Genes that Regulate Both Development and Longevity in *Caenorhabditis elegans*

Pamela L. Larsen,¹ Patrice S. Albert and Donald L. Riddle

Molecular Biology Program and Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

Manuscript received November 11, 1994
Accepted for publication January 11, 1995

**ABSTRACT**

The nematode *Caenorhabditis elegans* responds to conditions of overcrowding and limited food by arresting development as a dauer larva. Genetic analysis of mutations that alter dauer larva formation (**daf** mutations) is presented along with an updated genetic pathway for dauer vs. nondauer development. Mutations in the **daf-2** and **daf-23** genes double adult life span, whereas mutations in four other dauer-constitutive genes positioned in a separate branch of this pathway (**daf-1, daf-4, daf-7** and **daf-8**) do not. The increased life spans are suppressed completely by a **daf-16** mutation and partially in a **daf-2; daf-18** double mutant. A genetic pathway for determination of adult life span is presented based on the same strains and growth conditions used to characterize Daf phenotypes. Both dauer larva formation and adult life span are affected in **daf-2; daf-12** double mutants in an allele-specific manner. Mutations in **daf-12** do not extend adult life span, but certain combinations of **daf-2** and **daf-12** mutant alleles nearly quadruple it. This synergistic effect, which does not equivalently extend the fertile period, is the largest genetic extension of life span yet observed in a metazoan.

**POSTEMBRYONIC** development of *Caenorhabditis elegans* proceeds through four larval stages (L1–L4) to the adult when environmental conditions are favorable for growth and reproduction. A developmentally arrested dispersal stage, the dauer larva, may form at the second molt in response to high population density, as measured by a constitutively produced dauer-inducing pheromone, and limited food (Golden and Riddle 1984a). The L1 responds to a high pheromone/food ratio by molting to a predauer stage called the L2d, which feeds and stores nutrients in intestinal and hypodermal granules. Under continuing dauer-inducing conditions, L2d larvae molt to the dauer stage, shrink radially and become resistant to detergent treatment. The nonfeeding dauer larva has unique metabolic (Wadsworth and Riddle 1989) and morphological characteristics (Albert and Riddle 1983) that are reversed when development resumes in response to a low pheromone/food ratio.

Mutations in genes involved in dauer larva formation are divided into two main classes, dauer-defective mutations (**daf-d**) that prevent dauer development, and dauer-constitutive mutations (**daf-c**) that mandate entry into the dauer stage (Riddle et al. 1981). Expression of the Daf-d phenotypes is nonconditional, whereas mutations in most **daf-c** genes are temperature sensitive (Swanson and Riddle 1981). Based on interpretation of epistatic relationships between **daf-c** and **daf-d** mutations, the genes have been ordered in various genetic pathways that share the broad representation of sensory reception of environmental cues followed by neural signal transduction and subsequent morphogenetic response (Riddle 1988; Vowels and Thomas 1992; Thomas et al. 1993; Gottlieb and Ruvkun 1994). Sequence analysis of the **daf-c** genes cloned thus far indicates that they play roles in protein growth factor-mediated intercellular signal transduction (Georgi et al. 1990; Estevez et al. 1993; Lim 1993).

The proposed genetic pathways differ from each other in gene order and organization into multiple branches. Separate branches of the pathways are partially redundant and are thought to act together to control dauer larva formation. Genes positioned early in the pathways involve pheromone production (Golden and Riddle 1985) and response to environmental signals by chemosensory neurons. Amphid neurons are implicated in the latter function because the dendritic processes of these neurons are structurally abnormal in some Daf-d mutants (Lewis and Hodgkin 1977; Albert et al. 1981; Perkins et al. 1986) and because killing a subset of the amphid neurons in wild-type L1 larvae with a laser microbeam leads to constitutive dauer larva formation (Bargmann and Horvitz 1991). Vowels and Thomas (1992) proposed that the **daf-c** gene **daf-11** is directly involved in chemosensory transduction, whereas other **daf-c** genes act downstream of the chemosensory step. Thomas et al. (1993) then grouped all of the genes known to affect amphid structure or function in a separate branch of their pathway based on gene interactions and phenotypic criteria. Gottlieb and Ruvkun (1994) have reported genetic interactions be-
between three genes, daf-2, daf-23 and daf-16, which make up a branch of the pathway acting in parallel with or downstream of other branches.

As regulators of dauer larva morphogenesis, daf genes control functions that enable the efficient life maintenance necessary to be an effective dispersal stage. The dauer stage has been termed nonaging because the mean postdauer life span and reproductive capacity are not affected by a prolonged dauer stage of up to 60 days (Klass and Hirsh 1976). The apparent resilience of dauer larvae to the passage of time may be in part due to external and intracellular resistance to damage. Increased resistance to oxidative damage-inducing agents and increased levels of superoxide dismutase are correlated with increased life span in the C. elegans age-1 mutant (Larsen 1993; Vanfleteren 1993) and in dauer larvae (Anderson 1982; Larsen 1993). It was known that the effects of some daf mutations are not limited to dauer larva development because there are adult phenotypes affecting egg laying, chemotaxis and body size (Riddle 1988). In principle, adult life span might be increased in daf-c mutants if these mutations inappropriately induce dauer-related nonaging functions in adult animals.

Recently, Kenyon et al. (1993) observed that daf-2 mutants have doubled adult life spans and that the daf-2(e1370) longevity phenotype is suppressed by a daf-d mutation in daf-16, which was previously shown to suppress the Daf-c phenotype of daf-2 (Riddle et al. 1981). These results suggest that mutations in daf-2 extend life span by allowing inappropriate activation of daf-16 function (Kenyon et al. 1993). We are interested in defining the extent of genetic parallels between dauer formation and adult longevity because such parallels could represent heterochronic expression of dauer-specific life-maintenance functions that promote adult longevity.

We have examined daf genes that function late in the genetic pathway. Although we placed daf-12 and daf-2 in separate branches of the dauer pathway (Riddle et al. 1981), our recent studies reveal a complex pattern of allele-specific interactions in daf-2; daf-12 double mutants. Also, there is a synergistic effect of daf-12 mutations on increased adult longevity that was specific to daf-2(e1370) and not seen with daf-2(m41). These interactions parallel the allele-specific affects on the Daf phenotype. The daf-c mutations in genes that increase adult life span define a separate branch of the dauer formation pathway from those that do not. Interpretation of epistatic relationships reveals a genetic hierarchy for determination of adult life span.

**MATERIALS AND METHODS**

**Culture methods and mutations used**: Nematodes were grown on NG agar medium seeded with Escherichia coli strain OP50 (Brenner 1974) and maintained at 15° except as indicated below. All mutants were derived from the wild-type N2 strain (Brenner 1974). Genetic nomenclature follows guidelines described by Horvitz et al. (1979). Phenotypes are abbreviated as Daf-c (dauer-constitutive), Daf-d (dauer-defective), Dpy (dumpy, short body), Egl (egg-laying defective), Fer (fertilization defective), Sma (small adult size). Unc (uncoordinated movement) and t(s) (temperature sensitive). Mutations used are listed by linkage group as follows: LG I: dpy-1(e21), daf-8(m85ts), unc-29(e1072), daf-16(m26 and m27), unc-75(e50), LG II: fer-15(b626s), age-1(hx346), unc-4(e120), daf-19(m80ts), daf-23(m333), rol-1(e91), daf-5(e1386), unc-52(e144 and m250ts), mgl-10(e128), unc-52(e144); LG III: daf-7(e1372 and m969s), daf-2(e1370ts, m41ts and m65), daf-4(e1364ts and m72ts), unc-32(e189), qcl[dpv-19(e1295ts) gpl-1(q33)]; LG IV: daf-1(m40ks), daf-18(e1375), dpy-9(e12), dpy-13(e184ts), daf-14(m77ts), unc-22(e77); LG X: daf-3(e1376), dpy-3(e27), unc-27(e155), daf-12(m20, m25, m116 and m583), unc-58(e656d), egl-15(n484).

**Complementation and mapping data**: Observations made while testing daf-d mutants daf-12(m20) X and daf-20(m25) X for genetic interactions with Daf-c mutants led us to repeat complementation tests with these two alleles and test two others, each of which has slightly different genetic or phenotypic (daf-d or variably long adult) properties. All were subsequently shown to be allelic, so the daf-20 name is no longer used. For complementation testing, unc-27 daf-12(m20) hermaphrodites were mated with males hemizygous for daf-12(m20, m25, m116 or m583) or with wild-type males as a control. Several 1.4 hermaphrodite cross-progeny were placed singly on plates (20°), and the resulting populations were screened visually for the presence of dauer larvae (or dauer-like larvae) ~5 days after the bacteria were depleted, then were rinsed into 13-mm diameter round-bottom tubes and treated with 1% sodium dodecyl sulfate (SDS) by the method of Cassada and Russell (1975). Similar complementation tests were conducted with males hemizygous for daf-12(m20) or daf-20(m25) hermaphrodites. Wild-type, homozygous mutant, and m20/+ controls were scored in parallel with experimental populations. Only the wild-type and m20/+ controls yielded large numbers of SDS-resistant animals (dauer larvae). The m25 control population produced some dauer-like larvae that initially were resistant to SDS (30 min), but during a 2.5-hr period they ceased vigorous movement and some died. Dauer-like larvae also were observed in populations started with an animal heterozygous for m25. No dauer-like animals were observed on any other plates.

