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ORIGINAL ARTICLE

Mapping phenotypic plasticity and genotype–environment interactions affecting life-history traits in *Caenorhabditis elegans*

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Phenotypic plasticity and genotype–environment interactions (GEI) play an important role in the evolution of life histories. Knowledge of the molecular genetic basis of plasticity and GEI provides insight into the underlying mechanisms of life-history changes in different environments. We used a genomewide single-nucleotide polymorphism map in a recombinant N2 × CB4856 inbred panel of the nematode *Caenorhabditis elegans* to study the genetic control of phenotypic plasticity to temperature in four fitness-related traits, that is, age at maturity, fertility, egg size and growth rate. We mapped quantitative trait loci (QTL) for the respective traits at 12 and 24°C, as well as their plasticities. We found genetic variation and GEI for age at maturity, fertility, egg size and growth rate. GEI in fertility and egg size was attributed to changes in rank order of reaction norms. In case of age at maturity and growth rate, GEI was caused mainly by differences in the among-line variance. In total, 11 QTLs were detected, five QTL at 12°C and six QTL at 24°C, which were associated with life-history traits. Five QTL associated with age at maturity, fertility and growth rate showed QTL × environment interaction. These colocalized with plasticity QTL for the respective traits suggesting allelic sensitivity to temperature. Further fine mapping, complementation analyses and gene silencing are planned to identify candidate genes underlying phenotypic plasticity for age at maturity, fertility and growth.
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Introduction

Phenotypic plasticity is the ability of a genotype to produce different phenotypes in response to changing environmental conditions (Bradshaw, 1965). It is a widespread phenomenon and occurs throughout several kingdoms. Some well-known examples are temperature-dependent sex determination in reptiles (Crews *et al*., 1994), seasonal polyphenism in various species of butterflies in response to temperature and/or humidity (Roskam and Brakefield, 1996, 1999), or 'shade-avoidance' developmental response in several types of plants (Pigliucci, 1996). Plasticity can be visualized by plotting measurements for the same trait in different environments for a given genotype, also called the norm of reaction. Genotype-by-environment interactions (GEI) can become manifest if there is more genetic variance in one environment than the other (Kassen and Bell, 2000). On the other hand, GEI can be generated if the genotypes respond inconsistently to environmental variation that is visualized by the crossing of norms of reaction.

Phenotypic plasticity and GEI play an important role in the evolution of life histories (Roff, 2002). Knowledge of the genetic basis of plasticity and GEI provide insight into the underlying mechanisms of such life-history changes. A few detailed molecular studies have unravelled specific genes underlying plasticity such as the heat-shock response in *Drosophila* (Bettencourt *et al*., 2002) and recently Promislow (2005) unravelled a gene regulatory network of plasticity in yeast. Yet, knowledge about the genetic mechanisms of plasticity and GEI in life-history traits, which are complex and polygenic, is scant. Via *et al*. (1995) presented a relatively simple concept of the genetic control of phenotypic plasticity and GEI. They distinguished two classes of genetic models: regulatory loci that alter gene expression across environments, or loci displaying environmentally based allelic sensitivity. Quantitative trait loci (QTL) mapping offers a powerful tool for studying the genetic control of plasticity and GEI (Ungerer *et al*., 2003). Previously, a number of QTL studies focused on GEI and plasticity in single life-history traits in various species for lifespan in *Drosophila* (Vieira *et al*., 2000), inflorescence development in *Arabidopsis* (Ungerer *et al*., 2003) and various life-history traits in *Arabidopsis* (Hausmann *et al*., 2005).

In this paper, we investigate the genetic control of phenotypic plasticity to temperature of four fitness-related life-history traits in an N2 × CB4856 mapping population of the bacterivorous nematode *Caenorhabditis elegans*. Both parental strains have contrasting life-history traits (Hodgkin and Doniach, 1987). The objectives were: (i) to study genetic variation (GEI) in phenotypic plasticity, (ii) to map genomic regions associated with plasticity in the life-history traits and (iii) to study QTL–

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environment interactions. We found that the wild strains N2 and CB4856 differed in their plastic responses to temperature for a number of life-history traits. After crossing these strains, we obtained genetically homozygous recombinant inbred (RI) strains by a selfing procedure. As these strains can be maintained virtually indefinitely, this allows for the use of 'clones' in different environments and in different experiments in time. We analyzed a set of 80 RI strains at two temperatures (12 and 24°C) that reflected the environmental extremes for the phenotypic characteristics of several life-history traits. The strains have been single-nucleotide polymorphism (SNP) genotyped genomewide making it possible to perform a full genome coverage of QTL involved in phenotypic plasticity. Although several studies using *C. elegans* as a model organism have looked at the effect of the environment on life-history traits before (Johnson and Hutchinson, 1993; Shook *et al*., 1996; Shook and Johnson, 1999), none of these have analyzed plasticity by regarding it as a separate trait.

