 males were incubated for 4 days with 20 *lon-2(e678)* males. Forty-four such males were examined and none received mating plugs on their heads, although in almost all cases clumps of males were observed. In five control sets of 18–20 AB2 males, 1–4 males with mating plugs were seen (mean: 2.4/set, or 12.6%). Given a

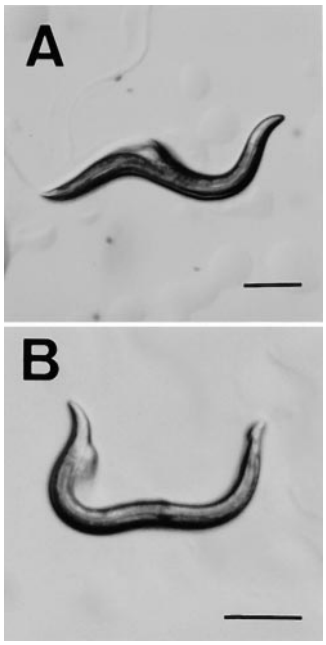

Figure 3.—Mating plugs on the AB2 wild isolate. (A) A mated hermaphrodite exhibiting the mating plug deposited over the vulva. (B) An AB2 male with a mating plug deposited on the ventral side of the head. Bar, 0.2 mm.

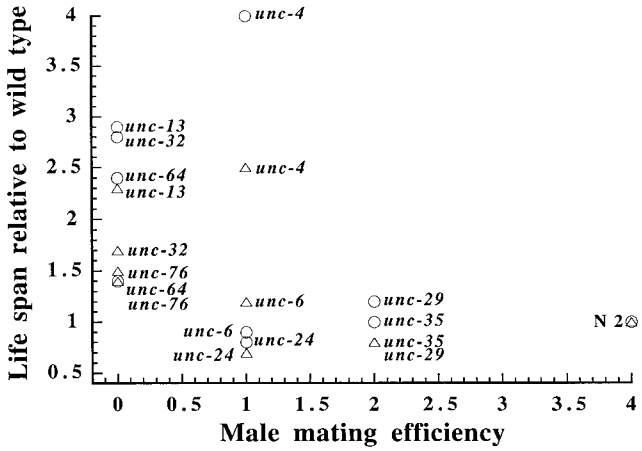

Figure 4.—Inverse relationship between mating efficiency and longevity in grouped Unc male populations. Median life span (circles) and maximum life span (triangles) relative to wild type. When longevity was increased relative to N2, median life span was increased more than maximum life span.

12.6% chance that each male will receive a mating plug, the probability of none of the 44 males getting plugged is $P = 0.0026$ (binomial test). Mating plugs were not seen on the heads of the N2-derived, Lon males either. In a further test, 25 AB2 males were incubated with 25 *lon-2(e678) unc-10(e102)* males. Mating plugs were seen only on the AB2 males (two trials, 2 and 4 plug-headed AB2 males seen). These results suggest that AB2 males are attracted to one another, but not to N2-derived males, and deposit plugs upon one another's heads, rather than upon their own. Although head-plugging was observed only in the AB2 strain, it nevertheless suggests the possibility that attempted mating via the excretory pore occurs in males of other strains, including N2. This may be deleterious.

Extended life span in *unc* males, but not hermaphrodites: Uncoordinated (*unc*) mutants were employed to characterize male aging in the absence of male mating behavior. Unexpectedly, the life spans of a number of different *unc* mutant males exceeded that of solitary N2 males, suggesting that male life span is shortened not only by male-male interactions but also by wild-type behavior in solitary males.

Nine randomly selected *unc* mutants were used, with a range of effects on male ME, which is measured on a scale of 0 (incapable of siring progeny) to 4 (wild type; Hodgkin 1983). An inverse relationship was seen between life extension and male mating efficiency (Figure 4), suggesting that mating behavior and/or the increased motility of males reduces life span in grouped populations. *unc-4(e120)* (ME1), *unc-13(e51)* (ME0), and *unc-32(e189)* (ME0) exhibited large increases in life span; *unc-64(e246)* (ME0) and *unc-76(e911)* (ME0) males exhibited intermediate increases; and *unc-6(e78)* (ME1), *unc-24(e138)* (ME1), *unc-29(e1072)* (ME2), and *unc-35(e259)* (ME2) males exhibited close to wild-type life span (Table 2).

Of the nine *unc* mutations, only *unc-64(e246)* increased hermaphrodite life span (Table 2). At 25°, median and maximum life spans were increased in *fer-15(b26); unc-64(e246)* hermaphrodites by a factor of 1.6 and 1.7, respectively, relative to N2 (four trials, 18–25 animals per trial). *fer-15(b26)* blocks fertilization at that temperature and does not affect life span (Friedman and Johnson 1988).

Life span was measured in solitary *unc-4*, *unc-13*, and *unc-32* males to exclude the possibility of male-male interactions. *unc-4* animals move forward, but cannot back; *unc-32* animals are more severely affected, moving little and tending to coil; and *unc-13* animals are paralyzed. Solitary *unc* males exhibited a 50–90% increase in median life span relative to solitary N2 males (Figure 5; Table 3). Male life spans were not significantly different from one another, despite the range in reduction of movement among the *unc* mutants, with the exception of the median life span of *unc-4*, which was significantly shorter than that of *unc-13* and *unc-13; fog-2* males ($P < 0.005$). Slight increases in *unc* hermaphrodite maximum life span were observed relative to N2 (Table 3). Note that since the estimates of grouped male life span (Table 2) and solitary male life span (Table 3) were not carried out simultaneously, and since N2 hermaphrodite controls are not comparable, these two tables should not be compared directly to give an indication of the effect of grouping in Unc male life span.

