# Genotype-Environment Interaction for Quantitative Trait Loci Affecting Life Span in *Drosophila melanogaster*

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ABSTRACT

The nature of genetic variation for Drosophila longevity in a population of recombinant inbred lines was investigated by estimating quantitative genetic parameters and mapping quantitative trait loci (QTL) for adult life span in five environments: standard culture conditions, high and low temperature, and heat-shock and starvation stress. There was highly significant genetic variation for life span within each sex and environment. In the analysis of variance of life span pooled over sexes and environments, however, the significant genetic variation appeared in the genotype × sex and genotype × environment interaction terms. The genetic correlation of longevity across the sexes and environments was not significantly different from zero in these lines. We estimated map positions and effects of QTL affecting life span by linkage to highly polymorphic *roo* transposable element markers, using a multiple-trait composite interval mapping procedure. A minimum of 17 QTL were detected; all were sex and/or environment-specific. Ten of the QTL had sexually antagonistic or antagonistic pleiotropic effects in different environments. These data provide support for the pleiotropy theory of senescence and the hypothesis that variation for longevity might be maintained by opposing selection pressures in males and females and variable environments. Further work is necessary to assess the generality of these results, using different strains, to determine heterozygous effects and to map the life span QTL to the level of genetic loci.

LIMITED life span and senescence, the progressive decline in survivorship and fertility with advancing age, are near-universal characteristics of eukaryotic organisms. However, there is great variation in total life span and rate of aging between and within species (Finch 1990). Variation in life span within natural populations is partly attributable to both genetic and environmental effects (Tower 1996; McClearn *et al.* 1997). Determining the nature of, and interactions between, the genetic and environmental factors affecting aging is important from two rather different perspectives: human health and evolutionary theory. As the human population rapidly grows older (Martin and Preston 1994), population aging is becoming one of the most important social and health problems facing the next half century (Rowe 1997). The evolutionary enigma is why organisms age at all, and why genetic variation for life span segregates within populations.

The classical evolutionary explanations for aging are based on the reduction of the strength of natural selection as organisms grow older (Medawar 1952; Williams 1957; Charlesworth 1980). The mutation-accumulation hypothesis (Medawar 1952) assumes that mutations that are neutral early in life but that have variable late-age-specific deleterious effects on viability will escape the vigilance of natural selection and cause both aging and variation in aging. The antagonistic pleiotropy hypothesis proposes that mutations with beneficial effects on survival and/or fertility early in life have deleterious effects later on, and vice versa, leading to an equilibrium situation in which both classes of alleles remain segregating (Williams 1957; Rose and Charlesworth 1980; Kirkwood and Rose 1991). Additionally, hypotheses for the maintenance of genetic variation for quantitative traits in general apply to longevity. These include unconditionally deleterious mutation-selection balance (Houle *et al.* 1996) and genotype × environment interaction (GEI). GEI can maintain quantitative genetic variation if heterozygotes are less sensitive to environmental variation than homozygotes (Gillespie and Turelli 1989) and if there are reversals in effects of alleles affecting the life history trait in different environments (Levene 1953). None of these hypotheses are mutually exclusive.

Attempts to discriminate among the various evolutionary hypotheses using *Drosophila melanogaster* as a model system, by testing whether genetic variation in fitness

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components increases with age, as predicted by the mutation-accumulation hypothesis (Rose and Charlesworth 1980; Engström *et al.* 1989; Hughes and Charlesworth 1994; Hughes 1995; Promislow *et al.* 1996; Tatar *et al.* 1996), and testing for negative genetic correlations between longevity and other life history traits, as predicted by the antagonistic pleiotropy hypothesis (Rose and Charlesworth 1981a,b; Rose 1984; Luckinbill *et al.* 1984; Engström *et al.* 1992; Partridge and Fowler 1992; Roper *et al.* 1993; Service 1993; Stearns and Kaiser 1993; Force *et al.* 1995; Zwaan *et al.* 1995), have produced mixed results. Given that the various studies did not have the same power to detect differences in variance, the potential for real genetic heterogeneity among the base populations used, and different population sizes and environments, such inconsistency is perhaps not surprising. Further, estimates of genetic correlation are only informative with respect to the net effects of all segregating loci and can conceal considerable heterogeneity in effects across loci. The evolutionary question is not whether each of the potential mechanisms operates, but rather at what quantitative trait loci (QTL) do alleles affecting variation in life span segregate, and at what fraction of the QTL is variation attributable to the segregation of rare, unconditionally deleterious alleles, alleles with late-age effects, alleles with opposing pleiotropic effects on early fitness components, and alleles that exhibit GEI.

What genes are likely to affect senescence and life span? Traditional screens for mutations altering longevity have not been considered feasible for a variety of reasons:

1. Mutations in almost any gene will have a deleterious pleiotropic effect on life span, but this does not imply the genes so identified directly affect life span.
2. Screens for mutations increasing life span are logistically difficult, because each potential mutation must be preserved prior to scoring the phenotype (date of death).
3. Chemical mutagenesis induces multiple mutations simultaneously; therefore, long-lived mutations will be difficult to discern given the background noise of mutations decreasing life span.
4. Single *P*-element mutagenesis in Drosophila is not a solution to the problem of deleterious background mutations, since the transgenic *P* element carries a dominant wild-type selectable marker gene and the host strain is homozygous for a mutant allele for the marker. The host strain thus has reduced fitness (and longevity) that can be "rescued" by many *P*-element insertions, which do not affect loci with direct effects on life span (Lyman *et al.* 1996; but see Lin *et al.* 1998).

In *Caenorhabditis elegans*, however, mutations in genes affecting dauer-larva formation, fertility, and biological rhythm have been found to have extended longevity phenotypes (Lithgow 1996). Several of these genes have been cloned and encode phosphatidyl inositol 3-kinase (*age-1*/*daf-23*; Morris *et al.* 1996), an insulin-like receptor (*daf-2*; Kimura *et al.* 1997), a member of the HNK-3/forkhead transcription factor family (*daf-16*; Lin *et al.* 1997), and a short protein with an 82-amino-acid tandem repeat that is homologous to yeast *CAT5/COQ7* (*clk-1*; Hekimi *et al.* 1998). These genes strongly implicate the glucose metabolism pathway in organismal aging. These observations are consistent with observations of increased glycogen and lipid content of Drosophila lines selected for postponed senescence relative to the unselected controls (Service 1987; Graves *et al.* 1992); an increase in frequency of the most active electromorph of the glycolytic enzyme phosphoglucomutase in the same long-lived Drosophila strains compared to the base population controls (Deckert-Cruz *et al.* 1997); and the effect of severe caloric restriction on extended life span in mice (Harrison and Archer 1987).

Long-lived strains of flies and worms also exhibit increased resistance to starvation, desiccation (Service *et al.* 1988; Graves *et al.* 1992; Lin *et al.* 1998), and thermal and UV-irradiation stress (Lithgow 1996; Lin *et al.* 1998), implicating oxidative stress and heat-shock response genes as candidates. Numerous observations support this hypothesis. The more active superoxide dismutase (*Sod*) electromorph allele is at a higher frequency in the long- than in the short-lived replicates of one set of Drosophila selection lines (Deckert-Cruz *et al.* 1997), while in an independent set of lines, *Sod*, catalase (*Cat*), and xanthine dehydrogenase (*Xdh*) have higher mRNA and enzyme activity levels in the long-lived strains (Force *et al.* 1995). Long-lived mutant strains of *C. elegans* have elevated *Sod* and *Cat* enzyme activities (Lithgow 1996). Transgenic Drosophila strains that jointly overexpress both *Sod* and *Cat* have extended life spans (Orr and Sohal 1994). Heat-shock proteins (*Hsp*s) are produced in response to thermal and other stress factors (Burton *et al.* 1988). Not only is the ability to induce *Hsp*s reduced in senescent animals (Niedzwiecki *et al.* 1991), but a brief, sublethal heat shock to young animals has been reported to extend life span (Lithgow *et al.* 1995; Khazaeli *et al.* 1997). Additional candidate genes affecting longevity are those associated with DNA repair and replication (Woodhead *et al.* 1985; Harrison 1990; Yu *et al.* 1996) and telomere length (Bodnar *et al.* 1998).

Analysis of effects of mutations at candidate genes and their expression patterns in young and old animals does not, however, directly address the question of whether allelic *variation* at these loci in natural populations causes quantitative *variation* in longevity. For this, allelic association studies are necessary, in which polymorphism(s) at the candidate gene(s) are identified, and the association between the allelic state of the polymorphism and longevity in a natural population is evaluated statistically (Finch and Tanzi 1997; see also

Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998). This approach is potentially exceedingly powerful but is limited by the sheer number of putative candidate genes to be tested, our restricted knowledge of all relevant candidates, and the difficulty in assessing effects of the candidate gene alleles in a range of environments if individuals are sampled from nature.

QTL analysis of naturally occurring modifiers of life span directly addresses the issue of what genetic regions are associated with variation in longevity between the two parental strains used to generate the mapping population and what are their effects. This approach complements association studies at candidate loci, because it can refine the list of candidate genes to be investigated to those that map to the same location as the QTL and can point to genomic regions containing no known candidate genes as worthy of further study. Previously, we mapped five autosomal QTL affecting *D. melanogaster* life span that segregated between two inbred strains that had not been selected for life span, using a panel of 98 recombinant inbred (RI) lines derived from the parental strains and a dense cytogenetic marker map (Nuzhdin *et al.* 1997). The effects of longevity QTL, estimated in a single environment, were highly sex-specific. Here, we have reared the same set of RI lines under standard and stressful environmental conditions to determine whether longevity QTL exhibit GEI. The stressful conditions—high and low temperature, a brief, sublethal heat shock early in adult life, and starvation—were chosen on the basis of reports in the literature of direct and correlated effects of these treatments on life span. Surprisingly, all significant genetic variation among these RI lines is associated with GEI: longevity QTL exhibit sex- and environment-specific effects, as well as reversal in the signs of effects across sexes and environments.

## MATERIALS AND METHODS

**Drosophila stocks:** The parental lines used were isogenic derivatives of two unrelated strains, Oregon-R (Lindsley and Zimm 1992) and 2b (Pasyukova and Nuzhdin 1993). These strains were not selected for life span. $F_1$ progeny were backcrossed to 2b, and the backcross progeny randomly mated for four generations. A total of 98 RI lines were constructed by 25 generations of full-sib mating (Nuzhdin *et al.* 1997; Gurganus *et al.* 1998).

