

TESTING LIFE-HISTORY PLEIOTROPY IN *CAENORHABDITIS ELEGANS*

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Abstract.—Much life-history theory assumes that alleles segregating in natural populations pleiotropically affect life-history traits. This assumption, while plausible, has rarely been tested directly. Here we investigate the genetic relationship between two traits often suggested to be connected by pleiotropy: maternal body size and fertility. We carry out a quantitative trait locus (QTL) analysis on two isolates of the free-living nematode *Caenorhabditis elegans*, and identify two body size and three fertility QTLs. We find that one of the fertility QTLs colocalizes with the two body size QTLs on Chromosome IV. Further analysis, however, shows that these QTLs are genetically separable. Thus, none of the five body size or fertility QTLs identified here shows detectable pleiotropy for the assayed traits. The evolutionary origin of these QTLs, possible candidate loci, and the significance for life-history evolution are discussed.

Key words.—body size, fertility, introgression, life-history traits, pleiotropy, QTL mapping, Tc1 elements.

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A central assumption of life-history theory is the existence of alleles that pleiotropically affect two or more life-history traits. Sometimes the nature of these pleiotropic effects is made explicit. For example, Williams (1957) proposed that alleles that affect senescence should also have antagonistic pleiotropic effects upon early-life fitness. More frequently, claims about pleiotropy are couched in phenotypic or mechanical terms—such as the many proposals that some trait is bound by a “trade-off” to another (Williams 1966; Roff 1992; Stearns 1992). It was in this spirit that Darwin (1859) observed that it is difficult to get a cow to give much milk and fatten at the same time.

Much evidence shows that pleiotropies of the sort required by life-history theory do exist. One kind of evidence comes from mutations of large effect. For example, consistent with the antagonistic pleiotropy theory of aging, many mutations in the nematode *Caenorhabditis elegans* that increase longevity cause a decline in fertility (Leroi 2001). It is sometimes even possible to determine the correlation between two traits for allelic effects at a particular locus. Across 16 alleles of the *C. elegans* gene *daf-2*, for example, there is a striking negative genetic correlation between longevity and fecundity (Gems et al. 1998; Leroi 2001). Similar examples can be found for alleles affecting many other organisms and traits. Such studies have the virtue of revealing the mechanisms underlying the pleiotropy. For example, *daf-2* is an insulin-like growth factor-receptor (IGFR; Kimura et al. 1997); the effects of this gene on fecundity and longevity tell us that IGF signaling can regulate these traits in opposition to each other.

But novel mutations of large effect do not necessarily reveal anything about the pleiotropic effects of the alleles relevant to evolutionary theory—those that segregate in natural populations and that contribute to evolutionary responses (Rose 1991). For this reason, many tests of life-history theory have been based on searches for genetic correlations among life-history traits in natural or experimental populations (Rose 1984; Roff 1996, 2000; Reznick et al. 2000). Again, genetic correlations consistent with postulated pleiotropies

have often been found (Rose 1991; Stearns 1992; Roff 1992, 1996, 2000). Genetic correlations, however, have the disadvantage that they may be due to linkage disequilibrium rather than pleiotropy. If genetic correlations are due to linkage disequilibrium among linked alleles without pleiotropic effects, then they will only affect evolutionary trajectories transiently. In other words, they will not cause fundamental mechanistic constraints of the sort that might account for the interspecific patterns of life-history diversity that we see (Roff 1992; Stearns 1992). This is a particular consideration for traits such as body size and fertility, both of which are thought to be influenced by many loci.

An understanding of the genetic relations among life-history traits requires, then, that we study variation in natural populations so that pleiotropy can be clearly distinguished from linkage disequilibrium. One way in which this might be done is to identify quantitative trait loci (QTLs) for life-history traits in order to determine whether the QTLs for the traits in question co-localize. If QTLs are mapped with sufficiently high resolution it should be possible to distinguish the effects of even tightly linked loci without molecular cloning of the genes in question.

Experimental Outline

Here we begin by identifying QTLs determining the difference in body size and fertility between two strains of the nematode *Caenorhabditis elegans*. We then ask whether there is any evidence for colocalization of QTLs that might be indicative of pleiotropy. Our analysis is based on recombinant inbred lines (RILs) derived from BO and N2 and developed by Johnson and Wood (1982). Previous studies of BO and N2 have identified QTLs for longevity, fertility and other life-history traits (Ebert et al. 1993; Shook et al. 1996; Shook and Johnson 1999). Here we identify a single novel body size QTL on chromosome IV. We reanalyse the fertility data of Shook et al. (1996) and show that one of the fertility QTLs identified by these workers colocalizes with the novel body size QTL. Finally, we undertake fine-scale mapping by introgression of genomic regions containing the QTLs from one strain into the other, in order to clarify the relationship between these QTLs. We show that the single body size QTL

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can be resolved into two QTLs of near opposite effects, each of which is genetically separable from the fertility QTL. Our study demonstrates how simple genetic techniques can be used to dissect the genetic architecture of fitness related traits; it also provides an initial step towards the molecular cloning of life-history QTLs.

MATERIALS AND METHODS

C. elegans Strains and Culturing

In our experiments we used four natural isolates of *C. elegans*: N2 (collected in Bristol, UK), the canonical "wild-type" strain used in laboratories throughout the world, BO (Bergerac, France), DH424 (El Prieto Canyon, CA), and TR403 (Madison, WI) (Hodgkin and Doniach 1997). These strains differ substantially in the number of copies of the Tc1 transposable element: N2 has about 30 copies, whereas DH424, TR403, and BO have about 200, 300, and 500 copies, respectively (Emmons 1988; Williams et al. 1992; Egilmez et al. 1995; Korswagen et al. 1996). Furthermore, Tc1 elements actively transpose in the germ-line of BO and TR403, but are inactive in N2 and DH424 (Emmons 1988).

For the QTL analyses we used a collection of 79 RILs constructed from an N2 × BO cross by many generations of selfing (Johnson and Wood 1982; Shook et al. 1996). T. E. Johnson (Univ. of Colorado, Boulder, CO) made available the genotypes for 36 Tc1 markers for each RIL, covering about two thirds of the *C. elegans* genetic map to within 5cM (Table 1). It is possible that Tc1 elements are actively transposing in some of the RILs or introgressed strains (less likely in the latter), but we have no direct evidence of this. All experiments were conducted within a few generations of reception or construction of a strain, and all strains were maintained at high population size, in order to minimize the probability of fixation of new mutations arising from germ-line transposition of Tc1 elements.

All strains were obtained from the *Caenorhabditis* Genetics Center (CGC, Univ. of Minnesota, Minneapolis, MN) except the versions of N2 and BO used for introgression, which were obtained directly from T. E. Johnson (originally used to construct the RILs; Johnson and Wood 1982). During the experiments, each strain was maintained in one NGM agar plate with a lawn of *E. coli* (OP50 strain, obtained from the CGC) at 20°C and high population density (Sulston 1988). Every week a chunk of medium containing hundreds of individuals was sampled from each plate and transferred to a fresh NGM plate, a procedure referred to as "chunking."