These results suggest that some element of behavior in wild-type solitary males shortens life span. This behav-

TABLE 2

Life span of grouped Unc males and hermaphrodites

Strain	Median life span	Maximum life span	Life span relative to wild type[a]	No. of trials
N2 hermaphrodites	15.2 ± 0.2	20.5 ± 2.5	1.0, 1.0	2 (59, 56)
N2 males	10.1 ± 0.0	25.0 ± 0.0	1.0, 1.0	2 (80, 60)
unc-4(e120) hermaphrodites	12.4 ± 2.0	24.5 ± 1.2	0.8, 1.2	4 (106, 97)
unc-4(e120) males	40.8 ± 2.5	62.3 ± 2.1	4.0, 2.5	4 (95, 70)
unc-6(e78) hermaphrodites	10.9 ± 2.2	19.5 ± 1.8	0.7, 1.0	4 (112, 106)
unc-6(e78) males	9.3 ± 0.4	30.0 ± 2.5	0.9, 1.2	4 (115, 107)
unc-13(e51) hermaphrodites[b]	—	—	—	4 (117, 0)
unc-13(e51) males	29.6 ± 1.2	56.5 ± 4.4	2.9, 2.3	4 (84, 68)
unc-24(e138) hermaphrodites	10.8 ± 1.1	20.8 ± 0.8	0.7, 1.0	4 (111, 104)
unc-24(e138) males	7.7 ± 1.3	17.8 ± 1.7	0.8, 0.7	4 (99, 88)
unc-29(e1072) hermaphrodites	8.2 ± 0.9	18.0 ± 1.5	0.5, 0.9	4 (114, 109)
unc-29(e1072) males	12.4 ± 2.1	20.5 ± 2.5	1.2, 0.8	2 (50, 38)
unc-32(e189) hermaphrodites	14.0 ± 0.5	20.5 ± 0.5	0.9, 1.0	4 (110, 105)
unc-32(e189) males	29.1 ± 0.8	43.5 ± 1.2	2.8, 1.7	4 (102, 98)
unc-35(e259) harmaphrodites	12.5 ± 0.4	18.3 ± 0.6	0.8, 0.9	4 (104, 99)
unc-35(e259) males	9.6 ± 0.3	20 ± 0.7	1.0, 0.8	4 (106, 91)
unc-64(e246) hermaphrodites	20.4 ± 1.6	32.8 ± 0.8	1.3, 1.6	4 (108, 105)
unc-64(e246) males	24.5 ± 3.0	35.5 ± 6.5	2.4, 1.4	3,2 (102, 45)
unc-76(e911) hermaphrodites	8.7 ± 0.7	13.5 ± 1.5	0.6, 0.7	2 (55, 51)
unc-76(e911) males	13.7 ± 0.7	37.5 ± 5.5	1.4, 1.5	2 (46, 39)

Life span is expressed as the mean of estimates from several trials, with the standard error given. In parentheses are the total sample sizes of animals in the starting populations, and of those, the number dying of old age. Animals were maintained on live *E. coli* at 20°.

[a] Median, maximum life span.

[b] All animals died from internal hatching of larvae.

ior is blocked by the three *unc* mutations employed, unmasking a greater potential male longevity. By this view, *unc-4(e120)*, *unc-13(e51)*, and *unc-32(e189)* are life-extending mutations that are sex specific in action because they reveal the underlying sex-specific differences in constitutional longevity. Taking the average of the median male life span of these strains, 33.8 days, our results indicate that wild-type solitary male behavior reduces life span by 38% and male-male interactions reduce it by a further 33% (or 53% of solitary wild-type male life span).

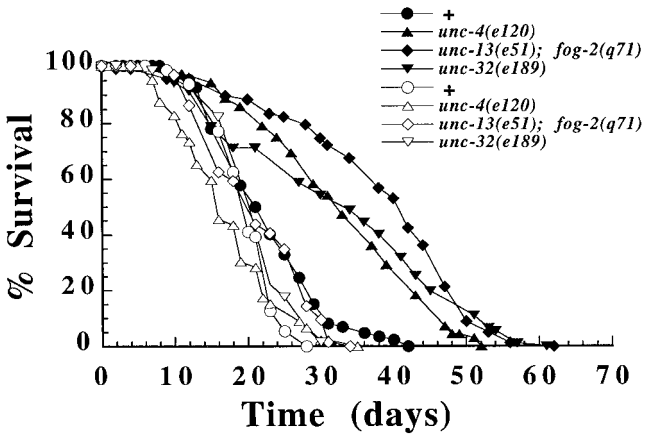

Figure 5.—Three *unc* mutations extend solitary male, but not hermaphrodite, life span at 20°. Solid symbols, males; open symbols, hermaphrodites. All mutant strains were backcrossed three times to male stock DRM to reduce possible variation in genetic background that might influence life span. The N2 wild-type strain used was the Riddle lab (DR) male stock, identical with the Caenorhabditis Genetics Center N2 male stock.