**Life span phenotypes:** Adult longevity was measured for all 98 RI and the two parental lines in five environments. All flies were reared from egg to adult on 10 ml standard cornmeal-agar-molasses medium at 25° in shell vials. The density of the stocks was controlled for three generations prior to the start of the longevity assays by restricting egg laying to 3 days and initiating the cultures with 10 pairs of flies. For each assay performed, 20 virgin males and females per line were collected in a 24-hr period. Assays were begun with 2-day-old flies housed in replicate vials with 10 same-sex individuals per vial. The five environments were as follows: control (C), 5 ml cornmeal-agar-molasses medium at 25°; high temperature (HT), 5 ml cornmeal-agar-molasses medium at 29°; low temperature (LT), 5 ml cornmeal-agar-molasses medium at 14°; heat shock (HS), 37° heat shock for 30 min followed by maintenance in the C environment; and starvation (S), 5 ml of 1.5% agar medium at 25°. To provide nutrition and humidity during the 30-min HS treatment, flies were held in vials containing a layer of filter paper soaked in a 25% sucrose solution. Numbers of dead flies were scored daily for the first four treatments, until all animals were dead, and vials were replaced weekly. The numbers of dead flies were scored every 8 hr for the starvation treatment. The five assays were conducted sequentially over a 2-mo period.

**Phenotypic analyses:** Variation in male and female life span in each of the five environments was partitioned into sources attributable to line ($L$), replicate ($R$) within line, and error by random effects analysis of variance (ANOVA), according to the model $y = \mu + L + R(L) +$ error. $\mu$ is the overall mean and nested effects are in parentheses. Variation in life span for the full data set was partitioned into sources attributable to environment ($E$), sex ($S$), and $L$ according to the mixed-model ANOVA: $y = \mu + E + S + L + E \times S + E \times L + S \times L + E \times S \times L + R(E \times S \times L)$ + Error. $E$, $S$, and $E \times S$ are fixed effects; the rest are random. Tests of significance of $F$-ratios and estimates of variance components of the random effects were obtained using SAS procedures GLM and VARCOMP, respectively (SAS Institute 1988). Type III sums of squares were used in these analyses, because the design was not completely balanced at the lowest level. Genetic correlations between the sexes ($r_{GS}$) and across pairs of environments for each sex ($r_{GE}$) were computed as $\mathrm{cov}_{12}/\sigma_{L1}\sigma_{L2}$, where $\mathrm{cov}_{12}$ is the covariance among line means between males and females or pairs of environments, and $\sigma_{L1}$ and $\sigma_{L2}$ are the square roots of the respective variance components among lines from the reduced-model analyses by sex and environment (Robertson 1959). All phenotypic analyses were performed on untransformed life span records as well as on ln-transformed data.

**Molecular marker map:** Highly polymorphic cytological insertion sites of high copy number *roo* transposable elements were used as molecular markers. *roo* element insertion sites in the RI lines were determined as described by Nuzhdin *et al.* (1997). There were a total of 76 informative markers, including the recessive mutation *spa*$^{pol}$, which also segregated among the RI lines. The average spacing between the markers on the standard Drosophila map (Lindsley and Zimm 1992) was 3.2 cM (Nuzhdin *et al.* 1997), but the additional generations of recombination during the construction of the RI lines resulted in an expanded genetic map. The map positions of the markers ($d$ cM) on the expanded map were estimated from the observed recombination frequencies ($r$) using the Kosambi mapping function: $100d = 0.25 \ln[(1 + 2r)/(1 - 2r)]$. The insertion sites of the informative markers and their estimated map positions were as follows: 1B(1-0.00), 3E (1-14.85), 4F (1-33.16), 5D (1-39.36), 6E (1-42.43), 7D (1-57.28), 7E (1-58.30), 9A (1-76.81), 10D (1-96.29), 11C (1-99.36), 11D (1-102.43), 12E (1-106.52), 14C (1-126.01), 15A (1-127.03), 17C (1-131.16), 19A (1-134.27), 21E (2-0.00), 22F (2-8.59), 27B (2-61.19), 29F (2-85.53), 30AB (2-87.65), 30D (2-89.77), 33E (2-126.77), 34EF (2-135.75), 35B (2-140.15), 38A (2-146.00), 38E (2-148.49), 43A (2-152.16), 43E (2-153.38), 46C (2-154.52), 48D (2-163.49), 49D (2-169.92), 50B (2-172.04), 50D (2-175.15), 50F (2-178.47), 57C (3-0.00), 57F (3-7.65), 60E (3-39.23), 61A (4-0.00), 63A (4-16.72), 65A (4-43.80), 65D (4-67.70), 67D (4-83.06), 68B (4-103.00), 68C (4-114.92), 69D (4-124.71), 70C (4-142.04), 71E (4-146.59), 72A (4-148.85), 73D (4-153.26), 76A (4-156.76), 76B (4-160.18), 77A (4-162.34), 82D (4-164.65), 85F (4-169.26), 87B (4-177.28), 87E (4-180.67), 87F (4-181.81), 88E (4-192.05), 89B (4-196.56), 91A (4-207.31), 91D (4-215.33), 92A (4-218.65), 93A (4-230.09), 93B (4-231.15), 94D (4-243.34), 96A (4-260.05), 96F (4-273.56), 97D (4-284.86), 97E (4-287.06), 98A (4-295.83),

99A (4-313.98), 99B (4-326.17), 99E (4-337.22), 100A (4-338.28), *spa* (5-0.00). Cytological divisions 1-20, 21-60, and 61-100 correspond to Drosophila chromosomes *1*, *2*, and *3*, respectively, on the standard map (Lindsley and Zimm 1992). Note that chromosome *2* was formally split into two linkage groups due to free recombination between markers 50F and 57C. All markers were scored as homozygous Oregon, homozygous 2b, or heterozygous (Nuzhdin *et al.* 1997).

**QTL mapping:** Multiple-trait composite interval mapping (Jiang and Zeng 1995) was used to test the hypotheses that an interval flanked by two adjacent markers contains QTL affecting the trait, and that it accounts for a QTL × trait interaction, while statistically accounting for the effects of additional segregating QTL by multiple regression on markers outside the tested interval. The analysis of one trait in multiple environments and the cross-environment genetic correlation is formally equivalent to the analysis of multiple genetically correlated traits in a single environment (Falconer 1952). The likelihood-ratio test statistic, LR, is $-2 \ln (L_0/L_1)$, where $L_0$ is the maximum likelihood under the null hypothesis that $a_1 = a_2 = \ldots = a_i = 0$, where $a_i$ is the additive effect of the QTL in environment $i$; and $L_1$ is the maximum likelihood under the alternative hypothesis that at least one $a_i \neq 0$. In other words, this is a test for the presence of QTL in one or more environments. The test statistic at a genomic location is asymptotically distributed as $\chi^2$ with $i + 1$ d.f. (The additional degree of freedom is because one tests for a location parameter in addition to the $i$ QTL effects.) The likelihood-ratio test for QTL × environment interaction evaluates the ratio of the maximum likelihood under the null hypothesis that $a_1 = a_2 = \ldots = a_i$, to that under the alternative hypothesis of $a_1 \neq a_2 \neq \ldots \neq a_i$. If this test is performed only in the regions where QTL were detected in the joint mapping analysis, the test statistic is asympotically distributed as $\chi^2$ with $i - 1$ d.f. under the null hypothesis. If a genome-wide scan for interaction effects is performed, the asymptotic distribution is $\chi^2$ with $i$ d.f.

The appropriate threshold for significance of each test must be adjusted for the number of independent tests in the genome-wide scan. Although there are 76 markers, there are not 76 independent tests, because the markers on the same chromosome are correlated. Under composite interval mapping (Zeng 1994), the effective number of independent tests in the search for QTL is determined by the size of the genome region to either side of the test interval in which marker cofactors are not fitted (the "window size"). In these analyses, we used a window size of 30 cM and thus conservatively estimate that adjacent regions of 50 cM may be regarded as independent. We estimate the number of independent tests as $\Sigma_i [(T_i/50) + 1]$, where $T_i$ is the total estimated map length in centimorgans of the $i$th linkage group. For these data, there are 3.7, 4.6, 1.8, and 7.8 independent tests for each of the four linkage groups, respectively; or ~20 independent tests in total. Consequently, a type I error rate of $\alpha = 0.0025$ was used in the joint mapping analyses and genome-wide screens for interaction. For tests of QTL × environment interaction that were conditional on the presence of significant QTL, a conventional 5% significance level is appropriate. To be conservative, we used a type I error rate of $\alpha = 0.01$ in conditional tests for interaction. Seven joint composite interval mapping analyses were performed: one for each of the five environments, estimating male and female QTL effects separately, joint QTL, and QTL × sex effects; and one for each sex, estimating joint QTL and QTL × environment effects. All analyses used a window size of 30 cM and 15 background markers, selected by stepwise multiple regression. Heterozygous genotypes were treated as missing data. Analyses were performed on life span and on ln(life span).

## RESULTS

**Life span phenotypes:** Mean life spans of the two parental lines, Oregon and 2b, and of the 98 RI lines derived from them, are given for each of the five environments in Table 1. The results of tests of significance of differences in life span between environments and sexes are presented in Table 2. Mean life spans of the RI lines ranged from a low of 2.3 days in the starvation treatment to a high of 80 days at low temperature. Relative to the control environment, the heat-shock treatment prolonged average life span by 6 days in males only, and the high temperature environment reduced average life span by 3.6 days in males and females. These treatment effects are consistent with previous reports documenting an inverse relationship between life span and temperature (*e.g.*, Zwaan *et al.* 1995) and demonstrating protective effects of early, sublethal heat shock on Drosophila longevity (Lithgow *et al.* 1995; Khazaeli *et al.* 1997). Mean differences in life span between all pairs of treatments were highly significant as was the overall effect of environment (Table 2). Females had significantly longer mean life spans than males in the control, high temperature, and starvation environments, but not under heat-shock and high temperature conditions; over all environments, sexual dimorphism for life span was only significant at $P < 0.05$ (Table 2). Differences in the magnitude of sexual dimorphism for life span across environments are reflected in highly significant ($P < 0.0001$) sex × environment interaction terms between the heat-shock and control, heat-shock and high temperature, and heat-shock and starvation environments, as well as between the high temperature and starvation environments (data not shown). Consequently, the sex × environment interaction term approached formal significance in the analysis over all five environments (Table 2).