Body Length Assays

In each experimental block, the chosen strains were sampled by chunking. After 48 h, 10 adult hermaphrodites were put into a drop of a 5% alkaline sodium hypochlorite solution (Sulston 1988). This "bleaching" dissolves the adults and any contaminants present in the culture but does not harm the eggs. The individuals hatched from these eggs (about 50–200) were allowed to grow and were used to produce the experimental individuals by a second round of bleaching. A fixed time after bleaching (96 h), 10 arbitrarily chosen adult hermaphrodites were photographed under a dissecting mi-

TABLE 1. Map positions of the 31 Tc1 markers used in QTL analyses (Fig. 2). All Tc1 insertions are present in BO (Williams et al. 1992).

Chromosome	Marker	Position (cM)
I	<i>stP124</i>	0.4
	<i>TCbn2</i>	18.5
II	<i>stP100</i>	-19.0
	<i>stP196</i>	-13.1
	<i>stP101*</i>	-4.5
	<i>stP50*</i>	-1.2
	<i>stP98*</i>	1.8
	<i>maP1*</i>	4.3
III	<i>stP19</i>	-5.1
	<i>mgP21</i>	-0.3
	<i>stP127</i>	1.4
IV	<i>stP13</i>	-2.6
	<i>stP51**</i>	2.2
	<i>stP44†</i>	3.3
	<i>sP4</i>	5.3
	<i>stP5</i>	7.0
	<i>stP35</i>	10.0
V	<i>stP3</i>	-6.2
	<i>stP192</i>	0.0
	<i>bP1**</i>	3.0
	<i>stP6**</i>	5.8
	<i>stP18**</i>	9.7
	<i>stP108**</i>	12.8
X	<i>stP105**</i>	17.5
	<i>stP41**</i>	-18.8
	<i>stP40**</i>	-12.5
	<i>stP156</i>	-5.4
	<i>stP33</i>	-2.8
	<i>stP103</i>	-1.3
	<i>stP129</i>	2.0
	<i>stP72</i>	6.6
	<i>stP2</i>	24.1

* Insertion present in BO and DH424, but not TR403 (Egilmez et al. 1995).

** Insertion present in BO, DH424 and TR403 (Egilmez et al. 1995).

† Insertion informative, and therefore used as a marker, only in the introgression analysis.

croscope at × 25 magnification, using a JVC KY-F50 video camera attached to a PowerMacintosh. The body length of each worm was measured from the mouth to the base of the tail.

RILs.—N2, B0, and all RILs were assayed in seven blocks, including subsamples of approximately 50 strains, one or two replicate plates per strain. Due to poor survival, two strains (TJ246 and 258) were only present in two blocks, whereas 64% of strains were present in four or more blocks; every strain was represented by at least three replicate plates in total. From each replicate, 10 worms were measured 96 h after bleaching.

The growth dynamics were studied in N2 and a subset of 16 genotypically diverse RI strains (TJ113, 120, 124, 127, 130, 147, 216, 225, 235, 241, 251, 258, 264, 280, 292, and 297). Two replicate plates per strain were set up and 10 worms were photographed at the following times (after bleaching): 48 h, 72 h, 96 h, 120 h, 168 h, and 264 h.

Natural isolates.—Four replicate plates for each wild strain were set up and 10 worms were photographed at 96 h after egg laying.

QTL Introgression

Backcrossing.—Crosses were between single late L4 hermaphrodites and three to four males generated from N2 in a

small (~1 cm²) area of bacteria. At each generation mated hermaphrodites layed for at least 24 h before the males were killed and the hermaphrodites picked individually to PCR tubes for genotyping. This was not necessary after the first cross where the mated hermaphrodites were fully BO.

For each successful and appropriate cross (i.e., where approximately 50% of the offspring were male and BO markers were found in the mother) one or more offspring was picked to an individual plate and allowed to self. Offspring were then crossed again as described above. Backcrossing was continued for eight generations (i.e., eight times through the selfing step). When complete, strains were selfed (still picking individuals) for four or five further generations. Homozygosity was confirmed by PCR genotyping 11 arbitrarily picked individuals. A positive result for all markers in all individuals gave more than 95% confidence in the strain's homozygosity, and subsequent culture was on population plates maintained by chunking as described above. Genotypes for all strains were reconfirmed when they were assayed for size and fertility.

Whenever crossovers were detected (i.e., where the genotype contained some but not all of the Tc1 markers of the previous generation) backcrossing was continued with those strains separately, alongside those for the full region. At each generation 10–12 crosses and subsequent PCR reactions were carried out per strain using worms from 4–5 selfings.

Assays.—Populations of each parental and introgression strain were synchronised by two generations of bleaching (see above) and experimental worms collected over a 30-min-hatch period. After, 24 h, five worms per strain were transferred to individual plates for fertility assays and 15–20 to a single plate for the body size assay.

Worms in the fertility assay were transferred to new plates 48 h after hatching and then subsequently at 24 h intervals until 144 h after hatching. Worms in the body size assay were transferred to a new plate once, 72 h after hatching. Once worms were transferred, fertility assay plates were left at 20°C for approximately 36 h before refrigeration at 4°C for a maximum of 48 h. Offspring were then counted (killing worms with a soldering iron). Ten to 12 worms from each strain in the body size assay were photographed as in the body length assay above, 96 h and 120 h after hatching. Areas (*A*) and lengths (*L*) of worms were measured and aspect ratios were calculated as L^2/A . This assay was replicated four times, with a further two replicates assayed for body size alone.

Genotyping

Templates for PCR were prepared by boiling 10–20 adult nematodes in 20 μ l of water for 10 min. The PCR reactions were performed according to the protocols described in Williams et al. (1992). We failed to confirm Shook's genotypes for six strains (TJ103, 131, 146, 202, 211, and 221) which were excluded from the analyses. This may have resulted from contamination or mislabeling before or after we received the strains from the CGC.

Introgression worms were genotyped by single worm PCR in 20 μ l reactions according to Williams (1995), using a multiplex of primers to score all six chromosome IV Tc1 markers listed in Table 1.

TABLE 2. Random model ANOVA for body length at 96 h. Variance components were estimated using restricted maximum likelihood (REML).

Source	SS	df	<i>F</i>	Variance component
Line	2.200	72	5.44***	0.00750
Block	0.832	6	24.71***	0.00412
Error	1.325	236		0.00561

*** $P < 0.001$.

Software

Image analysis was done using Object-Image version 1.6 (Vischer et al. 1994). Descriptive statistics and correlation coefficients were calculated, and tests of normality and homogeneity of variance, analyses of variance, and multiple regression were done using JMP versions 3.2 and 4.0 (SAS Institute NC). QTL maximum likelihood interval mapping and permutation tests were performed using QTL Cartographer version 1.13d (Basten et al. 1994). Programs for regression-based QTL models, permutation tests and bootstrapping were written in Visual Basic. Mapping and genomic information was obtained from ACeDB data version WS2.4-17 (R. Durbin and J. Thierry-Mieg, unpubl. data).

RESULTS

Genetic Variation in Body Size and Fertility

Adult hermaphrodites of the N2 strain were approximately 30% longer than BO worms at 96 h. In addition, N2 showed about a threefold higher hermaphrodite self-fertility. The variation in body length at 96 h among RILs was investigated by ANOVA on the means of replicate plates, with random effects of Line and Block. The residuals were normally distributed (Shapiro-Wilk test, $P > 0.5$) and the variances were homogeneous among strains and blocks (Levene's test, $P > 0.1$). The model showed highly significant variation among Lines and Blocks (Table 2).

