

Quantitative Trait Loci Affecting Survival and Fertility-Related Traits in *Caenorhabditis elegans* Show Genotype-Environment Interactions, Pleiotropy and Epistasis

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ABSTRACT

We have identified, using composite interval mapping, quantitative trait loci (QTL) affecting a variety of life history traits (LHTs) in the nematode *Caenorhabditis elegans*. Using recombinant inbred strains assayed on the surface of agar plates, we found QTL for survival, early fertility, age of onset of sexual maturity, and population growth rate. There was no overall correlation between survival on solid media and previous measures of survival in liquid media. Of the four survival QTL found in these two environments, two have genotype-environment interactions (GEIs). Epistatic interactions between markers were detected for four traits. A multiple regression approach was used to determine which single markers and epistatic interactions best explained the phenotypic variance for each trait. The amount of phenotypic variance accounted for by genetic effects ranged from 13% (for internal hatching) to 46% (for population growth). Epistatic effects accounted for 9–11% of the phenotypic variance for three traits. Two regions containing QTL that affected more than one fertility-related trait were found. This study serves as an example of the power of QTL mapping for dissecting the genetic architecture of a suite of LHTs and indicates the potential importance of environment and GEIs in the evolution of this architecture.

LIFE history theory suggests that the evolution of genes influencing aging is strongly dependent on, and in fact may be largely an accidental by-product of, selection of other life history traits (LHTs). Such traits include age of sexual maturity and fertility (for reviews, see Rose 1991; Roff 1992; Stearns 1992; Martin *et al.* 1996). The specific environment(s) in which a population evolves will determine the selective pressures on LHTs; genes that experience little or no selective pressure in the evolutionary environment may have strong genetic effects in a new environment. Alternatively, there may be trade-offs [genotype-environment interactions (GEIs)] that evolved due to the need to adapt to two environments, such that one allele is selectively favored in one environment, while a different allele is favored in a second environment (see Via and Lande 1987; Gillespie and Turelli 1989 for reviews). Thus variations in environment, past or present, allow alleles that limit survival in some environments to accumulate in the population.

Selection favors alleles that allow the individual to survive long enough to reproduce competitively, as determined by other LHTs. The absence of significant

selective pressure for the continued survival of the organism after reproduction allows mutations that increase the probability of death beyond this age to accumulate in the population. Alternatively, genes specifying one LHT may act pleiotropically on another LHT, such that selection of the first LHT can result in changes in the second, confounding or enhancing the effects of selection on that gene. Genes for a given LHT may also interact epistatically, confounding or enhancing the effects of selection for that trait on a given gene. Thus, an understanding of a species' LHTs, the effects of LHT genes in different environments, and the genetic architecture of these LHTs helps to clarify the evolutionary constraints limiting species survival and longevity.

Previous approaches to these issues have focused on genome-wide assessments. The existence of GEIs for fitness and the basis for their maintenance has been studied by comparing genetically distinct populations across environments (see Fry *et al.* 1996, and references therein). Evidence for pleiotropy among LHTs has been sought, either by comparing the responses of one LHT to artificial selection on another (*e.g.*, Rose and Charlesworth 1981a,b; Luckinbill *et al.* 1984; Zwaan *et al.* 1995) or by comparing species that have evolved in different environments with presumed different selection regimes (*e.g.*, Austad 1993; Keller and Genoud 1997).

The approach taken here, quantitative trait locus (QTL) mapping, in the nematode *Caenorhabditis elegans*,

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allows us to address these issues at individual genetic loci. *C. elegans* is a useful model system for studying LHTs, and aging in particular, due to its short generation time and life span, small size, and well-characterized genetics. We use QTL mapping strategies (Lander and Botstein 1989; Haley and Knott 1992; Tanksley 1993; Zeng 1993, 1994) to map loci showing allelic variation for LHTs within our test population, a series of recombinant inbred (RI) strains generated from two "wild-type" strains [Bristol (N2) and Bergerac (BO)]. Loci affecting LHTs can be identified and their GEIs or pleiotropic effects at single loci, and their epistatic interactions between loci, can be analyzed. These loci are good starting points for identifying genes important in the specification of LHTs. Each QTL represents a large genetic region containing many genes, such that GEIs, pleiotropic effects, or epistatic interactions involving specific genes must be established by further genetic characterization.

Three previous studies reported mapping QTL for survival in *C. elegans*. All of these studies examined survival in liquid media; the first two examined individual worms at the F₆ generation from N2-Bristol by Bergerac (Ebert *et al.* 1993) or N2-Bristol by DH424 (Ebert *et al.* 1996) crosses. A third study (Shook *et al.* 1996) used N2-Bristol by Bergerac (BO) RI strains, also used in the current study, and found QTL for survival in liquid media and for hermaphrodite self-fertility on solid media as well as QTL for internal hatching (bagging). Nuzhdin *et al.* (1997) have also used QTL mapping methods to find loci affecting longevity in *Drosophila*.

This study extends the previous results in *C. elegans* by localizing QTL for a suite of survival and fertility-related traits, all on solid media. This allows a comparison of our results for survival-related traits with our results for fertility-related traits in the same environment. This is important, because, as argued above, genes may have different effects in different environments, and any attempt to understand the genetic architecture of LHTs (and any pleiotropic interactions in particular) requires assessment in a common environment. We map the survival-related traits—life span and internal hatching—and the fertility-related traits—total fertility, fraction of fertility accrued at early ages, age at sexual maturity, and the Malthusian rate of population growth. Hodgkin and Barnes (1991) demonstrated that the onset or amount of early fertility can be a more important criterion for population growth (a fundamental measure of fitness) than total fertility. We have therefore assessed all these traits and consider the correlations among them, in addition to mapping QTL for each.

