Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6603–6607, November 1982
Genetics

Genetic analysis of life-span in *Caenorhabditis elegans*

(aging/senescence/nematode development/heterosis/heritability)

THOMAS E. JOHNSON*†‡ AND WILLIAM B. WOOD*

*Department of Molecular, Cellular and Developmental Biology and †Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado 80309

Contributed by William B. Wood, July 14, 1982

ABSTRACT **Crosses between Bristol and Bergerac strains of the self-fertilizing hermaphroditic nematode *Caenorhabditis elegans* do not show the heterosis effects for life-span that complicate analysis of interstrain crosses with *Drosophila* or mice. Instead they yield F_1 progeny with life-spans similar to those of the parent strains. By analysis of life-span variation among progeny F_2 populations from such crosses and by two independent analyses of life-spans among recombinant inbred lines derived from F_2 individuals by 18 rounds of self-fertilization, we estimate that the heritability of life-span in *C. elegans* is between 20% and 50%. Recombinant inbred lines show a range in mean life-spans of 10 days to 31 days compared to life-spans of about 18 days for each of the two parental strains. We conclude that life-span variation in *C. elegans* has a substantial genetic component and that this organism offers promising opportunities for selective breeding of longer-lived strains and genetic analysis of senescence.**

The extent to which genes determine life-span in animals remains controversial. For example, two recent studies suggest little or no genetic control of life-span in the Oregon R strain of *Drosophila* (1, 2). In contrast, another investigation led to estimates as high as 79% for heritability of life-span in mice (3). Direct genetic analysis of this question has been prevented so far by failure to identify specific life-span mutants. At the same time, selective breeding for naturally occurring life-span differences between laboratory strains of common experimental organisms has been hampered by heterosis effects: that is, the hybrid progeny from interstrain crosses live significantly longer than does either parent (3–5).

An accepted explanation for the heterosis effects seen, for example, with *Drosophila* and mice is that being inbred, laboratory strains have become homozygous for recessive alleles that shorten life-span but are not selected against under laboratory conditions. Interbreeding of these strains restores heterozygosity in the F_1 progeny, resulting in increased life-span. In hermaphroditic species that are predominantly or solely inbred, such as some snails (6) and nematodes (7), wild animals that are already homozygous at most or all loci might be expected to have evolved optimal life-spans. Therefore, maintenance of small laboratory populations should not markedly shorten life-span by inbreeding, and crosses between laboratory strains should show little or no heterosis effect.

In this study we developed methods that improve the reproducibility of life-span determination in *Caenorhabditis elegans*. Using these methods we have demonstrated that no heterosis effects are observed in crosses between two strains, var. Bristol and var. Bergerac, which have been maintained as laboratory stocks since the 1940s. This characteristic simplifies estimation of life-span heritability, the component of life-span variation that is due to genetic rather than environmental factors. We have made heritability estimates from analysis of the increased variation in life-span among progeny F_2 populations from interstrain crosses and from two separate analyses of life-span in recombinant inbred (RI) lines derived from F_2 individuals by repeated self-fertilization. The three analyses suggest that heritability of life-span is between 20% and 50%. The RI lines from these crosses include longer-lived strains that may be useful for further, more direct, genetic analysis of aging.

MATERIALS AND METHODS

Strains, Media, and General Techniques. Stocks of *C. elegans* were maintained at 20°C as described by Brenner (7) unless otherwise indicated. Wild-type strains included the N2 strain of var. Bristol (N7) and var. Bergerac (8) obtained from D. Hirsh. *Escherichia coli* strains used as food sources for *C. elegans* were OP50 (for growth on NGM agar plates) and B/r (for growth in liquid culture). Male and hermaphrodite master stocks were maintained for up to 8 months as dauerlarvae (9) on NGM plates sealed with Parafilm and kept at 16°C.

Establishment of Survival Populations. Dauerlarvae were allowed to mature and reproduce for one generation on NGM agar plates, and at the fourth larval stage their F_1 progeny were transferred to fresh NGM plates. Two days later these adult worms were used to start synchronous aging populations by transfer of appropriate parents (hermaphrodites and males for crosses; hermaphrodites alone for selfing) to fresh NGM plates preseeded with *E. coli* OP50. After 4–12 hr of egg laying, the adults were removed, and the synchronized F_2 progeny were allowed to mature. This procedure was designed to eliminate possible effects of differences in parental age on progeny life-span. After 3 days, F_2 progeny in groups of 50 worms were suspended in S basal medium plus cholesterol (7) and transferred to Falcon plastic Petri plates containing 4 ml of the same medium with 10^9 *E. coli* B/r or OP50 per ml. Maintenance temperature and bacterial concentration were chosen to optimize fecundity (10). These survival populations were transferred daily for 5 successive days, by which time almost all worms are past reproductive age. Thereafter, populations were transferred thrice weekly until all worms had died.

