Longevity-Determining Genes in Caenorhabditis elegans: Chromosomal Mapping of Multiple Noninteractive Loci

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ABSTRACT

We have used chromosome mapping with polymorphic markers to define genetic components governing life span in the nematode Caenorhabditis elegans. A complex recombinant-inbred population was derived from an interstrain cross, yielding >1000 genotypes, each a composite of homozygous segments from the two parental strains. Genotypes were analyzed for the last-surviving 1–5% of worms in aging cohorts, and for young controls, by multiplex polymerase chain reaction using polymorphic markers to distinguish the parental alleles. We identified five regions of the genome at which one parental allele was significantly enriched in long-lived subpopulations. At four of five loci, the same alleles were selected in aging cohorts maintained under two different conditions, implying that these genes determine life span in differing environments.

Genetic factors affecting life span have been inferred from species-specific life spans (FINCH 1990) and longevity differences among related species (COMFORT 1979; KIRKWOOD 1985). Results of interstrain crosses in mice (YUNIS et al. 1984; GELMAN et al. 1988) and in the nematode Caenorhabditis elegans (JOHNSON and WOOD 1982; JOHNSON 1986, 1987) indicate multiple longevity-determining genes distributed over several chromosomes. These genes have eluded subsequent characterization due to difficulties inherent to the analysis of polygenic traits by traditional means.

Here we describe a general approach to determine the genetic components governing C. elegans life span, derived from seminal studies of JOHNSON and WOOD (1982). They found that hybrid progeny (generation F1) of crosses between Bergerac-BO (BO) and Bristol-N2 (N2) strains of C. elegans have a mean life span similar to those of the parental strains (JOHNSON and WOOD 1982), demonstrating the absence of heterosis or "hybrid vigor," which has impeded the genetic analysis of life span in other animals. In contrast, F2 and subsequent generations obtained by self-fertilization have widely dispersed longevities (JOHNSON and WOOD 1982; JOHNSON 1986, 1987), relative to the parental strains and the uniformly heterozygous F1 hybrids, presumably due to varied segregation of multiple genes determining life span.

In the present experiments a heterogeneous recombinant-inbred (RI) population was generated from N2 x BO hybrids by interbreeding worms over five generations to accumulate recombinations, followed by 5–7 generations of self-fertilization to achieve 98–99.6% homozygosity. The resulting F10–12 worms thus comprise many different chromosomal mosaics of homozygous regions from the two parental strains. By synchronously aging such recombinant-inbred populations to ≥95% mortality, and analyzing genotypes of young worms vs. longest-lived worms using the polymerase chain reaction (PCR) with strain-specific markers, we have obtained an initial chromosomal mapping of life span-determining genes in C. elegans.

MATERIALS AND METHODS

Strains: C. elegans var. Bristol-N2 was isolated in England (BRENNER 1974). C. elegans var. Bergerac was isolated in France by NIGON in 1949; after maintenance in several laboratories, a sample of Bergerac-BO was sent to the Caenorhabditis Genetics Center in 1980 (for details see MOERMAN and WATERSTON 1984). Stock samples of both strains were obtained from the Caenorhabditis Genetics Center (St. Paul, Minnesota).

General methods: Strains were propagated at 20 ºC on NGM/agar plates [1.7% agar in Nematode Growth Medium (SULSTON and HODGKIN 1988) with 1 µg/ml uracil] unless otherwise noted. All plates were seeded with Escherichia coli strain OP50 (a leaky uracil-auxotrophic strain), added to the center of each plate as described by SULSTON and HODGKIN (1988).

Generation of the RI population: C. elegans reproduces primarily by self-fertilization of hermaphrodites (XX), but rare males (XO, ~0.2% in most strains) arise by nondisjunction of the X chromosome (WOOD 1988). Interbreeding can be promoted by keeping males in excess, or prevented by isolating hermaphrodites just prior to maturity (SULSTON and HODGKIN 1988). There were three stages in the gen...
eralization of F_{10-12} R1 worms: an initial cross, random mating for four generations, and inbreeding for seven generations.