Based on two-factor data, m25 was previously positioned to the right end of X, left of unc-3 (Vowels and Thomas 1992). The m20 allele of daf-12 has been positioned within a two-map unit interval between unc-27 and egI-15 (Yin 1991). Current work confirms that the m25 allele is in the same interval. Twelve F1 progeny of 10 Unc non-Egl and 10 Egl non-UnC recombinants picked from + daf-12(m25) / unc-27 + eg1-15 parents were placed individually on seeded plates (20°) and allowed to starve. One of 10 Egl recombinants and eight of 10 Unc recombinants carried the m25 allele, resulting in a gene order of unc-27(17/20) m25 (3/20) eg1-15. The numbers in parentheses indicate the position and frequency of recombination events. The m25 allele also was mapped relative to unc-58(e657), which conveys a dominant shaker phenotype. Non-Unc progeny segregated from + daf-12(m25)/ unc-58 are expected to be either homozygous for m25 (the parental chromosome) or m25/+ (the non-UnC recombinant class). Non-Unc adults were placed individually on seeded plates (20°), the populations allowed to starve, and scored for dauer or dauer-like larvae visually and by SDS treatment as described above. Appropriate control populations
were also tested. Only one of 67 non-Unc animals from + daf-12 (m25)/ unc-58/+ produced fully SDS-resistant dauer larvae upon starvation, placing daf-12 (m25) close to unc-58, consistent with its position in the unc-27 egf-15 interval.

Construction of linked daf-c; daf-d double mutants: The daf-c; daf-d strains used in a previous study (RIDDLE et al. 1981) also were homozygous for non-Daf visible markers used to facilitate strain construction. In some instances of such markers decreased the penetrance of the daf mutation. To avoid this, strains constructed for this study contained only daf mutations. The method of strain construction used was determined by whether the daf mutation was expected to suppress the daf-c mutation (RIDDLE et al. 1981) and whether the two genes were linked.

In the case of suppressed, unlinkd double mutants, daf-d males were mated with ts daf-c hermaphrodites at 20°, then shifted to restrictive temperature (25.5°). Several L4 progeny were transferred singly to seeded plates and allowed to self at 25.5°. Homozygous F2 Daf-c animals were identified either as dauer larvae, Egl adults (TRENT et al. 1983), or in the case of daf-d, Sma adults (ESTEVEZ et al. 1995). The dauer larvae were allowed to resume development at 15°, then the progeny of postdauer, Egl or Sma adults were monitored for growth at 25.5°. Stocks of double mutants were established from a population on which all or most of the animals grew to the adult. When the penetrance of suppression was <60%, dauer or dauer-like animals were allowed to mature and their progeny were restested at 25.5° to verify the genotype. If the putative double-mutant strain was not homozygous for the daf-c gene, then one-quarter of the restested individuals would have been expected to produce all dauer progeny.

For strains in which the daf-c mutation was unlinked to the daf-d mutation and not suppressed by it, secondary phenotypes were used in some cases to score the double mutants. These include the dauer-like larval arrest exhibited by certain daf-2; daf-12 strains and the dauer-like larvae formed by several daf-c; daf-16 or daf-c; daf-18 strains (VOWELS and THOMAS 1992). If there were no secondary phenotypes, a marker was used in trans to the daf-d mutation and subsequently segregated away. For example, daf-5 II males were mated to unc-52(e444) II; daf-2(1370) III hermaphrodites. Heterozygous F1 progeny were allowed to segregate F2 dauer larvae at 25.5°, and these animals were shifted to 15° to resume development. Adults were then transferred singly to plates (15°) and the F3 generation scored for the absence of unc-52.

Construction of daf-c; daf-d mutant genotypes: In most cases it was necessary to confirm the homozygosity of the daf-c mutation by resegregating constitutively formed dauer larvae from each of 4-6 hermaphrodites derived by mating putative double-mutant hermaphrodites with wild-type, dpy-13/+ or unc-22/+ males. The paternal markers allowed identification of F1 cross-progeny. For the double-mutant strains in which the daf-c mutation was not suppressed, the homozygosity of the daf-d mutation was confirmed by a modified complementation strategy. In the case of unlinked mutations, this involved mating Daf-d males with individual putative double-mutant hermaphrodites carrying the same daf-d mutation. Three hermaphrodites were tested for each double mutant strain. Several F1 progeny from each initial cross were selfed at 25.5° to distinguish cross progeny (daf-c+/ +; daf-d) from the suppressed double mutant based on the percentage of dauer larvae present. Twenty nondauer F2 animals from a single F1 from each of the three initial crosses were transferred singly to individual plates at 25.5°. Five to seven of each set of 20 F2 populations lacked constitutively formed dauer larvae in the first generation. These were later scored for staryation-induced dauer larvae, the absence of which from all test plates confirmed that the putative double mutant was homozygous for the daf-d mutation.

Construction of linked daf-c; daf-d double mutants and confirmation of genotypes: Several methods were used to construct double mutants carrying linked daf-c and daf-d mutations. Because daf-18 and daf-14 are not closely linked, the double mutant was constructed as described for unlinked double mutants, except that the 25.5° progeny of a larger number of post-dauer adults were examined for the presence of dauer-like animals (homozygous for both mutations) segregated from a recombinant parent. Construction of daf-8 daf-16 was facilitated by selecting non-Unc recombinant dauer larvae segregated at 25.5° from daf-8 unc-29 +/+ + daf-16 heterozygotes. Individual dauer larvae were allowed to resume development and self at 15°. Non-Unc segregants from daf-8 + daf-16/ daf-8 unc-29 +/+ + daf-16 heterozygotes were transferred singly to fresh plates at 15°. The double-mutant stock was started from a wild-type animal that failed to segregate Unc progeny and also formed dauer-like larvae at 25.5°.

The daf-23 daf-5/ mnC1 (dpy-10 unc-52) strain was constructed by mating daf-23 (e1386) males with daf-23 (m333) unc-52 (sa25/8c5) hermaphrodites, the latter having been identified as maternally rescued Egl Unc progeny of a daf-23 unc-52 +/ unc-52 parent. Individual Egl non-Unc recombinants were picked from daf-23 + unc-52 +/- daf-5 + hermaphrodites and mated with unc-4/ mnC1 males to balance the recombinant chromosome. Cross progeny were placed singly on fresh plates and allowed to self. The daf-23 daf-5/ mnC1 animals, identified based on the segregation of both Egl (daf-23) and Dpy Unc (mnC1) adults, were tested for the presence of daf-5 by reisolating the dauer-defective mutant. Unc non-Rol recombinants from three unc-4 +/- rol-1 +/+ + daf-23 + daf-5 hermaphrodites were picked, and 20 Unc non-Rol segregants from each of several recombinants were tested for the Daf phenotype. The absence of starvation-induced dauer larvae in some of the populations indicated the presence of daf-5 in the original strain.

Construction of daf-c; daf-d double mutants carrying a nonconditional daf-c allele: The daf-2 (m65) III mutation results in nonconditional dauer larva formation. Consequently, construction of the double mutant carrying daf-181(e1375) IV utilized qC1 dpy-19 (e1259s) glp-1(q339) to balance m65. The first step involved construction of daf-2 (m65)/ qC1 III; dpy-9 IV. Construction steps were performed at 20°, at which the Dpy-19 and Dpy-9 phenotypes are easily distinguished. Males of genotype daf-2/ qC1 were mated with dpy-9 hermaphrodites and individual F1 males of genotype daf-2/ +; dpy-9/+ or qC1/ +; dpy-9/+ were then backcrossed with daf-2/ qC1 hermaphrodites. F1 animals of genotype daf-2/ qC1; dpy-9/ + were identified based on F2 phenotypes. A Dpy-9 segregant was selected to establish a temporary stock of daf-2/ qC1; dpy-9.

The second step involved introducing daf-18 by mating daf-2/ qC1; dpy-9 hermaphrodites with daf-18 males. F1 males (daf-2/ +; dpy-9/+ + daf-18 and qC1/ +; dpy-9/+ + daf-18) were then backcrossed with daf-2/ qC1; dpy-9 hermaphrodites and wild-type L4 progeny were placed individually on fresh plates to identify daf-2/ qC1; dpy-9/+ + daf-18 animals by observing their progeny. The daf-2/ qC1; daf-18 stock was started from a hermaphrodite that did not segregate dpy-9 progeny. The presence of daf-18 in the m65 genetic background was confirmed in that the strain segregates constitutively formed, dauer-like larvae (they exhibit sporadic pharyngeal pump-
ing), whereas m65 alone results only in the formation of nonfeeding dauer larvae.

The protocol for construction of daf-16(m26) I; daf-2(m65) III was based on the prediction that daf-16 would suppress the Daf-c phenotype of m65, as was observed with daf-2(e1370). Markers used to facilitate construction included unc-32(e189), to balance m65, and dpy-5(e61) to follow segregation of m26. The first step was construction of daf-16+/ +; daf-2+/ +; unc-32. Males homozygous for daf-16 were mated with dpy-5; unc-32 hermaphrodites. The F1 males then were mated with daf-2+/ +; unc-32 hermaphrodites and wild-type L4 progeny were picked individually to fresh plates. Genotypes of these animals were determined based on the phenotypes of their progeny. Suppression by daf-16 was assessed by scoring the progeny of individual daf-16+/ +; daf-2+/ +; unc-32 hermaphrodites. A stock was established from an animal that produced neither Unc adults nor constitutive dauer larvae. The daf-16; daf-2 genotype was verified by complementation testing.

