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-

Corresponding author: Donald L. Riddle, Molecular Biology Program, 311 Tucker Hall, University of Missouri, Columbia, MO 65211.
E-mail: riddle@biosci.mbp.missouri.edu

[1] *Present address*: Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

tween 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 ts (temperature sensitive). Mutations used are listed by linkage group as follows: LG I: *dpy-5(e61), daf-8(m85*ts), *unc-29(e1072), daf-16(m26* and *m27), unc-75(e950)*; LG II: *fer-15(b26*ts), *age-1(hx546), unc-4(e120), daf-19(m86*ts), *daf-23(m333), rol-1(e91), daf-5(e1386), unc-52(e444* and *su250*ts), *mnC1[dpy-10(e128) unc-52(e444)]*; LG III: *daf-7(e1372*ts and *n696*ts), *daf-2(e1370*ts, *m41*ts and *m65), daf-4(e1364*ts and *m72*ts), *unc-32(e189), qC1[dpy-19(e1259*ts) *glp-1(q339)]*; LG IV: *daf-1(m40*ts), *daf-18(e1375), dpy-9(e12), dpy-13(e184*sd), *daf-14(m77*ts), *unc-22(s7)*; LG X: *daf-3(e1376), dpy-3(e27), unc-27(e155), daf-12(m20, m25, m116* and *m583), unc-58(e665*d), *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 L4 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 *m20* or *m583* mated with *dpy-13; daf-12(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 *egl-15* (YEH 1991). Current work confirms that the *m25* allele is in this same interval. Twelve F_1 progeny of 10 Unc non-Egl and 10 Egl non-Unc recombinants picked from + *daf-12(m25)* + / *unc-27* + *egl-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) *egl-15.* The numbers in parentheses indicate the position and frequency of recombination events. The *m25* allele also was mapped relative to *unc-58(e665)*, 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 egl-15* interval.

Close linkage of *daf-1* to *daf-18* thwarted attempts to construct a double mutant. A total of 208 Egl (*daf-1*) adults segregated from *daf-1* + /+ *daf-18* heterozygotes were transferred to separate plates at 25.5°. No adults or dauer-like progeny characteristic of *daf-18* were observed on any of the plates over a 6-day period.

Construction of unlinked *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 use of such markers decreased the penetrance of the *daf-c* 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-d* 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, unlinked 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 F_2 Daf-c animals were identified either as dauer larvae, Egl adults (TRENT *et al.* 1983), or in the case of *daf-4*, Sma adults (ESTEVEZ *et al.* 1993). 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 retested at 25.5° to verify the genotype. If the putative double-mutant strain were not homozygous for the *daf-d* gene, then one-quarter of the retested 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 F_1 progeny were allowed to segregate F_2 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 F_3 generation scored for the absence of *unc-52*.

Confirmation 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 heterozygotes derived by mating putative double-mutant hermaphrodites with wild-type, *dpy-13*/+ or *unc-22*/ + males. The paternal markers allowed identification of F_1 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 F_1 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 F_2 animals from a single F_1 from each of the three initial crosses were transferred singly to individual plates at 25.5°. Five to seven of each set of 20 F_3 populations lacked constitutively formed dauer larvae in the first generation. These were later scored for starvation-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* + 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-5(e1386)* males with *daf-23(m333) unc-52(su250*ts) 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-d 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-18(e1375) IV* utilized *qC1*[*dpy-19(e1259*ts) *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 F_1 males of genotype *daf-2*/ +; *dpy-9*/ + or *qC1*/ +; *dpy-9*/ + were then backcrossed with *daf-2*/ *qC1* hermaphrodites. F_2 animals of genotype *daf-2*/ *qC1*; *dpy-9*/ + were identified based on F_3 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. F_1 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 F_1 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 F_1 L4 larvae on fresh plates at 25.5°, and selecting F_2 Unc dauer larvae, which were transferred to 15° to resume development. One Unc Egl Daf 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 F_2 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 constitutively formed dauer larvae from five of five heterozygotes.