The N2 × BO cross was initiated by placing one virgin BO hermaphrodite at L4, and three N2 males (L4 to young-adult), onto each of ten 60-mm NGM/agar plates. When males are mated to virgin hermaphrodites, their spermatozoa preferentially fertilize hermaphrodite oocytes (WARD and CARREL 1979). Nevertheless, a small proportion of progeny may arise by self-fertilization of hermaphrodites, which would slightly reduce the total recombination accrued in these experiments. After 32 hr, worms were transferred to fresh plates and allowed to lay eggs for 24 hr. To ensure successful outbreeding, only F1 worms from those matings producing ~50% male progeny were sib-mated, at a 1:1 ratio of males to hermaphrodites.

F2 eggs were recovered from F1 hermaphrodites by alkaline hypochlorite lysis [5 min in 0.5% sodium hypochlorite, 0.5 N NaOH (modified from EMMONS, KLAAS and HIRSH 1979)], yielding ~8-10 fertilized but unlaid eggs per worm. Eggs were rinsed three times in S-basal [0.1 M NaCl, 0.05 M potassium phosphate pH 6.0 (BRENNER 1974)], and allowed to hatch on fresh NGM/agar plates. Isolation of unlaid eggs serves to keep the population synchronous, while reducing selection by fecundity. In addition, it generates a surplus of worms over requirements for the next cross. F3-F5 crosses were initiated at a 2:1 male:hermaphrodite ratio. To set up a cross of ~300 males and ~150 hermaphrodites, plates were marked that contained approximately 150 individuals of each sex. An additional 150 males were then picked onto "holding" plates. Worms from the marked plates and the holding plates were washed off, rinsed in S-basal and placed on fresh NGM/agar plates to mate. This serves to minimize selection by developmental rate since males were picked over periods of ~9 hr and were added to plates containing unselected males and hermaphrodites. Two days after each mating worms were washed off the plates and lysed with alkaline-hypochlorite solution.

The number of breeding worms was gradually increased to ~1000 by the F1 generation (males:hermaphrodites at F2 = 300:150, at F3 = 500:250, at F4 = 1000:500). At F5, 1000 L4 hermaphrodites were separated from males by manual transfer onto holding plates. These worms were propagated by self-fertilization for seven generations, with alkaline hypochlorite lysis of gravid hermaphrodites every fifth day, maintaining ≥3000 worms. At generation F_{29}, vials containing ~3000 L1 larvae were stored under liquid nitrogen. Worms were thawed at room temperature on modified NGM/agar plates (1% peptone), with recovery consistently >95%.

**Survival procedures:** Synchronously aging cohorts of N2, BO and F12 (N2 × BO) worms were maintained by a hand-transfer procedure (modified from JOHNSON and WOOD 1982). The N2, BO and smaller F12 survival populations (n < 150) were initiated from eggs laid by parental strains over 5- to 16-hr periods on 60-mm NGM/agar plates, or from eggs laid by F1 worms in individual wells of 24-well plates. To eliminate effects of differential fertility, single F12 progeny were picked from ≥100 isolated F11 parents. To generate the larger hand-transferred F12 aging cohorts (Figure 1, n = 449, 504 and 539), gravid F1, hermaphrodites were washed free of laid eggs and lysed in alkaline hypochlorite solution; fertilized eggs were rinsed and allowed to hatch on 100-mm NGM/agar plates (at ≤500 eggs/plate). To assess survival, groups of 40 worms were transferred as young adults to 60-mm Petri dishes containing 3-ml liquid survival medium (S-basal solution with 10^6 OP50/ml and 10 μg/ml cholesterol), maintained at 20°, and transferred to fresh medium daily while fertile (days 3-9 post-hatch) and every 2-3 days thereafter.