Materials and methods

Nematode culturing and construction of RI strains

Male and hermaphrodite cultures for *C. elegans* strains N2 (Bristol) and CB4856 (Hawaii) were started 6 months before breeding of the RI strains. Cultures were maintained at 15 or 20°C on standard nematode growth medium with *Escherichia coli* strain OP50 as food source (Lewis and Fleming, 1995). RI lines were grown by putting on each of 10 Petri dishes one L4 hermaphrodite of strain N2 with five males of strain CB4856, and *vice versa* on each of 10 other Petri dishes. We used a male:hermaphrodite ratio of 1:1 in the F_1 as criterion for successful mating. Around 1300 F_1 hermaphrodites were transferred to individual dishes and allowed to mature in three days at 20°C. We subsequently transferred a single mature hermaphrodite to a new dish and repeated this procedure for 20 generations. Lines that were suspected to have reproduced sexually (with a male) were excluded from subsequent transfer during the first three generations. Dishes from each previous inbreeding generation were kept at 10°C as back up to prevent lines from being lost. Following inbreeding, RI strains were stored at −80°C using standard protocols (Lewis and Fleming, 1995) before being used in experiments.

Phenotypic analysis of strains

Before being used in experiments, strains were cultured at 15°C for at least 4 weeks after recovery from −80°C. Only strains for which at least 20 nematodes within the defrosted sample recovered were used. Each parental strain and 80 RI strains were analyzed for age at maturity, fertility, growth rate and initial egg size at 12 and 24°C as described below. All experiments were carried out in Elbanton climate chambers (Elbanton, Kerkdriel, NL, Netherlands) and during each experiment temperature was monitored with a Tinytag Transit temperature logger (Gemini Data Loggers, UK). Between experiments, strains were kept at 15°C and transferred at regular intervals of 3–4 weeks. A full table of phenotypic values (and replicate numbers) for all strains can be found as Supplementary information.

Age at maturity: RI strains were synchronized by 2.5 h of egg laying by four gravid adults. Juvenile nematodes were individually transferred at room temperature to separate 3 cm Petri dishes after 24 h (24°C) or 96 h (12°C) from the start of the experiment. Dishes were randomized and returned to 12 or 24°C. Inspection of dishes for age at maturity started after 38 h, with subsequent intervals of 1.5 h for 24°C, and after 145 h, with subsequent intervals of 4 h for 12°C. Maturation was defined as the first moment at which one or more eggs per individual are laid on a dish. For matured individuals, the time of observation was registered and worms were immediately put at −20°C to prevent further development. Age at maturity was calculated as the difference between the registered time of maturation and the average of the start and end of the egg-laying period.

Fertility: Strains were synchronized by bleaching (Emmons *et al*., 1979). Bleached eggs from each strain were divided over four Petri dishes, of which two were put at 12°C and two at 24°C. After 36 h (24°C) or 72 h (12°C) for each strain, 14 L4 juveniles were individually transferred to separate dishes at room temperature and subsequently allowed to mature at 24°C or 12°C. After maturation, adults were transferred daily (24°C) or every 5 days (12°C) at room temperature to new dishes until the end of reproduction (i.e., when worms stopped laying fertilized egg sizes). Offsprings were allowed to develop to the L3–L4 stage and were subsequently killed with 70% ethanol or put at 2°C to prevent further development. Only this offspring was counted to determine fertility.

Egg size: Strains were synchronized by bleaching. Bleached eggs from each strain were divided over four Petri dishes, of which two were put at 12°C and two at 24°C. Worms were then allowed to mature. After 3 days (24°C) or 7 days (12°C), pictures were taken from eggs with a CoolSnap camera (Roper Scientific Photometrics, Tucson, AZ, USA) at ×43.75 magnification. Area and perimeter of each egg were measured automatically with Image Pro Express 4.0 (Media Cybernetics, Silver Spring, MD, USA). Using a measurement ocular, we calibrated 10 000 pixels^3 as 7591 μm^3. Using an estimated radius r (in pixels) for each egg, we calculated the volume V_{EGG} with the function:

$$V_{EGG} = \frac{4}{3}\pi r^3 \quad (1)$$

In subsequent analyses, V_{EGG} was used as input value for egg size.