Role of *daf-2* in greater male longevity: Several genes identified in *C. elegans* have a dual role in regulating dauer larva development and adult longevity (reviewed in Kenyon 1997). The dauer larva is a long-lived, developmentally arrested alternative third larval stage, which forms under conditions of starvation and crowding (Riddle and Albert 1997). Mutation of the *daf-2* gene results in constitutive dauer larva development (the Daf-c phenotype) and up to a tripling of adult life span (Kenyon *et al.* 1993; Gems *et al.* 1998). This gene encodes an insulin receptor-like protein (Kimura *et al.* 1997). Mutation of *daf-16*, on the other hand, results in the inability to form dauer larvae (the dauer-defective, or Daf-d, phenotype) and suppression of both the Daf-c and Age phenotypes of *daf-2* (Riddle *et al.* 1981; Kenyon *et al.* 1993). *daf-16* encodes a Forkhead transcription factor family member (Lin *et al.* 1997; Ogg *et al.* 1997) required for dauer larva formation and increased adult longevity, the activity of which may be regulated by *daf-2*. The established roles of *daf-2* and *daf-16* in regulating life span suggest the hypothesis that

TABLE 3

Effects on life span of gender and mutations that reduce motility

Genotype	Sex	Median life span (days)	Maximum life span (days)	Life span relative to wild type[a]	No. of trials
+	Hermaphrodite	19.4 ± 0.5	25.7 ± 0.6	1.0, 1.0	12 (240, 173)
	Male	21.1 ± 1.8	36.5 ± 3.5	1.0, 1.0	2 (450, 86)
fog-2(q71)	Female	14.9 ± 0.1***	21.0 ± 1.0***	0.8, 0.8	4 (80, 60)
	Male	23.0 ± 0.2	37.0 ± 1.0	1.1, 1.0	2 (400, 81)
unc-4(e120)	Hermaphrodite	16.6 ± 1.3	29.4 ± 1.9*	0.9, 1.1	5 (220, 80)
	Male	30.6 ± 0.6*	52.0 ± 0.0*	1.5, 1.4	2 (300, 72)
unc-13(e51)	Hermaphrodite[b]	—	—	—	4 (80, 0)
	Male	37.3 ± 0.9**	56.0 ± 0.0*	1.8, 1.5	2 (80, 68)
unc-13(e51); fog-2(q71)	Female	19.7 ± 1.3	30.3 ± 0.3***	1.0, 1.2	4 (80, 63)
	Male	40.1 ± 1.9**	54.5 ± 1.5**	1.9, 1.5	2 (100, 79)
unc-32(e189)	Hermaphrodite	20.2 ± 0.4	27.8 ± 1.0	1.0, 1.1	4 (80, 70)
	Male	33.4 ± 2.7**	53.5 ± 1.3*	1.6, 1.5	4 (120, 91)

Life span on live *E. coli* at 20°, expressed as the mean of estimates from several trials, with the standard error given. In parentheses are the total sample sizes of animals in the starting populations, and of those, the number dying of old age. Animals were maintained with one male, or 10 hermaphrodites or females per plate. *fog-2(q71)* produced a slight but significant reduction in life span in females but not males. This could potentially mask a small extension of median hermaphrodite life span by *unc-13(e51)*. * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$ (Student's *t*-test), where *P* expresses the probability of having the same life span as the wild type of the same gender.

[a] Median, maximum life span.

[b] All animals died from internal hatching of larvae. To prevent this the *fog-2(q71)* mutation was employed, which eliminates spermatogenesis in hermaphrodites but not males (Schedl and Kimble 1988).

the increased life span of males may result from down-regulation of DAF-2 and/or increased DAF-16 activity. If this were so, we would predict that *daf-2* mutant males and hermaphrodites should have the same life span, and the increased longevity of males should be suppressed by mutation of *daf-16*.

Our results indicate that the increased longevity of males is not mediated by *daf-2*. Male and hermaphrodite life spans were compared in a *daf-2(m577*ts*)* strain. *m577* is a class 1 allele, resulting in Daf-c and Age traits, but not the numerous pleiotropic traits seen in class 2 mutants (Gems *et al.* 1998). At 20°, *daf-2(m577)* males formed homosexual clumps, and male life span was reduced to a similar degree as in N2 males (data not shown). Thus, animals were maintained in isolation to avoid the life-shortening effects of male-male interactions and were also maintained on UV-killed *E. coli* to reduce the negative effects of live bacteria on longevity. Male *m577* longevity was greater than that of hermaphrodites, and the ratio of male to hermaphrodite life span was comparable to that seen in N2 controls (Figure 6A; Table 4). Furthermore, the increased longevity resulting from *m577* is similar in both sexes (Table 5).

If mutation of *daf-2* and maleness extend life span by distinct mechanisms, then the increased longevity of male *daf-2* mutants relative to wild-type hermaphrodites should resemble the product of the two effects, and this is the case (Table 5). We conclude that the life span of *daf-2* mutant males exceeds that of *daf-2* hermaphrodites to the same extent as in the wild type, indicating that the greater longevity of wild-type males is not mediated by a reduction of *daf-2* activity, but rather, by a distinct mechanism.