**Genetic variance in life span:** The among-line and residual variance components estimated from ANOVA for each sex in each of the five environments are given in Table 1. In all but one case, there is highly significant variation among the RI lines. (The exception is for female longevity at low temperature, for which the among-line variance component is not quite formally significant.) All among-line variance components were significant for the ln-transformed data (not shown). If one assumes that gene frequencies at all segregating loci affecting the trait are 0.5, then the variance among the RI lines is an estimate of the genetic variance, $V_G$, between the parental inbred lines (Falconer and Mackay 1996). Because the mean life span ($\overline{\times}$) of the RI lines varies across treatments, the coefficient of genetic variation $CV_G = 100\ (V_G)^{1/2}/\overline{\times}$ is an appropriate statistic to compare relative magnitudes of genetic variance in the different environments (Houle 1992). Estimates of $CV_G$ ranged from 14–28% in males and 13–20% in females.

**TABLE 1**

**Summary statistics of life span in different environments**

| $E^a$ | Sex | Mean[b] ± SE P1 | Mean[b] ± SE P2 | Mean[b] ± SE RI | $V_L{}^c$ | $V_R{}^d$ | $P$ (GSI)[e] | $r_{GS}{}^f$ ($L_1$,$L_2$)[g] |
|---|---|---|---|---|---|---|---|---|
| C | ♂ | 33.6 ± 3.09 | 37.5 ± 3.22 | 34.1 ± 1.06 | 36.5**** | 262 | *** | 0.23 (0.03, 0.41) |
| | ♀ | 39.5 ± 3.43 | 28.9 ± 4.28 | 38.8 ± 1.09 | 61.5**** | 308 | | |
| HS | ♂ | 49.0 ± 3.81 | 49.4 ± 3.29 | 40.5 ± 0.87 | 48.5**** | 187 | **** | 0.23 (0.03, 0.41) |
| | ♀ | 33.9 ± 5.19 | 39.0 ± 2.92 | 39.7 ± 1.04 | 66.3**** | 307 | | |
| HT | ♂ | 36.3 ± 1.05 | 23.2 ± 2.69 | 31.1 ± 0.53 | 19.0**** | 77.7 | **** | 0.45 (0.27, 0.59) |
| | ♀ | 33.9 ± 2.33 | 28.6 ± 1.99 | 34.6 ± 0.79 | 37.2**** | 185 | | |
| LT | ♂ | 77.5 ± 10.9 | 45.8 ± 0.27 | 78.7 ± 2.98 | 489**** | 1649 | NS | 1.23 (Undefined) |
| | ♀ | 48.3 ± 10.3 | 77.0 ± 10.5 | 82.2 ± 2.93 | 216* | 3337 | | |
| S | ♂ | 2.65 ± 0.09 | 1.52 ± 0.07 | 2.29 ± 0.04 | 0.129**** | 0.138 | ** | 0.94 (0.72, 0.96) |
| | ♀ | 2.64 ± 0.18 | 2.88 ± 0.10 | 2.60 ± 0.04 | 0.116**** | 0.258 | | |

NS, $P > 0.05$; *$0.05 < P < 0.10$; **$0.01 < P < 0.05$; ***$0.0001 < P < 0.001$; ****$P < 0.0001$.

[a] E, environments; C, control; HS, heat shock; HT, high temperature; LT, low temperature; S, starvation.

[b] P1, Oregon; P2, 2b; RI, recombinant inbred lines. Units are days.

[c] Among-line variance component from analyses of variance (ANOVA) of life span in the RI lines for each sex separately. $P$ values are from $F$-ratio tests of significance of the line term in these analyses.

[d] The sum of the between-replicate and within-replicate (error) variance components from the ANOVAs.

[e] $P$ (GSI) values are from $F$-ratio tests of significance of the sex × line interaction term from combined-sex ANOVAs and indicate whether the genetic correlations are significantly different from unity.

[f] The genetic correlation between the sexes.

[g] Lower and upper confidence limits for $r_{GS}$.

The ratio of $V_G$ to ($V_G + V_R$), where $V_R$ is the sum of the variance between and within replicates, indicates the extent to which variation in phenotypes in the population of RI lines is due to variation in genotypes. This ratio is 0.17, averaged over males and females in all but the starvation environment. The relatively large effect of uncontrolled environmental factors on individual phenotype is expected for life history traits such as life span. However, $V_G/(V_G + V_R)$ was much higher for the starvation treatment: ~0.40 averaged over the sexes. Ratios of $V_G$ to $V_G + V_R$ were reduced on average by 70% in the analyses of ln(life span) (not shown).

**Genotype × environment interaction for life span:** We assessed the extent to which alleles at loci affecting variation in life span among the RI lines cumulatively exhibited genotype × sex (GSI) and GEI interaction by determining the significance of these interaction effects in mixed model ANOVAs of life span among the RI lines within each environment, pooled across sexes, as well as pooled across environments. Highly significant GSI was observed for the control, heat-shock, and high temperature treatments; GSI was nominally significant in the starvation environment but not the low temperature environment (Table 1). The genotype × sex interaction effect was also significant in the analysis considering all five environments (Table 2). There was highly

**TABLE 2**

**ANOVA of life span for the RI lines, pooled over environments and sexes**

| Source | d.f. | Mean square | $F$ | $P$ | Variance component |
|---|---|---|---|---|---|
| Environment ($E$) | 4 | $2.960 \times 10^6$ | 554 | **** | Fixed |
| Sex (S) | 1 | $2.313 \times 10^4$ | 6.69 | ** | Fixed |
| $E \times S$ | 4 | 5004 | 2.14 | * | Fixed |
| Line ($L$) | 97 | 8379 | 1.30 | * | 9.787 |
| $L \times E$ | 388 | 5351 | 2.29 | **** | 75.23 |
| $L \times S$ | 97 | 3459 | 1.48 | *** | 10.47 |
| $L \times E \times S$ | 388 | 2338 | 0.99 | NS | −0.2680 |
| Replicate ($L \times E \times S$) | 979 | 2359 | 5.55 | **** | 195.1 |
| Error | 17,236 | 424.9 | | | 429.1 |

NS, $P > 0.05$; *$0.05 < P < 0.10$; **$0.01 < P < 0.05$; ***$0.001 < P < 0.01$; ****$P < 0.0001$.

significant GEI for life span among the five environments considered here (Table 2). Although the three-way genotype × sex × environment term was not significantly different from zero in the overall analysis, this term was highly significant in the pairwise control-starvation, heat shock-starvation and high temperature-starvation analysis (data not shown). Thus, the genetic variation in sexual dimorphism for life span is itself sensitive to environmental conditions. The significant genotype × sex and genotype × environment interaction variances observed on the natural scale are also significant in the analyses on ln(life span) and are thus not an artifact of scale (data not shown).

Measures of the importance of GSI and GEI are $r_{GS}$ and $r_{GE}$: the cross-sex and cross-environment genetic correlations, respectively. These correlations indicate the extent to which the same genes affect male and female life span within each environment, or the life span of males or females in different environments. Correlations approaching 0 indicate different constellations of genes affect the trait in the sexes or environments, and correlations approaching |1| indicate the same genes are responsible for variation in the trait (Falconer 1952) in males and females or treatments. The correlation in mean life span of RI lines with the same genotype across both sexes and all environments is $r_{GSE} = V_G/(V_G + V_{GSI} + V_{GEI})$, where $V_G$ is estimated by the among-line variance component and $V_{GSI}$ and $V_{GEI}$ are, respectively, the GSI and GEI variance components from the mixed-model ANOVA pooled over environments and sexes (Bulmer 1985). For the untransformed data, $r_{GSE} = 0.10$ (Table 2) and for the ln-transformed data, $r_{GSE} = 0.13$ (data not shown). All of the significant genetic variation in life span among these RI lines is tied up in the interaction terms!

We computed cross-sex genetic correlations and cross-environment genetic correlations for pairs of environments from variance components using the method of Robertson (1959). Approximate confidence limits for these correlations were estimated using Fisher's *z* transformation (Sokal and Rohlf 1981), which can be used to infer whether a given correlation is significantly different from zero or unity. The ANOVA results can also be used in this regard: cross-sex or cross-environment genetic correlations are significantly different from 1 if the GSI or GEI interaction terms from the pooled analysis are significant, and are significantly different from 0 if the among-line variance from the same analysis is significant. Estimates of $r_{GS}$ within each environment are presented in Table 1. The cross-sex genetic correlations in the control and heat-shock environments are not significantly different from 0 and are highly significantly different from 1, indicating that different QTL affect the life span of the two sexes in these environments. In the low temperature environment, the cross-sex genetic correlation is not significantly different from unity, suggesting that the same genes affect variation in life span in this environment. Intermediate situations are represented by the high temperature and starvation treatments, where the genetic correlations of life span between the sexes are significantly different from both 0 and 1. Cross-sex genetic correlations can depart from unity if there are changes in the rank order of line mean longevities in males and females (crossing of reaction norms) and if there are changes in the among-line variance of life span in the two sexes. In all cases for which the sex × line interaction is significantly different from 0, >90% of the interaction variance is attributable to changes in rank order of life span between males and females. Consistent with this analysis, estimates of cross-sex genetic correlations of ln-transformed data are very similar to the estimates obtained on the natural scale (not shown).

Estimates of $r_{GE}$ between pairs of environments and confidence limits of the estimates are given in Table 3. Cross-environment genetic correlations fall into four categories. The first group are high and positive; these are highly significantly different from zero and either not significantly different from unity or only nominally so. In this group are the control-high temperature and control-heat-shock environment pairs. Largely the same genes affect variation in life span in these environments. The second group has a single representative: the cross-environment genetic correlation between the high temperature and heat-shock environments is intermediate, and significantly different from both zero and one. Some common as well as different loci affect variation in life span in these environments. Most of the correlations fall into the third category: highly significantly different from one and not significantly different from zero. This group includes all correlations with the starvation environment and all male correlations with the low temperature environment. Completely different sets of loci affect longevity in these environment pairs. The fourth group is somewhat anomalous and includes the correlations of low temperature with the control, high temperature and heat-shock environments in females. These correlations are not significantly different from one or from zero. We regard these correlations as poor estimates; not unexpected given that the among-line variance component for females in the low temperature treatment was not formally significantly different from zero. The differences between cross-environment genetic correlations of ln-transformed compared to untransformed data are quantitative, not qualitative. Control, heat-shock, and high temperature treatments are more highly correlated with each other than with the low temperature treatment (particularly for males), and the correlation between starvation and the other treatments remains low (data not shown). Scale effects (changes in variance) are not the main cause of the observed departures of cross-environment genetic correlations from unity.