MATERIALS AND METHODS

General methods, media, and strains: Both survival and fertility assays were performed on NGM agar plates (Sulston and Hodgkin 1988) spotted with OP50. We examined 81 RI strains derived from two wild-type *C. elegans* varieties: Bristol

(N2) and Bergerac (BO) as described in Johnson and Wood (1982) and Shook *et al.* (1996). Stocks were maintained at 20°, and all manipulations were done in a 20° constant temperature room. Strains were axenized (made free of bacterial and fungal contamination; Emmons and Yesner 1984) and analyzed at least two generations postaxenization. Strains found to have become contaminated during the course of any phenotypic assay were not included in the results of that assay.

Survival assay: Life span ("survival") was assessed using one cohort for each strain by picking 25 third-larval-stage (L3) worms at random from age-synchronous populations laid during a 3- to 7-hr period. Plates were inspected and worms were transferred daily during the fertile period and were inspected three times weekly thereafter. Death was determined by absence of movement, pharyngeal pumping, or touch-response, as in Lithgow *et al.* (1994). This is different from our previous study (Shook *et al.* 1996) in which internal decay and lack of turgor pressure were also used as indicators of death; this may have slightly biased our assessment of survival time downward, but would have done so equally for all strains. Three terminal conditions in addition to senescent death were scored: death by internal hatching of progeny ("bagging"), death by desiccation on the wall of the plate ("desiccation"), and loss during handling. All terminal events other than senescent death were calculated as the fraction of the total starting population. Only strains for which at least five worms died senescent deaths were considered for further analysis of survival.

Fertility assay: The RI strains, N2 and BO, were assayed for age-specific fertility in four replicates, two on each of two dates. Five second-larval-stage (L2) or L3 worms were picked at random from age-synchronous populations laid during a 2-hr period by young gravid adults. Starting at 62 hr of age, observations were made every 2 hr to determine age of first reproduction ("alpha").

Age-specific fertility was measured by serial transfer of cohorts to fresh plates at 8 hr after worms with the earliest alpha started laying eggs, followed by two transfers at 8-hr intervals, then two at 12-hr intervals, and then daily until the end of each strain's fertile period. Adult survivorship was recorded at each transfer and censoring events were noted. Progeny produced was measured as the number of L2 to L3 stage larvae developed on the plate. The age-specific hourly fertility rate for a given interval was calculated as (number of progeny/number of parents at interval midpoint/interval width). Because parents that died of natural causes (senescence, bagging, or desiccation) were included, this measure gives the net fertility rate, or more specifically, the mean number of L2 to L3 progeny produced by a single worm that began the experiment as an L2 to L3.

Population growth rate (r) was determined by extrapolating the age-specific hourly fertility for several generations, assuming that each progeny from succeeding generations would have the same age-specific hourly fertility as its parent. In combination with survival data, this yields an expected number of worms alive at any time in the future. This approach is analogous to an iterated Leslie matrix (Carey 1993). r was determined from the equation $r = \ln(N_{x+t}/N_x)/t$, where N_x is the population at some time x , and N_{x+t} is the population size t hours later; for these analyses we took the average of r at several time points between 600 to 1200 hr, when the plot of population size with time began to visibly flatten (not shown, but see Figure 1). Because r was found not to be normally distributed, it was transformed as $e^{0.0r}$, which is normally distributed, and can be thought of as the factor by which population would increase in 100 hr (an approximate mean generation time) assuming an equilibrium age distribution and infinite resources. This value is called "population growth" in further analyses. "Early fertility" was determined by summing fertility prior to 100 hr; "late fertility" is the remainder. "Early fertility fraction" is early fertility divided by total fertility.

TABLE 1
Primers for new Tc1 polymorphisms

Marker/ primer	Position ^a	Primer sequence (5' to 3')
<i>pkP5062</i>	(I) + 11.35	CAA TGA CCC TTT ACA TGT TCA G
<i>pkP5036</i>	(I) + 26.76	ACA TTT GCG TCG GGA ATC AC
<i>zP598-L</i>	(III) - 2.5	GTA GGC TTT GTA ACA AAG TAG TG
<i>pkP580-U</i>	(X) + 15.10	CGT AGT CAA CTT CTG CAG
<i>Tc1.L1</i>		CAA GTC AAA TGG ATG CTT GAG
<i>Tc1.L3</i>		CAT TTC GCT TTA TGC ACA CGG

^a Estimated genetic map positions of Tc1 insertion polymorphisms based on physical map positions (ACEDB, Durbin and Mieg 1991), except for *zP589-L*, which is based on recombinations with flanking markers in our RI strains, as calculated by Map Manager (Manly and Cudmore 1994). Note that recombination distances observed in these RI strains are substantially different from those based on normal crosses (Shook *et al.* 1996).

Molecular markers: Genotype was assessed as described in Shook *et al.* (1996); six additional Tc1-based markers were scored in this study. Two of these (*stP17* and *stP128*) are from the original set developed by Williams *et al.* (1992), while four others are based on Tc1 polymorphisms found by the Plasterk lab [*pkP5062* (I), *pkP5036* (I), and *pkP580-U* (X); R. Korswagen, personal communication] or other Tc1 inserts not previously characterized [*zP589* (III)]. See Table 1 for new primer sequences. The primers for both *pkP5062* and *pkP5036* recognize sequences on the left flank of the Tc1 inserts, while all other markers used in this study recognize sequences on the right flank of the Tc1 inserts. The new markers were assayed as for other markers used in Shook *et al.* (1996), with the following exceptions: *pkP5062* was amplified using 1.2 mM MgCl₂ and internal Tc1 primer *Tc1.L3* (which is left facing); *pkP5036* was amplified using 1.6 mM MgCl₂ and internal Tc1 primer *Tc1.L1* (also left facing); and *zP589-L* was amplified with an annealing temperature of 48°. The new markers had nonpolymorphic background bands that acted as internal controls to ensure that amplification had occurred.