To construct the desired triple mutant, *daf-2; 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 selfing. 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-12(m20)* background (GOTTLIEB and RUVKUN 1994). Duplicate crosses between *daf-2(e1370)* males and putative triple-mutant hermaphrodites were started at 15° and shifted to 25.5° the next day. The P_0's 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 F_1, 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-d* 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 60 hr is required for a mutant animal to become a dauer larva and recover to the L4 stage (BYERLY *et al.* 1976; SWANSON and RIDDLE 1981; GOLDEN and RIDDLE 1984b). Hence, L4 larvae and adults scored 50 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-c* double mutants: Males heterozygous for the *daf-c* mutations were mated with *fer-15(b26ts)* raised at 25.5°, a temperature at which *b26* fails to produce sperm. Single F_1 hermaphrodites were placed on plates at 25.5° and F_2 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 F_3 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 adults. Each *fer-15; daf-c* stock was started from one animal that had been maintained at 15°. The *fer-15; daf-4* double mutant was constructed by placing F_1 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-75(e950); fer-15(b26) age-1(hx546)*, and a ts sterile F_2 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 plated 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

TABLE 1

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

daf-c mutation	*daf-d* mutation							
	daf-3(e1376) X	*daf-5(e1386) II*	*daf-12(m20) X*	*daf-12(m25) X*[a]	*daf-12(m116) X*	*daf-16(m26) I*	*daf-16(m27) I*	*daf-18(e1375) IV*
daf-1(m40) IV	100 (416)	90 (283)	100 (318)	77 (217)	100 (271)	14 (195)	0 (121)	ND
daf-4(e1364) III	99 (257)	100 (447)	92 (181)	16 (280)	100 (177)[b]	9 (348)	0 (386)	1 (229)
daf-7(e1372) III	100 (482)	98 (670)	100 (254)	8 (557)	100 (273)	5 (229)	0 (178)	0 (230)
daf-8(m85) I	100 (584)	98 (710)	100 (403)	80 (627)	100 (245)	0 (194)	0 (276)	0 (335)
daf-14(m77) IV	100 (428)	99 (257)	97 (360)	96 (340)	ND	47 (419)	1 (252)[c]	0 (200)
daf-2(m41) III	0 (404)[d]	ND	100 (642)	ND	13 (213)[e]	99 (726)	ND	ND
daf-2(e1370) III	0 (314)	0 (377)	0 (398)[f]	0 (371)[f]	0 (158)[f]	91 (388)	99 (373)	3 (319)
daf-23(m333) II[g]	0 (533)	0 (264)	0 (309)[h]	0 (220)	ND	98 (262)	ND	100 (113)[i]

ND, not determined

[a] This allele of *daf-12* was previously referred to as *daf-20* (RIDDLE *et al.* 1981).

[b] Animals were scored 68 hr after the middle of the egg-laying period.

[c] Animals were scored 72 hr after the middle of the egg-laying period.

[d] Unlike *daf-2(m41)* or *daf-3(e1376)* single mutants, this double mutant forms some transient dauer-like animals at 15°.

[e] By 72 hr 100% of the animals were L4 larvae or adults.

[f] The mutant exhibited a dauer-like larval arrest.

[g] The *daf-23* mutant is a nonconditional, maternal-effect mutant that primarily forms constitutive nonfeeding dauer-like larvae.

[h] Homozygous mutant adults segregated from *daf-23/mnC1*[*dpy-10 unc-52*]; *daf-12* do not lay eggs; consequently, the animals used in this study were cut in half to release eggs.

[i] Many of the double-mutant adults had a protruding vulva.

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).

Reproductive capacity and schedule: Hermaphrodites were raised at 15° until the L4 stage, then shifted to 25.5°. Each individual was transferred to a new plate every 24 hr for 10 days. The difference in age of the individual animals could have varied as much as 15 hr (the duration of the L4 stage). The progeny were raised at 15° and counted when they became L3 or L4 larvae.

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°), 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). This pathway applies to the formation of dauer larvae and not to long-term maintenance of the dauer state, because it is based on observations of synchronous populations 50 hr after the midpoint of a 4-hr egg-laying period.