**Life span QTL:** Cross-sex and cross-environment ge-

**TABLE 3**

**Cross-environment genetic correlations (confidence limits) of life span in RI lines**

| | C | HS | HT | LT | S |
|---|---|---|---|---|---|
| C | | 1.04<br>(UN)<br>NS | 0.72<br>(0.61, 0.81)<br>* | 0.01<br>(−0.19, 0.20)<br>**** | −0.21<br>(−0.39, −0.01)<br>*** |
| HS | 0.72<br>(0.61, 0.81)<br>** | | 0.77<br>(0.67, 0.84)<br>*** | −0.08<br>(−0.28, 0.12)<br>**** | 0.09<br>(−0.11, 0.28)<br>**** |
| HT | 0.82<br>(0.75, 0.88)<br>* | 0.64<br>(0.51, 0.75)<br>**** | | −0.08<br>(−0.27, 0.11)<br>**** | 0.35<br>(0.16, 0.51)<br>**** |
| LT | 0.91<br>(0.84, 0.94)<br>NS | 0.81<br>(0.73, 0.87)<br>NS | 0.31<br>(0.12, 0.48)<br>* | | −0.28<br>(−0.45, −0.08)<br>**** |
| S | 0.05<br>(−0.15, 0.25)<br>**** | 0.08<br>(−0.12, 0.28)<br>**** | 0.15<br>(−0.05, 0.34)<br>**** | −0.23<br>(−0.41, −0.03)<br>** | |

Abbreviations for the five environments are given in the Table 1 footnote. Entries above the diagonal are for males and below the diagonal are for females. *P* values are from *F*-ratio tests of significance of the line × environment interaction term from ANOVAs for pairs of environments and indicate whether the cross-environment genetic correlations are significantly different from unity. UN, undefined; NS, $P > 0.05$; *$0.05 < P < 0.10$; **$0.01 < P < 0.05$; ***$0.0001 < P < 0.001$; ****$P < 0.0001$.

netic correlations that are significantly less than one could arise if some loci are conditionally expressed in different sex or treatment environments, or if the effects change sign across environments (a kind of antagonistic pleiotropy). To determine the extent to which loci contributing to GSI and GEI exhibit these properties, we mapped the QTL affecting adult longevity and GSI and GEI for adult longevity in this set of RI lines, using multiple trait composite interval mapping (Jiang and Zeng 1995). Several analyses were done. First, we mapped QTL affecting life span and GSI for life span within each treatment environment. The LOD thresholds for significance of the main QTL effect, the conditional test for GSI, and the genome-wide scan for GSI were 3.11, 1.44, and 2.60, respectively. Second, we mapped QTL affecting life span and GEI for life span within each sex across treatment environments. It was necessary to exclude the starvation treatment from these analyses: there were convergence problems, most likely because this trait was not correlated with the others. The LOD thresholds for significance of the main QTL effect, the conditional test for GEI, and the genome-wide scan for GEI were 3.99, 2.46, and 3.56, respectively. A third analysis was performed for females, including only the control, heat-shock, and high temperature treatments, because the genetic variance among lines in the low temperature treatment was not significant in females. The LOD score threshold for the main QTL effect in this analysis was 3.56 and for the genome-wide scan for GEI was 3.11.

All composite interval mapping analyses were done on life span and on ln(life span). The results of these analyses on the untransformed scale are summarized in Table 4 and are depicted graphically in Figures 1–6. We have presented the results of the analyses on the natural scale for several reasons: (1) the ln-transformation did not improve the fit of the line means to a normal distribution; (2) the genetic variance was greater, relative to the total variance, for the untransformed data, thus giving greater power to detect QTL; and (3) there were few differences between the two sets of analyses. These differences are also summarized below.

We mapped 29 QTL affecting life span in the separate analyses; however, several QTL map to the same location. Conservatively, we estimate that a total of 17 QTL affect variation in life span in these lines, and have named them *Ls1–Ls17* for the purposes of discussion and future reference. QTL detected in different analyses were considered to be the same if the map positions were the same or the interval in which the LOD score exceeded the threshold value overlapped, and the estimated effects were similar. Two QTL were not clearly the same or different based on these criteria. To be conservative, they were classified as being the same as others detected in the gene region, but with the suffix "*a*" to indicate the ambiguity. A brief description of each QTL follows.

*Ls1* has a female-specific main effect, and female-specific GEI considering the control, heat-shock, and high temperature environments. The GEI is partly attributable to conditional expression (there is a highly significant positive effect in the control environment) and antagonistic pleiotropy ( the effect in the high temperature environment is negative).

**TABLE 4**

**Life span QTL**

| Traits | Map position (cM) | | Map position (Cyto)[a] | | Peak LOD[b] | | Male effect[c] | | | | | Female effect | | | | | QTL name |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Range | Peak | Range | Peak | Total | QTL × *E* | C | HS | HT | LT | S | C | HS | HT | LT | S | |
| F, 3T | 1-0; 1-10 | 1-0 | 1B–3E | 1B–3E | 4.56 | 4.50 | 2.12 | 1.47 | 0.34 | 4.10 | 0.175 | 5.99 | 1.18 | −2.59 | 4.81 | 0.126 | *Ls1* |
| HT | 1-15; 1-22 | 1-15 | 3E–4F | 3E–4F | 3.92 | 3.90 | 0.55 | 1.99 | 2.13 | 9.76 | 0.117 | −0.95 | 0.10 | −3.99 | 6.76 | 0.133 | *Ls2* |
| S | 1-32; 1-33 | 1-33 | 3E–4F | 3E–4F | 3.41 | 0.11 | −1.35 | −1.10 | 1.16 | 2.52 | 0.381 | −3.03 | −1.24 | −3.05 | 0.55 | 0.349 | *Ls3* |
| F, 4T | 1-43; 1-69 | 1-54 | 6E–9A | 6E–7D | 7.09 | 6.08 | 0.19 | 1.30 | 1.64 | 0.28 | 0.020 | −12.65 | −3.67 | −2.99 | −5.64 | 0.101 | *Ls4* |
| C | 1-43; 1-85 | 1-54 | 6E–10D | 6E–7D | 6.43 | 3.90 | | | | | | | | | | | *Ls4* |
| C | 1-43; 1-85 | 1-66 | 6E–10D | 7E–9A | 5.41 | 4.90 | 4.87 | 3.28 | 0.20 | −3.80 | −0.009 | −8.86 | −5.05 | −1.93 | −1.84 | 0.055 | *Ls5* |
| M, 4T | 1-77; 1-92 | 1-85 | 9A–10D | 9A–10D | 4.90 | 4.85 | 8.03 | 4.80 | −0.46 | 2.68 | −0.139 | −3.52 | −3.29 | −1.07 | 9.71 | −0.105 | *Ls5* |
| F, 4T | 1-131; 1-134 | 1-133 | 17C–19A | 17C–19A | 4.71 | 4.69 | −5.39 | 2.39 | 3.61 | −1.04 | 0.157 | −11.31 | 11.27 | 2.65 | −6.66 | 0.128 | *Ls6* |
| HS | 2-100; 2-109 | 2-105 | 30D–33E | 30D–33E | 2.85 | 2.67 | −1.20 | −4.88 | 1.94 | 5.81 | 0.127 | 0.53 | 5.91 | 0.80 | −11.75 | −0.049 | *Ls7* |
| S | 2-112; 2-144 | 2-126 | 30D–38A | 30D–33E | 5.01 | 5.30 | −3.59 | −3.03 | 0.80 | 5.75 | 0.180 | 0.87 | 4.12 | −0.94 | −7.00 | −0.051 | *Ls7a* |
| M, 4T | 2-148; 2-150 | 2-149 | 38A–43A | 38E–43A | 4.38 | 4.38 | −7.30 | −5.82 | 1.19 | 5.70 | 0.355 | 5.77 | 1.89 | 4.13 | 4.99 | 0.232 | *Ls8* |
| C | 2-149 | 2-149 | 38E–43A | 38E–43A | 3.11 | 3.11 | | | | | | | | | | | *Ls8* |
| S | 2-148; 2-161 | 2-154 | 38A–48D | 43E–46C | 5.64 | 0.23 | −5.16 | −6.72 | 0.71 | −0.30 | 0.485 | 5.06 | 2.20 | 5.75 | 1.94 | 0.414 | *Ls8a* |
| S | 3-0; 3-36 | 3-12 | 57C–60E | 57F–60E | 9.36 | 0.65 | −1.64 | −0.97 | 1.23 | −3.00 | 0.466 | 3.94 | −0.46 | 1.89 | 4.29 | 0.434 | *Ls9* |
| HT | 4-17; 4-27 | 4-20 | 63A–65A | 63A–65A | 3.54 | 3.52 | 2.99 | 0.81 | −1.67 | −1.03 | −0.104 | 0.93 | 0.00 | 4.54 | 4.69 | −0.197 | *Ls10* |
| LT | 4-52; 4-84 | 4-68 | 65A–68B | 65D–67D | 6.98 | 0.13 | −0.75 | −4.66 | −0.47 | −23.70 | 0.132 | 4.01 | 0.89 | 0.53 | −27.86 | 0.047 | *Ls11* |
| F, 4T | 4-60; 4-78 | 4-68 | 65A–67D | 65D–67D | 5.65 | 5.51 | | | | | | | | | | | *Ls11* |
| M, 4T | 4-68; 4-75 | 4-71 | 65D–67D | 65D–67D | 3.87 | 3.79 | −0.71 | −4.89 | −0.44 | −23.56 | 0.133 | 4.19 | 0.54 | 0.46 | −27.49 | 0.051 | *Ls11* |
| C | 4-107; 4-122 | 4-114 | 68B–69D | 68B–68C | 4.79 | 1.48 | −2.83 | 2.05 | 0.68 | −5.05 | 0.058 | −9.11 | −0.16 | −4.19 | −10.80 | 0.057 | *Ls12* |
| HT | 4-111; 4-114 | 4-114 | 68B–68C | 68B–68C | 2.66 | 2.64 | | | | | | | | | | | *Ls12* |
| F, 4T | 4-112; 4-122 | 4-114 | 68B–69D | 68B–68C | 4.39 | 3.79 | | | | | | | | | | | *Ls12* |
| F, 4T | 4-125; 4-128 | 4-125 | 69D–70C | 69D–70C | 4.09 | 3.88 | 0.91 | 4.72 | 1.75 | −3.52 | 0.160 | −7.61 | 3.69 | −2.34 | −1.45 | 0.126 | *Ls13* |
| M, 4T | 4-133; 4-146 | 4-142 | 69D–71E | 69D–70C | 6.39 | 6.26 | 6.36 | 8.55 | 3.23 | −3.83 | 0.204 | −3.89 | 4.88 | −0.96 | 11.11 | 0.064 | *Ls14* |
| S | 4-143; 4-148 | 4-146 | 70C–72A | 70C–71E | 4.80 | 4.74 | 6.38 | 9.01 | 3.25 | −0.61 | 0.290 | −1.21 | 5.33 | 0.17 | 14.96 | 0.098 | *Ls14* |
| HS | 4-134; 4-146 | 4-146 | 69D–71E | 70C–71E | 4.44 | 0.53 | | | | | | | | | | | *Ls14* |
| F, 4T | 4-162; 4-163 | 4-162 | 76B–82D | 76B–77A | 4.10 | 4.06 | 10.51 | 2.41 | 1.49 | −2.47 | 0.120 | −5.86 | −1.10 | 4.33 | 4.59 | 0.183 | *Ls15* |
| C | 4-154; 4-172 | 4-163 | 73D–87B | 77A–82D | 4.84 | 4.72 | 10.39 | 2.13 | 1.34 | −2.92 | 0.135 | −5.41 | −0.76 | 4.12 | 3.43 | 0.188 | *Ls15* |
| F, 4T | 4-168; 4-173 | 4-171 | 82D–87B | 85F–87B | 4.34 | 4.05 | 6.91 | 1.00 | 0.45 | −0.70 | 0.091 | −5.89 | −4.38 | 1.72 | −5.75 | 0.128 | *Ls16* |
| HS | 4-274 | 4-274 | 96F–97D | 96F–97D | 3.22 | 2.21 | 2.31 | −0.32 | 1.47 | −1.78 | 0.180 | −4.17 | −8.39 | −3.95 | −7.08 | 0.216 | *Ls17* |