Statistical analyses: Distribution statistics for all traits were determined using SPSS 5.0 (SPSS 1992). The proportion of phenotypic variance for survival due to genotype was computed as $V_G / (V_G + V_E)$, where V_G (σ_B^2) is the component of variance between strains and V_E (σ_W^2) is the component of variance within strains.

To detect QTL we used a composite interval mapping approach (Zeng 1993, 1994), using model five of QTL Cartographer (Basten *et al.* 1996) with a window size of 20 cM. Model five takes into account genetic background effects due to the two most significant markers on each of the chromosomes other than the one being analyzed, as well as markers on the same chromosome farther away than the window size; this allows the genetic effect of the locus under consideration to be assayed in relative isolation. A window size of 20 was chosen to prevent markers near the locus being analyzed from being considered as background; nearby markers will have similar genetic effects due to linkage, which should not be considered as background. One thousand permutations of the strain phe-

notypes with respect to genotypes were considered by QTL Cartographer (Basten *et al.* 1996) for each trait to give experiment-wide critical significance levels, based on the method of Churchill and Doerge (1994), and interpreted by the standards of Lander and Kruglyak (1995); an effective $P = 0.10$ was considered suggestive, while an effective $P = 0.05$ was significant. Additive effect sizes at the position of the QTL mapped by the interval mapping approach were calculated by QTL Cartographer (Basten *et al.* 1996) and normalized by the standard deviation of the entire population; these estimates of QTL effect are likely to be biased upward. Positive additive effects indicate that Bergerac has the higher mean.

GEIs for survival were analyzed for each marker nearest a QTL for survival in either environment using a regression model that included a marker genotype term, an environment term (both coded as "1" or "-1") and the GEI term, which was the product of the two. We used a Bonferroni correction for the number of independent markers we tested (Belknap 1992) to evaluate the significance of the regression coefficient for the GEI term at a given marker.

Epistasis among markers for a given trait was tested for by two-factor ANOVA (Kshirsagar 1983) of strain means by all possible marker pairs, with marker genotypes as the two factors. F -scores resulting from 10,000 permutations of strain phenotypes with respect to genotypes were generated for each trait by a C program (available on request) written by D.R.S. to determine critical experiment-wide significance levels after the methods of Churchill and Doerge (1994). Epistatic effect (i) was calculated as $(A + D - B - C) / SD$ (Mather and Jinks 1977), where A and D represent the means of strains with the homotypic cases (either N2 or BO alleles at both markers), B and C represent the means of the heterotypic cases (N2 allele at one marker, BO allele at the other marker), and SD is the standard deviation of the whole population. A multiple regression approach was used to find those individual markers and epistatically interacting pairs of markers that significantly accounted for the phenotypic variance observed for each trait. All 40 markers were entered into the model at the start along with those interacting pairs of markers that had been shown previously to be suggestive or significant. Markers were removed from or reentered into the model using the stepwise method for regression (SPSS 1992), with a regression coefficient significance cutoff that was chosen to reflect the appropriate experiment-wide significance cutoffs as determined by permutation testing for single-marker linear regression by QTL Cartographer (see above); in practice this was $\sim P = 0.003$. The significance cutoff for the epistatically interacting marker pairs found by permutation analysis (see above) was somewhat lower than this, but we started only with those pairs that we had already shown were at least suggestive. Determination of the proportion of phenotypic variance due to epistatic interactions was determined by comparing adjusted R^2 scores for regressions with and without epistatic interactions. The markers that interacted epistatically were also included individually in these regressions, so that only the interaction variance from these markers was represented by the difference between adjusted R^2 scores. Markers were coded as 1 (for BO genotype) or -1 (for N2), and epistatic interactions were represented as the product of the two markers for each strain.

RESULTS

Measurements of life history traits: Survival time was assayed in a single replicate for all 79 RI strains. The fraction of the initial population of worms dying due to bagging or desiccation was also calculated. The distribution statistics for survival, bagging, and desiccation

are presented in Table 2. Age-specific fertility data were collected from four replicate assays and several fertility-related traits were subsequently derived from these data: early, late, and total fertility, early fertility fraction, and alpha. Total fertility was not significantly affected by date of assay ($F = 0.12$, $P = 0.73$) or by assayers ($F = 0.10$, $P = 0.75$), and all four replicates were highly correlated (lowest $r = 0.87$, 79 cases). Both alpha and population growth were significantly different between dates of assay ($F = 25.14$, $P < 0.001$; $F = 14.65$, $P < 0.001$, respectively), but not between assayers. The mean alpha for the second assay date was ~ 3 hr earlier, reflecting an overall systematic bias. Correlations between dates of assay for both alpha and population growth were highly significant ($r = 0.75$ and 0.84 , respectively), and correlations among the four replicates were also high (lowest $r = 0.67$ for alpha, 0.79 for population growth). Thus, we used the combined mean of all four replicates for further analyses. Distribution statistics for traits based on the fertility assay are also presented in Table 2. We calculated population growth based on age-specific fertility and survival data rather than direct measurements. Examples of projected population growth curves for the first 500 hr of culture for N2 and BO are presented in Figure 1.