The effect of maleness and *daf-2(m577)* on the daily rate of mortality was calculated (Figure 6B). At most ages, the mortality rate of males is less than that of hermaphrodites, and the mortality of *daf-2* adults is less than that of wild-type adults. In *m577* males, mortality increases exponentially with age until around day 36 and then levels off. A less marked deceleration in demographic aging at advanced ages is also seen in wild-type hermaphrodites and males. Thus, while the reversal in the direction of the change of mortality with age seen in grouped wild-type males (Figure 1B) is not seen in solitary wild-type males, the leveling off of mortality rate at advanced age is seen.

We modeled the effect of gender and *daf-2* on mortality in terms of the Weibull distribution. This was chosen rather than the more commonly used Gompertz model since only the former predicts mortality decelerations at advanced ages (Vanfleteren *et al.* 1998). The Weibull hazard function can be expressed as $m(x) = \alpha x^{\beta}$, where $m(x)$ is the mortality rate at age x, α is a scale parameter that reflects mean life span, and β is a shape parameter that reflects the acceleration of mortality. In both sexes of wild-type and *daf-2* strains, an excellent fit to the Weibull distribution was obtained (Table 6). In the Weibull plot shown in Figure 6C, the slopes of the plots correspond to the rate of acceleration of mortality with increasing age. In the case of wild-type animals, the hermaphrodite mortality acceleration is significantly greater than that of males ($P < 0.05$).

Life span in class 2 *daf-2* males: A much greater difference between male and hermaphrodite life spans was observed in *daf-2(m120)* and *daf-2(e1370)*. These are class 2 mutants, which exhibit numerous defects in addition to the Daf-c and Age phenotypes (Gems *et al.* 1998). At 25.5°, class 2 *daf-2* mutant males are severely Unc and unable to mate (Gems *et al.* 1998). No male clumping was observed, and males of both mutants were much longer-lived than hermaphrodites (Figure 6D; Table 4). In the case of *daf-2(m120)*, median male life span was ~270% greater than that of hermaphrodites, and males lived up to 96 days. The *daf-2* Unc phenotype, like the *unc-4*, *unc-13*, and *unc-32* Unc phenotype, may itself increase male longevity.

A further contributing factor to the large difference between class 2 *daf-2* male and hermaphrodite median life spans may be premature hermaphrodite mortality at 25.5° (Gems *et al.* 1998). Median life span was reduced relative to maximum life span, and the abnormal, sick appearance of these mutants suggested that, despite living longer than wild type, many die prematurely from the deleterious effects of class 2-specific traits. However, class 2 *daf-2* mutations did not cause significant premature mortality in males. In this study, the ratios of median to maximum hermaphrodite life span in *daf-2(m120)* and *daf-2(e1370)* were 0.33 and 0.57, respectively, compared to 0.66 in N2 hermaphrodites. By contrast, the ratios of *daf-2(e120)* and *daf-2(e1370)* median male life span to maximum life span were 0.64 and 0.77, respectively. Furthermore, the males appeared healthy. There was no indication of premature mortality in *m577* class 1 mutant adults of either sex in the above-mentioned trial. Here the ratios of median to maximum life span were 0.63 and 0.7 in males and hermaphrodites, respectively. By comparison, the ratios in the N2 control were 0.60 and 0.63 in males and hermaphrodites, respectively.

Role of *daf-16* in the greater longevity of males: We measured *daf-16* adult life span in the presence or absence of *unc-4(e120)* or *unc-32(e189)* at 20°(Figure 7; Table 7). The presence of *daf-16(m26)* reduced life span in both sexes in all genetic backgrounds. Relative to N2, median life span was reduced by 34 and 25% in *daf-16* non-Unc males and hermaphrodites, respectively. *daf-16* largely suppressed the increased longevity of males (Table 7). However, when *unc-4* or *unc-32* was present, male maximum life span was significantly greater than that of hermaphrodites ($P < 0.0005$, Student's *t*-test). Overall, these results suggest that the increased life span of males is largely attributable to in-