F, female; M, male; T, treatment. See Table 1 footnote for other abbreviations.

[a] The interval bracketing the peak LOD position is given. Where the estimated map position of the QTL is closer to one or the other cytological markers, this marker is indicated by underscoring.

[b] LOD scores underscored exceed the significance threshold (see text for further explanation).

[c] Effects are in days. Numbers underscored are significant for the treatment and sex indicated.

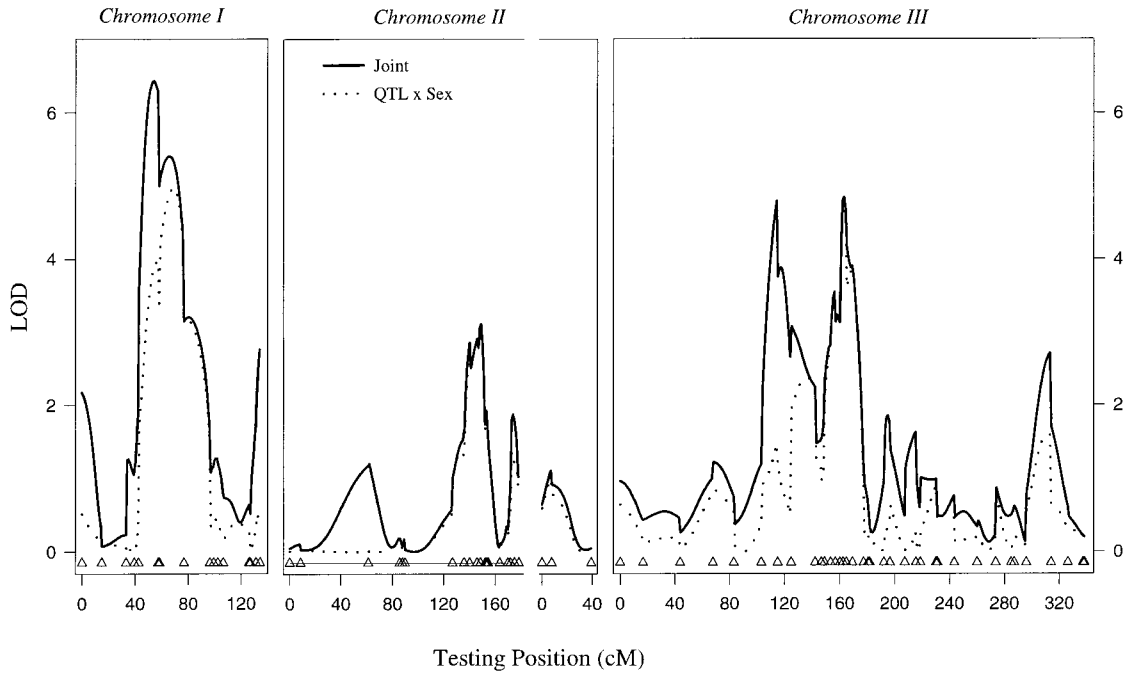


Figure 1.—Plot of LOD scores against estimated map position from multiple-trait composite interval mapping of life span in the control environment. The marker positions are indicated by triangles along the abscissa. Results are shown for the joint analysis pooled across sexes and the QTL × sex interaction.

*Ls2* is specific to the high temperature environment. The significant GSI effect is caused by antagonistic pleiotropy.

*Ls3* is a starvation-specific QTL that is expressed in both sexes.

*Ls4* is significant in both the control environment and in females over the four treatments. There is highly significant GSI and GEI due to conditional expression only in females in the control environment.

*Ls5* is significant in the control environment and in

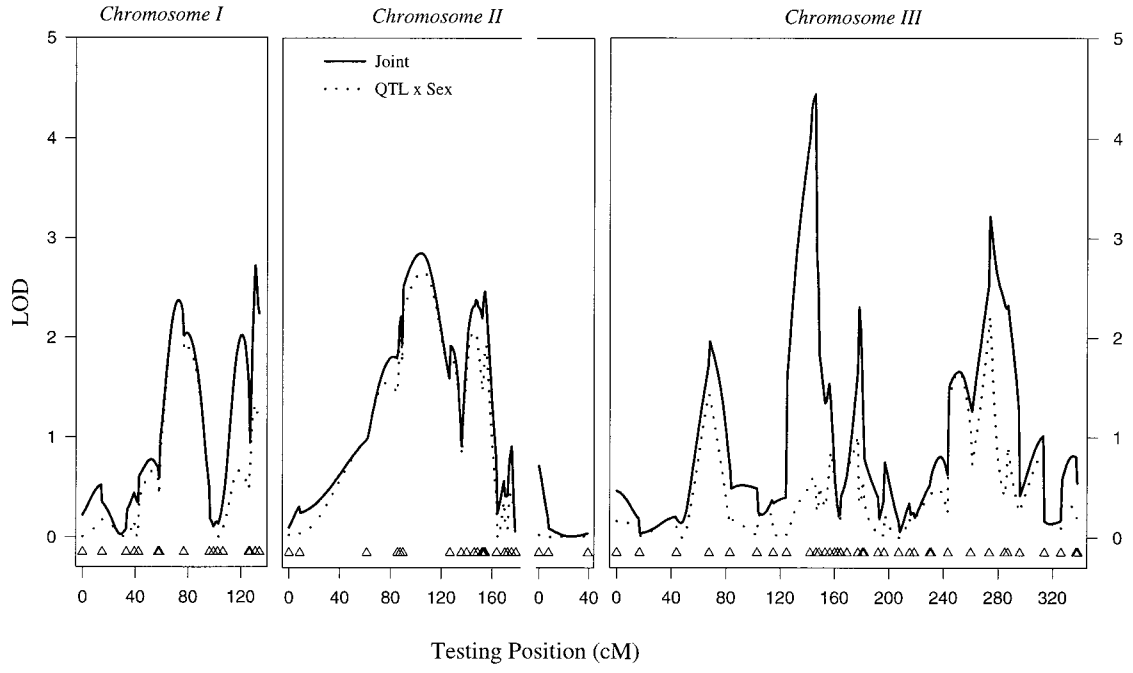


Figure 2.—Plot of LOD scores against estimated map position from multiple-trait composite interval mapping of life span in the heat-shock environment.

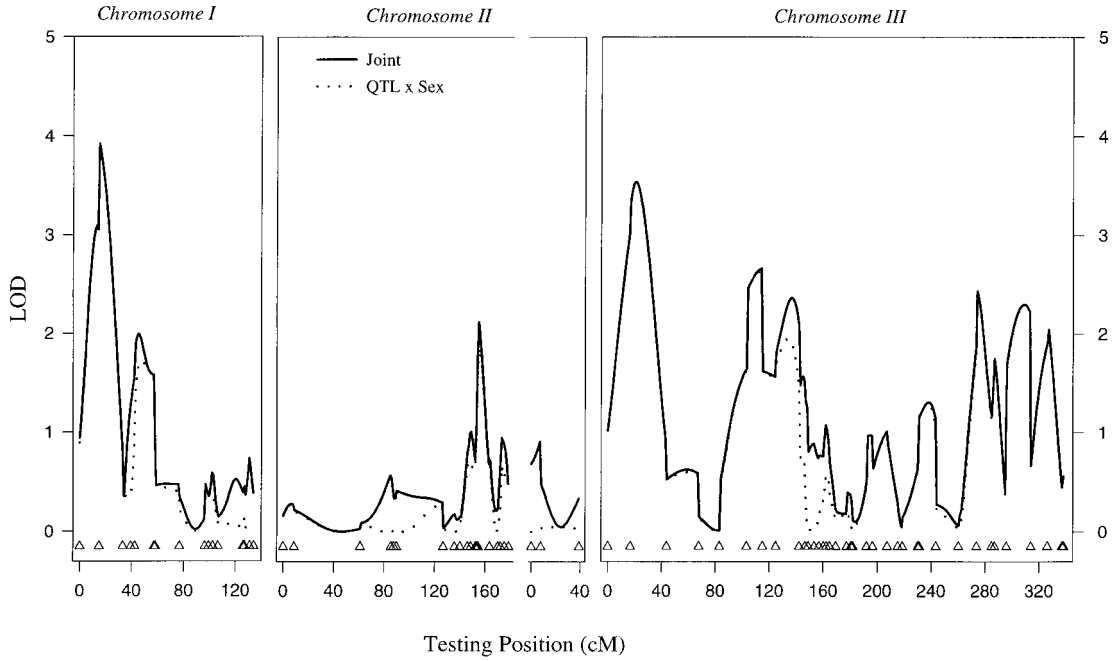


Figure 3.—Plot of LOD scores against estimated map position from multiple-trait composite interval mapping of life span in the high temperature environment.

males over the four treatments. The significant GSI effect is attributable to antagonistic pleiotropy, and the GEI effect is due to conditional expression in males of the control environment.