Criteria of Death. At each transfer of survival populations, the numbers of worms alive, dead, or lost were recorded. Criteria of death were: (*a*) lack of spontaneous movement, (*b*) no response when touched with a probe, (*c*) visible tissue degeneration, and (*d*) lack of osmotic turgor. Osmotic turgor was assayed by cutting worms that showed criteria *a* through *c*; cut living worms extrude body contents as a result of turgor pressure. The "lost" category included worms killed erroneously in checking for turgor pressure or unintentionally as a result of poor technique, worms killed by premature hatching of eggs

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: RI, recombinant inbred.

‡Current address: Dept. of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

within the body of the mother, and worms lost during transfer or incubation.

Data Storage and Analysis. Data were recorded manually and then transferred to computer files for maintenance. Software for entering of data by workers unfamiliar with file manipulation and management was developed by M. Raines. Data were stored as obtained and were checked against original notes before being incorporated into data sets compatible with the standard Statistical Package for the Social Sciences (Gehan) software (11) or with a customized software package for performing log-rank statistics (12). Both Gehan and log-rank statistics are designed especially for use in survival comparisons.

Identification of Hybrid F_1 Progeny. Hermaphrodites produce self as well as outcross progeny after mating. Outcross progeny of interstrain crosses were identified by taking advantage of the natural recessive uncoordinated movement (Unc) and temperature-sensitive sterile characters in the Bergerac strain. Bergerac hermaphrodites at the fourth larval stage were incubated with Bristol males for 2 days and then transferred to fresh plates for egg laying. After a few hours the adults were removed and the newly laid eggs were shifted to 25°C for maturation. F_1 hybrid worms, identified as being non-Unc and fertile, were shifted to 20°C for survival analysis. Control experiments showed that maturation at 25°C instead of 20°C did not significantly affect the outcome of these experiments.

RESULTS

Reproducibility. Study of life-span as a quantitative trait requires that it be measurable accurately and reproducibly. To establish the reproducibility of our life-span measurements, we compared the results of three survival experiments carried out simultaneously by different individuals. The Bristol stock was used to start three separate populations containing both males and hermaphrodites, which were maintained separately. As shown in Fig. 1, the three hermaphrodite populations showed similar mean life-spans, as did the three male populations. In addition, both the overall shapes of the survival curves and the maximum life-spans were similar. Survival curves for the three hermaphrodite populations show no statistically significant differences. Nor are the three male populations statistically different by either the Gehan or log-rank test. However, the hermaphrodites lived significantly ($P < 0.001$) longer than the males (mean life-spans 19.9 and 17.7 days, respectively). This sex-related difference was observed consistently and will be discussed in more detail elsewhere. Same-sex comparisons demonstrate the reproducibility of these life-span measurements. Reproducibility tests were included in most experiments and typically showed no significant differences between genetically identical populations.

Some variability in mean life-span was observed with apparently identical populations in experiments done at different times. Because the life-spans of different stocks covary, we ascribe these variations to still uncontrolled environmental effects on survival. This variation complicates comparisons of mean life-spans determined on different occasions. We have avoided the need for such comparisons by including control survival analyses on appropriate populations in each experiment.

Heterosis Effects. There was only a slight (although reproducible) difference in life-spans between Bristol and Bergerac hermaphrodites (Fig. 2A). F_1 hybrid hermaphrodite progeny of a Bristol × Bergerac cross had a life-span distribution similar