Large aging cohorts were maintained by a mass-aging procedure (modified from GANDI et al. 1980). N2×BO F2 worms were thawed and, when gravid, lysed as described above for F11 worms. F2 eggs were hatched overnight in 30 ml of S-basal medium and the resulting L1s (n = 160,000) were vigorously shaken (125 rpm) at 20° in 50 ml liquid survival medium. After ~55 hr, 100 μm each of 5-fluOrO-2'-deoxyuracil (FUdR, Sigma) and uridine monophosphate (UMP, as 2', 3' mixed isomers, Sigma) were added to L4/young-adult worms. OP50 was replenished daily to ~10^6/ml, monitored by absorption at 600 nm. FUdR and UMP were supplemented every second day to 75 μM; every 4 days, worms were settled, rinsed and placed in fresh medium containing 100 μM FUdR/UMP.

**Sampling of worms for PCR:** Young-control worms were taken randomly at five days post-hatch when all worms were gravid adults. All of the last 5% of worms surviving in hand-transfer populations were analyzed by PCR. For the mass-aging group, a random sample was taken from the last surviving ~1%. PCR results for a given worm were included in the data set only if reactions were successful for all five marker sets.

**PCR conditions:** Individual worms were lysed in 15 μl of lysis buffer (50 mM KCl, 10 mM Tris pH 8.3, 2.5 μM MgCl2, 0.45% NP40, 0.45% Tween-20, 0.01% gelatin, 60 μg/ml proteinase K), and processed as described (WILLIAMS et al. 1992). EDTA [15 μl of 1.25 mM stock (pH 7.9)] was then added and samples were stored at 4°. PCR reactions, in 10 μl, contained 2.5–5 μl (0.08–0.17 worm equivalents) of

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**Figure 1.**—Survival curves for N2, BO and N2×BO F12 populations. Percentage of surviving worms, as a function of days since hatching, is indicated for synchronous aging cohorts of C. elegans. Data points show cumulative percentage alive, without interpolation. (A) Survivals of strains Bergerac-BO (n = 71, 82 and 34, from left to right at 50% survival) and Bristol-N2 (n = 84, 74, and 55*). (B) Survivals of BOXN2 F_{12} progeny (n = 72, 504, 531, 449, 133, 169 and 83). Survivals were performed as described in MATERIALS AND METHODS, with the exception of one Bristol-N2 survival (*) for which worms were maintained on 60-mm NGM/agar plates and transferred daily while gravid.

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Diluted lysate; amplification comprised 40 cycles as described previously (Williams et al. 1992) in an aluminum-block (Perkin-Elmer/Cetus) or hot-air (Idaho Technology) thermal cycler. The loci tested, and sizes of amplified bands, are listed in the Figure 2 legend. DNA products were separated on 12% polyacrylamide gels (1 hr, 10 V/cm) or 1% agarose/3% NuSieve (FMC) agarose gels (2 hr, 7 V/cm), and stained in 0.5 μg/ml ethidium bromide.

RESULTS

Life span and the variance of mortality: Survival curves are shown in Figure 1 for three BO, three N2 and seven F12 populations. The BO strain had a mean life span of 16.5 ± 0.8 days (mean ± SEM; medians in three experiments = 15.0, 17.0 and 17.5 days), which is slightly less than that of N2, 19.8 ± 0.6 days (medians of 19, 19.5 and 21.0 days), consistent with earlier data (Johnson and Wood 1982). The F12 progeny had a mean life span of 18.2 ± 0.3 days (range of medians for seven survivals = 16.8–19.5 days), intermediate between parental strains.

As illustrated by the reduced slopes of the F12 survival curves in Figure 1, the variance of mortality for the F12 worms (39.0, n = 1941) was 2.3 times that determined for the BO parental strain (16.7, n = 187) and 1.9 times that of the N2 parental strain (20.8, n = 213). Both increases were significant (P < 0.01 by F-test). This striking increase in the variability of longevity reflects the heterogeneity of these RI populations for genetic determinants of life span. The broad-sense heritability of life span, calculated as the ratio of the genetic component of variance to total variance, is 52% based on these data. For comparison, life spans of F2 progeny (which are only ~50% homozygous) from similar crosses were reported to have a 60% increase in variance over parental strains, and a broad-sense heritability of 39% (Johnson and Wood 1982).