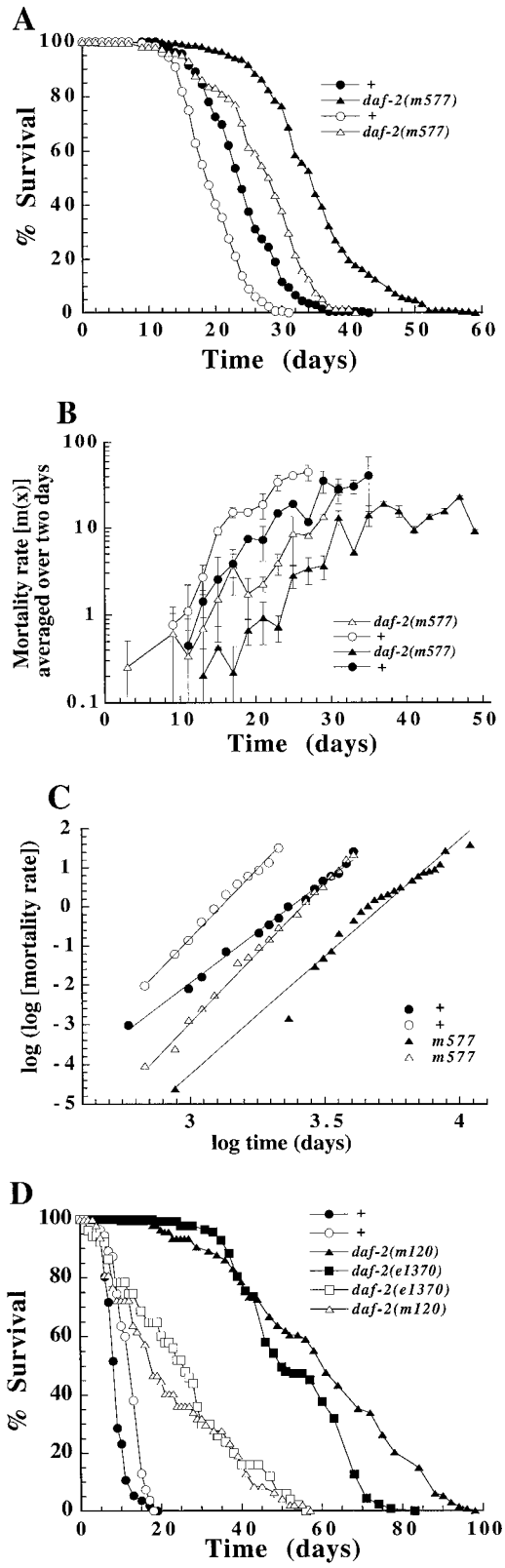

Figure 6.—Effect of gender on life span in *daf-2* mutants. Open symbols, hermaphrodites; solid symbols, males. (A) Survival of class 1 mutant *daf-2(m577)*. Both sexes were maintained in isolation on UV-killed bacteria at 20°. (B) Variation of mortality rate with age in wild-type and *daf-2(m577)* adults. Plot shows 2-day averages of daily mortality with standard error. (C) Weibull plot of daily mortality. (D) Survival of two class 2 *daf-2* mutants at 25°. Both sexes were maintained in groups on live *E. coli* lawns. At 25°, class 2 *daf-2* animals are Unc, and males are ME0 (Gems *et al.* 1998).

TABLE 4

Adult life span of *daf-2* males and hermaphrodites

Strain	Median life span (days)	Maximum life span (days)	No. of trials[a]
	20°, solitary animals, UV-killed bacteria		
N2 males	23.4 ± 0.7	39.0 ± 3.0	2 (300, 198)
N2 hermaphrodites	18.3 ± 0.7	29.0 ± 1.0	2 (205, 166)
Relative male longevity[b]	1.28	1.34	
daf-2(m577) males	34.3 ± 0.5	54.5 ± 3.5	2 (400, 208)
daf-2(m577) hermaphrodites	28.0 ± 0.0	40.0 ± 0.0	2 (200, 134)
Relative male longevity[b]	1.22	1.36	
	25°, grouped animals, live bacteria		
N2 males	8.2 ± 0.0	15.3 ± 1.2	3 (166, 148)
N2 hermaphrodites	12.8 ± 0.2	19.5 ± 1.0	4 (110, 108)
Relative male longevity[b]	0.64	0.79	
daf-2(m120) males	60.4 ± 1.2	94.0 ± 2.0	2 (144, 133)
daf-2(m120) hermaphrodites	16.2 ± 1.5	50.0 ± 5.0	2 (49, 47)
Relative male longevity[b]	3.73	1.88	
daf-2(e1370) males	53.1 ± 4.7	69.3 ± 5.0	3 (196, 184)
daf-2(e1370) hermaphrodites	25.7 ± 1.2	47.3 ± 5.0	4 (108, 102)
Relative male longevity[b]	2.07	1.47	

[a] In parentheses are the total sample sizes as described in Table 1.
[b] Ratio of male to hermaphrodite life span.

creased *daf-16* gene activity, but other factors may also be involved.

DISCUSSION

Our results suggest that the life-span potential of *C. elegans* males is greater than that of hermaphrodites by a factor of 1.7–2.2. Wild-type male life span is reduced by male-male interactions and some other unknown aspects of behavior that are prevented by *unc* mutations. This greater male life-span potential does not appear to be mediated by modulation of *daf-2*, yet is under the control of *daf-16*.

Biodemographic effects of gender: An exponential increase of the rate of mortality with increasing age is a common feature of animal aging, as first noted in humans by the actuary Benjamin Gompertz (Finch 1991). However, a relentless exponential increase in mortality in later life is not a universal feature of aging in animal species, and the mortality rate acceleration may level off and even reverse at advanced ages, in both insects (Rockstein and Lieberman 1959; Carey *et al.* 1992; Curtsinger *et al.* 1992) and humans at very advanced ages (Vaupel 1997; Vaupel *et al.* 1998).