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(m116* and *m583)*, the *daf-c* mutations *daf-8(m85*ts), *daf-2(m41*ts and *m65)* and *m333,* the original *daf-c* mutation defining *daf-23.* The *daf-8(m85)* mutant is more penetrant than the *e1393* reference allele used in previous work. The *daf-2(m41*ts) allele has different properties from the *e1370*ts 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-12(m20)* allele. The *daf-23(m333)* 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 predauer L2d larvae that no longer feed. At 25.5° the percentage of dauer larvae formed ranges from 15% (4 days) to 30–50% (10 days), whereas fewer than 3% of larvae raised at 15 or 20° undergo radial body shrinkage

by day 20. Hence, the terminal stage of developmental arrest is somewhat ts 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 *m116)*, 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-14* 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 *m25* 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° and the other 9% arrested development as L1 or L2 larvae. Although this strain was viable at 15°, mutant larvae that matured at 25.5° 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(e1375)* 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°, 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 ~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°, *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°.

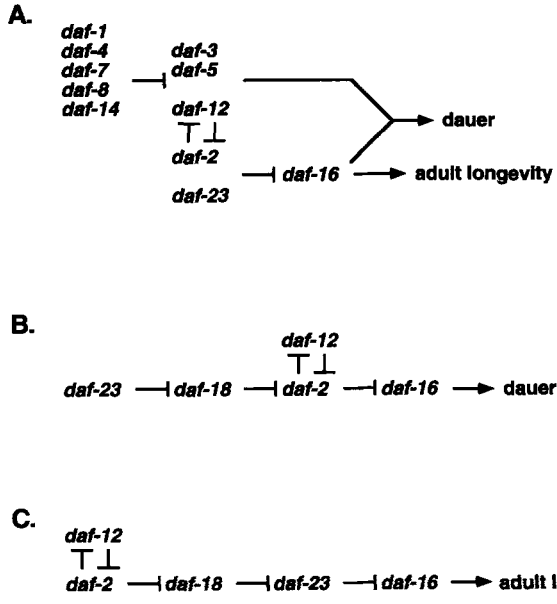

FIGURE 1.—Genetic pathways for dauer larva formation and adult life span. The pathways are drawn to depict wild-type gene functions that inhibit subsequent steps. The *daf-2* and *daf-12* genes are represented in a mutually antagonistic relationship. The *daf-c* genes are *daf-1, daf-2, daf-4, daf-7, daf-8, daf-14,* and *daf-23,* mutations in which result in constitutive dauer larva development. The *daf-d* genes are *daf-3, daf-5, daf-12, daf-16,* and *daf-18,* mutations in which prevent dauer larva development in response to the dauer-inducing pheromone. The *daf-1* and *daf-2* branches of the pathway are both required for dauer larva development, because mutations in either branch result in a Daf phenotype. Only the *daf-2* branch is required to promote adult longevity (because the *daf-1* group of dauer-constitutive mutants do not increase adult life span), yet *daf-12* mutations modulate the longevity phenotype of *daf-2* mutants. (A) A partial pathway that omits *daf-18* and 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 reinterpretation of two sets of epistasis data and is presented in the DISCUSSION. (B) The *daf-2* branch of the dauer formation pathway with *daf-18* included. 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%). (C) A 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.

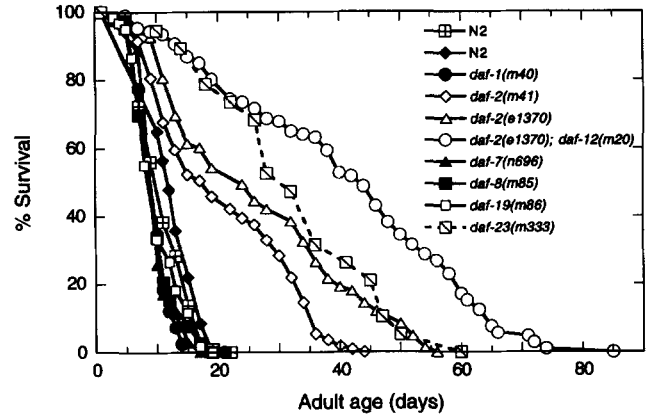

FIGURE 2.—Survival curves for *daf-c* mutants. Populations of animals were raised at 15° and then shifted to 25.5° either as young adults (closed symbols) or as L4 larvae (open symbols). Day 1 is the first day of adulthood.