The body length at 96 h (means and standard errors, SEs) for the parental strains and each RIL, was estimated from the linear model as described above, but including the parental strains (results not shown). Most RILs showed body sizes and fertilities within the range of the parental strains (Fig. 1). The distributions of strain means for each trait were significantly skewed (body length: $g_1 = -0.570$, $P < 0.05$; fertility: $g_1 = -0.522$, $P < 0.05$; Snedecor and Cochran 1989). Body size and fertility among the RILs were positively correlated: Kendall's rank correlation coefficient $\tau = 0.236$, $n = 72$, $P < 0.005$ (Fig. 1) and each was negatively correlated with the genetic distance to N2: $\tau = -0.167$, $n = 72$, $P < 0.05$, and ($\tau = -0.245$, $n = 80$, $P < 0.005$) respectively (genetic distance to N2 of each strain was defined as the number of BO alleles divided by 35, the number of informative loci).

Growth Dynamics

To test whether body length at 96 h is a good indicator of final size, we fitted the following logistic model to each replicate by non-linear least-squares regression:

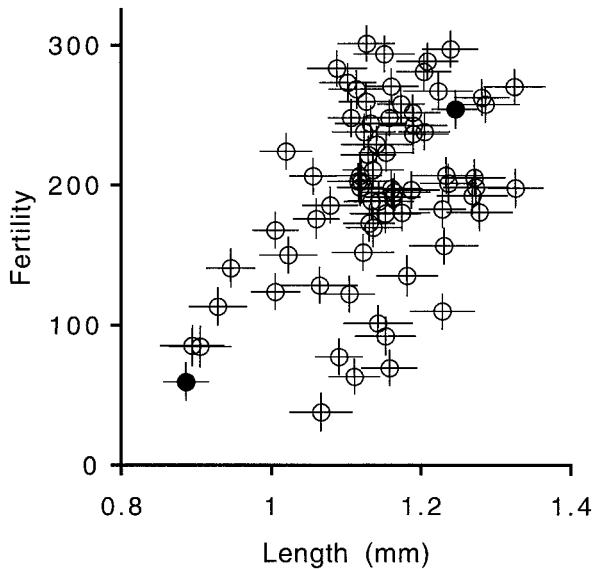


FIG. 1. Relationship between length (at 96 h) and fertility for each RIL (open circles) and parental strain (closed circles). Values are means (\pm SE). Fertility data from Shook et al. (1996).

$$L(t) = \frac{L_{\max}}{1 + a \cdot b^t}$$

where $L(t)$ is body length at time t , L_{\max} is the asymptotic body length, and a and b are the location and rate parameters, respectively. Growth rate was estimated from the fitted curves as the time required to attain 50% of L_{\max} :

$$T_{50\%} = -\frac{\ln(a)}{\ln(b)}$$

Both L_{\max} ($F_{[16,17]} = 18.24$, $P < 0.001$) and $T_{50\%}$ ($F_{[16,17]} = 3.28$, $P = 0.01$) varied significantly among strains. L_{\max} was significantly correlated among strains with body length at all times (Pearson correlation coefficient $r \geq 0.79$, $n = 17$, $P < 0.001$), but not with $T_{50\%}$ ($r = -0.006$, $n = 17$). Therefore, body length at 96 h is a good indicator of final size in these strains.

Interval Mapping

QTLs for body length and fertility were mapped by maximum likelihood interval mapping (Lander and Botstein 1989). This method tests the hypothesis that an interval flanked by two adjacent markers contains, at a given location, a QTL affecting the trait, and estimates its genetic effect. The test statistic is the likelihood ratio $LR = -2 \ln(L_0/L_1)$, where L_0 is the likelihood under the null hypothesis that there is no QTL in the interval and L_1 is the likelihood under the alternative hypothesis that there is a QTL in the interval. The analyses were done on strain means (least-squares mean body lengths obtained from the ANOVA model described earlier, $n = 72$; mean fertility, $n = 80$, from Shook et al. 1996) using the genotypes for 31 markers and the positions listed in Table 1. Markers spaced less than 1.5 cM relative to other markers (*hP4*, *stP36*, *stP33*, and *stP61*) and uninformative markers (*stP44*) were excluded from the analyses. The QTLs were

TABLE 3. QTLs detected by interval mapping, values of the likelihood-ratio statistic (LR), the proportion of the among-strain variance accounted for by the QTL (r^2), and the magnitude of the effect of a single BO QTL allele.

Map position	Nearest marker	LR	r^2 (%)	Effect
Length				
3.7 (IV)	<i>stP51</i>	15.44**	25	-0.056 mm
Fertility				
3.9 (II)	<i>maP1</i>	11.56*	14	-26 progeny
-5.1 (III)	<i>stP19</i>	10.97*	13	-22 progeny
2.2 (IV)	<i>stP51</i>	20.48***	23	-46 progeny

Experiment-wise significance levels from 10,000 permutations: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

fitted in steps of 1 cM, assuming a Haldane map function and a selfing RIL design. Empirical genome-wide significance thresholds (accounting for multiple tests and marker correlation) were obtained by randomly permuting the phenotypic data among strains 10,000 times and calculating the maximum LR across the whole genome, for each trait in turn (Churchill and Doerge 1994). A peak in LR was taken to indicate a significant QTL if $P < 0.05$ ($LR > 10.0$). We found one significant linked QTL for body length on chromosome IV with an additive genetic effect of about 90% the phenotypic standard deviation and explaining about 25% of the among line variance (Table 3; Fig. 2). We also found three significant unlinked QTLs for fertility with the data from Shook et al. (1996) (Table 3; Fig. 2).

Other peaks in the likelihood ratio for each trait in regions of the genome unlinked to the detected QTLs, were all non-significant ($P > 0.4$).

Pleiotropy

Because body size and fertility showed a consistent pattern of variation and significant QTLs for each trait were close to *stP51* on chromosome IV (Table 3; Fig. 2), body size and fertility may be under pleiotropic control by a single QTL. Empirical confidence intervals (CIs) for the positions of the QTLs for length and fertility on chromosome IV were calculated from 1000 joint bootstrap samples from the 72 strains scored for both traits (Visscher et al. 1996). Each bootstrap sample was subjected to regression interval mapping (Haley and Knott 1992; Martinez and Curnow 1992) in steps of 0.25 cM, assuming a Haldane map function and a selfing RIL design. The results from regression and maximum likelihood interval mapping analyses were highly correlated (in 1 cM steps, $n = 12$: likelihood ratios LR for length, Pearson correlation coefficient, $r = 0.974$, and for fertility, $r = 0.997$; additive effects b for length, $r = 0.970$, and for fertility, $r = 0.992$). Only bootstrapped samples which contained at least one significant QTL ($LR > 10.0$) for each trait were retained (Lebreton and Visscher 1998), and the position of the highest peak in the LR was noted for each trait. The empirical 95% CIs for the estimated positions of the body length and fertility QTLs overlapped by over 1 cM (Fig. 3). The difference (D) between the positions of the QTLs was calculated from each bootstrap sample. The 95% CI interval of D ranged from 0 to 7.25; because it included 0 we cannot reject the hypothesis