Growth rate: Strains were synchronized by bleaching. Following bleaching, eggs were divided over four replicates dishes for each strain for both temperatures. Starting 20 h (24°C) or 65 h (12°C) after bleaching, pictures were taken at regular intervals for four (24°C) or 10 (12°C) subsequent days using a CoolSnap camera (Roper Scientific Photometrics). Pictures were taken at ×7.25 magnification at room temperature. Area (R) and perimeter (P) of each worm were measured in pixels automatically with Image Pro Express 4.0 (Media Cybernetics, USA) and used to calculate nematode volume with $V_{GRO}(t) = \pi R^2/(2P)$ at time t. These values

were used to calculate the growth rate parameter C, which determines the slope of the curve, with the Gompertz growth model (Gompertz, 1825; Helmink *et al*., 2000):

$$V_{GRO}(t) = A \cdot e^{-e^{B-C \cdot t}} \quad (2)$$

where t is the time from bleaching, A is the estimated asymptotic (maximal) volume and B is a parameter determining the starting point of logistic growth rate. C was used as input for growth rate in subsequent analyses.

Statistics and computations

Life-history trait analysis: Split over both temperature and strains, and for growth rate also split over age at measurement, all data was compared against a normal distribution using a one-sample Kolmogorov–Smirnov test (two-tailed, significance cutoff value of $P<0.05$). For both temperatures, mean values of age at maturity, fertility and egg size were calculated to be used in QTL analysis. Growth rate parameters A, B and C were estimated by non-linear regression of Equation (2) using the Levenbergh–Marquardt algorithm with at maximum 999 iterations and parameter convergence at 10^{-10} as implemented by SPSS (2001). To obtain starting values for A, B and C for analysis per strain, we first analyzed for both temperatures all data for all strains using $A=50\,000$, $B=5$ and $C=1$.

Genotype–environment interaction: To determine the effect of temperature and strain on the traits age at maturity, fertility and egg size, we performed two-way analysis of variance (ANOVA) using the univariate generalised linear model (GLM)-procedure of SPSS, with each time one of the traits as dependent variable, and temperature and strain as factor. The factor strain was considered random. For growth rate, we could not perform a straightforward ANOVA as the data used for obtaining the growth rate parameters depended in more than one way (both size and developmental time) on temperature. However, as growth rate is based on the development of maximum body size during time the results of the ANOVA of age at maturity will indicate the effect of temperature on growth rate. In addition, to get even more insight of the influence of temperature and genotype on growth rate, we compared for several time points the maximum body sizes with which growth rate eventually was calculated. To make these measurements comparable between temperatures (as one day of development at 12°C does not correspond to one day at 24°C), we divided the age at measuring by the calculated age (using data for all strains) at which 50% of the asymptotic size would have been reached. Using these values as dependent variables and temperature and strain as factors, we performed ANOVA as described above.

Significant GEI can result from two sources: deviation from unity of the cross-environment genetic correlation and differences in the among-line variance in the different environments. The proportion of each of these sources can be derived from the equation (Robertson, 1959) $V_{GxE}=0.5(\sigma_{G1}-\sigma_{G2})^2+\sigma_{G1}\sigma_{G2}(1-\rho_{G1G2})$, where $V_{G\times E}$ is the genotypic GEI variance, σ_{G1} and σ_{G2} are the genetic standard deviations of a trait expressed in environments 1 and 2, respectively, and ρ_{G1G2} is the genetic correlation of that character across environments 1 and 2. The second term corresponds to the lack of perfect correlation and first term to differences in the among-line variance.

Phenotypic plasticity

For QTL mapping purposes, we defined phenotypic plasticity for all traits, except growth rate as the difference between the mean trait values at 12 and 24°C. Since we only have measurements at two temperatures, this method of determining plasticity is similar to both a character state approach as well as a reaction norm approach (Via *et al*., 1995). Plasticity in growth rate, however, was defined as the ratio of the values for growth rate at 12 and 24°C since growth rate was a derived rather than an absolute measurement for which it seemed inappropriate to use the definition of plasticity as carried out for the other traits.