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 = 1290) and *m41* formed 16% ± 3.4 dauer-like larvae (n = 1377) 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

TABLE 2

Adult life spans of dauer-constitutive mutant strains at 25.5°

Strain	Life span (days)[a]	% of N2 last quartile
N2[b]	12.3 ± 0.51 (62)	100
daf-1(m40)	10.3 ± 0.39 (53)	73
daf-7(n696)	10.4 ± 0.41 (39)	73
daf-8(m85)	10.3 ± 0.51 (41)	73
daf-19(m86)	10.3 ± 0.47 (63)	87
N2[c]	12.7 ± 0.37 (66)	100
fer-15(b26)	14.3 ± 0.31 (47)	107
fer-15(b26); daf-1(m40)	13.4 ± 0.23 (112)	107
fer-15(b26); daf-4(m72)	11.7 ± 0.32 (108)	93
fer-15(b26); daf-7(n696)	12.2 ± 0.35 (97)	107
fer-15(b26); daf-8(m85)	11.7 ± 0.25 (112)	93
N2[b]	11.2 ± 0.37 (109)	100
daf-2(m41)	20.9 ± 1.07 (109)	213
daf-2(e1370)	26.7 ± 1.62 (83)	253
daf-2(e1370); daf-12(m20)	41.4 ± 1.87 (114)	387
daf-23(m333)	32.7 ± 3.07 (19)	300

[a] Values are means ± SE with no. of animals in parentheses.
[b] The last quartile (25%) of the population remained alive on day 15.
[c] The last quartile remained alive on day 14.

be the most likely to do so. The life spans of adults carrying ts *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°), then placed at the restrictive temperature (25.5°) 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.* 1983), 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-15*ts*; 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° (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°. When the animals were raised at 15°, then shifted to 25.5°, 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°, 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° 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° 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

TABLE 3

Adult life spans of dauer-constitutive, dauer-defective mutant strains at 25.5°

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

[a] Values are means ± SE with no. of animals in parentheses.
[b] The last quartile remained alive on day-14.
[c] The last quartile remained alive on day-15.
[d] The last quartile remained alive on day-17.
[e] 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.
[f] The last quartile remained alive on day-16.

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°, 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 *m116)*, 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 *fer-15(b26) age-1(hx546)* at 25.5° 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) I; fer-15 age-1 II* was reduced to 15.8 ± 0.35 days. The control *daf-16; fer-15* strain had a mean life span of 12.7 ± 0.27 days at 25.5°.

Life spans of *daf-2* single and double mutants at 15°: Because the *daf-2* mutants are fully ts for dauer for-