*Ls6* is a female-specific QTL with antagonistic pleiotropic effects in the control and heat-shock environments.

*Ls7* has opposite effects in males and females in the heat-shock environment. The GSI effect is significant at the level of a genome wide-scan, but the main QTL

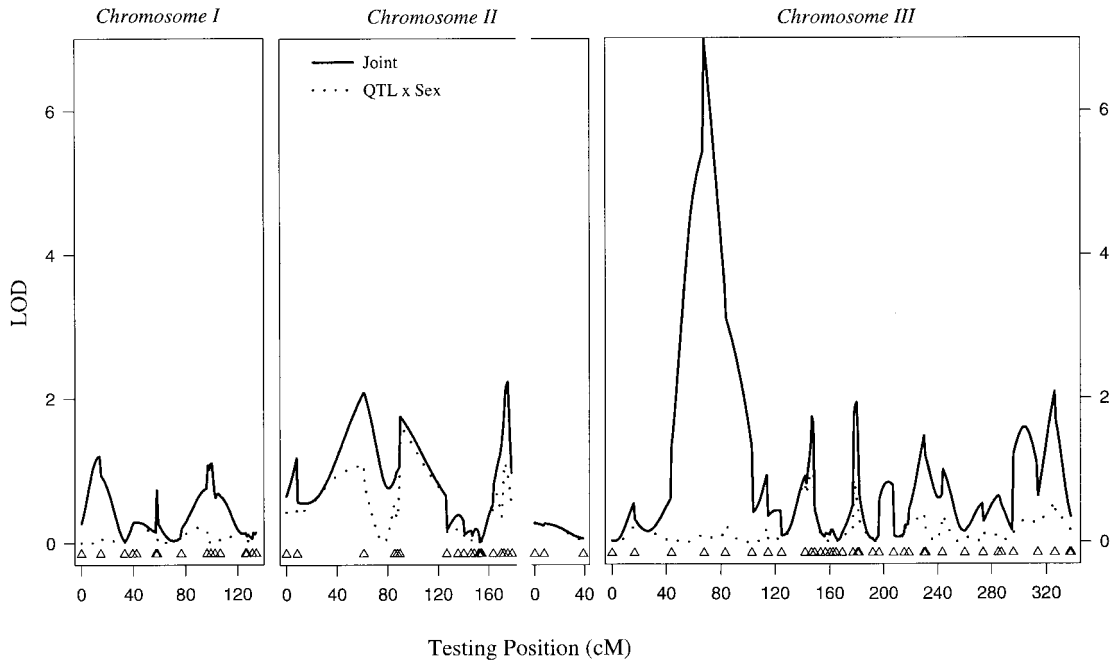


Figure 4.—Plot of LOD scores against estimated map position from multiple-trait composite interval mapping of life span in the low temperature environment.

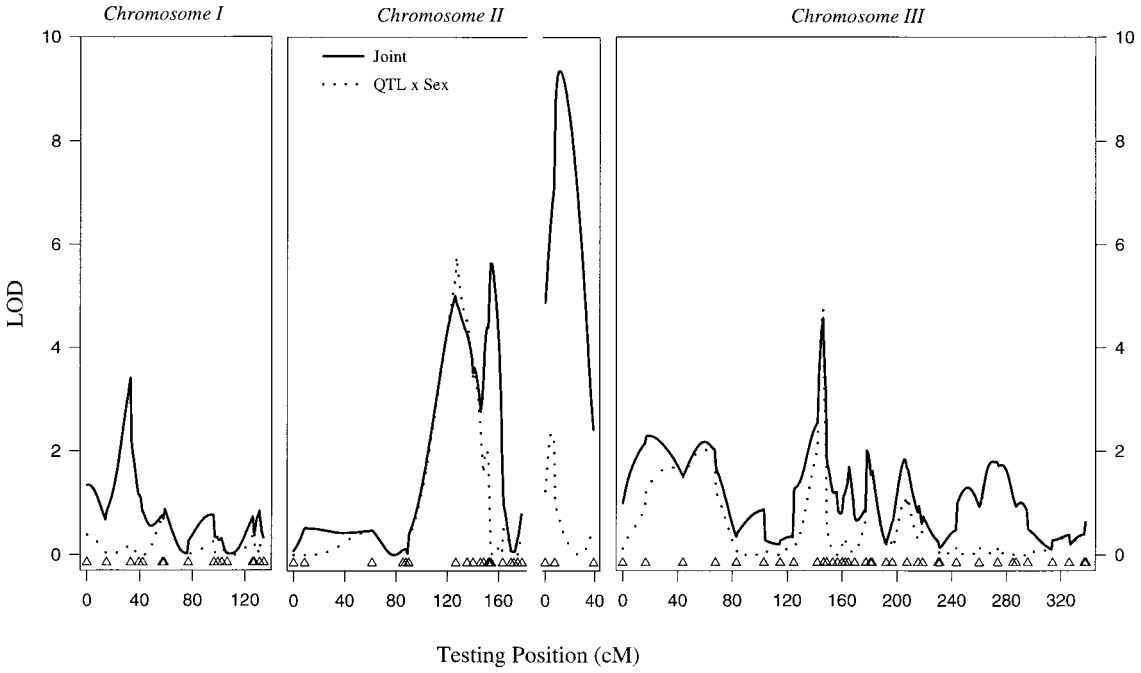


Figure 5.—Plot of LOD scores against estimated map position from multiple-trait composite interval mapping of life span in the starvation environment.

effect is not significant. *Ls7a*, which may be the same QTL, is significant in the starvation environment and has a significant GSI effect attributable to conditional expression in males. The effects in the heat-shock and starvation environments are of opposite sign.

*Ls8* is significant in males in the four environment analyses, and in the control environment alone. The significant GEI effect is due to the large conditional effect in the control (and to some extent, the heat-shock) environment. The significant GSI effect is because the effects change sign in males and females. *Ls8a* maps to the same region and may be the same QTL. It

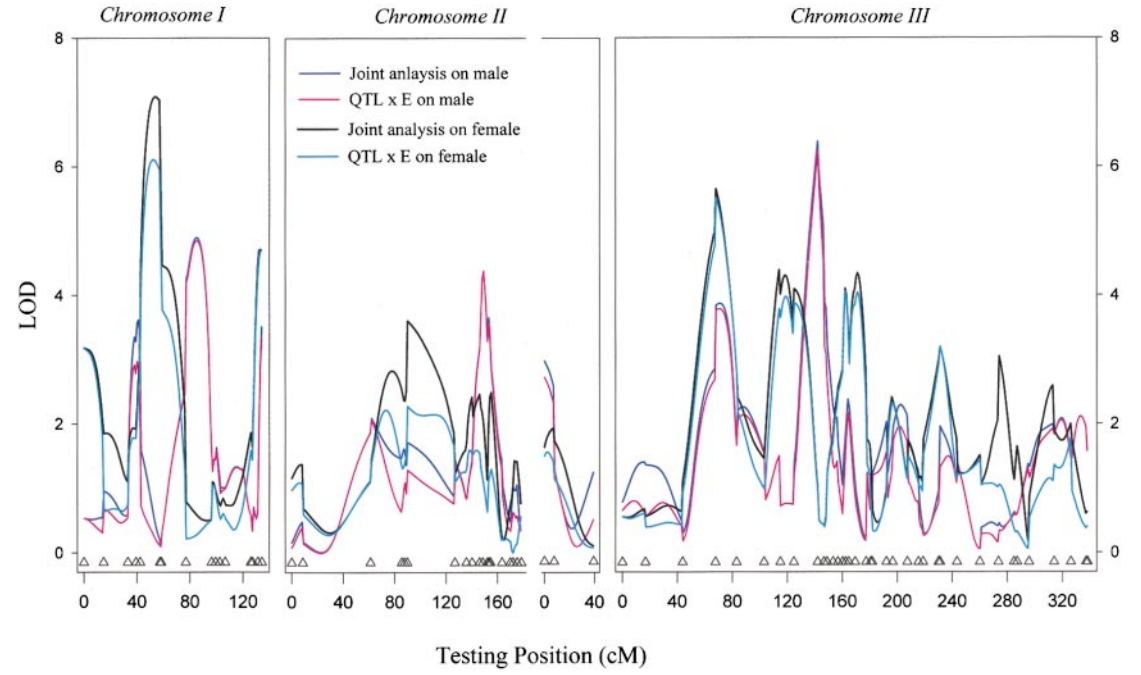


Figure 6.—Plot of LOD scores against estimated map position from multiple-trait composite interval mapping of male and female life span. The joint analysis considers the control, heat shock, high temperature, and low temperature environments. Total QTL and QTL × environment interaction LOD scores are shown.

is specific to the starvation environment; this effect is in the opposite direction to the control and heat-shock effects in males.

*Ls9* is a QTL with a very large effect in the starvation treatment only, expressed in both sexes.

*Ls10* has a main and a GSI effect in the high temperature environment. This QTL has opposite effects in males and females.

*Ls11* is expressed in both sexes in the low temperature environment. This large-effect QTL contributes to the main and GEI effect in the female four-treatment analysis, and a GEI effect in the male four-treatment analysis. The GEI effects are attributable to the conditional expression in a single environment.

*Ls12* has a large female-specific effect in the control environment, contributing to conditional GSI and conditional GEI in the four-treatment analysis. It also has a significant GSI effect in the high temperature environment, also due to conditional expression in females.

*Ls13* is a female-specific QTL with GEI in the four-treatment analysis. The effects of this QTL change sign between the control and high temperature environments.

*Ls14* is a male-specific QTL in the four-treatment analysis, with significant GEI due to conditional expression in the control and heat-shock environments. The heat-shock effect is also significant pooled over both sexes. This QTL also has a male-specific effect in the starvation treatment.

*Ls15* is significant in the female four-treatment analysis and in the control. It contributes to GEI by antagonistic pleiotropic effects in the control and high temperature environments, and to GSI through sexually antagonistic effects in the control environment.