Construction of daf-16(m26); daf-2(e1370); daf-12(m20):

Preliminary steps for constructing the triple mutant involved heat-shock induction (SULSTON and HODGKIN 1988) of daf-16(m26) I; daf-2(e1370) III and daf-2(e1370) III; daf-12(m20) X males. A daf-16 I; daf-2 III; unc-27 egl-15 X strain then was constructed in two phases by mating daf-2+/ +; males with unc-27 egl-15 hermaphrodites, putting individual wild-type F1 L4 larvae on fresh plates at 25.5°, and selecting F2 Unc dauer larvae, which were transferred to 15° to resume development. One Unc Egl daf-16 adult was chosen to establish a daf-2; unc-27 egl-15 stock.

In the second phase of construction, daf-16; daf-2 males were mated with the daf-2; unc-27 egl-15 hermaphrodites at 20°. Several L4 cross progeny, homozygous for daf-2, were put individually on fresh plates at 15° until the adult stage to bypass dauer formation and increase fertility, then shifted to 25.5° to select for daf-16 segregants based on suppression of daf-2 dauer formation. A daf-16; daf-2; unc-27 egl-15 stock was established from an Unc Egl F2 adult that did not segregate dauer larvae at 25.5°, so the presence of daf-2 was confirmed by mating with wild-type males and resegregating constitutive dauer larvae from five of five heterozygotes.

To construct the desired triple mutant, daf-2; unc-27 daf-12 males were mated with the daf-16; daf-2; unc-27 egl-15 hermaphrodites at 15°. Individual L4 cross progeny were transferred to fresh plates at 15° and after reaching adulthood, were shifted to 25.5° for selling. A non-Unc animal that grew to the adult at 25.5° and did not segregate either Unc Egl animals or dauer larvae was used to establish a daf-16; daf-2; daf-12 stock.

The presence of daf-2 and daf-16 mutations was confirmed based on complementation (daf-2) and the ability of daf-16 to dominantly suppress the Daf-c phenotype of daf-2 in a daf-16(m20) background. Duplicated crosses between daf-2(e1370) males and putative triple-mutant hermaphrodites were started at 15° and shifted to 25.5° the next day. The F1s were transferred to fresh plates daily for 2 more days and the progeny from each day's brood scored after 3 days. Both dauer larvae and adult males were observed in the F1, indicating the presence of both daf-2 and daf-16 in the triple mutant.

The presence of daf-12 was determined by resegregation of the X-linked Daf-d trait. First, males of genotype daf-16+/ +; daf-2+/ +; daf-12/0 were mated with unc-27 egl-15 hermaphrodites. Wild-type L4 progeny were then transferred individually to fresh plates at 25.5° to identify those not segregating constitutively formed daf-2 dauer larvae. Progeny of one such animal were put on fresh plates to identify populations in which Unc Egl adults were absent, and therefore presumed to be homozygous for daf-12. Fifteen such populations were scored later for the presence of starvation-induced dauer larvae. Their absence from all test plates confirmed that the triple mutant was homozygous for daf-12.

Daf phenotypes: The percentage of L4 and adult animals formed by the double mutants and the triple mutant at 25.5° was based on observations of synchronous populations at specific intervals during a 4–5-day period. Approximately 20–24 adult hermaphrodites were allowed to lay eggs on seeded NG plates for ~4 hr at 20° and then were removed. The plates were shifted to 25.5° and the populations monitored for dauer and nondauer development starting 48–50 hr after the midpoint of the egg-laying period, and at least daily thereafter. Fourth-stage larvae and adults were removed as necessary to prevent contamination by offspring. The daf-c controls were assayed simultaneously. The only known daf-14 mutation is not 100% penetrant at 25.5°. Consequently, the percentage of nondauer development for nearly all daf-14; daf-2 double mutants (Table 1) was reduced by 1–3% to reflect the background growth by daf-14 alone. The percentage for daf-16(m26); daf-14 was reduced by 18%, because 18% of the daf-14 control animals grew to the adult in that experiment.

From the time of egg laying a minimum of 69 hr is required for a mutant animal to become a dauer larva and recover to the L4 stage (BERLY et al. 1976; SWANSON and RIDDLE 1981; GOLDEN and RIDDLE 1984b). Hence, L4 larvae and adults scored 30 hr after the midpoint of the 4-hr egg-laying period resulted from nondauer development rather than recovery from a dauer larva. This focuses the study on entry into the dauer state.

Construction of fer-15; daf-4 double mutants: Males heterozygous for the daf-4 mutations were mated with fer-15( e2686) raised at 25.5°, a temperature at which b26 fails to produce sperm. Single F1 hermaphrodites were placed on plates at 25.5° and F2 dauer larvae (homozygous daf-c) were transferred to 15° to resume development. To test for fer-15, individual adults were allowed to lay eggs overnight at 15° and then were transferred to fresh plates. The F1 progeny from this egg-laying period were shifted to 25.5° after the temperature-sensitive period for dauer larva formation (SWANSON and RIDDLE 1981), and subsequently checked for oocytes laid by the adult. Each fer-15; daf-4 stock was started with one animal that had been maintained at 15°. The fer-15; daf-4 double mutant was constructed by placing F1 hermaphrodites at 15° and selecting individual small adults (homozygous daf-4), which were subsequently tested for Fer-15.

Construction of a daf-16; age-1 strain: Strains carrying age-1 also carry the closely linked fer-15 mutation because age-1 was identified in this genetic background (FRIEDMAN and JOHNSON 1988). Hence, the ts Fer-15 phenotype was used to follow age-1 in strain constructions. Males homozygous for daf-16 were mated with dpy-5(e61) unc-73(e950); fer-15(b26) age-1( hs546); and a sterile F2 derivative that did not segregate Dpy Unc progeny was saved as a stock for life-span analysis.

Adult life span: The animals for life-span analysis were raised at 15°. For experiments at the restrictive temperature, animals were placed at 25.5° as L4 larvae or young adults. The first day of adulthood is day 1 in the survival curves presented. The adult life span of homozygous daf-23(m333) could be determined only with maternally rescued animals, the progeny of which nonconditionally arrest development. Mutant populations were assayed in parallel with N2 to control for possible environmental fluctuations. Only twelve to eighteen adults were placed per 60-mm plate to avoid overcrowding and competition for food. During the reproductive period, adults were transferred daily to fresh plates and thereafter approximately every 10 days. Animals were scored as alive, dead, or lost at least every other day. Animals that neither fed nor moved in response to stimulation (prodding with the tip of a platinum wire) were scored as dead. At least
two independent trials were performed for each strain, and the figures show the data from representative trials. Statistical analyses were performed using SAS version 6.04 (SAS Institute, Cary, NC). Values reported are the mean ± SE, and the last quartile (25% survival).

**RESULTS**

**Epistasis analysis of dauer-constitutive, dauer-defective double mutants:**

Based primarily on double-mutant phenotypes, **daf** genes have been ordered in pathways thought to represent the hierarchy of steps involved in dauer larva formation (Riddle et al. 1981; Vowels and Thomas 1992; Thomas et al. 1993; Gottlieb and Ruvkun 1994). However, the gene order interpreted from the mutant analyses differs. We have conducted a new epistasis analysis using tightly synchronized populations of **daf-c**, **daf-d** mutant strains that do not carry visible marker mutations (see MATERIALS AND METHODS). The phenotype exhibited by a double mutant at the restrictive temperature (25.5°C), generally either **Daf-c** or **Daf-d**, is that of the epistatic gene, which is interpreted to define a later step in the genetic pathway (Riddle et al. 1981).

Some of the epistasis data reported here corroborates that previously published. In addition, we include data pertaining to six new alleles: the **daf-d** mutations **daf-12** (ml16 and m58?), the **daf-c** mutations **daf-8** (m85ts), **duf-2** (m4lts and m65) and **m???,** the original **daf-c** mutation defining **daf2?**. The **duf-8** (m85) mutant is more penetrant than the **e1?w** reference allele used in previous work. The **duf-2** (m4lts) allele has different properties from the **e1370ts** reference allele, and the **daf-2** (m65) allele results in a nonconditional Daf-c phenotype. Homozygous **m65** dauer larvae do not exit the dauer stage, so the mutation is maintained either in a balanced heterozygote or in a double mutant with **daf-16**. The **daf-12** alleles fail to form either dauer or dauer-like larvae, like the reference **daf-l2(rn20)** allele. The **daf-2?** (m3??) mutation results in a maternally rescued Daf-c phenotype; homozygous mutant progeny segregated from a heterozygous parent become Egl adults. Progeny of maternally rescued homozygous parents unconditionally arrest development either as dauer or pre-dauer L2d larvae that no longer feed. At 25°C, the percentage of dauer larvae ranges from 15% (4 days) to 30-50% (10 days), whereas fewer than 3% of larvae raised at 15 or 20°C undergo radial body shrinkage.