Genetic constitution of RI strains

The genetic analysis of the RI strains is described elsewhere in detail (Kammenga *et al*., submitted). Briefly, the average distance between two SNP markers was 835 kbp or 2.38 cM with an average chromosomal coverage of 95.9% if measured in bp and 95.3% if measured in cM. Compared to the Wormbase F_2-derived genetic maps (http://www.wormbase.org/, release WS106), the genetic maps showed on average an ample twofold expansion. This is common for RI strains and can be explained by the multiple rounds of meiosis that occurred during breeding (Dixon, 1993). Overall marker frequencies were 52.9% for the N2 allele and 47.1% for the CB4856 allele. We used 121 markers evenly distributed across the genome (20 markers on chromosomes *I*, *II*, *III*, *V*, *X*, and 21 markers on chromosome *IV*. Marker segregation analysis indicated segregation distortion toward the N2 allele on chromosome *I*, which is probably caused by selection for a gene or genes involved in reproduction at approximately −6.10 cM (Kammenga *et al*., submitted).

QTL mapping

QTL mapping was performed with QTL Cartographer (Wang *et al*., 2001). We analyzed the data by composite interval mapping assuming multilocus control of the trait. We used default settings in all analyses (walk speed 2 cM, model number 6, five background parameters, window size 10 cM and automatic selection of the background parameters). The experiment-wise threshold significance level for each trait was determined by performing 10 000 permutations on the data (Churchill and Doerge, 1994). Ninty-five percent confidence intervals for QTL were calculated according to Dupuis and Siegmund (1999).

QTL–environment interaction

All individual genotypic markers (located in either significant or nonsignificant QTL) were analyzed for QTL–environment interaction by performing an ANOVA on the trait means for all traits with temperature (12 or 24°C) and genotype (a or b) at each marker position as explaining variables.

Results

Life-history trait responses

For nearly all strains, individual life-history traits were normally distributed. Also, the subsequently calculated mean trait values per strain as well as the measurements for plasticity were normally distributed (not shown). Table 1 shows the mean trait values for both parental strains and RI strains at both temperatures.

Parental strains

The two parental strains differed from each other at both temperatures for all traits except age at maturity. Fertility in strain N2 was significantly higher at 12°C than at 24°C (*t*-test, $P=0.003$). There was no difference in fertility in CB4856 between these temperatures. With regard to egg size, parental strains responded significantly different (ANOVA, $P_{G\times E}<0.001$, $P_E<0.001$). Egg sizes from CB4856 decreased by 21.3% in size in response to higher temperatures while N2 egg sizes decreased only by 8.3%. This may reflect a difference in trade-off strategy between N2 and CB4856. At both temperatures, CB4856 was able to keep fertility constant while egg size changed dramatically. In contrast, egg size in N2 did not change much while fertility did. Growth rate at 12°C was not different between both parental strains (Table 1), but at 24°C CB4856 worms grew faster than their N2 counterparts.

Genetic variation, plasticity and GEI

As generally observed in RI crosses between divergent strains, the mean trait values for many of the RI strains exceeded the mean value for either parental strain. This transgressive variation can often be explained by the accumulation of complementary alleles in the progeny or epistatic interactions (Lynch and Walsh, 1998). Positively and negatively acting alleles at loci affecting a trait are dispersed between the parental lines, but recombine to yield more extreme phenotypes in the segregants. With regard to age at maturity, higher temperatures obviously resulted in faster maturation for all strains. On average, strains grew more rapidly at higher temperature. Two-way ANOVA with main effects of RI strain and temperature and their interaction revealed significance of main effects and the interaction for age at maturity, fertility and egg size (Table 2a). For fertility we did not find a temperature effect.

With regard to growth rate, Table 2b shows for each temperature the selected ages used for comparison, and the results of the subsequent two-way ANOVA. All effects (strain, temperature and strain × temperature) for all measurements were significant at $P<0.001$ (Table 2b).

The results firstly point out that the RI strains are genetically different with regard to all traits. Secondly, they show that age at maturity, growth and egg size are plastic across temperatures, and thirdly the RI strains differ in their plastic response, that is, there is GEI for all traits. Table 3 shows that GEI in fertility and egg size was due to changes in rank order of reaction norms. In case of age at maturity and growth rate, GEI were caused mainly by differences in the among-line variance (Figure 1).