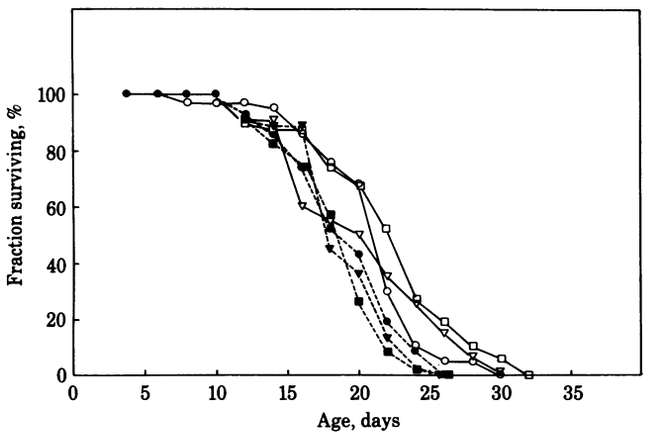

FIG. 1. Reproducibility of survival curves for three populations of Bristol hermaphrodites (open symbols) and three populations of Bristol males (solid symbols). Mean (±SEM) life-spans for the three hermaphrodite populations were 19.8 ± 0.7 (○), 18.8 ± 1.2 (▽), and 20.8 ± 1.0 (□) days; means for the three male populations were 18.1 ± 0.6 (●), 17.3 ± 0.6 (■), and 17.8 ± 0.6 (▼) days. Comparisons of the hermaphrodite means with each other by using the Gehan and log-rank tests yielded P values of 0.28 and 0.70, respectively. (P values >0.05 are taken throughout to indicate no significant differences between the populations tested.) Similar comparisons of the male means yielded P values of 0.69 and 0.20, respectively. However, comparison of the mean for the combined hermaphrodite populations with that of the combined male population yielded P values of <0.001 for both statistical tests.

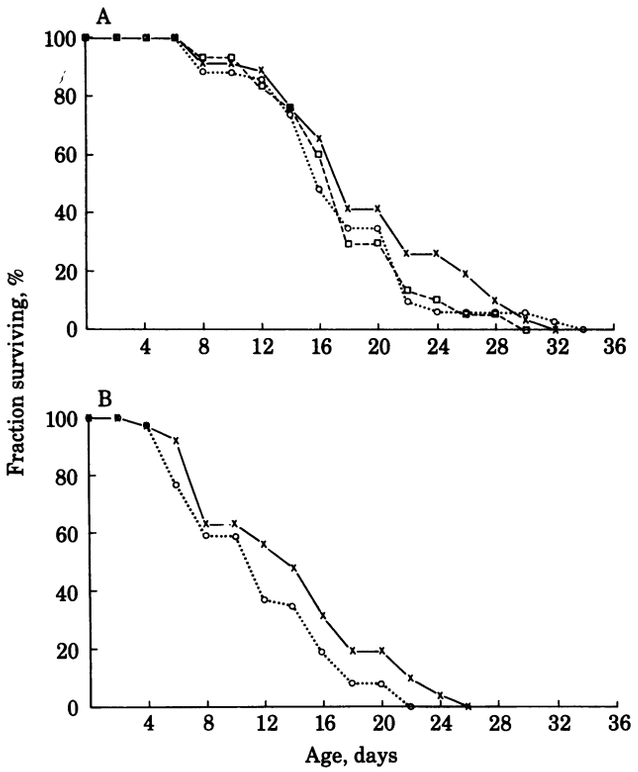

FIG. 2. Survival curves for parental and F_1 hybrid populations. (A) Hermaphrodites. ×——×, Bristol; □– – –□, Bergerac; ○···○, F_1 hybrid. (B) Males. ×——×, Bristol; ○···○, F_1 hybrid. Mean (± SEM) life-spans for the Bristol, Bergerac, and F_1 hybrid hermaphrodites were 18.2 ± 1.1, 16.6 ± 0.8, and 16.6 ± 1.1 days, respectively. Mean life-spans for Bristol and F_1 hybrid males were 12.9 ± 0.8 and 10.7 ± 0.7 days, respectively. Comparisons of the hermaphrodite means by Gehan and log-rank tests indicated no significant differences in life-span among the three populations ($P > 0.35$ in all pairwise tests). Similar comparison of the male means also showed no significant differences ($P = 0.08$ and 0.10 from Gehan and log-rank tests, respectively). However, all pairwise comparisons between a male and a hermaphrodite population showed significant differences ($P < 0.02$).

to distributions of the parental strains; the Bristol, Bergerac, and F_1 hybrid hermaphrodite survival curves show no statistically significant differences. Likewise, survival curves for F_1 hybrid males are not statistically different from those of the Bristol parental males (Fig. 2*B*). The Bristol and hybrid hermaphrodites, however, had significantly longer life-spans than did the Bristol and hybrid males.