**TABLE 1**

Percentages of L4 and adult animals formed at the restrictive temperature by dauer-constitutive, dauer-defective double mutants

<table>
<thead>
<tr>
<th><strong>daf/c</strong> mutation</th>
<th><strong>daf-3(e1376)</strong></th>
<th><strong>daf-5(e1386)</strong></th>
<th><strong>daf-12(m20)</strong></th>
<th><strong>daf-12(m25)</strong></th>
<th><strong>daf-12(m116)</strong></th>
<th><strong>daf-16(m26)</strong></th>
<th><strong>daf-16(m27)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>daf-1(m46) IV</strong></td>
<td>100 (416)</td>
<td>90 (283)</td>
<td>100 (318)</td>
<td>77 (217)</td>
<td>100 (271)</td>
<td>14 (195)</td>
<td>0 (121)</td>
</tr>
<tr>
<td><strong>daf-4(e1364)</strong></td>
<td>99 (257)</td>
<td>100 (447)</td>
<td>92 (181)</td>
<td>16 (280)</td>
<td>100 (177)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9 (348)</td>
<td>0 (386)</td>
</tr>
<tr>
<td><strong>daf-7(e1372)</strong></td>
<td>100 (482)</td>
<td>98 (670)</td>
<td>100 (254)</td>
<td>8 (357)</td>
<td>100 (273)</td>
<td>5 (229)</td>
<td>0 (178)</td>
</tr>
<tr>
<td><strong>daf-8(m58)</strong></td>
<td>100 (584)</td>
<td>98 (710)</td>
<td>100 (403)</td>
<td>80 (627)</td>
<td>100 (245)</td>
<td>0 (194)</td>
<td>0 (276)</td>
</tr>
<tr>
<td><strong>daf-14(m77)</strong></td>
<td>100 (429)</td>
<td>99 (257)</td>
<td>97 (360)</td>
<td>96 (234)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>ND</td>
<td>47 (419)</td>
<td>1 (252)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>daf-20(m41)</strong></td>
<td>100 (404)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 (377)</td>
<td>0 (396)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 (371)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 (158)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>91 (388)</td>
<td>99 (726)</td>
</tr>
<tr>
<td><strong>daf-20(e1376)</strong></td>
<td>0 (314)</td>
<td>0 (264)</td>
<td>0 (309)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 (220)</td>
<td>ND</td>
<td>98 (262)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined

<sup>4</sup>This allele of **daf-12** was previously referred to as **daf-20** (Riddle et al. 1981).

<sup>4</sup>Animals were scored 68 hours after the middle of the egg-laying period.

<sup>4</sup>Animals were scored 72 hours after the middle of the egg-laying period.

<sup>4</sup>Unlike **daf-20(m41)** or **daf-3(e1376)** single mutants, this double mutant forms some transient dauer-like animals at 15°C.

<sup>4</sup>By 72 hr 100% of the animals were L4 larvae or adults.

<sup>4</sup>The mutant exhibited a dauer-like larval arrest.

<sup>4</sup>The **daf-23** mutant is a nonconditional, maternal-effect mutant that primarily forms constitutive nonfeeding dauer-like larvae.

<sup>4</sup>Homozygous mutant adults segregated from **daf-23(mmc1)[dp-10 une-52]**; **daf-12** do not lay eggs; consequently, the animals used in this study were cut in half to release eggs.

<sup>4</sup>Many of the double-mutant adults had a protruding vulva.
by day 20. Hence, the terminal stage of developmental arrest is somewhat as for *daf-23(m333)*. The intestines of maternally rescued adults and developmentally arrested larvae appear darker than the surrounding tissue when viewed through the dissecting microscope, presumably as a result of the accumulation of food storage granules characteristic of dauer and predauer L2d larvae (Albert and Riddle 1988; Thomas et al. 1993).

Suppression of the Daf-c phenotype by an epistatic *daf-d* mutation in the double mutants divides the *daf-c* genes into two groups (Table 1). First, mutations in *daf-1, daf-4, daf-7, daf-8, and daf-14* were fully suppressed by *daf-d* mutations in *daf-3, daf-5, and daf-12 (m20 and n116)*, but were not efficiently suppressed by *daf-d* mutations in *daf-16* or *daf-18*. The range of suppression by *daf-12 (m25)* for the same *daf-c* mutations varied widely, from 96% for *daf-1* down to only 8% for *daf-7 (e1372)*. This incomplete suppression by *daf-12 (m25)*; previously referred to as *daf-20* (see MATERIALS AND METHODS), suggested that *n25* is a weak allele. Unlike other *daf-12* mutants, *m25* formed some dauer-like larvae upon starvation, and the adults were not long. Dauer-like larvae possess some, but not all, of the unique characteristics of dauer larvae. The *m25* dauer-like larvae closely resembled dauer larvae, but were not fully resistant to SDS treatment due to incomplete suppression of pharyngeal pumping (Vowels and Thomas 1992).

In contrast to the first group of *daf-c* mutations, *daf-2* and *daf-23* were not suppressed by *daf-3 or daf-5*, but were suppressed by *daf-16*. In addition, *daf-16 (m26)* was capable of suppressing *daf-2 (m65)*, a nonconditional Daf-c allele. Fifty hours after the midpoint of egg laying, 91% of *daf-16 (m26)*; *daf-2 (m65)* progeny developed into L4 larvae or adults (*n = 139*) at 25.5°C and the other 9% arrested development as L1 or L2 larvae. Although this strain was viable at 15°C, mutant larvae that matured at 25.5°C laid eggs that did not hatch. Hence, this strain is a ts embryonic lethal mutant.

Whereas *daf-16* was epistatic to both *daf-2* and *daf-23*, *daf-18* was epistatic to *daf-23*, but not *daf-2 (e1370)*. The *daf-18 (e1373)* mutation was previously reported (Vowels and Thomas 1992; Gottlieb and Ruvkun 1994) not to suppress *daf-2 (e1370)*. Our results are consistent with that, and show that 50 hr after egg laying only 3% of the double mutant progeny grew to the adult at 25.5°C, although 96% grew by day 5. The *daf-18* mutation also did not suppress *daf-2 (m65)*. The double mutant formed dauer-like larvae similar to those formed at low frequency by the *daf-18* strain; they were radially shrunken but fed sporadically. The percent suppression exhibited by *daf-23; daf-18* mutants depends on the *daf-23* allele. The three known *daf-23* alleles, *m333, mg44* and *mg55*, are all nonconditional and maternally rescued. Data pertaining to *m333* are presented in this study, and that for the other alleles is from Gottlieb and Ruvkun (1994). Based on the percentage of animals that complete dauer larva morphogenesis, *mg55* is the most severe allele. This is also true with regard to epistasis. Both *m333* and *mg44* were completely suppressed by *daf-18 (e1375)*, but only a small percentage of *daf-23 (mg55); daf-18 (e1375)* animals grew to the adult. However, *mg55* was induced by gamma irradiation and appears to be a complex rearrangement (Gottlieb and Ruvkun 1994). Therefore, other loci could be affected in addition to *daf-23*. Also, *daf-18 (e1375)* might be a weak allele; it is the only known mutation defining the gene. Until further information is obtained, we consider *daf-18* to be epistatic to *daf-23*.

In summary, there are two branches in the genetic pathway (Figure 1, A and B) because the *daf-d* mutations *daf-16* and *daf-18* are not epistatic to *daf-c* mutations in *daf-1*, *daf-4*, *daf-7*, *daf-8*, and *daf-14*, yet are epistatic to *daf-23*. In addition, the *daf-d* mutations *daf-3* and *daf-5* are epistatic to *daf-1*, *daf-4*, *daf-7*, *daf-8*, and *daf-14*, but not to *daf-23* or *daf-2*. We refer to these two branches of the pathway as the *daf-1* and *daf-2* branches, respectively.

**Novel phenotypes in double mutants:** Certain *daf-c; daf-d* mutants displayed phenotypes unlike those of either parent. The *daf-2 (e1370); daf-12* mutants displayed predauer or dauer-like arrest (Table 1). This was previously observed for the *daf-2 (e1370); daf-12 (m20 or m25)* and *daf-2 (e1286); daf-12 (m20)* strains (Yeh 1991; Vowels and Thomas 1992). We observed that *daf-2 (e1370); daf-12* double mutants constructed with four independent alleles of *daf-12* each arrested development with subtly different phenotypes. The *daf-2 (e1370); daf-12 (m25)* double mutant was most like *daf-2 (e1370)* alone, but formed a small percentage of SDS-sensitive animals with incomplete radial body shrinkage. Resistance to 1% SDS is normally acquired within 1 hr after radial shrinkage (Swanson and Riddle 1981). The *daf-2 (e1370); daf-12 (m20 and m116)* animals arrested at about the size and morphology of an L2d with only sporadic pharyngeal pumping. A few animals appeared to initiate, but not complete, radial shrinkage of the body. The *daf-2 (e1370); daf-12 (m583)* animals fed more and consequently grew larger than the other *daf-2 (e1370); daf-12* double mutants. Some initiated abnormal vulval differentiation. At 22.5°C, *daf-2 (e1370)* formed dauer and L2d larvae, but the four *daf-2 (e1370); daf-12* double mutants developed slowly into sterile adults, with the exception of an occasional *m583* double mutant that was fertile. Hence, the *daf-12* mutations tested were not epistatic to *daf-2 (e1370)*, but they produced novel, intermediate phenotypes. A ranking of *daf-12* alleles in order of increasing severity (*m25 < m20 = m116 < m583*) might be inferred from these results, but this order is not maintained with respect to the fecundity or life-span phenotypes.