*Ls16* is a female-specific QTL with GEI due to conditional expression in the control and heat-shock environments.

*Ls17* is a heat-shock-specific QTL with GSI due to conditional expression in females.

We detected 27 QTL mapping to 18 locations in the analyses of ln(life span). Twenty-one QTL mapping to 14 separate locations were the same in the two analyses. Of the 21 QTL common to both analyses, 17 exhibited the same QTL × environment interactions on both scales. The major differences between the two analyses were as follows:

1. Three highly significant QTL were detected in the analysis of the untransformed data that were not detected in the analysis of ln-transformed data: *Ls6*, *Ls10*, and *Ls13*.
2. The QTL detected between markers 38E–43A in the analysis of the untransformed control data appear between markers 34EF–35B in the analysis of the ln-transformed data. Such shifts in inferred QTL location are common using composite interval mapping analysis because the inferred locations are sensitive to the marker cofactors used.
3. Although QTL mapping to the locations of *Ls12* and *Ls15* were detected in both sets of analyses, the exact analyses in which the QTL were significant were not the same. The *Ls12* QTL detected in the C and female four-treatment (F, 4T) analyses of untransformed data were not detected in the ln-transformed data, and the *Ls15* QTL detected in the F, 4T analysis of untransformed data was not detected in the ln-transformed data. All remaining differences between the two analyses were attributable to appearance and disappearance in one or the other analysis of QTL significant for the interaction effect only; these could be false-positive results.

## DISCUSSION

We have examined the genetic architecture of Drosophila life span by estimating quantitative genetic parameters and mapping QTL for adult life span in a population of RI lines reared in five environments—standard culture conditions, high and low temperature, and heat-shock and starvation stress. Although there was highly significant genetic variation for life span among the RI lines within each sex and environment (with the exception of the female, low temperature analysis on the natural scale), the only significant genetic variation in the analysis pooled over all environments and the two sexes appeared in the GSI and GEI interaction terms. The genetic correlation of life span across sexes and environments was not significantly different from zero.

The expression of QTL affecting longevity in Drosophila is thus highly sensitive to environmental conditions. No QTL were expressed in all environments. Two QTL, *Ls4* and *Ls17*, were conditionally expressed in only one sex and environment. Four sex-specific QTL (*Ls1*, *Ls6*, *Ls13*, and *Ls16*) were expressed in two environments, and one sex-specific QTL (*Ls14*) was expressed in three environments. *Ls1*, *Ls6*, and *Ls13* exhibited antagonistic pleiotropic effects in the different environments, whereas the effects of *Ls14* and *Ls16* were in the same direction in the different treatments. Five QTL affected both sexes in only one environment: *Ls2* and *Ls10* had sexually antagonistic effects, and *Ls3*, *Ls9*, and *Ls11* had similar effects in both sexes and did not contribute to GSI. The remaining five QTL, *Ls5*, *Ls7*, *Ls8*, *Ls12*, and *Ls15*, had more complicated patterns of expression in both sexes and two or more environments. Each of these QTL had sexually antagonistic effects in one environment. In addition, *Ls5* and *Ls12* had sex-specific effects in the same direction in a second environment; and *Ls7*, *Ls8*, and *Ls15* had antagonistic pleiotropic effects in two or more environments that were expressed in only one sex.

Nuzhdin *et al.* (1997) mapped QTL for adult longevity using the same set of RI lines. In this experiment, animals were housed individually in small tubes rather than in groups of 10 in standard culture vials; otherwise the environment was equivalent to the control conditions reported here. Five sex-specific QTL were detected, four of which correspond in location to *Ls7*, *Ls8*, *Ls11*, and *Ls15*.

The use of RI lines is the best experimental design for investigating the genetic basis of variation for traits whose phenotypic expression is highly sensitive to uncontrollable environmental variation, such as life history traits (Soller and Beckmann 1990), and is also the optimal method for evaluating QTL × environment interactions. However, like all QTL mapping methods, these analyses are of necessity restricted to assessing the nature of the genetic variation that segregates between the two parental strains, and results from a single experiment beg the question of generality. Will the same QTL be found to segregate between different parental strains? To what extent are sex- and environment-specific effects of longevity QTL a peculiarity of the genetic constitution of the two parental lines used to establish the RI line mapping population studied here? These questions can only be addressed by extensive replication of the experimental design using different starting material or by introgressing gene regions containing the life span QTL from a large natural sample of chromosomes into a common genetic background (Lyman and Mackay 1998). If the same QTL are found in many parental lines, one would infer that alleles at these loci are at intermediate frequency in the natural population from which the strains were sampled; if not, a rare alleles model (Barton and Turelli 1989) of segregating variation is supported. There is some evidence that extensive GSI and GEI are general properties of longevity QTL. Maynard Smith (1958) undertook a quantitative genetic analysis of longevity in *D. subobscura*, and found that genes with sex-limited effects were responsible for variation in life span within and between populations of this species, and that the sex differences were temperature-dependent. Highly environment-specific effects of QTL affecting longevity pose a practical problem for the genetic dissection of this trait, for it is clearly critical to evaluate life span in a wide range of environments to obtain a complete picture of the factors affecting variation in this trait.

The common occurrence of QTL with opposite effects in males and females, and between environments, immediately suggests that genetic variation for life span could be maintained by the balancing selection mechanism proposed by Levene (1953), whereby the fitness effects of two alleles at a single locus change rank in alternate environments. Although the original formulation of the antagonistic pleiotropy hypothesis for aging was in terms of negative genetic correlations between fitness effects of genes expressed early and late in life (*i.e.*, negative correlations between alleles affecting life span and other life history traits), this hypothesis can be readily extended to opposite effects of alleles affecting life span in males and females or in different environments. These observations thus provide support for Williams' (1957) pleiotropy theory of senescence. An important caveat regarding the generality of inferences drawn from analyses of life history traits in RI lines is that these are nearly totally homozygous genotypes, a contrived genetic situation that does not exist in natural populations of outbreeding species like Drosophila. It is critical in the future to determine homozygous and heterozygous effects of QTL affecting life span, and QTL × environment interaction effects, in a largely heterozygous background genotype. However, if homozygous genotypes exhibit more GSI and GEI than heterozygotes, genetic variation would still be maintained at these loci via the mechanism proposed by Gillespie and Turelli (1989).

It is interesting to note that opposing QTL effects in males and females and across environments did not lead to significantly negative estimates of genetic correlations between the sexes or environments, although many of the correlations were not significantly different from zero. The genetic correlations represent the summation of effects over all contributing loci and can conceal considerable heterogeneity of individual QTL properties. Such heterogeneity can confound efforts to dissect physiological and genetic mechanisms responsible for variation in longevity based on correlated responses to selection for postponed senescence and may provide a partial explanation for the variable results obtained in different experiments (in addition to real genetic differences among the starting base populations). The long-lived lines of Rose (1984) had decreased early fertility and increased resistance to starvation and desiccation stress relative to the controls (Service *et al.* 1988). However, lines selected for postponed senescence by Partridge and Fowler (1992) did not differ in early female fertility from the control lines, and the long-lived line of Luckinbill *et al.* (1984) exhibited decreased early fertility and no difference in resistance to starvation and desiccation relative to the unselected line (Arking 1987; Force *et al.* 1995). These conflicting results can only be resolved by analyses at the level of individual QTL. For example, the genetic correlation between starvation resistance and life span in the other environments in the population of RI lines studied here was not significantly different from zero, and most QTL affecting variation in starvation resistance were correspondingly specific to this treatment. However, one QTL, *Ls14*, exhibited a positive correlation between starvation resistance and life span in the control and heat-shock environments.

Based on the results reported here, different environmental conditions might be expected to yield variable correlated responses to selection, given that the effects

of the same genotype on life span will vary according to the environment. Indeed, Buck *et al.* (1993) report that their long-lived phenotype is conditional on high larval density, and LeRoi *et al.* (1994) note that the genetic correlation between life span and early fecundity in Rose's long-lived and control lines can vary according to the conditions under which the phenotypes are assessed.

Many candidate genes affecting life span have been postulated: genes involved in resistance to heat shock and oxidative stress, DNA repair, cellular aging, metabolic energy storage, and loci with sex-specific effects on fertility and reproduction. With so many candidate genes and such a large fraction of the genome covered by QTL affecting life span, it is expected that many candidate gene and QTL map positions will overlap by chance. While fine-scale mapping is necessary before positional candidates can be proposed for further analysis, it is nevertheless interesting to note that life span QTL map to the same locations as genes encoding enzymes of carbohydrate metabolism (*Phosphogluconate dehydrogenase*, *Ls1*; *Phosphoglucose isomerase*, *Ls8a*); oxidative and heat-shock stress (*Sod* and the *small heat shock proteins*, *Ls11*; *Heat shock protein 70A*; *Ls16*); sex determination (*Sex-lethal*, *Ls4*; *sisterless-a*, *Ls5*); and sex-specific peptides (*accessory gland proteins B* (*AcpB*), *Ls7*; *AcpK*, *Ls8*).

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## LITERATURE CITED

Arking, R., 1987 Successful selection for increased longevity in *Drosophila*: analysis of the survival data and presentation of a hypothesis on the genetic regulation of longevity. Exp. Gerontol. **22:** 199–220.

Barton, N. H., and M. Turelli, 1989 Evolutionary quantitative genetics: how little do we know? Annu. Rev. Genet. **23:** 337–370.

Bodnar, A. G., M. Ouellette, M. Frolkis, S. E. Holt, C.-P. Chiu *et al.*, 1998 Extension of life-span by introduction of telomerase into normal human cells. Science **279:** 349–352.

Buck, S., M. Nicholson, S. Dudas, R. Wells, A. Force *et al.*, 1993 Larval regulation of adult longevity in a genetically-selected long-lived strain of *Drosophila*. Heredity **71:** 23–32.

Bulmer, M. G., 1985 *The Mathematical Theory of Quantitative Genetics*. Clarendon Press, Oxford.

Burton, V., H. K. Mitchell, P. Young and N. S. Petersen, 1988 Heat shock protection against cold stress of *Drosophila melanogaster*. Mol. Cell. Biol. **8:** 3550–3552.

Charlesworth, B., 1980 *Evolution in Age-Structured Populations*. Cambridge University Press, London.