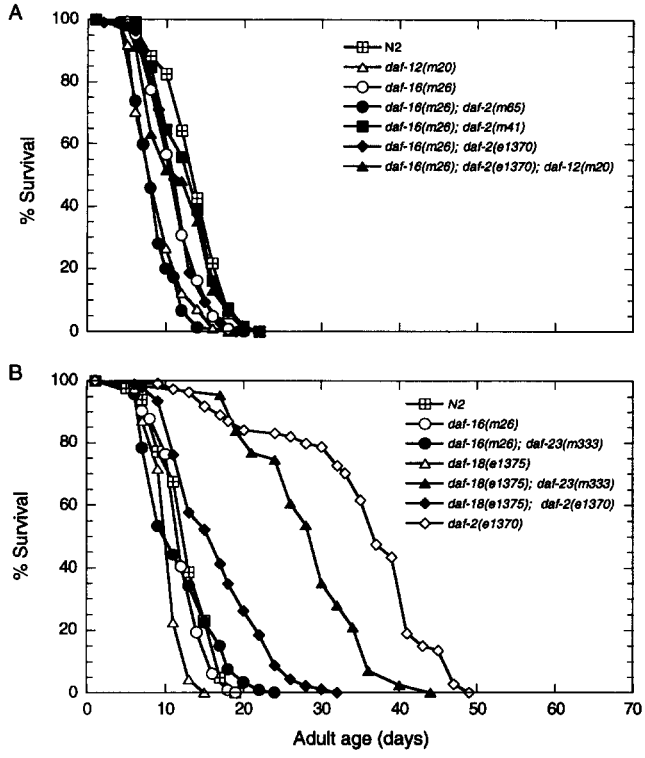

FIGURE 3.—Survival curves for *daf-c; daf-d* mutants in the *daf-2* branch of the genetic pathway for dauer formation. Populations were raised at 15° to the L4 stage, then shifted to 25.5°. (A) Strains carrying *daf-2* and *daf-16*. (B) Strains carrying *daf-16* or *daf-18*.

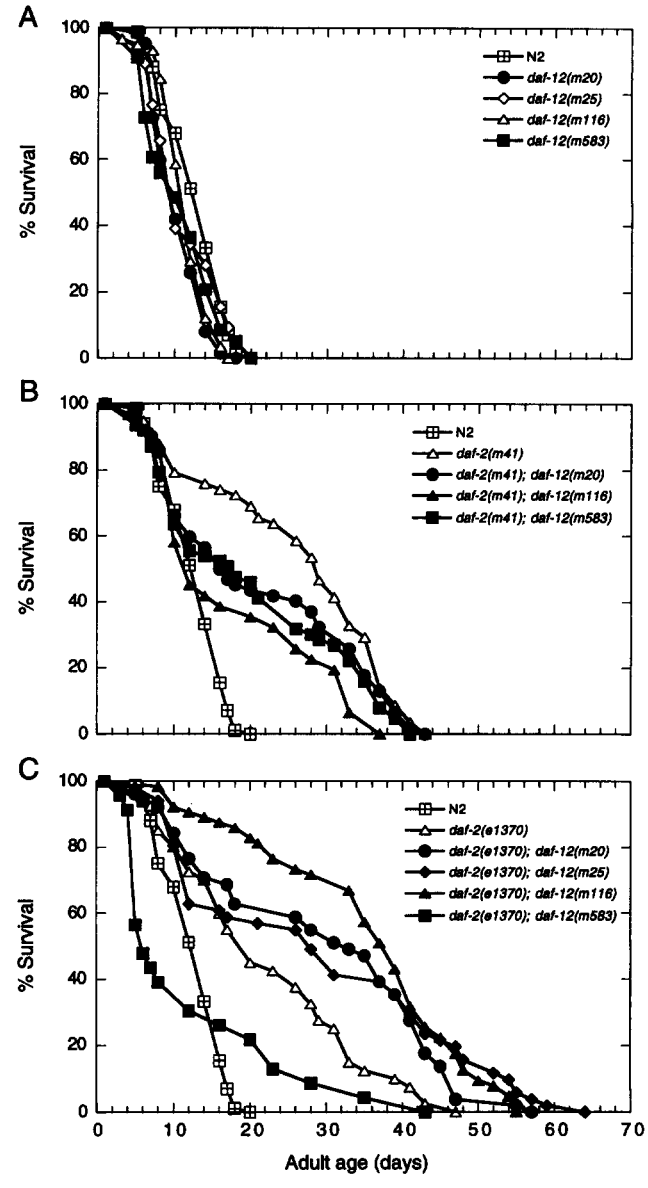

FIGURE 4.—Survival curves for *daf-2, daf-12,* and *daf-2; daf-12* adults at 25.5°. The animals were raised at 15°, then shifted to 25.5° as young adults. (A) Alleles of *daf-12*. (B) Strains bearing *daf-2(m41)*, alone and with alleles of *daf-12*. (C) Strains bearing *daf-2(e1370)*, alone and with alleles of *daf-12*.