Correlations among traits and estimation of V_G/V_P : Correlations among all traits measured in this study, as well as those measured in our previous study (Shook *et al.* 1996), are presented in Table 3. Note that survival on agar is not correlated with earlier survival results in liquid. Comparing survival and fertility traits, only late fertility and bagging on agar are significantly correlated. Unlike the survival traits, most fertility traits were highly correlated. Early fertility, late fertility, and alpha are statistically independent and they are highly correlated ($r \geq 0.65$). Early fertility is highly correlated with ($r = 0.99$) and is the major determinant of projected population growth rate. Alpha also shows a strong correlation ($r = -0.78$) with population growth, while late fertility is the least important of these three determinants ($r = 0.64$).

The proportion of phenotypic variance due to among-strain differences in survival was 0.33. Individual measures within strains were not available for other traits, and so proportion of phenotypic variance due to genetic effects could not be calculated using within-strain variances; however, see the section on multiple regression below.

Mapping QTL: A composite interval mapping method (Zeng 1993, 1994) using QTL Cartographer (Basten *et al.* 1996) was used to map QTL for all traits (Figure 2). Forty genetic markers [6 new and 34 reported previously (Shook *et al.* 1996)] were used to localize QTL. For survival (Figure 2A) one suggestive QTL was found on the X chromosome (near *stP129*; see Table 4 for effect sizes and positions). No QTL for bagging were found. For total hermaphrodite self-fertility (Figure 2B) two

TABLE 2
Descriptive statistics for life history traits

Trait	RI Strains									
	N2 Mean	BO Mean	Mean	S.D.	Minimum	Maximum	Kurtosis	Skewness	Median	RI Strains (N)
Survival traits										
Survival (days)	13.3	19.5	19.4	4.1	11.9	27.4	-0.66	0.24	19.4	67
Bagging (fraction)	0.40	0.08	0.08	0.10	0	0.40	1.5	1.5	0.04	79
Desiccation (fraction)	0.10	0.36	0.34	0.25	0	0.88	-0.97	0.48	0.32	79
Fertility traits										
Total fertility (progeny)	287	34.7	175	74	18	302	-0.91	-0.47	191	80
Early fertility (progeny)	175	12.8	100	45.8	10.0	186	-0.91	-0.15	104	80
Late fertility (progeny)	112	22.2	75.2	35.9	5.5	154	-0.72	-0.12	75.5	80
Early fertility fraction	0.61	0.37	0.57	0.10	0.34	0.80	-0.65	-0.06	0.59	80
Alpha (hours)	67.1	88.8	71	4.1	64	82	0.91	1.2	69	80
Population growth ^a (factor)	686	33.7	310	163	16.4	738	-0.74	0.08	319	80

^a Population growth is a derived, rather than measured, trait.

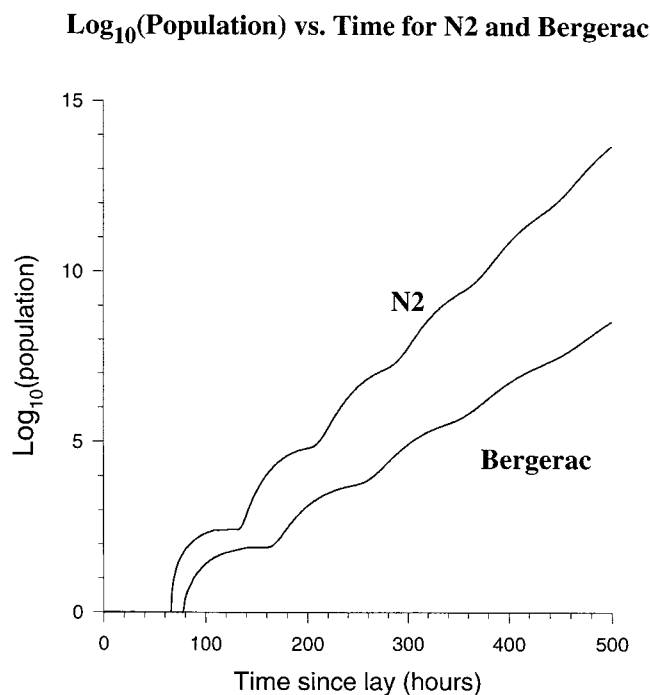


Figure 1.—Population size vs. time for N2 and BO, based on projected population growth rates. Calculations were based on age-specific fertility data.

significant QTL were found: one on *II* (near *maP1*) and one on *IV* (near *stP51*). For early fertility fraction (Figure 2C) one suggestive QTL was found on *II* (near *stP98*). For alpha (Figure 2D) two peaks were found on *II* (near *stP19* and *stP127*), which probably represents only one significant QTL, and one was found on *IV* (near *stP51*) as well as a suggestive QTL on *V* (near *stP18*). For population growth (Figure 2E) suggestive QTL were found on *II* (near *stP36* and *maP1*) and *III* (*stP19*), and one significant QTL was found on *IV* (*stP51*). Late fertility was found to have a QTL map very similar to that of total fertility, while the map for early fertility is nearly identical to that for population growth (data not shown).