The foregoing experiment has been repeated four times. In two experiments, the Bristol and Bergerac mating was done without temperature shifts in such a way that almost all the progeny were hybrid, as confirmed by the non-Unc phenotype; in these experiments all animals were maintained at 20°C throughout. The lower rearing temperature caused no significant change in the results. In all four experiments the hybrid progeny showed no significant increase in life-span over parental controls.

We conclude that there is no significant heterosis effect on life-span in crosses between Bristol and Bergerac varieties of *C. elegans*. Similar results have been obtained with F_1 hybrids resulting from crosses between four other wild-type strains and a mutant Bristol stock (data not shown).

Analysis of F_2 Populations. Life-span variations within parental Bristol and Bergerac populations, which should be genetically homogeneous, are assumed to result from environmental effects. Among the F_2 individuals resulting from self-fertilization of hybrid F_1s, parental genes will assort into new homozygous combinations. If some of these genes affect length of life, then assortment will increase the variation in life-span in the F_2 population compared to the parental population. Fig. 3 shows the distributions of deaths per day for two such comparisons. In both experiments, some F_2 individuals died at earlier times and some F_2 individuals lived longer than any individual in the parental populations; the total increase in variance was about 60% for both experiments (Table 1). Subtraction of the environmental variance (10.9 for experiment A) from the total F_2 variance (16.5 for experiment A) yields an estimate of 5.6 for the genetic component of variance. Heritability (broad sense; see *Discussion*) is defined as the ratio of the genetic component to the total variance. Calculation of this ratio from the

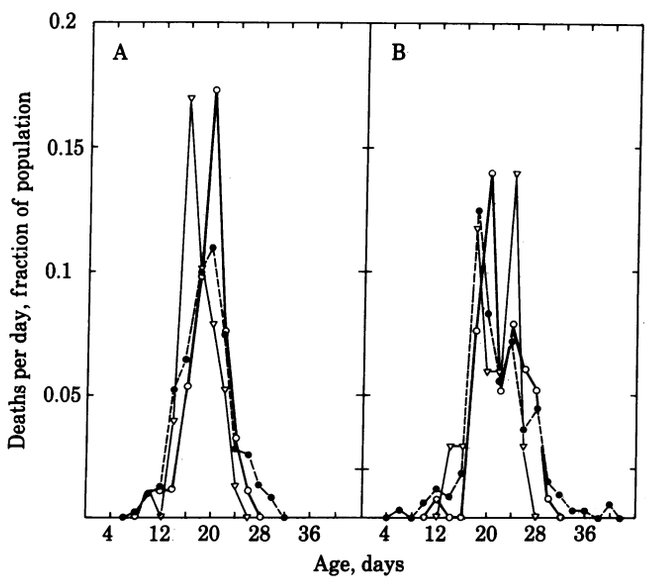

FIG. 3. Distributions of deaths as a function of age for parental and F_2 populations from a cross of Bristol males with Bergerac hermaphrodites. *Left* and *Right*, Results from separate duplicate experiments. ○——○, Bristol hermaphrodites; ▽——▽, Bergerac hermaphrodites; ●---●, F_2 hermaphrodites. See also Table 1.

Table 1. Variance comparisons of F2 and parental populations*

Experiment	Population	Mean life-span, days	Variance, days	*n*	*P*†
A 1	Bristol	20.2	10.4	46	
A 2	Bergerac	18.6	11.3	38	
A 3	F_2	19.8	16.5	249	<0.05
B 1	Bristol	22.7	16.0	57	
B 2	Bergerac	20.8	15.1	17	
B 3	F_2	22.2	28.0	167	<0.005

* Where populations larger than 50 are shown, several groups were assayed independently, checked by using Gehan statistics to establish lack of significant differences, and then combined for use in this analysis.

† *P* values are a measure of the significance of the increase in F_2 variance over parental strains. Each *P* value was obtained by using standard analysis of variance techniques from an *F* statistic calculated by comparing the F_2 variance with the pooled weighted variance of the two parental strains.

values in Table 1 yields heritability estimates of 34% ($P < 0.05$) for experiment A and 44% ($P < 0.005$) for experiment B. Such experiments have been repeated several times, both under similar and under different environmental conditions, and have given similar results.