We have shown that the pattern of age-specific mortality in grouped males is unusually complex, in contrast to the simple exponential increase in hermaphrodite age-specific mortality (Figure 1B). However, in the absence of male-male interactions the general pattern of mortality is similar to that of hermaphrodites (Figure 6B). A good fit was observed when mortality data from populations of solitary wild-type or *daf-2* adults of either

TABLE 5

Extension of adult life span by maleness and *daf-2(m577)*

	Relative median life spans	Relative maximum life spans
daf-2(m577)/N2, males	1.47	1.40
daf-2(m577)/N2, hermaphrodites	1.53	1.38
Mean 1	1.50 ± 0.03	1.39 ± 0.01
Males/hermaphrodites, N2	1.28	1.34
Males/hermaphrodites, *daf-2(m577)*	1.22	1.36
Mean 2	1.25 ± 0.03	1.35 ± 0.01
daf-2(m577) males/N2 hermaphrodites	1.87	1.88
Mean 1 × Mean 2	1.88	1.88

TABLE 6

Weibull analysis of survival of wild-type and *daf-2(m577)* adults of both sexes

	Fit to Weibull distribution		Weibull parameter estimates	
Strain	R^2	F	α^a	β^a
N2 hermaphrodite	0.992	1114.0*	24.19 (23.58, 24.83)	8.21 (7.14, 9.35)
N2 male	0.995	2558.3*	30.17 (29.18, 31.26)	5.68 (4.95, 6.45)
daf-2 hermaphrodite	0.996	3990.1*	32.09 (31.18, 33.02)	7.21 (6.19, 8.31)
daf-2 male	0.968	604.2*	41.70 (40.56, 42.87)	6.12 (5.47, 6.80)

* $P < 0.0001$.

[a] In parentheses, 95% confidence intervals, top and bottom, respectively.

sex maintained on killed *E. coli* were modeled in terms of the Weibull equation (Figure 6C; Table 6). In wild type, maleness primarily reduced the mortality rate acceleration (*i.e.*, reduced the rate of aging).

A pattern of sex-specific behavior and longevity similar to that described in this study has been reported in the housefly, *Musca domestica*. In this species, single-sex groups of females live longer than males (Rockstein and Lieberman 1959), but if male-male interactions (indiscriminate attempts at mating) are prevented, male longevity is increased to that of females (Ragland and Sohal 1973). Thus, life-span reductions resulting from attempted male mating may be common in single-sex groups of males of lower metazoan species in the laboratory. Whether this effect occurs or has any effect on fitness in the wild is unknown.

We previously found that the life span of mated N2 males is marginally greater than that of unmated, grouped males (Gems and Riddle 1996). From this it was inferred that *C. elegans* males do not exhibit a cost of mating in reduced longevity. It is clear from the current study that mating cost analysis in *C. elegans* males must be performed in the absence of other males. Preliminary evidence from studies of single N2 males exposed to hermaphrodites shows that their life spans are greatly reduced relative to those of solitary males (D. Gems, unpublished results).

Homosexual behavior among AB2 males: We have shown that males of the AB2 wild isolate deposit mating plugs upon one another over the excretory pore. Since mating plugs are produced only after ejaculation (Barker 1995), this suggests that males copulate with

TABLE 7

Effect of *daf-16(m26)* on the increased life span of males

Genotype	Median life span (days)	Maximum life span (days)	No. of trials[a]
N2 males	16.2 ± 0.5	31.5 ± 3.9	4 (850, 143)
N2 hermaphrodites	20.4 ± 0.5	26.3 ± 1.1	7 (140, 111)
Relative male longevity[b]	0.79	1.20	
daf-16(m26) males	10.7 ± 0.4	20.0 ± 1.1	4 (690, 197)
daf-16(m26) hermaphrodites	15.3 ± 0.6	18.5 ± 0.6	5 (100, 87)
Relative male longevity[b]	0.70	1.08	
unc-4(e120) males	26.6 ± 2.4	50.8 ± 0.3	4 (570, 144)
unc-4(e120) hermaphrodites	14.2 ± 2.9	29.5 ± 2.1	4 (200, 68)
Relative male longevity[b]	1.87	1.72	
unc-32(e189) males	28.0 ± 2.9	48.5 ± 1.6	4 (143, 120)
unc-32(e189) hermaphrodites	21.5 ± 0.8	29.8 ± 0.8	6 (150, 107)
Relative male longevity[b]	1.30	1.63	
unc-4(e120); daf-16(m26) males	16.2 ± 0.4	30.3 ± 1.3	4 (500, 190)
unc-4(e120); daf-16(m26) hermaphrodites	16.2 ± 0.6	21.2 ± 0.9	5 (180, 74)
Relative male longevity[b]	1.00	1.43	
unc-32(e189); daf-16(m26) males	15.7 ± 1.9	30.5 ± 2.3	4 (160, 148)
unc-32(e189); daf-16(m26) hermaphrodites	16.6 ± 0.4	22.9 ± 0.7	8 (160, 132)
Relative male longevity[b]	0.95	1.33	

All males were maintained in isolation on live *E. coli*. Life spans are means of values from all trials, with standard errors.

[a] In parentheses are the total sample sizes, as described in Table 1.

[b] Ratio of male to hermaphrodite life span.