Effects of environment: Environment had a highly significant effect on survival times ($t = 3.07$, $P = 0.003$) when considered alone. This reflects the 2.7-day difference in mean survival times on agar [22.1 days (Shook *et al.* 1996)] vs. in liquid (19.4 days, Table 2). The life spans for the parental strains N2 and BO also show a greater difference on agar than they do in liquid [13.3 and 19.5 days, respectively, on agar; 15.7 and 17.5 days in liquid (Shook *et al.* 1996)]. The cause of this variation in life span is unknown. The extent of the differences in worm “lifestyle” experienced in liquid vs. solid is not clear, but worms show far more rapid undulation behavior in liquid culture (Croll 1975; D. R. Shook and T. E. Johnson, personal observations) and have reduced food consumption (Avery and Thomas 1997—also indicated by our observation of greater bagging

TABLE 3

Correlations among phenotypes

	Survival in liquid	Survival on agar	Bag in liquid	Bag on agar	Desiccation	Total fertility 1	Total fertility 2	Alpha	Fraction early	Early fertility	Late fertility
Survival on agar											
Bag in liquid	0.02	0.23									
Bag on agar	-0.65**	-0.10	0.16								
Desiccation	0.21	-0.01	-0.19	-0.42**							
Total fertility 1	-0.05	-0.02	-0.04	0.14	-0.06						
Total fertility 2	0.02	-0.11	-0.10	0.20	-0.05	0.82**					
Alpha	0.02	-0.08	0.03	-0.19	0.06	-0.68**	-0.75**				
Fraction early	0.18	0.10	-0.18	-0.10	-0.04	-0.02	-0.06	-0.22*			
Early fertility	0.06	-0.12	-0.15	0.15	-0.10	0.78**	0.93**	-0.77**	0.27*		
Late fertility	-0.04	-0.08	-0.02	0.22*	0.02	0.71**	0.88**	-0.56**	-0.46**	0.65**	
Population growth ^a	0.05	-0.10	-0.13	0.11	-0.07	0.77**	0.92**	-0.78**	0.25*	0.99**	0.64**

^a“Survival in liquid,” “bag in liquid,” and “total fertility 1” are all from Shook *et al.* (1996); “total fertility 2” and all other traits are from this article. * $P \leq 0.05$; ** $P \leq 0.01$. Population growth is a derived, rather than measured, trait.

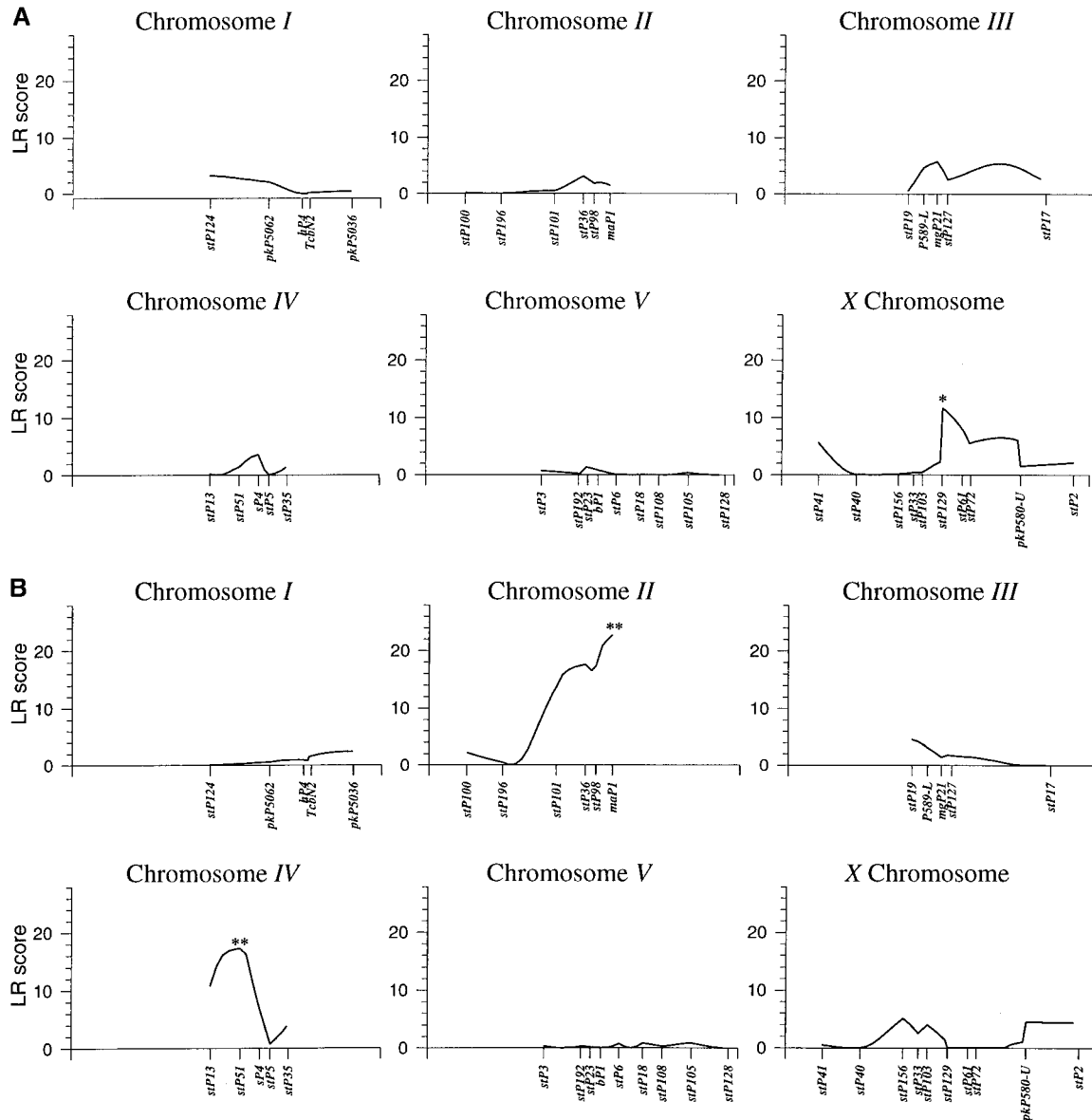


Figure 2.—QTL maps. Plots are from composite interval mapping by QTL Cartographer, model 5, with a window size of 20 cM. LR, likelihood ratio. (*) Suggestive (corrected $P < 0.10$) and (**) significant (corrected $P < 0.05$) composite interval mapping peaks, as determined by permutation testing. The number of RI strains used for each QTL map is indicated in Table 3. Updated marker positions can be found in ACEDB (Durbin and Mieg 1991). (A) Survival on agar (mean life span); (B) total fertility; (C) early fertility fraction (fraction of progeny produced before 100 hr); (D) alpha (time of onset of egg laying); and (E) population growth (factor of population increase over 100 hr, calculated using projected population growth rate).