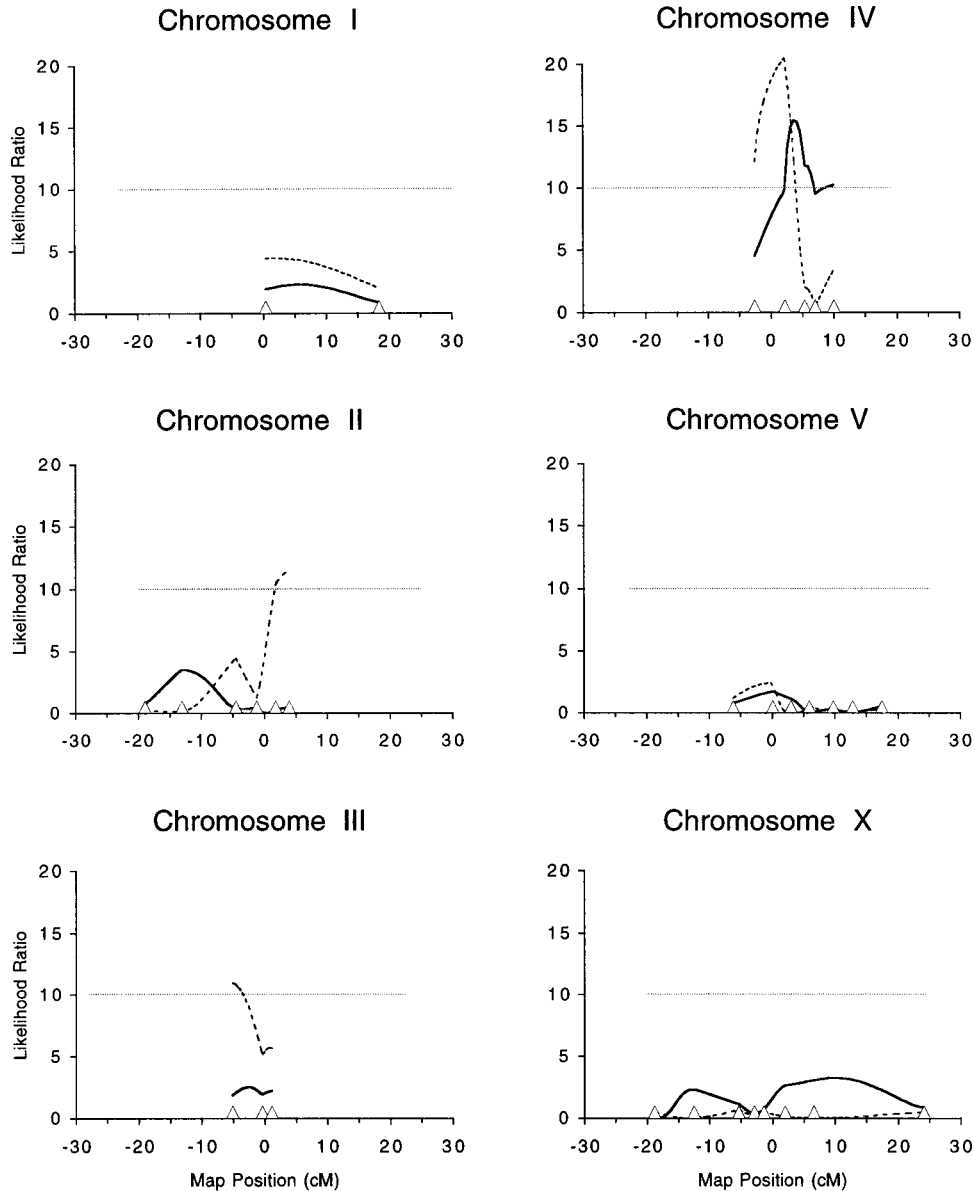


FIG. 2. Results of fitting a single QTL by maximum-likelihood interval mapping for body length (bold line) and fertility (dashed line). Empirical experiment-wise significance threshold for $P = 0.05$ and length of the chromosomes marked by bold gray lines. Marker positions are indicated by open triangles.

of pleiotropy at the 5% significance level (Lebreton et al. 1998).

Whereas the fertility bootstrapping distribution has one major peak, that for body size has two (Fig. 3). This could indicate two body size QTLs, as suggested by the small secondary peak crossing the significance threshold to the right of the first in the original analysis (Fig. 2). Fitting two QTLs simultaneously is one way to approach the question (Haley and Knott 1992; Martinez and Curnow 1992). However, the two peaks are in adjacent marker intervals, thus we cannot estimate their position by interval mapping (Whittaker et al. 1996). In fact, such multi-modal bootstrapping distributions can arise even in simulated data sets in a region containing only one QTL (Visscher et al. 1996). Thus, further genetic

dissection of the chromosome IV body size and fertility QTLs, required a more direct approach.

Genetic Dissection of the Chromosome IV QTL Region

The region on chromosome IV identified as containing the 95% CI on both body length and the putatively pleiotropic fertility QTL was backcrossed eight times to introgress the region from BO into the background of N2, resulting in strain X. In this process of backcrossing seven strains containing subsections of the region of interest were obtained and backcrossed to the same extent giving strains A–G (Fig. 4).

Fertility and body length at 96 h for N2, and strains A–G and X showed highly significant among strain variation (P

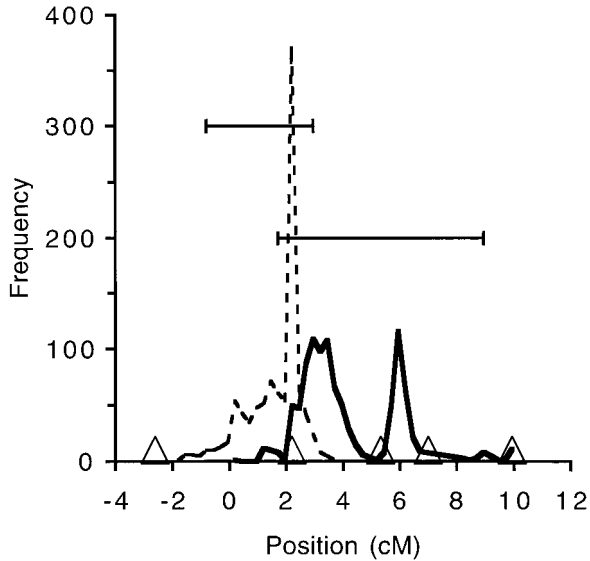


FIG. 3. Frequency distribution of the results of fitting a single QTL by regression interval mapping in chromosome IV for body length (bold line) and fertility (dashed line) in 1000 selected joint bootstrap samples of the original data. Error bars are empirical 95% CIs for each QTL. Marker positions indicated by open triangles.

< 0.0001). Each strain was compared with N2 by contrast analysis. Surprisingly the strains containing the full region or the left-hand end of the region from BO (X, A, B, C, and E) were all significantly longer than N2. Strains N2, D, and F did not differ significantly and averaged 1.32 mm long at 96 h. Strains X, A, B, C, and E were on average 34 μ m (2.5%) longer. Only one strain (G) containing the right hand end of the region was significantly smaller, averaging 92 μ m (7.0%) shorter than N2 at 96 h (Figs. 4, 5). This implies the existence of two body length QTLs but indicates that one is

of opposite effect to that expected (the ‘‘long QTL’’ favoring increased length in BO as opposed to the ‘short QTL’ involved in making BO shorter than N2). This pattern of two QTLs is confirmed by the measurements at 120 h, where the measurements for each genotype are even more tightly grouped.