QTL mapping of life-history traits and their plasticities

Table 4 shows the most likely locations of the QTL for all traits at 12 and 24°C. Also, the total proportion of phenotypic variance explained by the QTL detected is shown in this table as well as the additive effects. A positive effect reflects a larger effect of the N2 allele compared to the CB4856 allele, a negative effect indicates the opposite. Overall the number and location of QTL differed between the two temperatures. In total, 11 significant QTLs were detected, five QTL at 12°C and six QTL at 24°C, which were associated with life-history traits in the N2 × CB4856 mapping population. We found QTL at both temperatures for all traits, except egg size

Table 1 Life-history trait values for parental and RI strains for trait mean at 12 and 24°C

Trait[a]	*Strain*	*Mean ± s.e.*	
		12°C	*24°C*
Age at maturity	N2	7.02 ± 0.04	2.18 ± 0.02
	CB4856	7.01 ± 0.03	2.10 ± 0.03
	RI strains	7.09 ± 0.01	2.17 ± 0.00
H^2		0.70	0.54
Fertility	N2	234.3 ± 13.4	177.4 ± 10.4
	CB4856	159.9 ± 16.9	156.6 ± 12.8
	RI strains	172.7 ± 72.0	176.6 ± 62.4
H^2		0.21	0.20
Egg size	N2	13.0 ± 0.3	11.9 ± 0.3
	CB4856	14.4 ± 0.3	11.3 ± 0.2
	RI strains	13.2 ± 0.1	10.9 ± 0.1
H^2		0.33	0.23
Growth rate	N2	0.66 ± 0.05	1.68 ± 0.14
	CB4856	0.56 ± 0.05	2.53 ± 0.27
	RI strains	0.60 ± 0.01	1.70 ± 0.03
H^2		0.23	0.20

H^2 is broad-sense heritability that was computed as the ratio of among-RI variance component (V_G) divided by the total phenotypic variance (V_G+V_E).
[a]Trait units: age at maturity in days, egg size in ng, fertility in number of eggs and growth rate in day^{-1}.

Table 2a Results for ANOVA of age at maturity, fertility and egg size

Trait	*Source*	*d.f.*	*Mean squares*
Age at maturity	Temperature (*E*)	1	16 607***
	Strain (*G*)	81	0.772***
	G × *E*	81	0.602***
	Error	2831	0.087
Fertility	Temperature (*E*)	1	8300
	Strain (*G*)	80	14 672***
	G × *E*	78	14 426***
	Error	1750	3583
Egg size	Temperature (*E*)	1	2.209E+10***
	Strain (*G*)	81	1.967E+08***
	G × *E*	76	7.166E+07***
	Error	1467	2.435E+07

Abbreviations: ANOVA, analysis of variance; d.f., degrees of freedom.
*$P<0.05$.
**$P<0.01$.
***$P<0.001$.

Table 2b Results for ANOVA of data on which GRO parameters were based

Age		*Source*	*d.f.*	*Mean squares*
12°C	*24°C*			
3.16	1.08	Temperature (*E*)	1	5 487 424***
		Strain (*G*)	70	134 0869***
		$G \times E$	57	381 483*
		Error	781	276 799
4.69	1.50	Temperature (*E*)	1	1.159E+09***
		Strain (*G*)	33	3.597E+07***
		$G \times E$	32	2.815E+07***
		Error	558	1.216E+07
7.70	2.50	Temperature (*E*)	1	8.611E+09***
		Strain (*G*)	33	3.041E+08***
		$G \times E$	32	7.546E+07***
		Error	579	2.972E+07
9.00	2.83	Temperature (*E*)	1	7.575E+09***
		Strain (*G*)	76	3.267E+08***
		$G \times E$	72	1.160E+08***
		Error	1106	3.727E+07
12.15	3.83	Temperature (*E*)	1	9.116E+09***
		Strain (*G*)	78	4.109E+08***
		$G \times E$	69	1.381E+08***
		Error	699	5.315E+07

Abbreviations: ANOVA, analysis of variance; d.f., degrees of freedom; GRO, growth rate.
Measurements in time were made comparable between temperatures by dividing the age (in days, at measuring) by the calculated age at which 50% of the asymptotic size would have been reached (6.27 days for 12°C; 2.01 days for 24°C).
*$P<0.05$.
**$P<0.01$.
***$P<0.001$.