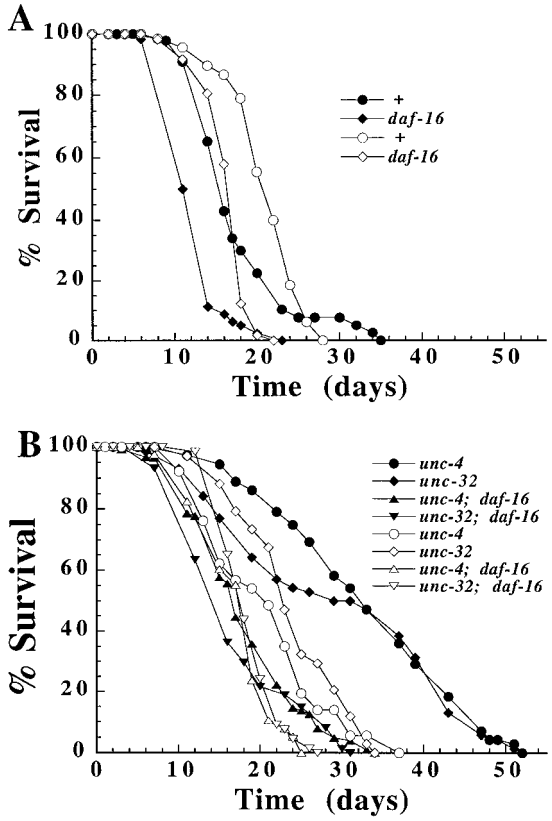

Figure 7.—The effect of *daf-16(m26)* on aging in solitary males. (A) *daf-16* reduces life span in both sexes, but *daf-16* male maximum life span still exceeds that of *daf-16* hermaphrodites. (B) *daf-16* partially suppresses the extended life span of Unc males. Animals were maintained in isolation on live *E. coli* at 20°. Open symbols, hermaphrodites; solid symbols, males.

one another via this orifice. Alternatively, AB2 males might differ from the plug depositing strain used in the Barker study (a derivative of the Cl2a wild isolate) in being able to deposit a mating plug without prior copulation. The fact that AB2 males do not deposit plugs on Bristol males suggests that they are not unusually attracted to other males, but rather they resemble hermaphrodites in some way, possibly at the excretory pore (although the duct and pore region of the cuticle appeared morphologically normal under Nomarski microscopy). It is possible that N2 males are similarly attracted to AB2 males. Whether attempted or actual copulation via the excretory pore occurs among N2 males, or whether this contributes to the life-shortening effects of male-male interactions, remains unclear. The ability of AB2 males to form mating plugs in the absence of hermaphrodites demonstrates that males secrete the plug material themselves rather than stimulating the hermaphrodite to do so.

Effects of *E. coli* on survival: Two observations suggest that adult males are more sensitive than hermaphrodites to variable properties of live bacterial lawns. Without transfers to fresh plates, median and maximum life spans were reduced in males but not hermaphrodites. In addition, maintenance of males on UV-killed *E. coli* reduced variation in life span.

Maintenance on UV-killed *E. coli* also increased median and maximum life spans of both males and hermaphrodites. This is reminiscent of the increases in longevity seen in axenic medium (Croll *et al.* 1977; Vanfleteren and De Vreese 1995). How the presence of *E. coli* reduces nematode life span is a question that has remained unresolved for many years. One possibility is that live *E. coli* are slightly detrimental to survival, either due to production of toxins (Hansen *et al.* 1964) or due to invasion and infection of elderly worms (Vanfleteren *et al.* 1998). Alternatively, nutrients may be limited in axenic medium, causing caloric restriction (CR) and life extension (Mitchell *et al.* 1979). Animals maintained on UV-killed *E. coli* are unlikely to experience CR since *E. coli* is abundant. Furthermore, CR results in a reduction of fertility, and this was not observed. This is consistent with the view that old *C. elegans* are killed by live *E. coli* either by means of infection or by toxins that build up in old bacterial lawns.

The mechanism of life extension in Unc males: The extension of longevity observed in Unc males cannot be explained entirely by prevention of male-male interactions, since Unc male life span in these cases greatly exceeds that of solitary wild-type males. There may be a single deleterious element of male behavior occurring in solitary wild-type males that increases in grouped males and is greatly reduced or blocked by some *unc* mutations. For example, young wild-type males frequently exhibit a reflexive mating behavior, such that the tail tracks over their own body in a continuous vulva-searching behavior. Potentially, this behavior is deleterious.

We propose that the life span of *unc-4*, *unc-13*, and *unc-32* mutant males represents the underlying potential or constitutional longevity of *C. elegans* males. Male activity may involve increased metabolic rate, resulting in decreased longevity. Alternatively, some component of male-specific behavior may result in mechanical damage leading to early death.

It seems unlikely that *unc* mutations directly affect the underlying process of aging in males, given that *unc-4*, *unc-13*, and *unc-32* are not known to act on related aspects of *C. elegans* biology. Furthermore, it is improbable that six out of nine randomly selected *unc* mutations would specifically affect the aging process in males. A second possibility is that feeding in males is easily disrupted by *unc* mutations, resulting in caloric restriction. However, if this were so, it would be expected that male life extension would be greater in the more severe *unc* mutations, such as *unc-13*, which greatly reduces the rate of pharyngeal pumping, and this is not the case. Furthermore, *eat-2* mutants, which extend life span by reducing dietary intake (Lakowski and Hekimi 1998), have a starved appearance, whereas the Unc males studied here do not.