The low fertility of strains containing the left hand end of the region confirmed the location of the fertility QTL to the left of *stP51* (Figs. 4, 5). However, the suggested pleiotropy with the nearby body size QTL (the long QTL) can be excluded since one strain (E) contains the long QTL, but not the low fertility one (Figs. 4, 5).

If we assume the 95% CI obtained by bootstrapping the QTL analysis to be valid, then the introgression analysis suggests that the fertility QTL is likely to be located between *stP13* and *stP51*. However, the possibility that the fertility QTL is outside the region of interest (to the left of *stP13*) could not be excluded by this assay. The short QTL is confirmed as being beyond the 95% CI previously obtained, to the right of *stP35*. The long QTL is located between *stP13* and *stP51*, specifically to between the (unknown) crossover points in strains A and E. On the genetic map these correspond to the positions: -0.8 to +2.5 cM for the fertility QTL, -1.6 to +2.5 cM for the long QTL, and to the right of +10.0 cM for the short QTL.

The only strain with a significantly different shape from N2 is G which has the short QTL. This strain is proportionately squatter, having an aspect ratio of 19.0 (\pm 0.1 SE) compared with N2’s aspect ratio of 20.3 (\pm 0.1 SE). Conversely, though all the strains with the long QTL have a significantly larger crosssectional area than N2, strain G (with the short QTL) has a crosssectional area not significantly different from N2. Thus, the short QTL simply changes the shape of the worm, whereas the long QTL makes the worms proportionately giant.

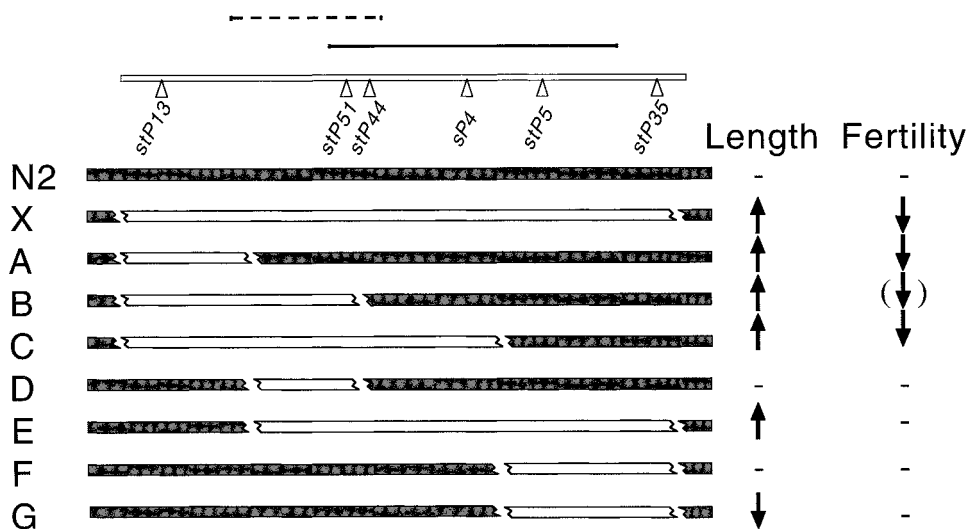


FIG. 4. The chromosome IV QTL region in the introgression strains. The bars at the top indicate the 95% CIs from the initial analysis on the QTLs for length (complete line) and fertility (dashed line) with the positions of markers used (triangles). For each of the strains (X and A–G) shaded bars indicate DNA from N2 and open bars indicate DNA introgressed from BO (boundaries are not shown explicitly since the only information on their position is the presence or absence of the markers indicated above). The columns labeled Length and Fertility are relative to N2: up arrows indicating significant increases in the trait at least at the 5% level, down arrows significant reductions and dashes indicating no significant difference. The bracketed arrow indicates a change significant at the 10% level.

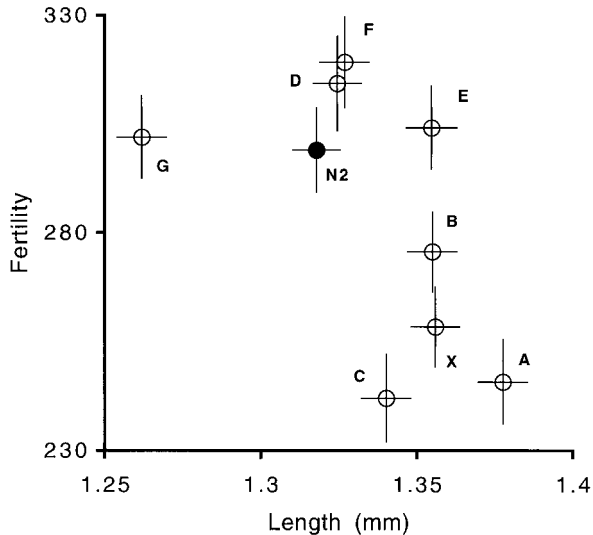


FIG. 5. The 96 h body lengths and total fertilities of the introgression strains and the parental N2 strain. Values are least-square means from the ANOVA (\pm SE)

Natural Isolates

ANOVA on the means of replicate plates shows that there was significant variation in body length at 96 h among natural isolates (mean \pm SE in mm of 4 replicates per strain: BO, 1.14 ± 0.02 ; DH424, 1.35 ± 0.01 ; TR403, 1.29 ± 0.01 ; N2, 1.32 ± 0.02 ; $F_{[3,12]} = 16.6$, $P = 0.0001$). BO worms were significantly shorter at 96 h than worms from all other strains (Tukey-Kramer HSD test, $P < 0.001$). Worms from N2, DH424 and TR403 did not differ significantly from each other in body length ($P > 0.5$).

DISCUSSION

Identification of Body Size QTLs

We have searched for body size quantitative trait loci (QTLs) using QTL analysis followed by introgression of genomic segments from one strain into the other. Our initial analysis revealed a single locus for body length on chromosome IV that accounted for 25% of the variance among RILs. This QTL affects body length independently of growth rate, the estimated effect being to make BO worms $92 \mu\text{m}$, or 7.0% shorter than N2 worms. Surprisingly, further mapping showed this QTL region contains two distinct QTLs—one which decreases the body length of BO worms by $61 \mu\text{m}$ or 4.6% and another which increases body size of BO worms by $34 \mu\text{m}$ or 2.5% relative to N2.

Although we have identified two QTLs that contribute to the difference in body size between N2 and BO, others undoubtedly exist that we have not detected. There are four reasons for thinking this. First, our study does not have the statistical power to detect QTLs explaining less than about 10% of the phenotypic variance (Table 3). Second, the Tc1 markers used do not cover about one third of the genetic map. Third, the QTL model we used does not allow for epistasis between QTLs. Indeed, most QTL experiments have low power to detect epistasis (Tanksley 1993; Liu 1998). Fourth,

the total effects of the detected QTLs are very different from the observed parental difference.