in liquid). Other explanations may include small differences in assay technique (see materials and methods) or differences in damage done to worms during transfer from plate to plate (by wire pick on solid media and by mouth pipette in liquid).

To test whether any of the four QTL for survival on agar (current study) and survival in liquid (Shook *et al.* 1996) were in fact specific to one environment, we tested for GEIs. Of the four markers most closely associated with these QTL, *stp5* had a significant GEI ($t = 2.86$, $P = 0.005$), *stp101* was suggestive ($t = -2.24$, $P = 0.026$), and the last two (*stp129*, $t = 0.58$, $P = 0.565$; and *stp61*, $t = 1.02$, $P = 0.308$) showed no GEI [assuming a Bonfer-

roni correction of 3, for three independent marker groups (*stp129* and *stp61* are separated by only 3.6 cM)]. The QTL mapping to *stp129* and *stp61* are right next to each other on the X chromosome, and both influence life span in the same direction (higher for BO), and so may in fact represent the same QTL mapped to slightly different positions in the two different environments. When environment was considered as a cofactor in an ANOVA of each marker's effect on survival in both environments, only *stp61* was suggestive ($F = 9.35$, $P = 0.003$).

Epistasis within traits: All markers were tested pairwise for each trait by two-way ANOVA to determine

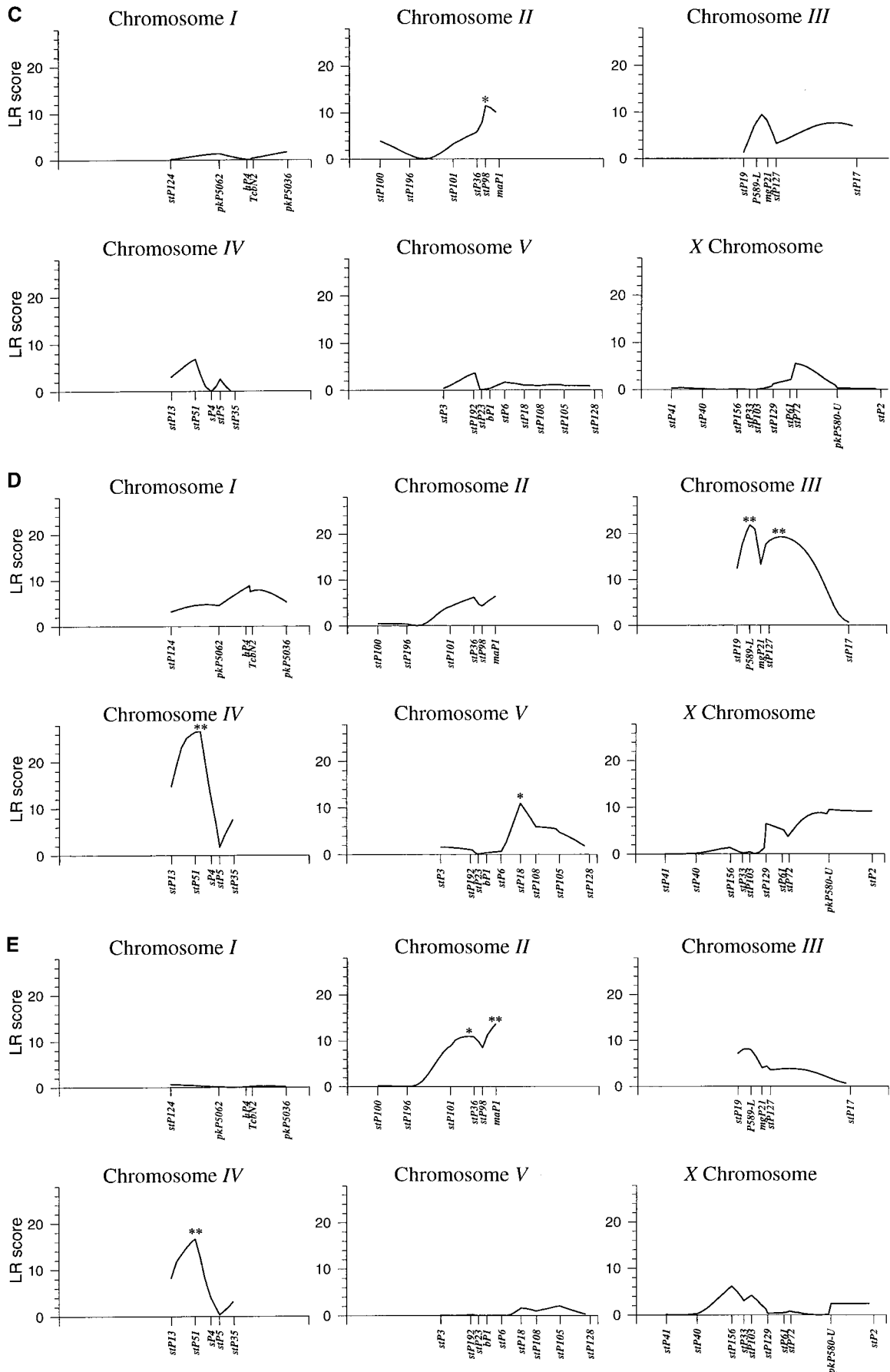


Figure 2.—(Continued)