***unc-64* regulates aging:** One of the nine *unc* mutations studied here, *unc-64(e246)*, increased hermaphrodite life span. The *unc-64* gene encodes a protein homologous to syntaxin, which is involved in synaptic vesicle fusion in neurons (Saifee *et al.* 1998). The *e246* allele has also been reported to act as an enhancer of the dauer-constitutive phenotype of *daf-28(sa191)*, a mutation that also extends adult life span (Malone *et al.* 1996). Thus, *unc-64* may, like *daf-2*, *age-1*, and *daf-16*, act in the genetic pathway regulating dauer larva formation and life span (Kenyon 1997). If *unc-64*, a neuronal function gene, controls adult longevity, this provides direct evidence for the control of aging by the nervous system in *C. elegans*. Neuronal control of aging is consistent with the observation that *daf-2* controls aging in a noncell autonomous fashion (Apfeld and Kenyon 1998). The role of *unc-64* in life-span determination and its site of action in the nervous system have recently been reported by Ailion *et al.* (1999).

***C. elegans* exhibits three patterns of longevity:** The character of long-lived *daf-2* mutants suggests that *C. elegans* may have two distinct developmental programs for longevity, that of the long-lived dauer larva and that of the shorter-lived adult hermaphrodite (Kenyon *et al.* 1993). The present study reveals a third pattern of longevity, that of the adult male. Our results suggest that the greater longevity of males is not determined by the *daf-2* gene. Thus, there appear to be two enhanced life-maintenance programs: one specifying dauer longevity that is regulated by *daf-2* and *daf-16* and one specifying male longevity that is regulated by *daf-16* but not *daf-2*. Understanding the biological basis of the surprisingly large difference in potential longevity between the sexes may provide insights into possible general mechanisms that determine the rate of animal aging.

Mutation of *daf-16* largely suppresses the increased longevity of males (this study) and it fully suppresses the increased longevity resulting from mutation of *daf-2* and *age-1* (Kenyon *et al.* 1993; Dorman *et al.* 1995; Larsen *et al.* 1995). Our results suggest that the increased life span of males is largely determined by upregulation of *daf-16* by some male-specific signal that is independent of *daf-2*. Alternatively, a life-shortening signal that antagonizes *daf-16* may be present in hermaphrodites but not males. This latter interpretation is supported by a recent study on the effect of signaling from the gonad on life span (Hsin and Kenyon 1999). Using laser microsurgery, it was shown that the presence of the germline shortens hermaphrodite life span, whereas the somatic gonad extends it. Hsin and Kenyon then observed that ablation of the germline extended life span in *daf-2* but not *daf-16* mutants. Thus, life extension by both maleness and ablation of the germline is independent of *daf-2* but dependent on *daf-16*. Since mutation of *daf-16* does not entirely suppress the increased longevity of males, there may be a second, *daf-16*-independent process extending male life span.

There are other possible interpretations of these gene interactions. *daf-16(m26)* alone reduces hermaphrodite life span (Kenyon *et al.* 1993; Larsen *et al.* 1995; Malone *et al.* 1996; Lakowski and Hekimi 1998; this study). Reductions in life span resulting from *daf-16(m26)* in *eat-2(ad465)* or *clk-1(e2519)* were similar to its reduction of wild-type life span (Lakowski and Hekimi 1998). From this it was inferred that the *daf-16* gene does not mediate the Age phenotype of *eat-2* or *clk-1* mutants. In the present study the picture is more complex. While *daf-16(m26)* only partially suppresses the increased longevity of males, the effect of *daf-16* on male life span is much greater than that on hermaphrodites. For example, *daf-16(m26)* reduces maximum life span in *unc-4(e120)* hermaphrodites and males by 28 and 40%, respectively. This could indicate that *daf-16* is one of several genes whose expression ensures the greater longevity of males. Alternatively, it could reflect the greater susceptibility of males to deleterious effects of *daf-16* mutations.

The effects of maleness on longevity: Our results show that if sex-related behavior is prevented in *C. elegans*, males are the longer-lived sex. Greater female longevity is seen in many species (Comfort 1979; Smith 1989), but in most cases it is unclear whether this reflects a greater female constitutional longevity or higher mortality in males resulting from reproductive activity or sex-related behavior. One way to block male sex-related physiology and behavior is castration, and this has been shown to increase male life span in species that undergo reproductive death, *e.g.*, the marsupial mouse (Diamond 1982) and the Pacific (kokanee) salmon (Robertson 1961), and also in domestic cats (Hamilton 1965; Bronson 1981a) and possibly dogs (Bronson 1981b). In one human study, castrated mentally handicapped men were found to live on average 13.5 years longer than intact controls (Hamilton and Mestler 1969), and furthermore, to live slightly longer than control, intact women. It is unclear whether constitutional longevity differs between sexes in higher animals.

Why should the constitutional longevity of *C. elegans* males exceed that of hermaphrodites? The life spans of mated males and hermaphrodites are similar (Gems and Riddle 1996). Possibly, if the constitutional longevity of males were as short as that of hermaphrodites, then male longevity would be so shortened by mating as to compromise fitness. Perhaps males have evolved a greater constitutional longevity to compensate for the greater cost of mating (Gems and Riddle 1996), an instance of counter-gradient selection (Berven *et al.* 1979). An alternative possibility is that greater male longevity is an evolutionary consequence of protandrous hermaphroditism, where unmated hermaphrodites become postreproductive at an earlier age than males. Consequently, late-acting deleterious mutations affecting males will be more strongly selected against, resulting in a slower rate of male aging.

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