The existence of other unseen body size QTLs and the question of epistasis may help resolve the apparent conflict between the loci identified in the initial QTL mapping (one locus decreasing size in BO) and the loci found by subsequent fine mapping (two loci of opposite effect with a net additive effect of much less than the original estimated effect). There are two simple hypotheses. First, epistasis between the two body size loci: the short QTL is what was originally detected, the long QTL was epistatically masked by it until it was separated from it in the fine mapping. Circumstantial evidence to support this is found in a reexamination of the original RILs—the only strain containing the BO marker nearest the long QTL (strain TJ148 and marker *stP51*) without the other BO chromosome IV markers, is one of the longest strains measured. We also note that the kind of epistasis required by this explanation (a short allele masking the effect of a long allele) is of a kind commonly seen in *C. elegans* mutations of large effect on body size (Z. Z. Shen and A. M. Leroi, unpubl. data). Second, linkage disequilibrium with another unseen body size QTL: the short QTL corresponds to the small secondary crossing of the significance threshold, to the right of the main peak in the initial analysis (Fig. 2). The main peak results from the combination of a strong QTL just outside the mapped region, reducing length, and the long QTL. Circumstantial evidence supporting the existence of strong unseen body size QTLs comes from the original RILs where the shortest strain measured (TJ130) had the N2 genotype at all chromosome IV markers. The first hypothesis could be tested by experimentally bringing the isolated long and short QTLs back onto the same chromosome: if it is correct, an inbred strain with both QTLs would be short relative to N2. The second hypothesis could be tested by finding markers outside the region currently mapped on chromosome IV and further QTL mapping.

Our study is not the first to attempt to map variation in body size among wild isolates of *C. elegans*, although it is the first to do so in any detail. Hodgkin and Doniach (1997) studied the genetic basis of the difference in body size between N2 and another strain, CB4855, and identified body size QTLs on chromosomes V and X. Because both of these regions were well covered by markers in our study and we did not identify body size QTLs on either, we conclude that CB4855 has acquired its small body size independently of BO. These observations, taken together with the finding of among-strain variation in body size in a collection of *C. elegans* isolates from Quebec (Kader and Côté 1996), suggest the existence of considerable variation for body size within and among natural populations of *C. elegans*.

Pleiotropic Relations between Body Size and Fertility

Life-history theory has repeatedly postulated the existence of positive pleiotropies between body size and fertility (Stearns 1992; Roff 1992, 2000). This seems reasonable since, a priori, it is easy to see how alleles that affect maternal body size might affect fecundity. Maternal body size might, for example, influence fertility via a physical restriction on gonad size, or body size and gonad size might both be directly

regulated by common developmental pathways. Consistent with the second idea, mutations of large effect which cause dwarfism also frequently cause low fecundity. For example, loss of function mutations in components of an insulinlike growth factor (IGF) signaling pathway in *Drosophila* give dwarf flies with low fertility (Fernandez et al. 1995; Chen et al. 1996; Böhni et al. 1999). In contrast, overexpression of growth hormones in fish and mice increases size, but also reduces fertility (Rollo et al. 1997; Rahman and Maclean 1999).

Many quantitative genetic studies have shown a positive genetic correlation between body size and fertility (Roff 2000), but for reasons discussed in the introduction, genetic correlations may frequently be a poor guide to pleiotropy. Although many studies have searched for either body size or fertility QTLs (Keightley et al. 1996; Shook et al. 1996; Li et al. 1998; Shook and Johnson 1999; Frary et al. 2000), few have simultaneously searched for both. Macdonald and Goldstein (1999) and True et al. (1996, 1997) have identified colocalized QTLs for tibia length (a measure of body size) and several male fertility traits in *Drosophila simulans* × *D. sechellia* and *D. simulans* × *D. mauritania* crosses, but did not attempt to determine if they were due to pleiotropy.

Using data from Shook et al. (1996), we implicated three QTLs in the three-fold difference in N2 and BO fertility. These results are consistent with the analysis of Shook et al. (1996) and Shook and Johnson (1999). One of these fertility QTLs, on chromosome IV, colocalized with the single body size QTL. The chromosome IV fertility QTL caused BO worms to have 52 fewer offspring than N2 (17%) over their lives.

Our initial analysis was consistent with the possibility that a single QTL on chromosome IV had pleiotropic effects on body size and fertility (Fig. 3). However, as discussed above, we were able to show that the body size QTL was composed of at least two loci of near opposite effect. Both of these are genetically separable from the fertility QTL. Of the five body size or fertility QTLs that we have examined here, none appears to have detectable pleiotropic effects on the other trait.

Our results are subject to the following caveats. First, the fact that we failed to find a pleiotropic effects of the QTLs on size and fertility does not mean that pleiotropic effects cannot occur in other environments. For example, body size might constrain fertility under caloric restriction. Second, it is possible that other QTLs that were not detected in our analysis show pleiotropy. Even so, suggestions that colocalizing QTLs of different traits are due to pleiotropy (e.g., Nuzhdin et al. 1999; Shook and Johnson 1999), should be treated with caution, even when there is a strong a priori expectation of the pleiotropy in question.

History

What is the origin of the QTLs identified in this study? It is likely that the QTLs identified here are due to mutations that occurred during the laboratory history of N2 and BO for three reasons. First, although both strains are derived from stocks kept at the *Caenorhabditis* Genetics Center, where they are stored inert at -90°C , both were kept in continuous culture in different laboratories for several years between iso-

lation from the wild and freezing (Hodgkin and Doniach 1997). Second, the very transposons which have made it possible to map the QTLs provide an obvious source for “unnatural” variation since isolation from the wild. Tc1 elements have been actively transposing in the BO lineage for an unknown amount of time and BO has about two orders of magnitude more copies of Tc1 than N2. Some Tc1 insertions fall in the short QTL region, although none are known in the long or fertility QTL regions (Williams et al. 1992; Korswagen et al. 1996). Third, the extremely low fertility of BO is unlikely to be maintained in the wild.

One way of investigating the antiquity of the QTLs is to compare N2 and BO with other high Tc1 copy number strains independently isolated from the wild, but which lack the long histories of laboratory culture of BO and N2. (Emmons 1988; Egilmez et al. 1995; Hodgkin and Doniach 1997). DH424 and TR403 share regions of particular chromosomes with BO, probably as a result of recombination (Egilmez et al. 1995). We found that DH424 and TR403 were longer than BO but did not differ significantly in size from N2. We also find that the Tc1 marker closest to (in fact, within) the long QTL region (*stP51*) is shared by BO, DH424, and TR403, but not N2. This is consistent with the hypothesis that the long QTL is an ancient one that exists in the wild. On the other hand, the Tc1 marker closest to the short QTL (*stP35*) is thus far unique to BO, consistent with the hypothesis that the short QTL arose in the laboratory (Egilmez et al. 1995). Hodgkin and Doniach (1997) found that DH424 and TR403 worms do not differ significantly in fertility from each other (~ 260 viable progeny), but, like BO, both strains have significantly lower fertility than N2 (~ 330). Again, the Tc1 insertion closest to the fertility QTL is *stP51* which is shared by DH424, TR403, and BO; and so it is possible that the chromosome IV fertility QTL exists in the wild. This line of argument is admittedly indirect—confirmation that the QTL alleles identified in BO exist also in other natural isolates requires QTL analysis of DH424 and TR403 with N2 and complementation tests of any identified QTL alleles with those of BO. In conclusion, we do not have enough information to decide whether the putative QTLs are natural in origin.