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-12* strains 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

TABLE 4

Adult life span of *daf-2* and *daf-12* single and double mutant strains at the permissive and restrictive temperatures

Strain	15°		25.5°	
	Life span (days)[a]	% of N2 last quartile[b]	Life span (days)[a]	% of N2 last quartile[c]
N2	21.6 ± 0.47 (86)	100	12.6 ± 0.42 (84)	100
daf-12(m20)	20.0 ± 0.41 (109)	88	10.4 ± 0.41 (62)	88
daf-12(m25)	20.0 ± 0.41 (112)	88	11.3 ± 0.54 (64)	100
daf-12(m116)	20.7 ± 0.69 (55)	88	11.5 ± 0.40 (58)	88
daf-12(m583)	21.1 ± 0.52 (96)	96	10.8 ± 0.57 (63)	88
daf-2(m41)	21.4 ± 0.43 (84)	92	26.4 ± 1.54 (58)	231
daf-2(m41); daf-12(m20)	25.2 ± 0.47 (98)	108	21.4 ± 1.57 (62)	219
daf-2(m41); daf-12(m116)	24.8 ± 0.60 (62)	116	17.8 ± 1.90 (31)	175
daf-2(m41); daf-12(m583)	22.9 ± 0.70 (73)	108	20.4 ± 1.53 (63)	206
daf-2(e1370)	34.9 ± 0.70 (113)	156	22.3 ± 1.83 (40)	200
daf-2(e1370); daf-12(m20)	30.5 ± 0.85 (100)	148	29.5 ± 2.13 (51)	269
daf-2(e1370); daf-12(m25)	38.8 ± 1.10 (92)	188	29.2 ± 2.47 (51)	269
daf-2(e1370); daf-12(m116)	37.5 ± 1.43 (49)	180	35.4 ± 1.63 (63)	281
daf-2(e1370); daf-12(m583)	30.8 ± 0.61 (128)	148	12.4 ± 2.32 (23)	125

[a] Values are means ± SE with no. of animals in parentheses.
[b] The last quartile of the N2 population remained alive on day 25.
[c] The last quartile of the N2 population remained alive on day 14.

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 *daf-2* life spans (KENYON *et al.* 1993). Of *C. elegans* mutations that affect fertility, only *spe-26* is known to increase life span (JOHNSON 1983; VAN VOORHIES 1992).

A remarkable exception to the predominant reproductive schedule, noted during the life-span analysis, was that all *daf-2(e1370)*-bearing strains intermittently produced a small number of progeny late in life. For instance, on day 31 of the experiment shown in Figure 4 there were two F_1s from 21 *daf-2(e1370); daf-12(m25)* adults, five F_1s from 45 *daf-2(e1370); daf-12(m116)* adults, and three F_1s from nine *daf-2(e1370)* adults. A few progeny were produced by adults more than 50 days old, 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° 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° than at 25.5°, 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°. 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* (ESTEVEZ *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