Deckert-Cruz, D. J., R. H. Tyler, J. E. Landmesser and M. R. Rose, 1997 Allozymic differentiation in response to laboratory demographic selection of *Drosophila melanogaster*. Evolution **51:** 865–872.

Engström, G., L.-E. Liljedahl, M. Rasmuson and T. Björklund, 1989 Expression of genetic and environmental variation during ageing. 1. Estimation of variance components for number of adult offspring in *Drosophila melanogaster*. Theor. Appl. Genet. **77:** 119–122.

Engström, G., L.-E. Liljedahl and T. Björklund, 1992 Expression of genetic and environmental variation during ageing. 2. Selection for increased life span in *Drosophila melanogaster*. Theor. Appl. Genet. **85:** 26–32.

Falconer, D. S., 1952 The problem of environment and selection. Am. Nat. **86:** 293–298.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics*, Ed. 4. Longman, Harlow, Essex, United Kingdom.

Finch, C. E., 1990 *Longevity, Senescence and the Genome*. University of Chicago Press, Chicago.

Finch, C. E., and R. E. Tanzi, 1997 Genetics of aging. Science **278:** 407–411.

Force, A. G., T. Staples, S. Soliman and R. Arking, 1995 Comparative biochemical and stress analysis of genetically selected *Drosophila* strains with different longevities. Dev. Genet. **17:** 340–351.

Gillespie, J. H., and M. Turelli, 1989 Genotype-environment interactions and the maintenance of polygenic variation. Genetics **121:** 129–138.

Graves, J. L., E. C. Toolson, C. Jeong, L. N. Vu and M. R. Rose, 1992 Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. Physiol. Zool. **65:** 268–286.

Gurganus, M. C., J. D. Fry, S. V. Nuzhdin, E. G. Pasyukova, R. F. Lyman *et al.*, 1998 Genotype-environment interaction for quantitative trait loci affecting sensory bristle number in *Drosophila melanogaster*. Genetics **149:** 1883–1898.

Harrison, D. E., 1990 *Genetic Effects on Aging II*. The Telford Press, Caldwell, NJ.

Harrison, D. E., and J. R. Archer, 1987 Genetic differences in effects of food restriction on aging in mice. J. Nutr. **117:** 376–382.

Hekimi, S., B. Lakowski, T. M. Barnes and J. J. Ewbank, 1998 Molecular genetics of life span in *C. elegans*: how much does it teach us? Trends Genet. **14:** 14–19.

Houle, D., 1992 Comparing evolvability and variability of quantitative traits. Genetics **130:** 195–204.

Houle, D., B. Morikawa and M. Lynch, 1996 Comparing mutational variabilities. Genetics **143:** 1467–1483.

Hughes, K. A., 1995 The evolutionary genetics of male life-history characters in *Drosophila melanogaster*. Evolution **49:** 521–537.

Hughes, K. A., and B. Charlesworth, 1994 A genetic analysis of senescence in *Drosophila*. Nature **367:** 64–66.

Jiang, C., and Z.-B. Zeng, 1995 Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics **140:** 1111–1127.

Khazaeli, A. A., M. Tatar, S. D. Pletcher and J. W. Curtsinger, 1997 Heat-induced longevity extension in Drosophila. I. Heat treatment, mortality, and thermotolerance. J. Gerontol. **52:** B48–B52.

Kimura, K. D., H. A. Tissenbaum, Y. Liu and G. Ruvkun, 1997 *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science **277:** 942–946.

Kirkwood, T. B. L., and M. R. Rose, 1991 Evolution of senescence: late survival sacrificed for reproduction. Philos. Trans. R. Soc. Lond. B Biol. Sci. **332:** 15–24.

Lai, C., R. F. Lyman, A. D. Long, C. H. Langley and T. F. C. Mackay, 1994 Naturally occurring variation in bristle number and DNA polymorphisms at the *scabrous* locus of *Drosophila melanogaster*. Science **266:** 1697–1702.

LeRoi, A. M., A. K. Chippendale and M. R. Rose, 1994 Long-term laboratory evolution of a genetic life-history trade-off in *Drosophila melanogaster*. I. The role of genotype-by-environment interaction. Evolution **48:** 1244–1257.

Levene, H., 1953 Genetic equilibrium when more than one ecological niche is available. Am. Nat. **87:** 331–333.

Lin, K., J. B. Dorman, A. Rodan and C. Kenyon, 1997 *daf-16*: an HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. Science **278:** 1319–1322.

Lin, Y.-J., L. Seroude and S. Benzer, 1998 Extended life-span and stress resistance in the *Drosophila* mutant *methuselah*. Science **282:** 943–946.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of* Drosophila melanogaster. Academic Press, San Diego.

Lithgow, G. J., 1996 Invertebrate gerontology: the age of mutations of *Caenorhabditis elegans*. BioEssays **18:** 809–815.

Lithgow, G. J., T. M. White, S. Melov and T. E. Johnson, 1995 Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc. Natl. Acad. Sci. USA **92:** 7540–7544.

Long, A. D., R. F. Lyman, C. H. Langley and T. F. C. Mackay, 1998 Two sites in the *Delta* gene contribute to naturally occurring variation in bristle number in *Drosophila melanogaster*. Genetics **149:** 999–1017.

Luckinbill, L. S., M. J. Arking, M. J. Clare, W. C. Cirocco and S. Buck, 1984 Selection for delayed senescence in *Drosophila melanogaster*. Evolution **38:** 996–1003.

Lyman, R. F., and T. F. C. Mackay, 1998 Candidate quantitative trait loci and naturally occurring variation for bristle number in *Drosophila melanogaster*: the *Delta-Hairless* gene region. Genetics **149:** 983–998.

Lyman, R. F., F. Lawrence, S. V. Nuzhdin and T. F. C. Mackay, 1996 Effects of single *P* element insertions on bristle number and viability in *Drosophila melanogaster*. Genetics **143:** 277–292.

Mackay, T. F. C., and C. H. Langley, 1990 Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. Nature **348:** 64–66.

Martin, L. G., and S. H. Preston, 1994 *The Demography of Aging*. National Academy Press, Washington, DC.

Maynard Smith, J., 1958 Sex limited inheritance of longevity in *Drosophila subobscura*. J. Genet. **56:** 227–235.

Medawar, P. B., 1952 *An Unsolved Problem of Biology*. H. K. Lewis, London.

McClearn, G. E., B. Johansson, S. Berg, N. L. Pederson, F. Ahern *et al.*, 1997 Substantial genetic influence on cognitive abilities in twins 80 or more years old. Science **276:** 1560–1563.

Morris, J. Z., H. A. Tissenbaum and G. Ruvkun, 1996 A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature **382:** 536–539.

Niedzwiecki, A., A. M. Kongpachith and J. E. Fleming, 1991 Aging affects expression of 70-kDa heat shock proteins in *Drosophila*. J. Biol. Chem. **266:** 9332–9338.

Nuzhdin, S. V., E. G. Pasyukova, C. L. Dilda, Z.-B. Zeng and T. F. C. Mackay, 1997 Sex-specific quantitative trait loci affecting longevity. Proc. Natl. Acad. Sci. USA **94:** 9734–9739.

Orr, W. C., and R. C. Sohal, 1994 Extension of life span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science **263:** 1128–1130.

Partridge, L., and K. Fowler, 1992 Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution **46:** 76–91.

Pasyukova, E. G., and S. V. Nuzhdin, 1993 *Doc* and *copia* instability in an isogenic *Drosophila melanogaster* stock. Mol. Gen. Genet. **240:** 302–306.

Promislow, D. E. L., M. Tatar, A. A. Khazaeli and J. W. Curtsinger, 1996 Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. Genetics **143:** 839–848.

Robertson, A., 1959 The sampling variance of the genetic correlation coefficient. Biometrics **15:** 469–485.

Roper, C., P. Pignatelli and L. Partridge, 1993 Evolutionary effects of selection on age at reproduction in larval and adult *Drosophila melanogaster*. Evolution **47:** 445–455.

Rose, M. R., 1984 Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution **38:** 1004–1010.

Rose, M. R., and B. Charlesworth, 1980 A test of evolutionary theories of senescence. Nature **287:** 141–142.

Rose, M. R., and B. Charlesworth, 1981a Genetics of life-history in *Drosophila melanogaster*. I. Sib analysis of adult females. Genetics **97:** 173–186.

Rose, M. R., and B. Charlesworth, 1981b Genetics of life-history in *Drosophila melanogaster*. II. Exploratory selection experiments. Genetics **97:** 187–196.

Rowe, J. W., 1997 The new gerontology. Science **278:** 367.

SAS Institute, 1988 *SAS/STAT User's Guide, Release 6.03 Edition*. SAS Institute, Cary, NC.

Service, P. M., 1987 Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. Physiol. Zool. **60:** 321–326.

Service, P. M., 1993 Laboratory evolution of longevity and reproductive fitness components in male fruit flies: mating ability. Evolution **47:** 387–399.

Service, P. M., E. W. Hutchinson and M. R. Rose, 1988 Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. Evolution **42:** 708–716.

Sokal, R. R., and F. J. Rohlf, 1981 *Biometry*, Ed. 2. W. H. Freeman, New York.

Soller, M., and J. S. Beckmann, 1990 Marker-based mapping of quantitative trait loci using replicated progenies. Theor. Appl. Genet. **80:** 205–208.

Stearns, S. C., and M. Kaiser, 1993 The effects of enhanced expression of elongation factor EF-1α on life span in *Drosophila melanogaster*. Genetica **91:** 167–182.

Tatar, M., D. E. L. Promislow, A. A. Khazaeli and J. W. Curtsinger, 1996 Age-specific patterns of genetic variance in *Drosophila melanogaster*. II. Fecundity and its genetic covariance with age-specific mortality. Genetics **143:** 849–858.

Tower, J., 1996 Aging mechanisms in fruit flies. BioEssays **18:** 799–807.

Williams, G. C., 1957 Pleiotropy, natural selection, and the evolution of senescence. Evolution **11:** 398–411.

Woodhead, A. D., A. D. Blackett and A. Hollaender, 1985 *Molecular Biology of Aging*. Plenum Press, New York.

Yu, C.-E., Y. H. Oshima, E. M. Fu, F. Wijsman, R. Hisama *et al.*, 1996 Positional cloning of the Werner's Syndrome gene. Science **272:** 258–262.

Zeng, Z.-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

Zwaan, B., R. Bijlsma and R. F. Hoekstra, 1995 Direct selection on life span in *Drosophila melanogaster*. Evolution **49:** 649–659.

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