Protocols for maintaining aging cohorts: Two distinct methods were used to generate and maintain synchronous aging populations of F10-12 hybrid worms: (1) Small populations of F12 young-adult worms (3 cohorts of 600 worms each) were carried in groups of 40, in 3-ml liquid survival medium (see MATERIALS AND METHODS for details). Daily for the first 7 days, and at 2- to 3-day intervals thereafter, they were counted, assessed for survival and separated from larvae while transferring to fresh medium; cultures were maintained until mortality reached ~95%.

(2) A large population (~160,000) of F10 worms was maintained in 50-ml vigorously aerated liquid survival medium containing 100 μM 5-fluorodeoxyuridine (FUDR) to inhibit generation of progeny (see MATERIALS AND METHODS for details). Worms were rinsed and placed in fresh medium at 4-day intervals. Cultures were examined daily, replicate samples counted and bacteria (E. coli strain OP50) replenished as needed, until only ~1% of worms remained alive.
Analysis of genotypes by multiplex polymerase chain reaction: We examined the DNA of many individual worms, both young controls and the longest-lived subsets of each aging cohort, using PCR to amplify markers which distinguish between the parental strains. This genotype analysis takes advantage of a >10-fold difference between the two strains in copy number of the transposon Tcl: BO has ~500 Tcl copies/haploid genome (Mori, Moerman and Waterston 1988, and our unpublished results), while N2 has only ~30 copies (Liao, Rosenzweig and Hirsh 1983). The presence or absence in an F_{10-12} worm of a differential Tcl element at a given locus, can thus identify the parental origin of that region of DNA. Individual worms were lysed and their DNA was examined at 25 loci polymorphic for Tcl. Five multiplex PCR sets (modified from Williams et al. 1992) each comprised five Tcl-flanking primers—selected from several linkage groups—and a single opposing primer within the Tcl element. Up to 5 Tcl-adjoining genomic sequences are thus co-amplified in each reaction, dependent upon the presence of Tcl elements at sites previously mapped in the C. elegans genome (Williams et al. 1992). At each marker, the BO allele generates a band of characteristic interprimer length, which will be absent in worms homozygous for the corresponding N2 allele.

Figure 2A illustrates PCR results obtained using DNA from the parental-strain worms. With each of the primer sets, five bands were amplified from BO DNA, whereas none were generated from N2 DNA. Genotypes of individual F_{12} worms are thus indicated by the bands produced (Figure 2B). In repeat assays performed on DNA samples from the same worms, duplicates agreed for >97% of bands (n = 300).

Estimation of total recombinations accrued between markers: PCR analyses of the young-control population allowed us to calculate [using Mapmaker (Lander et al. 1987)] the extent of marker resolution actually achieved during the five interbreeding generations. Effective recombination distances accumulated in the population (Figure 2C) averaged 3.5-fold greater (range: 1.4- to 7.8-fold) than corresponding distances in the C. elegans genetic map previously calculated from recombination in single-generation crosses (Williams et al. 1992).

The mean distance between recombinations, detected in the RI worms, was estimated by counting the number of recombinations per chromosome for individual control worms (n \geq 138). This value, corrected for the proportion of the chromosomes covered by the markers, averaged 22.7 map units (m.u.), equivalent to ~1.2 recombinations/chromosome.

Chromosomal mapping of reproductive-fitness loci: Genetic-map histograms in Figure 3, A–L, indicate the allele fraction—the proportion of RI worms with the BO-derived (Tc1^{+}) allele—at each marker, before (cross-hatched bars) and after (solid bars) selection for longevity. For each of the six linkage groups, corresponding to chromosomes I–V and X, two panels are presented: the left panel in each pair shows PCR results for three hand-transferred aging groups, while the right panel represents data from a mass-culture grown with FuDR. Data for young adults are the same within each pair of panels, with F_{10} and F_{12} control data pooled since these did not differ significantly.