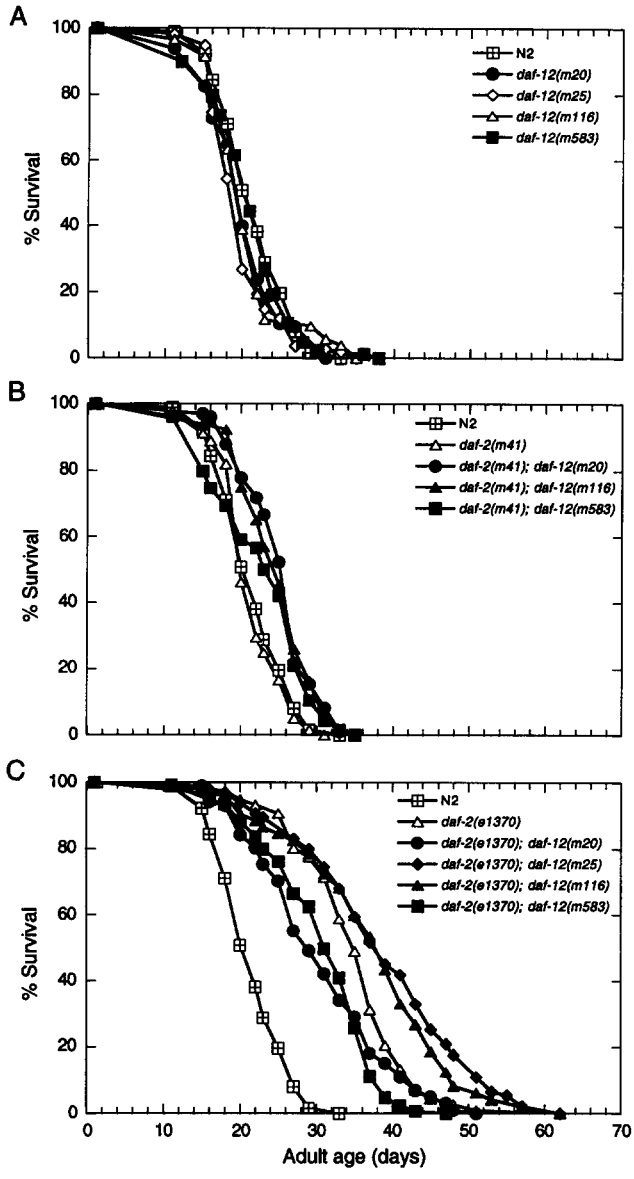

FIGURE 5.—Survival curves for *daf-2, daf-12,* and *daf-2; daf-12* adults at 15°. (A) Alleles of *daf-12.* (B) Strains bearing *daf-2(m41)*, alone and with alleles of *daf-12.* (C) Strains bearing *daf-2(e1370)*, alone and with alleles of *daf-12.*

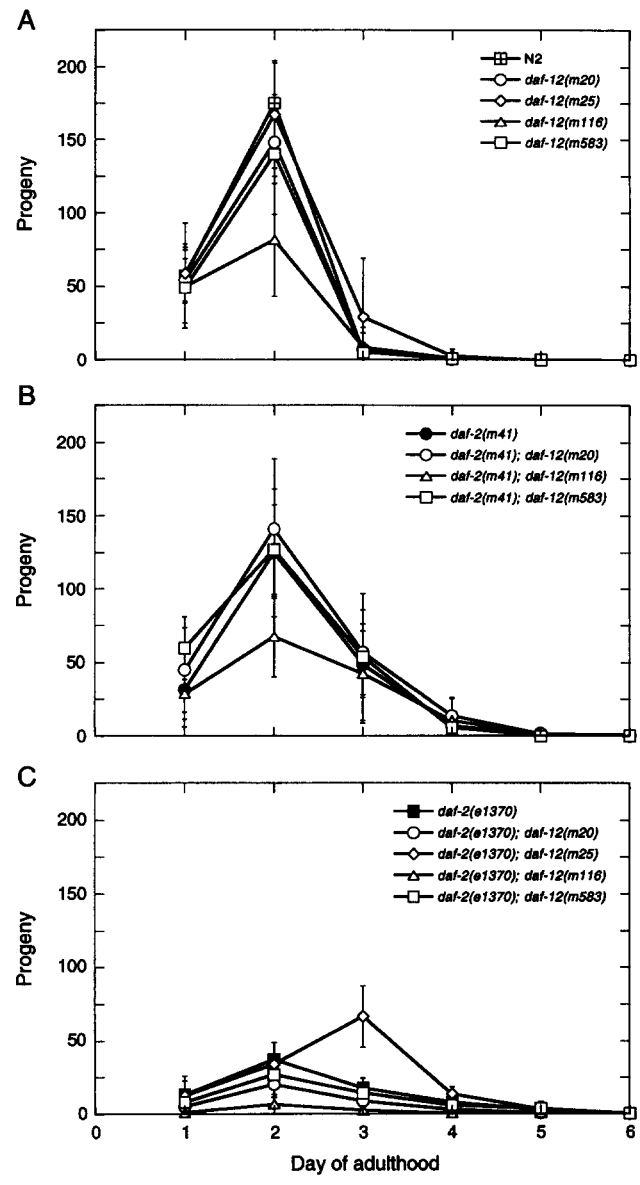

FIGURE 6.—Profiles of the fertile periods for *daf-2, daf-12,* and *daf-2; daf-12* adults at 25.5°. The average number of progeny per animal per day is shown for (A) Alleles of *daf-12.* (B) Strains bearing *daf-2(m41)*, alone and with alleles of *daf-12.* (C) Strains bearing *daf-2(e1370)*, alone and with alleles of *daf-12.* The average brood was 0–0.1 progeny per animal on day 7, and no progeny were observed on days 8–10 for any strain. Vertical bars represent SD.