Whereas *daf-2 (e1370)* was not suppressed by *daf-12* mutations, the *daf-2 (m41)* allele was suppressed well by *daf-12 (m20, m116 or m583)*. Double mutants displayed the *daf-12* phenotype of nondauer development at 25.5°C.
for dauer larva development, because mutations in either of the upstream genes that have pleiotropic effects on amphid sensory functions. Omitting daf-18 reconciles the dauer and life span pathways as shown. A common pathway for both dauer formation and longevity that incorporates daf-18 requires re-interpretation of two sets of epistasis data and is presented in the Discussion. The pathway represents epistatic interactions observed at 25.5° between daf-d mutations and daf-c mutations as scored in double mutants 50 hr after the midpoint of a 4-hr egg-laying period. Suppression of the Daf-c phenotype by an epistatic daf-d mutation is defined as growth of at least two-thirds of the experimental animals to adults (Table 1, values >67%). The pathway for determination of adult life span that includes data for daf-18. The data are presented in Tables 3 and 4 and in Figures 3 and 4.

The daf-c phenotype of daf-2(m41) was not weaker than that of e1370. All animals of both mutants developed into dauer larvae when raised at 25.5°. The phenotypes differed only slightly at 20°; e1370 formed 0.6% ± 0.9 dauer larvae (n = 1280) and m41 formed 16% ± 3.4 dauer-like larvae (n = 1577) that had radially shrunken bodies but exhibited small pharyngeal movements. Neither mutant forms dauer larvae at 15°. In terms of direct nondauer development at 20°, e1370 is more wild type than m41.

The daf-12 mutations tested did not suppress daf-23 (m333). However, the daf-23 (m333); daf-12 (m20) mutant also exhibited a novel phenotype, as was the case with daf-2 (e1370); daf-12 double mutants. Unlike daf-23 in a daf-12 (+) genetic background, maternally rescued adult double-mutant animals segregated from daf-23/mnC1; daf-12 did not lay eggs, even though they each possessed a vulva. Their arrested progeny, unlike daf-23 arrested larvae, eventually became less dark-bodied and underwent some additional gonadal cell division. This gonadal development, visible in DAPI-stained animals, could not be observed using Nomarski optics due to the presence of intestinal granules responsible for the dark-intestine phenotype. In a double mutant constructed with daf-12 (m20) and the weaker daf-23 (mg44) allele, ~30% of the animals slowly develop into late larvae or sterile adults lacking the dark intestine (Gottlieb and Ruvkun 1994).

In summary, the daf-23 (m333); daf-12 (m20) novel Egl and gonadal phenotypes did not involve entry into dauer formation as defined here because the double mutant progeny were similar in phenotype to daf-23 at 50 hr after the eggs were obtained. The position of daf-12 is either upstream of daf-23 or in a different branch of the pathway. The data on daf-12 and daf-2 do not permit unambiguous ordering of these two genes, because all tested daf-12 alleles are epistatic to m41, but not to e1370, and novel phenotypes were produced in the various daf-2 (e1370); daf-12 double mutants. We represent these genetic interactions as a mutually antagonistic relationship between the daf-2 and daf-12 gene products (Figure 1, A and B) similar to that proposed by Gottlieb and Ruvkun (1994).

Adult life spans of Daf-c mutants: To investigate the overlap in genetic regulation of dauer larva formation and adult life span, the same strains and temperatures used to analyze dauer formation were used to determine life spans. If adult life span can be extended by induction of dauer-related life maintenance mechanisms, mutations that result in a Daf-c phenotype would
be the most likely to do so. The life spans of adults carrying *daf-c* mutations were determined at a temperature at which the Daf phenotype is fully penetrant. The animals were allowed to progress through development at the permissive temperature (15°C), then placed at the restrictive temperature (25.5°C) to determine adult life span. Some mutations did not increase life span, whereas others doubled it.

Five *daf-c* mutations, *daf-1(m40)*, *daf-4(m72)*, *daf-7(n696)*, *daf-8(m85)*, and *daf-19(m86)* did not increase adult life span (Figure 2 and Table 2). These mutants have a strong Egl phenotype (TRENT et al. 1985), so eggs hatch internally and the developing larvae eventually kill the mother. Animals killed in this manner during the experiment presented in Figure 2 were 8, 46, 95, 61, and 56% of the populations for N2, *daf-1(m40)*, *daf-4(m72)*, *daf-7(n696)*, and *daf-8(m85)*, respectively. Animals that died due to internal hatching of eggs were considered to have died from matricide, not senescence. These matricidal deaths before day 7 of adulthood were excluded from all the survival curves shown. Inclusion of such deaths would have decreased the means, in some cases to a large extent. To avoid internal hatching of eggs, *fer-15ts; daf-c* double-mutant strains were constructed and their life spans were determined. The ts *fer-15* mutation causes sterility, but does not increase life span (FRIEDMAN and JOHNSON 1988). The mean life spans of the sterile *fer-15*; *daf-c* double mutants were like that of wild type (Table 2), confirming that exclusion of matricidal deaths prevented the skewing of mean life spans relative to those of control strains.

The *daf-c* mutations that increased adult life span were *daf-23* and the two alleles of *daf-2(m41)* and *e1370* that we tested (Figure 2 and Table 2). The *daf-2* alleles *sa189*, *sa193* and *e1370* also increase adult life span at 20°C (KENYON et al. 1993). The *daf-2(e1370)*; *daf-12(m20)* strain was included in the analysis because the *daf-12* mutation modifies the Daf-2 phenotype. Double-mutant animals constitutively arrested development as L2d or dauer-like larvae at 25.5°C. When the animals were raised at 15°C, then shifted to 25.5°C, the *daf-12(m20)* mutation nearly doubled *daf-2(e1370)* adult longevity (Figure 2). The 41-day mean adult life span for this double mutant is the largest genetic increase observed to date for *C. elegans* (Table 2). One animal lived 85 days at 25.5°C, more than seven times the mean wild-type life span of 12 days (Figure 2).

**Epistasis analysis of adult life span:** We have deduced a genetic hierarchy for determination of life span from epistatic relationships in appropriate *daf-c; daf-d* double mutants. The life spans were analyzed for double-mutant strains carrying a long-lived *daf-c* mutation, *daf-23* or *daf-2*, and a *daf-d* mutation that suppressed the Daf-c phenotype. KENYON et al. (1993) have shown that the *daf-16(m26)* mutation suppresses the *daf-2(e1370)* life span extension phenotype at 20°C using the *daf-16(m26); daf-2(e1370)* strain that we had constructed for analysis of the Daf phenotype. Our experiments with this strain were performed at 25.5°C because this is the temperature at which the Daf phenotypes were determined for the dauer pathway. As shown in Table 3 and Figure 3, the *daf-16(m26); daf-2(e1370)* life span was similar to that of *daf-16(m26)*. The life spans of *daf-d* strains *daf-16* and *daf-18* were reduced in comparison with wild type. Comparisons of the means using the log-rank test yielded a chi-square value of 31.2 for *daf-16* (*P* < 0.0001), and a chi-square value of 112 (*P* < 0.0001) for *daf-18* vs. wild type. The *daf-16* mutation also suppressed the extended life spans of the *daf-23* and *daf-2(m41)* mutants. Furthermore, we observed no extension of life span for the *daf-16(m26); daf-2(m65)* double mutant, in which the nonconditional *daf-2(m65)* allele is a putative null allele.

The *daf-18* mutation partially suppressed the life span extension of *daf-2(e1370)*, as shown in Figure 3B. The life span of the double mutant is intermediate between those of *daf-18* and *daf-2(e1370)*, but closer to that of *daf-18*. By contrast, extension of life span by *daf-23(m333)* is not suppressed by *daf-18*. A caveat of this experiment is that 99% of the *daf-18*; *daf-23* mutant animals die due to internal hatching of eggs, so we started the experiment with a very large synchronous population and selected for those rare animals that survived the reproductive period. A *fer-15* *daf-23* chromosome was not constructed because these genes are closely linked (less than two map units). In summary, observations of the longevity phenotype suggested a genetic hierarchy (Figure 1C) that involves the genes.
from the daf-2 branch of the dauer pathway (Figure 1B), but the order of these genes differs because of the results with the double mutants carrying daf-18. Alternate interpretations of daf-18 epistasis are presented in the DISCUSSION.

The life spans of seven daf-2; daf-12 double-mutant strains were analyzed to place daf-12 in the life-span pathway and to test for allele-specific effects similar to those observed in dauer larva development. As controls, the life spans of each of four daf-12 mutants were determined and found to be slightly shorter than that of N2 (Figure 4A). The small reduction in mean life span was less than that observed for daf-18. The maximum life spans of all the daf-2(m41); daf-12 animals were approximately double that of wild type and similar to that of m41 alone (Figure 4B). Although the mean life spans of these double mutants appeared to be slightly decreased relative to that of m41 (Table 4), the significance of these differences was difficult to interpret because the fraction of the m41 population that exhibited extended life varied in independent trials. The maximum life spans also fluctuated because they represented the survival of a single animal. In general, we used the last quartile for comparisons between independent trials of the daf-2 strains.

Mutants homozygous for daf-2(e1370) and one of four independent daf-12 alleles each displayed larval arrest phenotypes if hatched and raised at 25.5°C, although there were subtle qualitative differences in the terminal phenotypes. The shape of the adult survival curve also differed depending on the daf-12 allele (Figure 4C). The last quartile and maximum life spans of daf-2(e1370) were enhanced in double mutants with daf-12(m20, m25, or m16), but in the case of daf-2(e1370); daf-12(m583), 70% of the population died earlier than wild type, and the remainder lived longer (Table 4 and Figure 4C). Premature death was not seen in any other allelic combination tested, including daf-2(m41); daf-12(m583). These allele-specific mutant interactions suggest that in wild-type animals, the daf-2 and daf-12 gene products may interact to determine adult life span.