Table 3 Proportion of $V_{G \times E}$ that can be attributed to the deviation of the cross-environment genetic correlation from unity and to changes in the among-line variance in two temperatures

	Proportion explained by cross-environment correlation	*Proportion explained by changes in variance*	$r_{G \times E}$
Age at maturity	0.37	0.63	0.29
Fertility	0.99	0.01	0.02
Egg size	0.95	0.05	0.52
Growth rate	0.41	0.59	0.33

$r_{G \times E}$ is cross-environment genetic correlation calculated as $cov_{E1E2}/\sigma_{E1}\sigma_{E2}$. In the case of growth rate, we calculated the proportions on the basis of the average growth rate during the whole juvenile period.

and growth rate for which we did not detect QTL at 24°C. This could not be due to low heritabilites (at 24°C broad-sense heritability was 0.23 for egg size and 0.20 for growth rate), but may due to the relatively low number of mapping populations (Beavis, 1998). Failure to detect significant QTL was also found for *Arabidopsis* by Weinig *et al*. (2003b). As they mention, QTL explaining less than 5% of the total phenotypic variation will not be detected (Lynch and Walsh, 1998).

For age at maturity and fertility, QTLs were found on different chromosomes for each temperature. Three QTL associated with age at maturity (two QTL at chromosome *II*) and fertility (one QTL at chromosome *IV*) showed QTL × environment interaction (Figure 2), suggesting that the underlying genetic mechanism for these traits differs between the two temperatures.

Table 4 Positions of QTL maxima for age at maturity, fertility, growth rate parameter *C* and initial egg size size at 12 and 24°C

Trait	*QTL*			*Effect*	
	Map position[a]	*LOD*	R^2	*abs*	*%*
Age at maturity 12°C	II: −3.51***	3.86**	0.14	−0.1116	−1.58
	II: 0.45***	3.36*	0.13	−0.1052	−1.49
Age at maturity 24°C	I: −1.48	9.12***	0.28	0.0441	2.04
	I: 11.53	5.21***	0.19	0.0317	1.46
	III: −18.01	2.80*	0.07	−0.0196	−0.90
	IV: 10.22	3.15*	0.08	0.0211	0.97
	X: 3.04	3.54**	0.10	0.0221	1.02
Fertility 12°C	IV: −9.96**	5.84***	0.29	19.91	11.38
Fertility 24°C	V: 5.78	2.72*	0.12	11.33	6.38
Growth rate 12°C	I: 13.88	3.00*	0.12	−0.0881	−14.67
Egg size 12°C	IV: −21.28	3.02*	0.15	0.005	−3.64

Abbreviations: ANOVA, analysis of variance; d.f., degrees of freedom; GRO, growth rate; LOD, linkage of disequilibrium; QTL, quantitative trait loci.
No significant QTLs were found for growth rate at 24°C and egg size at 24°C. Significance for LOD scores (*$P<0.05$, **$P<0.01$, ***$P<0.001$) based on experiment-wise significance levels from 10 000 permutations. R^2 indicates the proportion of the among-strain variance explained by the QTL. A positive effect is a bigger effect of the N2 allele compared to CB4856, a negative effect means the opposite. Absolute effects for age at maturity in days, egg size in ng, for fertility in number of eggs, and for growth rate in $days^{-1}$.
[a]Marked QTL positions show significant QTL × *E* interaction (**$P<0.01$, ***$P<0.001$).

Figure 2 also shows five plasticity QTLs. Two associated with plasticity of age at maturity on chromosomes *II* and *IV*, one QTL associated with plasticity of fertility on chromosome *IV* and two plasticity QTLs for growth rate on chromosomes *II* and *III*. The markers for which we found interaction with the environment were harbored by the plasticity QTL for the respective traits (plasticity in growth rate: two QTLs showed QTL × environment interaction at chromosomes *II* and *III*). This suggests that phenotypic plasticity of age at maturity, fertility and growth rate to temperature is controlled by loci that are sensitive to temperature changes. Plasticity QTL overlapped (the 1 linkage of disequilibrium (LOD) support limits of the plasticity QTL overlap with the support limits for the single trait QTL; cf. Ungerer *et al*., 2003) with significant, or suggestive QTL in case of age at maturity (chromosome *IV*) and growth rate. This may not be surprising because plasticity loci are likely to have significant effects at either temperature.