The initial allele fraction—the proportion of Tc1^{+} alleles among control worms—was <0.5 at most marker loci (Figure 3). This suggests some residual bias favoring N2 alleles during the 10–12 generations of breeding, despite efforts to obviate fecundity-selection by recovering unlaid eggs from gravid hermaphrodites at each generation (see MATERIALS AND METHODS for details). Genes responsible for such bias can be localized near markers having the lowest percentage of BO (+Tc1) alleles among young controls, i.e., at chromosomal minima in the control histograms. Thus, genes for which the N2 allele conferred a selective advantage during the interbreeding and/or inbreeding generations—which we tentatively term "reproductive-fitness" genes (see DISCUSSION)—are implicated on linkage group (LG) II, corresponding to the second chromosome, at 36 \pm 7 m.u. (horizontal axis scale in Figure 3); on LG-IV at \leq 34 m.u.; on LG-V at 15–18 m.u.; and on LG-X near 19 m.u.

Mapping longevity-determining loci: At any Tcl-marker, significant enrichment of either allele in the longevity-selected group relative to the initial population indicates linkage of that marker to a polymorphic gene affecting life span. The map locations of genes responsible for long-lived phenotypes can thus be estimated. The [longest-lived/control] ratio of Tc1^{+} allele fractions was calculated, and is displayed above each pair of vertical bars in Figure 3. Alleles with differential effects on longevity should be indicated by ratios differing markedly from 1.0, and the variation in this ratio allows us to estimate locations of the implicated genes (horizontal bars below abscissas in Figure 3).

Significance values were calculated from \chi^2 values, independently for each marker, and separately for hand-transferred and mass-aged worms. Correcting for multiple comparisons (OTT 1991), by analogy with the analysis of LOD scores (Lander and Botstein 1989), significance levels of P \leq 0.004 for individual markers will ensure that the overall probability of false-positives is <0.05 in an experiment. Significance would be considerably greater for clusters of covarying markers, but requires calculation by maximum-likelihood algorithms [e.g., Mapmaker QTL (Lander and Botstein 1989)] currently being adapted to RI data. Hand-transfer and mass-aging P values, for
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**FIGURE 3.**—Analysis of genotypes by linkage group. Histograms summarizing PCR results at 25 loci, arranged by their genetic-map positions in six linkage groups (WILLIAMS et al. 1992). The horizontal scale represents genetic (recombinational) distance in map units (m.u.) or centimorgans; bar heights indicate the percent of worms with BO (+Tcl) genotype at each locus. Cross-hatched bars correspond to young-control worms at \( F_{10-12} \) (panels A-L, \( n = 277 \)) and solid bars represent the longest-lived ~5% of worms from 3 hand-transferred cohorts (panels A, C, E, G, I, K; \( n = 81 \)), or a random sample of the longest-lived ~1% from a mass-aging cohort (panels B, D, F, H, J, L; \( n = 140 \)). Error bars display the standard error of the mean (SEM) for each proportion; for the long-lived, hand-transferred worms (solid bars, panels A, C, E, G, I, K), they show the intergroup SEMs for three cohorts, which were larger than pooled-data SEMs. Numbers above bars show the ratios of allele fractions for each pair: values for the longest-lived worms (solid bars) divided by those for control worms (cross-hatched bars). Dotted horizontal lines indicate the 50% allele-fraction ratio. Solid horizontal bars below the abscissa highlight the genomic regions likely to contain significant longevity-determining loci, positioned by the relative strengths of longevity selection at included and adjacent markers.

those markers that showed a marginally significant shift in either environment, were multiplied to evaluate their significance when considered together.