The synergistically enhanced life span of daf-2(e1370); daf-12(m20) is completely suppressed by daf-16(m26) in a triple mutant (Figure 3A). These results indicate that daf-16 acts downstream of daf-2 and daf-12 with respect to life span. The dauer-like arrest phenotype is suppressed in this strain, and in a similar strain reported by Gottlieb and Ruvkun (1994). The daf-16 gene also acts downstream of age-1. The mean life span of frr-15(b26) age-1(hs546) at 25.5°C was 28.0 ± 0.64 days, consistent with that previously reported by Friedman and Johnson (1988), whereas the mean life span of daf-16(m26) frr-15 age-1 II was reduced to 15.8 ± 0.35 days. The control daf-16; frr-15 strain had a mean life span of 12.7 ± 0.27 days at 25.5°C.

**TABLE 3**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Life span (days)</th>
<th>% of N2 last quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>12.7 ± 0.37 (66)</td>
<td>100</td>
</tr>
<tr>
<td>daf-16(m26)</td>
<td>9.1 ± 0.26 (71)</td>
<td>71</td>
</tr>
<tr>
<td>daf-16(m26); daf-2(m65)</td>
<td>8.6 ± 0.29 (110)</td>
<td>71</td>
</tr>
<tr>
<td>N2</td>
<td>13.0 ± 0.37 (94)</td>
<td>100</td>
</tr>
<tr>
<td>daf-16(m26)</td>
<td>12.5 ± 0.27 (125)</td>
<td>93</td>
</tr>
<tr>
<td>daf-16(m26); daf-23(m333)</td>
<td>12.0 ± 0.41 (122)</td>
<td>100</td>
</tr>
<tr>
<td>daf-16(m26); daf-2(e1370)</td>
<td>14.0 ± 0.29 (125)</td>
<td>113</td>
</tr>
<tr>
<td>daf-18(e1375)</td>
<td>10.7 ± 0.19 (117)</td>
<td>73</td>
</tr>
<tr>
<td>daf-18(e1375); daf-2(e1370)</td>
<td>16.9 ± 0.60 (92)</td>
<td>147</td>
</tr>
<tr>
<td>daf-18(e1375); daf-23(m333)</td>
<td>35.3 ± 1.00 (114)</td>
<td>273</td>
</tr>
<tr>
<td>N2</td>
<td>14.8 ± 0.28 (118)</td>
<td>100</td>
</tr>
<tr>
<td>daf-18(e1375); daf-23(m333)</td>
<td>28.7 ± 1.00 (44)</td>
<td>200</td>
</tr>
<tr>
<td>N2</td>
<td>14.0 ± 0.33 (122)</td>
<td>100</td>
</tr>
<tr>
<td>daf-16(m26)</td>
<td>11.6 ± 0.28 (125)</td>
<td>88</td>
</tr>
<tr>
<td>daf-16(m26); daf-2(m41)</td>
<td>13.4 ± 0.34 (125)</td>
<td>100</td>
</tr>
<tr>
<td>daf-16(m26); daf-2(e1370); daf-12(m20)</td>
<td>12.2 ± 0.36 (148)</td>
<td>100</td>
</tr>
<tr>
<td>daf-12(m20)</td>
<td>9.2 ± 0.36 (98)</td>
<td>75</td>
</tr>
</tbody>
</table>

*Values are means ± SE with no. of animals in parentheses.

†The last quartile remained alive on day-14.

‡The last quartile remained alive on day-15.

§Animals were from eggs isolated by treatment of gravid adults with hypochlorite (Sulston and Hodgkin 1988). There were initially ~8,000 double-mutant adults; only those that did not die due to internal hatching of eggs were included in this analysis.

‖The last quartile remained alive on day-16.
mation (no constitutive dauer larvae develop at 15° and 100% form dauer larvae at 25.5°), a ts increase in longevity would further the parallels between dauer formation and life span. In control experiments, the life spans of each of the four independent daf-12 alleles were nearly like that of wild type at 15° (Figure 5A). Remarkably, the life span of daf-2(m41) was also like that of wild type (Table 4 and Figure 5B). The daf-2(m41); daf-12 mutants were also ts; they had only a very slightly increased life span at 15°. The daf-2(e1370) mutant was somewhat ts for life span (Figure 5C) in that the percent increase over wild type was less at 15° than at 25.5° (Table 4). Interestingly, both the premature death of daf-2(e1370); daf-12(m583) and the synergistically extended life spans of the other daf-2(e1370); daf-12strains at 25.5° were not apparent at 15°.

Reproduction in the long-lived Daf strains: We determined the length of the fertile period and the number of progeny produced by the daf-2 and daf-12 single and double mutants to assess whether these genetically altered strains may have “paid” for a greatly increased life span with a reduced reproductive capacity, as might be predicted from the evolutionary theory of aging. In this theory, each species evolved for optimal success of its reproductive strategy instead of affording the maximum possible protection to the soma (MEDAWAR 1952). Furthermore, cases of antagonistic pleiotropy may arise, in which a gene with an early benefit may be detrimental later, and thereby limit life (ROSE 1991).

The onset of reproduction was not delayed and the duration of the reproductive period was not equivalently extended in mutants exhibiting increased adult life spans (Figure 6). The total number of progeny at 25.5° was consistently reduced by daf-2(e1370) and daf-12(m116) relative to wild-type N2 (Table 5), and the daf-2(e1370); daf-12(m116) mutant was the most severely reduced. However, the mean brood size for daf-2(m41), which like daf-2(e1370) doubles mean life span, was reduced only 11% relative to wild type. The daf-12 mutant strains with nearly wild-type life spans had

Figure 3.—Survival curves for daf-c; daf-d mutants in the daf-2 branch of the genetic pathway for dauer formation. Populations were raised at 15° to the L4 stage, then shifted to 25.5°. (A) Strains carrying daf-2 and daf-16. (B) Strains carrying daf-16 or daf-18.

Figure 4.—Survival curves for daf-2, daf-12, and daf-2; daf-12 adults at 25.5°. The animals were raised at 15°, then shifted to 25.5° as young adults. (A) Alleles of daf-12. (B) Strains bearing daf-2(m41), alone and with alleles of daf-12. (C) Strains bearing daf-2(e1370), alone and with alleles of daf-12.
mean brood sizes as low as 59% of wild type, whereas the *daf-2(e1370); daf-12(m25)* strain, which had a similar brood size, nearly tripled life span. We conclude that a reduction in brood size was neither necessary nor predictive for an increased adult life span. Indeed, ablation of gonadal cells with a laser microbeam had no effect on wild-type or double mutant strains at 15°C showed that fecundity was ts for the *daf-2(e1370); daf-12(m116)* adults, and most of these late progeny were viable and fertile. This phenomenon is allele-specific, since no late progeny were observed during the life-span analysis of any *daf-2(m41)*-carrying strains.

Determination of brood sizes and reproductive schedules at 15°C showed that fecundity was ts for the *daf-2*, but not *daf-12*, mutations, as is the case for dauer larva development and adult life span. The *daf-2(e1370)* strain produced substantially larger broods at 15°C than at 25.5°C, but a similar effect was not observed for *daf-2(m41)* because the brood size was only slightly less than that of wild type at both temperatures (Table 5). The small *daf-12(m116)* brood size showed no improvement at the lower temperature; neither the dauer formation defect nor the reproductive defect is ts for any of the tested alleles of this gene. The timing of onset and cessation of reproduction is similar for all 14 strains tested (Figure 7). In summary, reproduction is neither delayed nor prolonged in mutants with extended adult life spans, except for the very few late progeny produced by *daf-2(e1370)*-bearing strains at 25.5°C. In some strains brood sizes were substantially reduced, but this was not predictive of increased longevity.

**DISCUSSION**

During the life history of *C. elegans*, daf genes are involved in processes as diverse as larval development, reproduction, and adult life span. The partial genetic pathway for dauer larva formation presented here has two branches. The *daf-c* mutations that increase adult life span are all positioned in the *daf-2* branch, but the gene order may differ depending on the interpretation of interactions with the *daf-d* gene, *daf-18*. The *daf-2* and *daf-12* genes interact in an allele-specific manner to affect both dauer larva formation and adult life span.

