Genetic Analysis of Life-Span in Caenorhabditis elegans

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Genetic analysis of life-span in *Caenorhabditis elegans*

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**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 F1 progeny with life-spans similar to those of the parent strains. By analysis of life-span variation among progeny F2 populations from such crosses and by two independent analyses of life-spans among recombinant inbred lines derived from F1 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 F1 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 F2 populations from interstrain crosses and from two separate analyses of life-span in recombinant inbred (RI) lines derived from F2 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 NMG agar plates) and B/r (for growth in liquid culture). Male and hermaphroditic master stocks were maintained for up to 8 months as dauerlarvae (9) on NMG plates sealed with Parafilm and kept at 16°C.

**Establishment of Survival Populations.** Dauerlarvae were allowed to mature and reproduce for one generation on NMG agar plates, and at the fourth larval stage their F1 progeny were transferred to fresh NMG 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 selling) to fresh NMG plates preseeded with *E. coli* OP50. After 4–12 hr of egg laying, the adults were removed, and the synchronized F2 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, F2 progeny in groups of 50 worms were suspended in 5 ml 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.

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**Abbreviation:** RI, recombinant inbred.

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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 F₁ 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. F₁ 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 reproducibility. 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). F₁ hybrid hermaphrodite progeny of a Bristol × Bergerac cross had a life-span distribution similar

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**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 survival curves 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 mean survival curves yields 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 F₁ hybrid populations. (A) Hermaphrodites. × × ×, Bristol; ○ ○ ○, Bergerac; ○ × ○, F₁ hybrid. (B) Males. × × ×, Bristol; ○ × ○, F₁ hybrid. Mean (±SEM) life-spans for the Bristol, Bergerac, and F₁ hybrid hermaphrodites were 18.2 ± 1.1, 18.6 ± 0.8, and 18.6 ± 1.1 days, respectively. Mean life-spans for Bristol and F₁ 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 > 0.38 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 F1 hybrid hermaphrodite survival curves show no statistical-
ly significant differences. Likewise, survival curves for F1
hybrid males are not statistically different from those of the
Bristol parental males (Fig. 2B). The Bristol and hybrid her-

morhphrodites, 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 phenotyp:
in these experiments all animals were maintained at 20°C
throughout. The lower rearing temperature caused no signif-
ificant change in the results. In all four experiments the hybrid
progeny showed no significant increase in life-span over paren-
tal 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 F1 hybrids
resulting from crosses between four other wild-type strains and
a mutant Bristol stock (data not shown).

Analysis of F2 Populations. Life-span variations within par-
ental Bristol and Bergerac populations, which should be ge-
netically homogeneous, are assumed to result from environ-
mental effects. Among the F2 individuals resulting from self-
fertilization of hybrid F1s, 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 F2 population compared to the parental population. Fig.
3 shows the distributions of deaths per day for two such com-
parisons. In both experiments, some F2 individuals died at ear-
lier times and some F2 individuals lived longer than any indi-

cidual 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 F2 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 com-
ponent to the total variance. Calculation of this ratio from the

Table 1. Variance comparisons of F2 and parental populations*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Population</th>
<th>Mean life-span, days</th>
<th>Variance, days²</th>
<th>n</th>
<th>P+</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1</td>
<td>Bristol</td>
<td>20.2</td>
<td>10.4</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>A 2</td>
<td>Bergerac</td>
<td>18.6</td>
<td>11.3</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>A 3</td>
<td>F2</td>
<td>19.8</td>
<td>16.5</td>
<td>249</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B 1</td>
<td>Bristol</td>
<td>22.7</td>
<td>16.0</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>B 2</td>
<td>Bergerac</td>
<td>20.8</td>
<td>15.1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>B 3</td>
<td>F2</td>
<td>22.2</td>
<td>28.0</td>
<td>167</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

* Where populations larger than 50 are shown, several groups were
  assayed independently, checked by using Gehan statistics to estab-
  lish lack of significant differences, and then combined for use in this
  analysis.
+ P values are a measure of the significance of the increase in F2 var-
  iance over parental strains. Each P value was obtained by using stan-
  dard analysis of variance techniques from an F statistic calculated
  by comparing the F2 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 sim-

ilar 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 F1 progeny. Individual F2 self-progeny of these F1 an-
imals were placed on separate plates and allowed to self-fertil-
ize; an F2 individual from each F2 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 ac-


Fig. 3. Distributions of deaths as a function of age for parental
and F2 populations from a cross of Bristol males with Bergerac her-
maphrodites. Left and Right. Results from separate duplicate experi-
ments. - Bristol hermaphrodites; Long dash, Bergerac hermaph-
rodites; Short dash, F2 hermaphrodites. See also Table 1.
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 F2 population from an interstrain cross can be obtained by subtracting the parental variance from the total F2 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 F2 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 F2 or F2 population. Better quantification 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 29%, suggesting about 40% heritability in wild populations of D. melanogaster. 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 F2 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 F₁ 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.

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