The BO allele was substantially and significantly enriched in the longest-lived subpopulation, at markers on the right-center of chromosome \( II \) (30-42 m.u.), ranging from 1.9- to 2.1-fold in Figure 3C (\( P < 0.001 \) at 2 markers), or 1.6- to 1.8-fold in Figure 3D (\( P < 0.004 \) at 2 markers). Both \( sod-I \), encoding Cu/Zn superoxide dismutase, and \( age-I \), a gene that extends life span when mutated (FRIEDMAN and JOHN-son 1988; JOHNSON and LITHgow 1993) map near the center of this cluster of markers (A \textit{C. elegans} Database 1993).

A pronounced survival benefit is also evident for worms having the BO-derived chromosome \( IV \), although the greatest shifts occurred at different markers in the two environments. In the hand-transfer population (Figure 3G), the enrichment of BO genotype was 2-fold at the 33 m.u. marker (\( P = 0.0005 \)), and 1.6-fold at the 44 m.u. marker (\( P < 0.003 \)). With mass aging (Figure 3H), only the 1.7-fold shift at the
44 m.u. marker was significant ($P < 0.0002$). This difference between experiments may be due to sampling error, or could arise from multiple longevity-selected loci on this chromosome, which differ in their response to the aging environment. Further data incorporating additional markers will be necessary to resolve this question.

Selection for longevity favored the N2 allele at the right-center of chromosome V, but only in the mass-aging cohort (Figure 3J), by a maximum of twofold ($P < 0.0003$) at 31 m.u. The two ends of the X chromosome were enriched for different parental components in the longest-lived worms (Figure 3, K and L). Greater longevity accompanied the BO allele at the right terminus, with 1.6-fold enrichment ($P < 2 \times 10^{-4}$, $P < 2 \times 10^{-5}$, in the two environments). At the left extreme of LG-X, however, increased longevity followed the N2 allele, with 1.7-fold enrichment in the hand-transferred groups ($P < 0.04$), and 1.5-fold for the mass-cultured worms ($P < 0.02$). The latter shift is significant, according to our stringent criteria, only when both experiments are considered together ($P < 0.001$).

**Multilocus interactions:** We tested for interactions between loci, occurring either at the level of genes (epistasis) or of phenotype (OTT 1991). Eleven effectively unlinked marker groups were defined on the six chromosomes (corresponding to clusters separated by >40 m.u. in Figure 2C) and were assessed for independent co-occurrence by Fisher's exact test on stratified 2×2 tables. If two loci act independently to affect a trait, the frequency of their joint occurrence should equal the product of their frequencies taken separately; over- or underrepresentation of any pairwise combination of alleles would imply cooperative or inhibitory interactions, respectively. Nonrandomness in the distribution of alleles within the initial (young-control) population indicated significant interactions between LG-I and LG-IV ($P < 10^{-4}$), and between the left-terminal marker on LG-X and both LG-III ($P = 0.0004$) and the right-central LG-II cluster ($P = 0.003$). We saw no interactions, however, specific to the longest-lived subset of worms; for this group, only an interaction between LG-I and LG-IV was significant ($P \approx 10^{-3}$ in each environment), and presumably was residual to the bias in the initial population from which they were selected. Apart from this, diallele frequencies in the longevity-selected worms appeared at roughly the product of the individual allele frequencies—indicating that these loci act independently on life span.

**DisCUSSION**

**Genes affecting life span:** These results constitute an initial mapping of five genomic regions at which the two alleles have differing effects on longevity. Two loci were significantly enriched for the N2 genotype in long-lived worms, while three loci were enriched for the BO genotype. Selection for longevity produced similar shifts, except on LG-V (see below), of the allele fraction in both the hand-transferred and mass-aging data sets. Each locus appeared to act independently on life span, which should greatly facilitate subsequent efforts to isolate the relevant genes.