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-

TABLE 5

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

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

[a] Values are means ± SD with no. of animals in parentheses.

ically. 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; CARLBERG *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°. The *daf-12* alleles result in a nonconditional Daf-d 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* mutants, 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-

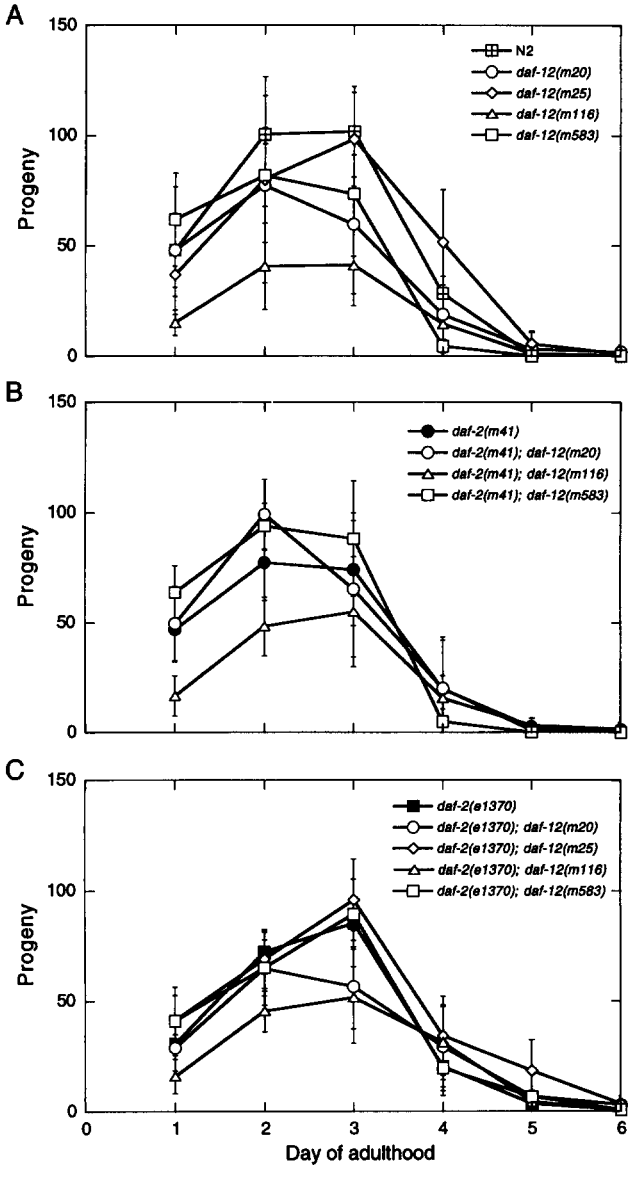

FIGURE 7.—Profiles of the fertile periods for *daf-2, daf-12,* and *daf-2; daf-12* adults at 15°. The average number of progeny per animal per day is shown for (A) Alleles of *daf-12.* (B) Strains bearing *daf-2(m41)*, alone and with alleles of *daf-12.* (C) Strains bearing *daf-2(e1370)*, alone and with alleles of *daf-12.* Vertical bars represent SD.

tended in any of the *daf-c* mutants in the *daf-1* 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-1* 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°, 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-c* phenotype is 100% penetrant at 25.5°. However, the survival curve for *daf-2(e1370)* at 20° 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 four-fold 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 (YEH 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° 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. HINDERKS 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.

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Communicating editor: I. GREENWALD