Analysis of RI Lines. RI lines were obtained by crossing Bristol males with Bergerac hermaphrodites and identifying hybrid F_1 progeny. Individual F_2 self-progeny of these F_1 animals were placed on separate plates and allowed to self-fertilize; an F_3 individual from each F_2 plate was placed on a new plate to self-fertilize, and so on for 17 additional generations of self-fertilization. The resulting RI lines differ in genotype according to the various assortments of parental genes among the F_2 individuals and their progeny. However, after 19 generations of inbreeding by self-fertilization each line should be homozygous at all loci to 1 in 5×10^5.

If life-span determination is partly genetic, then the mean life-span of an RI line should correlate with the life-span of the F_2 individual from which it was derived. Mean life-spans of 14 RI lines were compared by linear regression analysis with the previously determined life-spans of their respective F_2 founders (Fig. 4). The slopes of the resulting curves provide an independent set of estimates for heritability of life-span (narrow sense; see *Discussion* and ref. 13). Two experiments yielded estimates of 51 ± 9% ($P < 0.001$) and 38 ± 13% ($P < 0.01$), respectively.

Heritability (narrow sense) was estimated by a third independent method using analysis of variance techniques (13). Within-line variance of an RI line is assumed to represent the influence of environmental factors on life-span, whereas between-line variance provides an estimate of twice the additive genetic variance (13). The within-line and between-line components of variance were estimated with sets of 34 and 36 different RI lines, respectively, in two separate experiments (data not shown). Heritability was calculated as

$$100\% \times 0.5\,\sigma_B^2/(\sigma_W^2 + 0.5\,\sigma_B^2),$$

in which σ_B^2 and σ_W^2 are between-line and within-line variance, respectively (13). Both experiments yielded estimates of 19% heritability ($P < 0.001$). [Use of $(\sigma_W^2 + 0.5\sigma_B^2)$ in the denominator to approximate total variance assumes that only additive factors contribute to the genetic component. This approximation probably is valid because the RI lines, being homozygous at all loci, should show no dominant and little epistatic contribution to variance; see also *Discussion*.]

Like the parental Bristol and Bergerac strains, each RI line

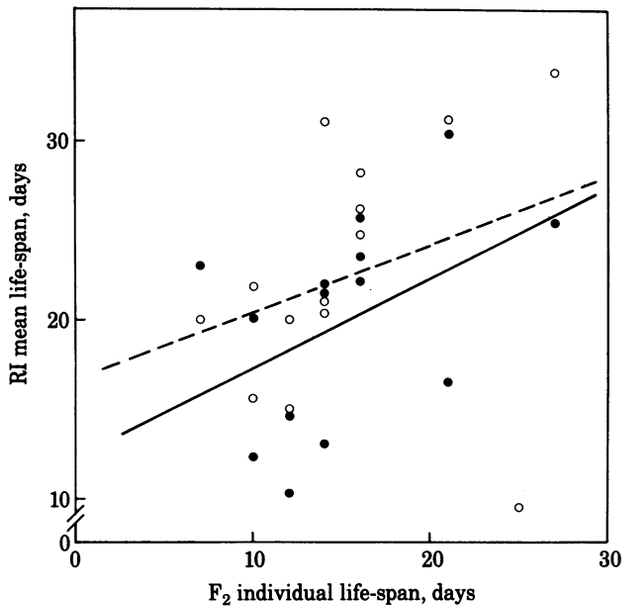

FIG. 4. Regression of mean life-span of 14 RI lines on the life-spans of the respective F_2 individuals from which each line was derived. Data from two separate experiments are shown (solid circles, solid curve; and open circles, dashed curve). The curves derived by linear regression from the plotted points have slopes of 0.51 ± 0.09 and 0.38 ± 0.13. For calculations (see ref. 13, pp. 165–181).

showed a characteristic, reproducible mean life-span. However, these life-spans differ strikingly from each other and from the parental life-spans. Mean life-spans of individual RI lines differ by as much as 21 days, ranging from 10 to 31 days (Fig. 4). By comparison, the two parental life-spans differ insignificantly, by no more than 2 days (19 and 17 days, respectively).

DISCUSSION

Reproducibility. The liquid culture method we have used in this study allows highly reproducible life-span determinations for *C. elegans*. The convenience of the method is such that we routinely maintain up to 300 separate survival assays simultaneously. The high reproducibility and ease of assay allow us to use life-span as a parameter for both quantitative and Mendelian genetic analyses.