**Reproductive-fitness effects:** The bias toward N2 at four loci in the young controls is not surprising in the context of the BO phenotype of low brood size and poor male fertility (LIAO, ROSENWEIG and HIRSH 1983). In addition, there may be several alternative explanations for the N2 bias in initial allele frequencies, which we termed putative "reproductive-fitness" effects, including the resistance of certain genotypes to alkaline hypochlorite treatment.

**Implications of the mutator phenotype:** Bergerac-BO is a mutator strain; i.e., it contains one or several mut loci that increase the Tcl transposition rate (MOERMAN and WATERSTON 1984; MORI, MOERMAN and WATERSTON 1988). MOERMAN and WATERSTON (1984) observed a forward mutation rate at the unc-22 locus of $1 \times 10^{-4}$ in this strain. EIDE and ANDERSON (1988) reported that germ-line reversion rates of various Tcl insertions in the unc-54 locus in the BO strain range from $<10^{-6}$ to $4 \times 10^{-5}$ per worm per generation, depending upon site, while the frequency of observable somatic-cell reversion varies from $\approx 2 \times 10^{-4}$ to $3 \times 10^{-2}$/worm (EIDE and ANDERSON 1988). Upon backcrossing into the nonmutator strain DH424, germ-line reversion decreased 60- to 80-fold while somatic reversion declined only ~3-fold (EIDE and ANDERSON 1988).

MORI, MOERMAN and WATERSTON (1990) and PLASTERK (1991) have shown that germ-line excision of Tcl from the unc-22 locus is elevated in F1 progeny of certain interstrain crosses, in which the hermaphrodite parent is a mutator and the gene in F1 hybrids is heteroallelic for Tcl insertion. We are currently testing the possibility that some Tcl excision might occur in this way during interbreeding, and could contribute to low Tcl-allele fractions at some loci. However, the observation of smooth gradations of young allele fractions (e.g., on LG-V and LG-X [cross-hatched bars in Figure 3 1–L]) implies gene selection rather than germ-line excision. Moreover, at many loci the allele fraction is close to 0.5, indicating that this effect is relatively minor or is restricted to a subset of the loci tested. Germine excision of Tcl from BO regions, in generations preceding the aging cohort, would slightly alter the observed shift in allele fraction, from young control to longest lived, so that the extent of life span selection may be somewhat under- or overestimated.

It is theoretically possible that the mutator pheno-
type of BO may be responsible for a reduction in life span among cross progeny, by analogy with other fitness effects of hybrid dysgenesis in Drosophila (Kidwell, Kidwell and Sved 1977; Bregliano et al. 1980). This is highly unlikely, however, since dysgenic effects on viability or fitness of cross progeny have not been observed in C. elegans (Liao, Roseweig and Hirsh 1983). Moreover, the increase in variance seen in the N2×BO F12 populations was also observed in F2 and later progeny of a cross between N2 and DH424, both nonmutator strains at 20°C (R. H. Ebert, N. K. Egilmez, S. Ruggles and R. J. Shmoookler Reis, unpublished data). It should also be noted that deleterious effects of Tcl transposition could not account for overrepresentation of the BO allele, in long-lived worms, at three of the five longevity-determining loci.

Effects of environmental variables: The two aging environments differed in many parameters which could influence life span. Even though the net effect was not pronounced, these factors could, in principle, favor different genes under the two protocols. FudR was utilized in the mass-aging protocol because manual separation of adults from their progeny is not feasible for large populations. FudR slightly extended (by ≤10%) median but not maximal life span of these F12 worms (data not shown); similar results were obtained previously for strain N2 (Mitchell et al. 1979). The mass-aging protocol also entailed agitation and aeration, which could affect selection by longevity. There was a considerably greater larval density in the mass-aging procedure (>5000 worms/ml in liquid survival medium) than in the hand-transfer procedure (≤500 worms per 15 ml of gelled NGM/agar medium in 100-mm plates). This is likely to be important since larval density has been shown to alter the life span of Drosophila imagos (Lints and Bourgeois 1985). In addition, the culture density was 240-fold greater for the mass-aged adults. In view of these differences, it is noteworthy that most of the loci enriched in the last-surviving worms' genotypes were selected with similar effect in both environments. Only a single region, the right-center of LG-V, demonstrated strong allelic selection limited to one of the environments. This consistency of outcomes indicates that most longevity-selected heteroalleles are unlikely to reflect adaptations to specific environments, but are of general relevance to aging per se, and supports the use of mass-aging cohorts for high-resolution mapping.