TABLE 4
QTL additive effect sizes and variances

Trait	QTL map position ^a			Genetic effect (2 <i>a</i> , SD units) ^b
	Chromosome	Centimorgans	Marker	
Survival	<i>X</i>	+2.05	<i>stP129</i>	+0.80
Total fertility	<i>II</i>	+4.45	<i>maP1</i>	-1.02
Total fertility	<i>IV</i>	+2.18	<i>stP51</i>	-1.46
Early fertility fraction	<i>II</i>	+1.85	<i>stP98</i>	+0.78
Alpha	<i>III</i>	-2.49	<i>zP589-L</i>	+0.85
Alpha	<i>III</i>	+3.40	<i>stP127</i>	+0.90
Alpha	<i>IV</i>	+2.18	<i>stP51</i>	+1.46
Alpha	<i>V</i>	+9.70	<i>stP18</i>	+0.61
Population growth	<i>II</i>	-0.53	<i>stP36</i>	-0.70
Population growth	<i>II</i>	+4.45	<i>maP1</i>	-0.87
Population growth	<i>IV</i>	+2.18	<i>stP51</i>	-1.29

^a Chromosome number and map position of peaks for QTL by composite interval mapping, with the closest Tc1 marker indicated.

^b Additive effects (2*a*) as calculated by QTL Cartographer.

whether they showed any epistatic interaction (Table 5). We found one case of significant epistasis for bagging between *stP124* and *stP3*, and one suggestive case between *stP41* and *stP2*. For total fertility we found two cases of significant epistasis between *maP1* and *stP127* and between *stP5* and *stP6*. For alpha we found one case of suggestive epistasis between *stP98* and *bp1*, and three cases of significant epistasis between *stP19* and *stP17*, between *zP589-L* and *sp4*, and between *stP5* and *stP6*. For population growth we found one case of suggestive epistasis between *stP5* and *stP6*, and one case of significant epistasis between *maP1* and *zP589-L*.

Multiple regression models: We used multiple regression analysis to determine the proportion of the total phenotypic variance for each trait explained by significant markers and epistatic interactions found in this study (Table 6). The amount of phenotypic variance accounted for ranged from 13% (for bagging) to 46% (for population growth). Epistatic variance accounted for ~10% of the phenotypic variance for total fertility, alpha, and population growth.

DISCUSSION

QTL: Over all life history traits, we found at least nine suggestive or significant QTL by composite interval mapping. Only one QTL for survival was found, while the other eight were associated with the fertility-related traits (two for total fertility, one for early fertility fraction, three for alpha, and two for population growth). The two peaks for alpha on chromosome *III* might reflect two QTL, but it seems more likely that *mgP21* is simply a poor indicator of genotype. A similar case is seen for population growth on chromosome *II* (Figure 2 and Table 4). The multiple regression analysis supports the existence of only one QTL in each case, be-

cause only the stronger of the two peaks is included in the final multiple regression model for each trait (Table 6).

While a higher trait value for survival, fertility, and population growth indicates higher fitness, the interpretation for bagging is ambiguous. A lower value for alpha and for early fertility fraction is generally considered to confer higher fitness for an organism with a life style like *C. elegans* (Hodgkin and Barnes 1991; Roff 1992; Stearns 1992). Overall, only the QTL for survival and early fertility fraction are associated with increased fitness for the Bergerac genotype. All other fertility-related traits showed increased fitness for the N2 genotype.

Effects of environment: We found no overall correlation between survival on agar and survival in liquid. Of the four QTL mapped for survival in the two environments, two show GEIs, indicating that these QTL affect survival differently in different environments. The existence of GEIs for LHTs has important implications in driving adaptation (and perhaps speciation) in specific environments and for maintaining genetic variation in varying environments. The existence of GEIs also suggests caution in studying pleiotropy among traits (they must be studied in the same environment for the pleiotropies to be evolutionarily relevant) and for studying the evolution of traits and their genetic architecture (they must be assessed in the environment in which evolution has or will take place for implications about the mechanisms of their evolution to be relevant). However, the assay of LHTs even in a completely artificial environment should help to elucidate the genetic pathways that determine LHTs, whether those genes played a direct role in the evolution of a given species or not.

Pleiotropy: Among the fertility-related traits, there are two cases of QTL that affect more than one trait mapping to the same region. The QTL near *stP51* affects