Heterosis Effects and Their Significance. In most organisms, hybrid F_1 individuals derived from crosses between two inbred strains show large heterosis effects for length of life (5, 14). We have found no evidence for such effects in crosses between different strains of *C. elegans*. Our findings support the view that *C. elegans*, as a self-fertilizing hermaphrodite, is sufficiently inbred in wild populations that laboratory cultivation does not lead to significant inbreeding depression of life-span.

Perhaps related to our finding are recent observations that there is little enzyme polymorphism among wild strains of both *C. elegans* (15) and hermaphroditic snails (6). These results suggest that populations of self-fertilizing hermaphroditic animals may show less genetic variation than do the more commonly studied populations of outbreeding animals. Similar conclusions have been drawn for plants, in which the lower levels of allozyme variation in inbreeding populations have been extensively studied (16, 17). A likely explanation for these differences in variability is that populations of outbreeding organisms maintain variant loci by selection for heterozygotes (18, 19), whereas populations of self-fertilizing hermaphroditic organisms are driven to homozygosity and consequently face strong selection against less-fit variants.

Estimates of Life-Span Heritability. The three different methods we have used for determining the portion of life-span variation under genetic control in *C. elegans* yield estimates ranging from 19% to 51%. One estimate is based on the assumption that the variance caused by genetic differences in an F_2 population from an interstrain cross can be obtained by subtracting the parental variance from the total F_2 variance. This difference can be used to estimate the so-called broad-sense heritability, which includes the additive, dominant, and epistatic genetic contributions to life-span variation. This estimate yielded heritability values of about 34% and 44%.

The other two estimates are made by using RI lines derived from F_2 individuals. After 19 generations of inbreeding by self-fertilization, each of these lines is homozygous for one or the other of any pair of alleles that differed in the two parental strains. Therefore, heritability measurements with RI lines estimate only the component of the total genetic variance that is expressed in homozygous individuals. This component is called the narrow-sense heritability, which includes only the additive genetic contributions. The range of estimates obtained from RI analysis (19% to 51%) is similar to the range of values obtained for broad-sense heritability. This similarity further confirms the absence of heterosis effects and supports our assumption that the genetic component of life-span variance in *C. elegans* is primarily in the form of simple additive factors, which should readily permit selective breeding of longer-lived strains.

Analysis of RI lines has several advantages over analysis of the individual animals in an F_1 or F_2 population. Better quantitation is possible, and replicate populations are readily available. Linkage of a new single-gene trait with other previously mapped traits can be established from the correlation of their appearance among many RI lines. For a polygenic trait, the number of genes involved can be estimated from distributions of the trait among RI lines. Homozygosity at all loci simplifies analysis by eliminating effects due to heterozygosity, such as dominance. The ease with which a large set of RI lines can be produced rapidly is another attractive feature of *C. elegans* for quantitative genetics.

Estimates of life-span heritability have been made for both *Drosophila* and mice. Maynard Smith (20) obtained full sib and parent–offspring correlations for length of life of about 20%, suggesting about 40% heritability in wild populations of *D. subobscura*. Tantawy *et al.* (21, 22), working on newly caught wild populations of *D. melanogaster* and *D. simulans*, obtained heritability estimates in the range of 11% to 19%. More recently, experiments with the Oregon R wild-type laboratory strain of *D. melanogaster* yielded the conclusion, based on inability to select longer-lived variants, that there is little heritability of life-span in this stock (1, 2). However, this result could be explained by loss of genetic variation during the inbreeding that occurs during laboratory propagation. Such loss of life-span heritability has been demonstrated directly by Tantawy and El-Helw (22).

In mice, Storer (23) obtained heritability estimates of 21% for males and 36% for females in a survey of 22 inbred mouse strains using analysis of variance techniques. Goodrick (3) obtained values of 79% and 48% as heritability estimates for life-span on F_2 populations of mice from crosses between a relatively short-lived strain (A/J) and a long-lived strain (C57BL/6J). However, the apparent presence of a single major gene affecting life-span in these crosses may have made the analysis invalid and led to artificially inflated estimates.