Conclusions: Selective genotyping of the longest-lived individuals in a population greatly increases the mapping information obtained, by focusing on individuals at or beyond the extremes of environmental variation (Lander and Botstein 1989; Ott 1991). Information from very short-lived worms was not utilized since these would include deleterious mutations over many loci, and worms injured during handling. This approach, combined with multigeneration accrual of recombinations, successfully resolved five genomic regions affecting life span. The actual number of longevity-determining genes may be much greater, primarily because only those that are polymorphic between the two parental strains would be detected. Map resolution can be improved in subsequent experiments through increases in the number of long-lived individuals genotyped, the density of polymorphic markers in selected areas, and the total amount of recombination accumulated.

This work was supported by National Institute on Aging grant RO1-AG09413. We thank B. D. Williams, B. Schrank and R. H. Waterston for sharing their Tcl-marker data with us prior to publication, and T. E. Johnson for many invaluable discussions. We also thank J. J. Thaden and N. K. Egilmez for critical reading of the manuscript. Worm stocks utilized were supplied by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources.

LITERATURE CITED


traits including mutation, sterility and male recombination. 

Kirkwood, T. B. L., 1985 Comparative and evolutionary aspects 

of longevity, pp. 27-44 in Handbook of the Biology of Aging, 

edited by C. E. Finch and E. L. Schneider. Van Nostrand 

Reinhold, New York.

Landers, E. S., and D. Botstein, 1989 Mapping Mendelian fac-

tors underlying quantitative traits using RFLP linkage maps. 

Genetics 121: 185–199.

Landers, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, 

et al., 1987 Mapmaker: an interactive computer package for 

constructing primary genetic linkage maps of experimental and 


Liao, L. W., B. Rosenzweig and D. Hirsh, 1983 Analysis 


Acad. Sci. USA 80: 3585–3589.

Lints, F. A., and M. Bourgois, 1985 Aging and life span in 

insects with special regard to Drosophila: review 1982–1984, 

pp. 61–84 in Review of Biological Research in Aging, Vol. 2, 


Mitchell, D. H., J. W. Stiles, J. Santelli and D. R. Sanadi, 

1979 Synchronous growth and aging of Caenorhabditis elegans 


Moermon, D. G., and R. H. Waterston, 1984 Spontaneous 


Mori, I., D. G. Moerman and R. H. Waterston, 1988 Analysis of 

of a mutator activity necessary for germline transposition and 

excision of Tcl transposable elements in Caenorhabditis elegans. 

Genetics 120: 397–407.


Univ. Press, Baltimore.

Plasterk, R. H. A., 1991 The origins of footprints of the Tcl 


Sulston, J., and J. Hodgkin, 1988 Methods, pp. 587–606 in The 


Harbor Laboratory, Cold Spring Harbor, N.Y.

Ward, S., and J. S. Carrel 1979 Fertilization and sperm com-

petition in the nematode Caenorhabditis elegans. Dev. Biol. 73: 

304–321.

Williams, B. D., B. Schrank, C. Huynh, R. Showkneek and R. 

H. Waterston, 1992 A genetic mapping system in Caenor-

habditis elegans based on polymorphic sequence-tagged sites. 

Genetics 131: 609–624.

Wood, W. B., 1988 Introduction to C. elegans biology, pp. 1–16 


Harbor Laboratory, Cold Spring Harbor, N.Y.


Bronson, et al., 1984 Traits that influence longevity in mice. 

Genetics 108: 999–1011.