The order of genes in the *daf-1* branch of the dauer formation pathway is consistent with the deduced functions of cloned genes. Those affecting chemosensory structure and function (not shown in Figure 1) are followed by three genes involved in signal transduction. Both *daf-1* (Georgi et al. 1990) and *daf-4* (Estévez et al. 1993) encode receptor serine/threonine kinases similar to the activin and transforming growth factor-β (TGF-β) receptors (Mathews and Vale 1991; Lin et al. 1992), and the *daf-7* gene encodes a novel member

**TABLE 4**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Life span (days)</th>
<th>% of N2 last quartile</th>
<th>Life span (days)</th>
<th>% of N2 last quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>21.6 ± 0.47 (86)</td>
<td>100</td>
<td>12.6 ± 0.42 (84)</td>
<td>100</td>
</tr>
<tr>
<td>daf-12(m20)</td>
<td>20.0 ± 0.41 (109)</td>
<td>88</td>
<td>10.4 ± 0.41 (62)</td>
<td>88</td>
</tr>
<tr>
<td>daf-12(m25)</td>
<td>20.0 ± 9.41 (112)</td>
<td>88</td>
<td>11.3 ± 0.54 (64)</td>
<td>100</td>
</tr>
<tr>
<td>daf-12(m116)</td>
<td>20.7 ± 0.69 (55)</td>
<td>88</td>
<td>11.5 ± 0.50 (58)</td>
<td>88</td>
</tr>
<tr>
<td>daf-12(m583)</td>
<td>21.1 ± 0.52 (96)</td>
<td>96</td>
<td>10.8 ± 0.57 (63)</td>
<td>88</td>
</tr>
<tr>
<td>daf-2(m41)</td>
<td>21.4 ± 0.43 (84)</td>
<td>92</td>
<td>26.4 ± 1.54 (58)</td>
<td>231</td>
</tr>
<tr>
<td>daf-2(m41); daf-12(m20)</td>
<td>25.2 ± 0.47 (98)</td>
<td>108</td>
<td>21.4 ± 1.57 (62)</td>
<td>219</td>
</tr>
<tr>
<td>daf-2(m41); daf-12(m116)</td>
<td>24.8 ± 0.60 (62)</td>
<td>116</td>
<td>17.8 ± 1.96 (31)</td>
<td>175</td>
</tr>
<tr>
<td>daf-2(m41); daf-12(m583)</td>
<td>22.9 ± 0.70 (73)</td>
<td>108</td>
<td>20.4 ± 1.53 (63)</td>
<td>206</td>
</tr>
<tr>
<td>daf-2(e1370)</td>
<td>34.9 ± 0.70 (113)</td>
<td>156</td>
<td>22.3 ± 1.88 (40)</td>
<td>200</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m20)</td>
<td>30.5 ± 0.85 (100)</td>
<td>148</td>
<td>29.5 ± 2.13 (51)</td>
<td>269</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m25)</td>
<td>38.8 ± 1.10 (92)</td>
<td>188</td>
<td>29.2 ± 2.47 (51)</td>
<td>269</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m116)</td>
<td>37.5 ± 1.43 (49)</td>
<td>180</td>
<td>35.4 ± 1.63 (63)</td>
<td>281</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m583)</td>
<td>30.8 ± 0.61 (128)</td>
<td>148</td>
<td>12.4 ± 2.32 (23)</td>
<td>125</td>
</tr>
</tbody>
</table>

*Values are means ± SE with no. of animals in parentheses.

The last quartile of the N2 population remained alive on day 25.

The last quartile of the N2 population remained alive on day 14.
of the TGF-β superfamily that is a putative ligand for these receptors (Lim 1993). A phenotypic difference between mutants in the daf-1 and daf-2 branches of the pathway is that nonconditional alleles of daf-2 and daf-23 exist, whereas all known alleles of the other daf-c genes are ts. The null phenotype of at least some genes in the daf-1 branch is ts, based on analysis of nonsense mutants (Golden and Riddle 1984b). Synergistic interactions between daf-c mutations led Thomas et al. (1993) to propose that daf-11 and daf-21 define a separate branch of the pathway that is partially redundant with the daf-1 branch. Both of these branches are upstream of daf-12.

The daf-12 and daf-2 genes cannot be ordered unambiguously based on current data. It appears that daf-12 function is required to complete dauer larva formation in daf-2 mutants, yet loss of daf-12 function does not suppress commitment of daf-2(e1370) to developmental arrest as it does with daf-2(m41). The allele-specific phenotypes indicate direct or indirect interactions between the daf-2 and daf-12 gene products. The daf-12 gene encodes a protein with Zn-finger motifs that are diagnostic of the steroid/thyroid hormone receptor superfamily (Yeh 1991). Direct protein-protein interactions between members of this receptor superfamily and with hsp90, other superfamily members and transcription factors have been demonstrated biochem-
Dauer Development and Adult Longevity

TABLE 5

Brood sizes for daf-2 and daf-12 single and double mutant strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>15°C Brood size*</th>
<th>% N2</th>
<th>25.5°C Brood size*</th>
<th>% N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>280 ± 38 (20)</td>
<td>100</td>
<td>239 ± 29 (14)</td>
<td>100</td>
</tr>
<tr>
<td>daf-12(m20)</td>
<td>209 ± 60 (9)</td>
<td>75</td>
<td>211 ± 25 (18)</td>
<td>88</td>
</tr>
<tr>
<td>daf-12(m25)</td>
<td>294 ± 76 (10)</td>
<td>105</td>
<td>259 ± 36 (22)</td>
<td>109</td>
</tr>
<tr>
<td>daf-12(m116)</td>
<td>122 ± 33 (11)</td>
<td>44</td>
<td>142 ± 70 (15)</td>
<td>59</td>
</tr>
<tr>
<td>daf-12(m583)</td>
<td>222 ± 49 (10)</td>
<td>79</td>
<td>196 ± 44 (17)</td>
<td>82</td>
</tr>
<tr>
<td>daf-2(m41)</td>
<td>225 ± 57 (9)</td>
<td>80</td>
<td>213 ± 54 (16)</td>
<td>89</td>
</tr>
<tr>
<td>daf-2(m41); daf-12(m20)</td>
<td>236 ± 56 (13)</td>
<td>85</td>
<td>259 ± 46 (14)</td>
<td>109</td>
</tr>
<tr>
<td>daf-2(m41); daf-12(m116)</td>
<td>141 ± 38 (11)</td>
<td>50</td>
<td>151 ± 32 (10)</td>
<td>63</td>
</tr>
<tr>
<td>daf-2(m41); daf-12(m583)</td>
<td>252 ± 32 (10)</td>
<td>90</td>
<td>247 ± 44 (18)</td>
<td>103</td>
</tr>
<tr>
<td>daf-2(e1370)</td>
<td>213 ± 17 (10)</td>
<td>76</td>
<td>81 ± 24 (22)</td>
<td>34</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m20)</td>
<td>190 ± 38 (10)</td>
<td>68</td>
<td>40 ± 18 (14)</td>
<td>17</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m25)</td>
<td>265 ± 54 (10)</td>
<td>95</td>
<td>132 ± 34 (10)</td>
<td>55</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m116)</td>
<td>150 ± 35 (11)</td>
<td>54</td>
<td>13 ± 15 (14)</td>
<td>5</td>
</tr>
<tr>
<td>daf-2(e1370); daf-12(m583)</td>
<td>223 ± 19 (10)</td>
<td>90</td>
<td>61 ± 14 (17)</td>
<td>25</td>
</tr>
</tbody>
</table>

*Values are means ± SD with no. of animals in parentheses.

physically. Furthermore, the particular pairings result in unique biological properties that can range from positive to negative regulation (Diamond et al. 1990; Picard et al. 1990; Carlb erg et al. 1993; Yao et al. 1993). These precedents suggest types of direct interactions that would be consistent with the molecular identity of DAF-12 and the allele-specific phenotypes of the daf-2; daf-12 double mutants.

The interactions observed in the daf-2; daf-12 double mutants may represent a mutually antagonistic relationship between DAF-2 and DAF-12 that integrates the two branches of the pathway (Gottlieb and Ruvkun 1994; Table 1). Activation of either branch of the pathway would trigger the activation of the other (both branches are required to promote dauer formation). Furthermore, the decision between dauer vs. nondauer development is a dynamic process (Golden and Riddle 1984a) and may involve a regulatory loop. The potential for nondauer development remains throughout the L1, L2d and dauer stages. Without such a regulatory loop, continual reassessment of the environment could lead to conflicting developmental responses, such that an animal might not develop according to either morphogenetic pathway. One hypothesis is that in cases where DAF-12 activity predominates, it remains subject to inhibition by DAF-2, which may be activated by food or reduced pheromone concentration. Once DAF-2 activity predominates, the daf-1 branch of the pathway becomes permanently inactivated to achieve developmental commitment to growth.

The alleles used to construct the daf-2; daf-12 double mutants are almost certainly hypomorphic (partial loss of function). The most common class of daf-2 mutation is nonconditional daf-c; that is, all homozygous animals enter the dauer stage irreversibly (Riddle 1988). The ts daf-2 mutations used in this work display less severe phenotypes and have sufficient gene function to execute nondauer development at 20°C. The daf-12 alleles result in a nonconditional Daf-c phenotype, but alleles differ from one another both in their suppression of mutations in daf-c genes and in their interactions with daf-2(e1370). These data indicate that at least two, if not all, of the tested daf-12 alleles are not null. It is possible that either daf-2 or daf-12, or both, may have essential functions, and that complete loss of activity would result in lethality. A low percentage of lethality (dead eggs and L1 arrest) at the restrictive temperature for the ts daf-2(e1370 and e1286) mutations has been reported (Vowels and Thomas 1992). Although the null phenotypes for daf-2 and daf-12 are unknown, this does not prohibit representing them in the pathway. A pathway for vulva induction has been defined, in part, with hypomorphic mutations in genes for which the null phenotype is lethal (Sternberg 1993).

There are numerous parallels between Daf phenotypes and adult life span. Mutations in daf-23 and daf-2 extend adult life span and result in constitutive formation of the dauer larva, a state characterized by efficient life maintenance. Both the Daf-c and adult life-span phenotypes are ts for daf-2 mutations, and there are allele-specific interactions between daf-2 and daf-12 for both adult life span and dauer formation. An important parallel is between the genetic pathways shown in Figure 1. A daf-16 mutation that suppresses the Daf-c phenotype of daf-2(e1370 and m41) and daf-23(m333) similarly suppresses the life extension phenotype (Figure 3; Kenyon et al. 1993). All of the genes in the daf-2 branch of the dauer formation pathway also affect adult life span. Furthermore, the adult life span is not ex-
tended in any of the *daf-c* mutants in the *daf-I* branch of the pathway when our data are taken together with the data of Kenyon et al. (1993), who examined *daf-7*, *daf-11* and *daf-14*. The *daf-c* mutations in the *daf-I* branch of the pathway would not be expected to prolong life if activation of *daf-16* is necessary for increasing longevity.