Genetic Analysis of Aging in *C. elegans*. Genetic variants that live longer than parental strains seem more likely than shorter-lived variants to be altered in primary rate-limiting processes that determine life-span. Longer-lived variants therefore may

allow genetic approaches to identifying and understanding these processes. In *C. elegans*, genetic factors appear to increase life-span variation approximately the same amount toward both longer and shorter life (Figs. 3 and 4). By contrast, genetic factors in mouse and *Drosophila* populations almost exclusively appear to increase variation toward life-spans shorter than those seen in F_1 hybrid individuals. Our observations suggest that breeding for longer-lived variants of *C. elegans* is likely to succeed. In fact, we already have obtained RI lines with mean life-spans of up to 31 days, an increase of >70% over the mean parental life-span of 18 days.

The lack of heterosis effects and associated inbreeding depression for life-span in *C. elegans* also will be advantageous for the analysis of single-gene mutants. In most organisms, a newly induced long-lived mutant could easily be lost due to heterosis effects in the backcrosses necessary to analyze the new mutation. Although this difficulty could be overcome by working within a single inbred genetic background, such a solution has the associated problem that selection for longer life might yield primarily mutations that restored normal hybrid vigor or that affected life-span only within the particular inbred background used.

In summary, many of the previous difficulties with genetic analysis of aging have been due to inbreeding depression and associated heterosis effects that are difficult to control in experiments with outbreeding organisms. Because *C. elegans* does not exhibit these effects it should provide an excellent system for further work on the genetic basis of senescence.

We are grateful to Gretchen McCaffrey, Bud Lashlee, and Cindy Cheng for excellent technical assistance, to J. C. DeFries for helpful suggestions and discussion on use of recombinant inbred strains, and to Michael Raines for development of computer software. This research was supported by National Institutes of Health Young Investigator Award (AG-01236) to T.E.J. and grant (HD-11762) to W.B.W.

1. Lints, F. A., Stoll, J., Gruwez, G. & Lints, C. V. (1979) *Gerontology* **25**, 192–204.
2. Flanagan, J. R. (1980) *Mech. Ageing Dev.* **13**, 41–62.
3. Goodrick, C. L. (1975) *J. Gerontol.* **30**, 257–263.
4. Clarke, J. M. & Maynard Smith, J. (1955) *J. Genet.* **53**, 172–180.
5. Clark, A. M. (1971) *Adv. Gerontol. Res.* **3**, 207–255.
6. McCracken, G. F. & Selander, R. K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 684–688.
7. Brenner, S. (1974) *Genetics* **77**, 71–94.
8. Wood, W. B., Hecht, R., Carr, S., Vanderslice, R., Wolf, N. & Hirsh, D. (1980) *Dev. Biol.* **74**, 446–469.
9. Klass, M. & Hirsh, D. (1976) *Nature (London)* **260**, 523–525.
10. Klass, M. R. (1977) *Mech. Ageing Dev.* **6**, 413–429.
11. Gehan, E. (1975) in *Cancer Therapy: Prognostic Factors and Criteria of Response*, ed. Staquet, M. J. (Raven, New York), pp. 231–257.
12. Peto, R., Pike, M. C., Armitage, P., Breslow, N. E., Cox, D. R., Howard, S. V., Mantel, N., McPherson, K., Peto, J. & Smith, P. G. (1977) *Br. J. Cancer* **35**, 1–39.
13. Falconer, D. S. (1960) *Quantitative Genetics* (Ronald, New York).
14. Comfort, A. (1956) *Ageing, The Biology of Senescence* (Holt, Rinehart and Winston, New York).
15. Butler, M. L., Wall, S. M., Luehrsen, K. R., Fox, G. E. & Hecht, R. M. (1981) *J. Mol. Evol.* **18**, 18–23.
16. Hamrick, J. L., Linhart, Y. B. & Mitton, J. B. (1979) *Annu. Rev. Ecol. Syst.* **10**, 173–200.
17. Brown, A. H. D. (1979) *Theor. Popul. Biol.* **15**, 1–42.
18. Tracey, M. L. & Ayala, F. J. (1974) *Genetics* **77**, 569–589.
19. Simmons, M. J. & Crow, J. F. (1977) *Annu. Rev. Genet.* **11**, 49–78.
20. Maynard Smith, J. (1959) *J. Genet.* **56**, 207–235.
21. Tantawy, A. O. & Rakha, F. A. (1964) *Genetics* **50**, 1349–1355.
22. Tantawy, A. O. & El-Helw, M. R. (1966) *Genetics* **53**, 97–110.
23. Storer, J. B. (1966) *J. Gerontol.* **21**, 404–409.