Candidate Genes

In searching for life-history QTLs we aim, ultimately, to identify at the molecular level the loci responsible for phenotypic variation within and among natural populations of *C. elegans*. One way that this might be done is to identify candidate genes located in the vicinity of QTLs of interest. This is a difficult task for body size, because it is probably affected by many genes. As an illustration of the complexity of body size control in *C. elegans*, alleles that visibly affect body size are known for several loci belonging to the following classes of *C. elegans* genes: feeding defects (*eats*), egg laying defective (*egls*), uncoordinated (*uncs*), and cuticle and body shape defects (*dpys*, *lons*, *rols*, *smas*, *sqts*) (Brenner 1974; Cox et al. 1980; Park and Horvitz 1986; Vonmende et al. 1988; Kramer et al. 1990; Avery 1993; Estevez et al. 1993; Reiner et al. 1995; Savage et al. 1996; Lee et al. 1997; Krishna et al. 1999; Suzuki et al. 1999; Z. Z. Shen and A. M. Leroi, unpubl. data; M. N. Patel and A. M. Leroi, unpubl. data).

The region highlighted by the QTL study for fine mapping is located near the chromosome IV gene cluster. The calculated 95% CI on the body length QTL analysis spans a 7cM region containing over 150 mutationally described genes. Introgressing subsections of this region from BO into N2 narrowed the interval down to 3.3cM for the fertility QTL, 4.1cM for the long QTL, and 9cM for the short QTL. The fertility and long QTL region contains 28 mutationally described genes, and the short QTL region contains eight. Of these genes, three have been cloned and are known to affect adult body length: one in the long QTL region, *dpy-13* (Vonmende et al. 1988), and two in the short QTL region, *dpy-4* (Cox et al. 1980), and *egl-23* (M. N. Patel and A. M. Leroi, unpubl. data). Additionally *gro-2*, a growth rate gene which was found by spontaneous mutation in an N2/BO cross maps close to this region (Hodgkin and Doniach 1997). Ten genes in the fertility QTL region are known to affect fertility: *evl-1*, *fem-1*, *let-278*, *let-279*, *let-280*, *let-281*, *let-282*, *let-284*, *let-288*, and *rme-2*. Thus, there are some candidate loci for all three QTLs. However, on the physical map, these regions contain several hundred predicted coding sequences which have not been studied in detail, implying that finer mapping is required before candidate loci can be fruitfully investigated.

Although we have not mapped QTLs with sufficiently high resolution to identify candidate loci which can be tested by physical mapping techniques, the molecular analysis of such life-history variation remains possible in *C. elegans*. The fully sequenced genome (Consortium 1998) is leading to the identification of large numbers of single nucleotide polymorphisms (SNPs), potentially providing a source of markers at whatever resolution is required (Jakubowski and Kornfeld 1999; Koch et al. 2000). Finer QTL mapping in *C. elegans* to the level at which physical mapping techniques can be brought to bear is, then, straightforward. We believe that molecular analysis of the loci responsible for life-history variation is central to the testing of the assumptions underlying life-history theory (Riska 1989; Leroi 2000, 2001). Such analysis may also point to the molecular causes of macroevolutionary variation in body size and fertility in nematodes (Skorping et al. 1991; Morand 1996).

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LITERATURE CITED

- Avery, L. 1993. The genetics of feeding in *Caenorhabditis elegans*. *Genetics* 133:897–917.
- Basten, C. J., B. S. Weir, and Z. B. Zeng. 1994. ZMAP—a QTL cartographer. Pp. 65–66 in C. Smith, J. S. Savora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, B. W. Kennedy, and E. B. Burnside, eds. Proceedings of the 5th world conference on genetics applied to livestock production. Vol. 22. Organizing Committee, 5th World Conference on Genetics Applied to Livestock Production, Guelph, Ontario, Canada.
- Böhni, R., J. Riesgo-Escovar, S. Oldham, W. Brogiolo, H. Stocker, B. F. Andruss, K. Beckingham, and E. Hafen. 1999. Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1–4. *Cell* 97:865–875.
- Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77:71–94.
- Chen, C., J. Jack, and R. S. Garofalo. 1996. The *Drosophila* insulin receptor is required for normal growth. *Endocrinology* 137:846–856.
- Churchill, G. A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971.
- Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282:2012–2018.
- Cox, G. N., J. S. Laufer, M. Kusch, and R. S. Edgar. 1980. Genetic and phenotypic characterization of roller mutants of *Caenorhabditis elegans*. *Genetics* 95:317–339.
- Darwin, C. 1859. The origin of species by means of natural selection. John Murray, London.
- Ebert, R. H. d., V. A. Cherkasova, R. A. Dennis, J. H. Wu, S. Ruggles, T. E. Perrin, and R. J. Reis. 1993. Longevity-determining genes in *Caenorhabditis elegans*: chromosomal mapping of multiple noninteractive loci. *Genetics* 135:1003–1010.
- Egilmez, N. K., R. H. Ebert, and R. J. S. Reis. 1995. Strain evolution in *Caenorhabditis elegans*—transposable elements as markers of interstrain evolutionary history. *J. Mol. Evol.* 40:372–381.
- Emmons, S. W. 1988. The Genome. Pp. 47–49 in W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, New York.
- Estevez, M., L. Attisano, J. L. Wrana, P. S. Albert, J. Massague, and D. L. Riddle. 1993. The *daf-4* gene encodes a bone morphogenetic protein-receptor controlling *C. elegans* dauer larva development. *Nature* 365:644–649.
- Fernandez, R., D. Tabarini, N. Azpiazu, M. Frasch, and J. Schlessinger. 1995. The *Drosophila* insulin-receptor homolog: a gene essential for embryonic-development encodes 2 receptor isoforms with different signaling potential. *Embo. J.* 14:3373–3384.
- Frary, A., T. C. Nesbitt, S. Grandillo, E. van der Knaap, B. Cong, J. P. Liu, J. Meller, R. Elber, K. B. Alpert, and S. D. Tanksley. 2000. fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289:85–88.
- Gems, D., A. J. Sutton, M. L. Sundermeyer, P. S. Albert, K. V. King, M. L. Edgley, P. L. Larsen, and D. L. Riddle. 1998. Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* 150:129–155.
- Haley, C. S., and S. A. Knott. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* 69:315–324.
- Hodgkin, J., and T. Doniach. 1997. Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* 146:149–164.
- Jakubowski, J., and K. Kornfeld. 1999. A local, high-density, single-nucleotide polymorphism map used to clone *Caenorhabditis elegans* *cdf-1*. *Genetics* 153:743–752.
- Johnson, T. E., and W. B. Wood. 1982. Genetic-analysis of life-span in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 79:6603–6607.
- Kader, N. A., and M. G. Côté. 1996. Isolation, identification and characterization of some strains of *Caenorhabditis elegans* (Mau-pas, 1900) from Quebec. *Fund. App. Nematol.* 19:381–389.
- Keightley, P. D., T. Hardge, L. May, and G. Bulfield. 1996. A genetic map of quantitative trait loci for body weight in the mouse. *Genetics* 142:227–235.
- Kimura, K. D., H. A. Tissenbaum, Y. X. Liu, and G. Ruvkun. 1997. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277:942–946.
- Koch, R., H. G. A. M. van Luenen, M. van der Horst, K. L. Thijssen, and R. H. A. Plasterk. 2000. Single nucleotide polymorphisms in wild isolates of *Caenorhabditis elegans*. *Genome Res.* 10:1690–1696.
- Korswagen, H. C., R. M. Durbin, M. T. Smits, and R. H. A. Plasterk.