In the pathway for adult life-span determination (Figure 1C), *daf-16* is positioned late because *daf-16(m26)* completely suppresses *daf-23(m333) and *daf-2(e1370, m41 or m65)*. The *daf-16* mutation also suppresses the extension of life span by *age-1*. The order of *daf-2*, *daf-18* and *daf-23* in the life span pathway is reversed from that in the dauer pathway (Figure 1B), suggesting that the functional relationships between the *daf* genes may differ between the two different processes. The gene order in the life span pathway is based on the observation that the *daf-18* mutation partially suppresses the longevity of *daf-2(e1370)* but does not suppress the longevity of *daf-23(m333)*. Conversely, in the dauer formation pathway the *daf-18* mutation suppresses *daf-23*, but not *daf-2*, when scored 50 hr after egg laying. By 96 hr, however, *daf-18* appears to suppress *daf-2(e1370)* because the double mutants eventually mature to the adult. This late time point measures maintenance or exit from the dauer state rather than entry into it, so the 50-hr data are used to draw the pathway for dauer formation. However, if *daf-18* were considered to be epistatic to *daf-2*, then the *daf-2* branch of the dauer pathway would have only two steps, one defined by *daf-2* and *daf-23*, and the subsequent one defined by *daf-16* and *daf-18*. This is not sufficient to reconcile the dauer and life span pathways because there is no obvious effect of *daf-18* on *daf-23* life span.

The simplest common pathway for both dauer formation and life span would be *daf-18*, followed by *daf-2* and *daf-23*, followed by *daf-16*. This pathway can be derived if *daf-23* were considered to be epistatic to *daf-18* for dauer formation, and if *daf-2* were considered to be epistatic to *daf-18* for life span. Supporting the former point, the *mg55* rearrangement is the most severe *daf-23* mutant, and its dauer formation is not well suppressed by the *daf-18* mutation (Gottlieb and Ruvkun 1994). For the latter point, the intermediate life span of the *daf-2; daf-18* double mutant could be interpreted as incomplete epistasis of *daf-2* (although the life span is closer to that of *daf-18* than *daf-2*). Regardless of epistatic ordering, molecular analysis can be used to sort out the relevant functions of these genes with respect to both dauer formation and adult life span. The processes downstream of *daf-16* presumably involve many parallel physiological changes associated with longevity. Genetic approaches may reveal the major downstream effectors.

The *daf-2* and *daf-12* genes are depicted (Figure 1) as acting negatively on each other. At 25.5°C, allele-specific effects of *daf-12* mutations were seen in combination with *daf-2(e1370)*. These effects on life span ranged from nearly doubling it to nearly halving it, and suggested that *daf-2* and *daf-12* interact to determine adult life span. For example, mutant variants of *DAF-12* that negatively titrate *DAF-2* activity more efficiently than wild type would enhance longevity, whereas variants that are less efficient than wild type would shorten life span. By contrast with the *daf-2(e1370); daf-12* double mutants, the *daf-2(m41); daf-12(m20, m116 or m583)* mutants exhibited the *daf-2* phenotype for maximum life span and the *daf-12* phenotype for dauer formation. Although such a result generally implies that the two genes are not
involved in the same pathway, in this case it may suggest that each of these genes is the major effector for one of these phenotypes. The mutant *daf-2* gene is the major effector for the longevity phenotype, whereas the mutant *daf-12* gene is the major effector for the Daf phenotype. The wild-type *daf-2* gene promotes nondauer development and limits adult life span, and the *daf-12* gene modulates these functions.

The survival curves for *daf-2* single mutants and the *daf-2; daf-12* double mutants appear to be bimodal, unlike the sigmoidal control curves and most other survival curves previously observed for *C. elegans* (Friedman and Johnson 1988; Van Voorhies 1992; Kenyon et al. 1993). In these bimodal survival curves one fraction of the population seems to exhibit wild-type survival characteristics and the other exhibits extended life span. All animals are genetically identical, yet some apparently fail to exhibit the mutant phenotype. This may indicate incomplete penetrance for life span extension, even though the *daf-* phenotype is 100% penetrant at 25.5°C. However, the survival curve for *daf-2(e1370)* at 20°C is not bimodal (Kenyon et al. 1993), suggesting complete penetrance at the lower temperature. It is possible that higher growth temperature may have a deleterious effect on the mutants such that extension of life is masked in a portion of the population. The *daf* mutations are known to affect a pathway of response to specific environmental cues, including temperature, and similar variation within a controlled environment is seen during wild-type dauer larva formation, in which individuals differ in their sensitivity to dauer-inducing pheromone (Golden and Riddle 1984a).

Although increased life span was not consistently associated with decreased fertility or delayed reproduction, the phenotypes described for the *daf-2* mutants are consistent with the evolutionary theory of aging and the hypothesis of antagonistic pleiotropy (Medawar 1952; Rose 1991). The early benefit of *daf-2*(+) activity is to allow animals to reach reproductive maturity quickly rather than to arrest development as a dauer larva. A trade-off for this benefit is a limited adult life span. There is precedent for a relationship between timing of reproduction and the rate of aging in Drosophila, in which strains selected for late reproduction also showed increased longevity (Hutchinson and Rose 1991). In *C. elegans* hermaphrodites, sperm production in the L4 stage limits the number of self-progeny. Sperm made during the L4 stage are stored and used to fertilize oocytes produced by the adult (Wood 1988). A strain that produces more self-progeny was found to be at a selective disadvantage because the time spent making additional sperm increases the generation time (Hodgkin and Barnes 1991). Thus, for this reproductive strategy the selective advantage is derived from the timing of reproduction (shortest generation time), not from a larger number of progeny per individual.

Coordinate regulatory changes may be essential to produce the very large positive effects on the life spans of *daf-2(e1370); daf-12* double mutants (up to a fourfold increase). Nevertheless, the organism’s adaptive limits have not been exceeded because these changes have not produced negative side effects sufficient to mask the life-lengthening phenotype. Altered regulation of transcription may coordinate multiple physiological changes that act together to prolong life, and at least one of the genes involved (*daf-12*) encodes such a regulatory protein (Yih 1991). In this model, an existing program for efficient life maintenance of the dauer larva state would be inappropriately expressed in *daf-2* and *daf-23* adults, and thereby prolong life. The program might involve dauer-related modifications to energy metabolism (Wadsworth and Riddle 1989) coupled with increased defense and repair capacity (Anderson 1982; Larsen 1993).

The *daf-2*(+) and *daf-23*(+) activity threshold necessary for life span limitation appears to be higher than that for nondauer development. One example of this is that mutant *daf-2(e1370)* animals grown at 15°C still exhibit increased longevity, but there is no Daf phenotype. Another example is that the nonconditional *daf-23(m333)* Daf-c phenotype is maternally rescued, but these adults have an extended life span similar to that of *daf-2* mutants. The genetic specification of adult life span does not completely overlap with that of dauer larva formation because *daf-2; daf-12* mutants are unable to complete dauer formation, yet they still have extended adult life spans. Thus, only a dauer subprogram for efficient life maintenance is necessary to increase adult life span in *daf-2* and *daf-23* mutants. For example, dauer larvae do not feed, and *daf-2(e1370)* adults were observed to decrease pharyngeal pumping considerably before death (Kenyon 1993), raising the possibility that increased longevity results at least partially from reduced caloric intake.

Alternatively, *daf*(+) gene products may function in distinct processes during development and adulthood. In this model, similar environmental cues (e.g., food and temperature) would trigger different intracellular processes depending upon the stage. For instance, dietary restriction promotes dauer formation in *C. elegans*, and it also prolongs adult life span in this and other organisms (Klass 1977). The mechanism for transducing nutritional information in *C. elegans* at different times is as yet undefined, but it is interesting to speculate that a specific signal transduction pathway, conserved in evolution, may control functions expressed in the adult that directly determine life span in response to nutritional level.

Molecular genetic analysis of relevant portions of the *C. elegans* dauer pathway provides the means to isolate causal factors for at least one mechanism of aging. Some
daf genes encode signal transduction components that are conserved between species to the degree that a human ligand (bone morphogenetic protein, BMP-4) can interact effectively with the nematode daf-4 receptor (Estevez et al. 1993). Hence, it seems possible that elucidation of efficient life maintenance mechanisms controlled by daf genes could reveal insights into mechanisms affecting human life span.

We thank M. L. Sundermeyer, B. A. Hinders and D. J. Riddle for technical assistance, D. C. Jamison, S. Shaw and R. Tsutakawa for help with statistics, I. M. Caldicott, D. C. Jamison, and C. V. Gunther for critical reading of the manuscript, C. Kenyon for sharing results in advance of publication, the 17,686 worms that died for this study, and L. Hatley for processing the manuscript. Some strains were obtained from the Caenorhabditis Genetics Center, which is supported by the National Institutes of Health National Center for Research Resources. This work was supported by a postdoctoral fellowship from the University of Missouri Molecular Biology Program, and a grant from the American Federation of Aging Research to P.L.L. and Department of Health and Human Services grant HD-11239 to D.L.R.

LITERATURE CITED


Vowels, J. J., and J. H. Thomas, 1992 Genetic analysis of chemosen-


Communicating editor: I. Greenwald