TABLE 5
Epistasis between markers

First marker	Second marker	First marker allele	Second marker allele	
			N2	BO
A. Bagging				
			Fraction (<i>N</i>)	Fraction (<i>N</i>)
<i>stP124</i> (I) (<i>F</i> = 13.18, <i>P</i> < 0.05, 4 <i>i</i> = +1.84)	<i>stP3</i> (V)	N2	0.08 (34)	0.04 (27)
		BO	0.06 (8)	0.21 (9)
<i>stP41</i> (X) (<i>F</i> = 11.44, <i>P</i> < 0.10, 4 <i>i</i> = -1.74)	<i>stP2</i> (X)	N2	0.06 (41)	0.10 (18)
		BO	0.17 (10)	0.03 (9)
B. Total fertility				
			Progeny (<i>N</i>)	Progeny (<i>N</i>)
<i>maP1</i> (II) (<i>F</i> = 15.64, <i>P</i> < 0.05, 4 <i>i</i> = +1.62)	<i>mgP21</i> (III)	N2	212 (32)	157 (27)
		BO	81 (10)	147 (11)
<i>stP5</i> (IV) (<i>F</i> = 12.40, <i>P</i> < 0.05, 4 <i>i</i> = -1.73)	<i>stP6</i> (V)	N2	144 (22)	181 (39)
		BO	211 (10)	119 (9)
C. Alpha				
			Hours (<i>N</i>)	Hours (<i>N</i>)
<i>stP98</i> (II) (<i>F</i> = 10.74, <i>P</i> < 0.10, 4 <i>i</i> = -1.55)	<i>hP1</i> (V)	N2	69 (31)	71 (29)
		BO	74 (8)	71 (12)
<i>stP19</i> (III) (<i>F</i> = 13.51, <i>P</i> < 0.05, 4 <i>i</i> = -1.67)	<i>stP17</i> (III)	N2	69 (33)	72 (10)
		BO	75 (12)	71 (22)
<i>zP589-L</i> (III) (<i>F</i> = 14.24, <i>P</i> < 0.05, 4 <i>i</i> = +1.71)	<i>sP4</i> (IV)	N2	70 (29)	69 (13)
		BO	70 (26)	77 (9)
<i>stP5</i> (IV) (<i>F</i> = 16.42, <i>P</i> < 0.05, 4 <i>i</i> = +1.98)	<i>stP6</i> (V)	N2	71 (22)	70 (39)
		BO	67 (10)	74 (9)
D. Population growth				
			Factor (<i>N</i>)	Factor (<i>N</i>)
<i>maP1</i> (II) (<i>F</i> = 16.44, <i>P</i> < 0.01, 4 <i>i</i> = +1.69)	<i>zP589-L</i> (III)	N2	407 (32)	246 (25)
		BO	136 (10)	253 (10)
<i>stP5</i> (IV) (<i>F</i> = 11.35, <i>P</i> < 0.10, 4 <i>i</i> = -1.63)	<i>stP6</i> (V)	N2	258 (22)	325 (39)
		BO	405 (10)	198 (9)

F-scores from two-way ANOVA of strain trait means by both markers. *P* value levels based on experiment-wide significance level cutoffs, determined by permutation testing for each trait. Epistatic effects (4*i*, in standard deviation units) are based on genotypic means from each of the four cells of the interacting markers.

total fertility, alpha, and population growth while that near *maP1* affects both total fertility and population growth. For both QTL, the N2 genotype confers higher fitness for each of these traits. Because population growth is dependent on both alpha and total fertility, this set of positive fitness pleiotropies is not surprising. Also notable was the absence of QTL on chromosomes III and V for population growth (and early fertility), where QTL are found for alpha; this may indicate that some QTL for alpha control only the timing of the onset of fertility, while others control the number of progeny produced early.

Epistasis: We found a total of 10 suggestive or signifi-

cant epistatic interactions affecting different traits. Except for *maP1* for both total fertility and population growth and *zP589-L* for alpha, none of the markers nearest QTL for a given trait were involved in epistatic interactions for that trait. While 7 of the interactions are unique to a specific trait, the interaction between *stP5* and *stP6* is pleiotropic for total fertility, alpha, and population growth. The epistatic marker pairs remaining in the multiple regression models for total fertility, alpha, and population growth account for 9–11% of the phenotypic variance. This suggests that QTL modeling and analysis approaches would do well to include the detection and analysis of epistatically interacting

TABLE 6
Multiple regression models

Trait	Regression model	Adj. R^2	Δ Adj. R^2 for interaction terms
Bagging	<i>stP17</i>	0.13	—
Total fertility	<i>maP1</i> + <i>stP13</i> + (<i>maP1</i> × <i>mgp21</i>)	0.41	0.11
Early fertility fraction	<i>mgP21</i>	0.14	—
Alpha	<i>stP127</i> + <i>stP51</i> + (<i>zP589-L</i> × <i>sP4</i>)	0.41	0.11
Population growth	<i>maP1</i> + <i>stP51</i> + (<i>maP1</i> × <i>zP589-L</i>)	0.46	0.09

Multiple regression models. The Δ adjusted R^2 for interaction terms is found by taking the adjusted R^2 from the model plus all the interacting markers added individually, and then subtracting the adjusted R^2 for this model without the interaction terms.

QTL. *C. elegans* is a self-fertilizing hermaphroditic species and thus normally highly inbred. This may lead to coadapted gene complexes, which are detected as epistasis when divergent strains such as N2 and BO are crossed. Because gene interactions are of particular interest in understanding the genetic pathway of a given trait, inbreeding species may prove especially valuable for QTL mapping studies.

Comparisons with previous QTL mapping studies in *C. elegans*: The QTL of Ebert *et al.* (1993, 1996) for survival in liquid, which were at least partially in agreement with our previous results (see Shook *et al.* 1996 for discussion), are not replicated in our results for survival on solid media. This study did confirm two QTL for total fertility in the same regions (near *maP1* and *stP51*) as detected previously (Shook *et al.* 1996), supporting the replicability of the results, despite small differences in methods of assay. However, in the earlier study a QTL associated with *stP19* was found, which was not replicated here.

Conclusions: We have taken a first step in unraveling the genetic architecture of life history traits in a simple model organism, *C. elegans*; further studies using this system may eventually help us to understand the complex functional and genetic relationships among these traits as well as their evolution. These studies provide evidence for several QTL for survival and other life history traits. More QTL are suggested by analysis of epistatic interactions among traits; these interactions make up a substantial part of the genetic variance for these traits. We found GEIs for two survival QTL, indicating the importance of environment in considering the evolution of LHTs.

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