1996. Transposon Tc1-derived, sequence-tagged sites in *Caenorhabditis elegans* as markers for gene-mapping. *Proc. Natl. Acad. Sci. U.S.A.* 93:14680–14685.
- Kramer, J. M., R. P. French, E. C. Park, and J. J. Johnson. 1990. The *Caenorhabditis elegans* *rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. *Mol. Cell. Biol.* 10:2081–2089.
- Krishna, S., L. L. Maduzia, and R. W. Padgett. 1999. Specificity of TGF β signalling is conferred by distinct type I receptors and their associated SMAD proteins in *Caenorhabditis elegans*. *Development* 126:251–260.
- Lander, E. S., and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199.
- Lebreton, C. M., and P. M. Visscher. 1998. Empirical nonparametric bootstrap strategies in quantitative trait loci mapping: conditioning on the genetic model. *Genetics* 148:525–535.
- Lebreton, C. H., P. M. Visscher, C. S. Haley, A. Semikhodskii, and S. A. Quarrie. 1998. A nonparametric bootstrap method for testing close linkage vs. pleiotropy of coincident quantitative trait loci. *Genetics* 150:931–943.
- Lee, R. Y. N., L. Lobel, M. Hengartner, H. R. Horvitz, and L. Avery. 1997. Mutations in the alpha 1 subunit of an L-type voltage-activated Ca $^{2+}$ channel cause myotonia in *Caenorhabditis elegans*. *Embo. J.* 16:6066–6076.
- Leroi, A. M. 2000. The scale independence of evolution. *Evol. Dev.* 2:67–77.
- . 2001. Molecular signals versus the *Loi de Balancement*. *Tr. Ecol. Evol.* 16:24–29.
- Li, Z. K., S. R. M. Pinson, J. W. Stansel, and A. H. Paterson. 1998. Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). *Mol. Breed.* 4: 419–426.
- Liu, B. H. 1998. *Statistical genomics*. CRC Press, New York.
- Macdonald, S. J., and D. B. Goldstein. 1999. A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. *Genetics* 153:1683–1699.
- Martinez, O., and R. N. Curnow. 1992. Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. *Theor. Appl. Genet.* 85:480–488.
- Morand, S. 1996. Life-history traits in parasitic nematodes: a comparative approach for the search of invariants. *Funct. Ecol.* 10: 210–218.
- Nuzhdin, S. V., C. L. Dilda, and T. F. C. Mackay. 1999. The genetic architecture of selection response: inferences from fine-scale mapping of bristle number quantitative trait loci in *Drosophila melanogaster*. *Genetics* 153:1317–1331.
- Park, E. C., and H. R. Horvitz. 1986. *C. elegans unc-105* mutations affect muscle and are suppressed by other mutations that affect muscle. *Genetics* 113:853–867.
- Rahman, M. A., and N. Maclean. 1999. Growth performance of transgenic tilapia containing an exogenous piscine growth hormone gene. *Aquaculture* 173:333–346.
- Reiner, D. J., D. Weinshenker, and J. H. Thomas. 1995. Analysis of dominant mutations affecting muscle excitation in *Caenorhabditis elegans*. *Genetics* 141:961–976.
- Reznick, D., L. Nunnev, and A. Tessier. 2000. Big houses, big cars, superfleas, and the costs of reproduction. *Trends Ecol. Evol.* 15: 421–425.
- Riska, B. 1989. Composite traits, selection response, and evolution. *Evolution* 43:1172–1191.
- Roff, D. A. 1992. *The evolution of life histories; theory and analysis*. Chapman and Hall, New York.
- . 1996. The evolution of genetic correlations: an analysis of patterns. *Evolution* 50:1392–1403.
- . 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *J. Evol. Biol.* 13: 434–445.
- Rollo, C. D., J. Rintoul, and L. J. Kajiura. 1997. Lifetime reproduction of giant transgenic mice: the energy stress paradigm. *Can. J. Zool.* 75:1336–1345.
- Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* 38:1004–1010.
- . 1991. *The evolutionary biology of aging*. Oxford Univ. Press, Oxford, U.K.
- Savage, C., P. Das, A. L. Finelli, S. R. Townsend, C. Y. Sun, S. E. Baird, and R. W. Padgett. 1996. *Caenorhabditis elegans* genes *sma-2*, *sma-3*, and *sma-4* define a conserved family of transforming growth factor beta pathway components. *Proc. Natl. Acad. Sci. U.S.A.* 93:790–794.
- Shook, D. R., and T. E. Johnson. 1999. Quantitative trait loci affecting survival and fertility-related traits in *Caenorhabditis elegans* show genotype-environment interactions, pleiotropy and epistasis. *Genetics* 153:1233–1243.
- Shook, D. R., A. Brooks, and T. E. Johnson. 1996. Mapping quantitative trait loci affecting life-history traits in the nematode *Caenorhabditis elegans*. *Genetics* 142:801–817.
- Skorping, A., A. F. Read, and A. E. Keymer. 1991. Life-history covariation in intestinal nematodes of mammals. *Oikos* 60: 365–372.
- Snedecor, G. W., and W. G. Cochran. 1989. *Statistical methods*. Iowa State Univ. Press., Ames, IA.
- Stearns, S. C. 1992. *The evolution of life histories*. Oxford Univ. Press, New York.
- Sulston, J., and J. Hodgkin. 1988. *Methods*. Pp. 587–606 in W. B. Wood, ed. *The nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, New York.
- Suzuki, Y., M. D. Yandell, P. J. Roy, S. Krishna, C. Savage-Dunn, R. M. Ross, R. W. Padgett, and W. B. Wood. 1999. A BMP homolog acts as a dose-dependent regulator of body size and male tail patterning in *Caenorhabditis elegans*. *Development* 126:241–250.
- Tanksley, S. D. 1993. Mapping polygenes. *Annu. Rev. Genet.* 27: 205–233.
- True, J. R., B. S. Weir, and C. C. Laurie. 1996. A genome-wide survey of hybrid incompatibility factors by the introgression of marked segments of *Drosophila mauritiana* chromosomes into *Drosophila simulans*. *Genetics* 142:819–837.
- True, J. R., J. J. Liu, L. F. Stam, Z. B. Zeng, and C. C. Laurie. 1997. Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. *Evolution* 51:816–832.
- Vischer, N. O. E., Huls, P. G., Woldringh, C. L. 1994. Object-image: an interactive image-analysis program using structured point collection. *Binary* 6:160–166.
- Visscher, P. M., C. S. Haley, and R. Thompson. 1996. Marker-assisted introgression in backcross breeding programs. *Genetics* 144:1923–1932.
- Vonmende, N., D. M. Bird, P. S. Albert, and D. L. Riddle. 1988. *dpy-13*—a nematode collagen gene that affects body shape. *Cell* 55:567–576.
- Whittaker, J. C., R. Thompson, and P. M. Visscher. 1996. On the mapping of QTL by regression of phenotype on marker-type. *Heredity* 77:23–32.
- Williams, B. 1995. Genetic mapping with polymorphic sequence-tagged sites. Pp. 81–97 in H. F. Epstein and D. C. Shakes, eds. *Caenorhabditis elegans; modern biological analysis of an organism*. Academic Press, London, U.K.
- Williams, B. D., B. Schrank, C. Huynh, R. Shownkeen, and R. H. Waterston. 1992. A genetic-mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* 131:609–624.
- Williams, G. C. 1957. Pleiotropy, natural selection and the evolution of senescence. *Evolution* 11:398–411.
- . 1966. Natural selection, the costs of reproduction and a refinement of Lack's principle. *Am. Nat.* 100:687–690.