Discussion

Genetics of temperature–response traits

A principal goal of evolutionary biology is to gain insight into the mechanisms of adaptation to heterogeneous environments (Roff, 2002). We have studied the genetic control underlying plasticity and GEI in response to high and low temperature for four life-history traits in the nematode *C. elegans*. All traits showed plasticity and GEI

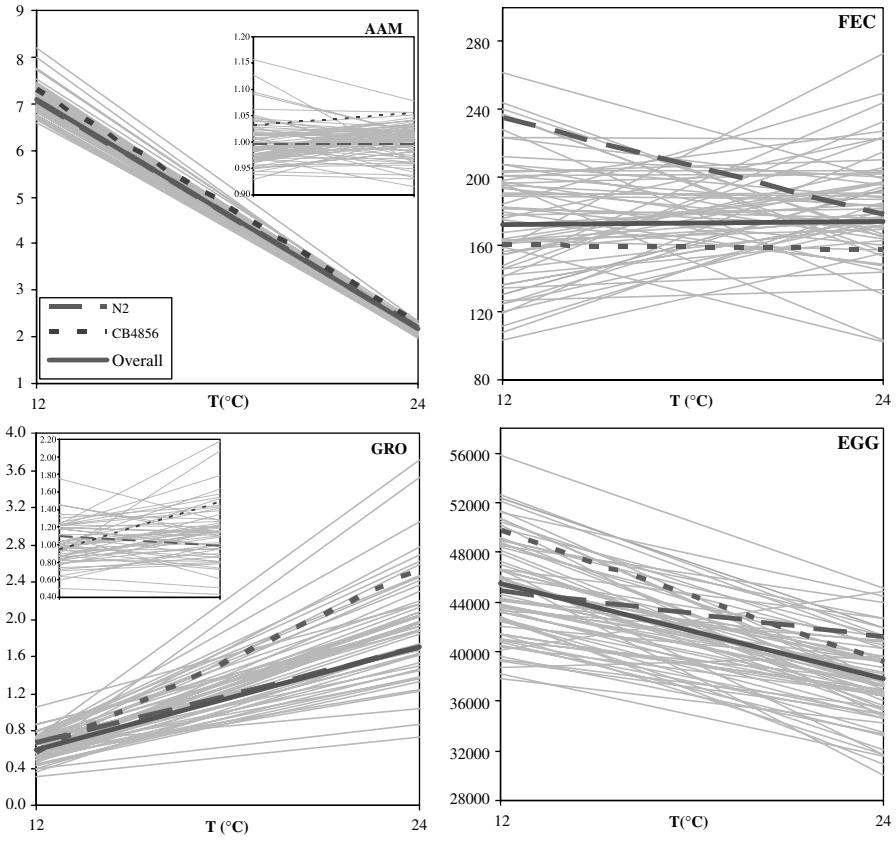

Figure 1 Reaction norms (plastic responses to temperature) for age at maturity (AAM), fertility (FER), egg size (EGG) and growth rate (GRO). Subfigures show absolute reaction norms, and insets in figures for age at maturity and growth rate show reaction norms relative to the overall mean (which equals 1 at both temperatures, lines for overall mean in insets not shown) for all strains for each trait.

with regard to changing temperature. For fertility and egg size, the GEI was mainly caused by changes in rank order expressed as crossing of reaction norms. Studying three temperature environments, Gurganus *et al*. (1998) also reported that GEI for bristle number in *Drosophila* was mainly attributed to changes in rank order of reaction norms.

Of all QTL detected, 27.3% exhibited a significant interaction with the environment. Gurganus *et al*. (1998) reported 70% of all QTL associated with bristle number exhibited GEI with sex and temperature. Using two environments, solid and liquid culture media Shook and Johnson (1999) found 50% of the four QTLs associated with *C. elegans*' lifespan showing a significant QTL–environment interaction. Instead, Vieira *et al*. (2000) reported that nearly all QTL affecting lifespan exhibited QTL–environment interaction in *Drosophila*. Dilda and Mackay (2002) reported 33–50% *Drosophila* bristle number QTLs having an interaction with temperature. QTL–environment interactions for life-history traits are of great importance for adaptive life-history changes in different environments and for maintaining genetic variation in changing environments. Shook and Johnson (1999) point out that the existence of QTL–environment interactions implies a cautious approach toward interpreting pleiotropic effects underlying life-history traits from an evolutionary point of view. Such effects should be studied under evolutionary relevant environmental conditions, thereby reducing the probability of recording false positives. We did not find QTL–environment interactions for other traits. Ayyadevara *et al*. (2003) also reported no environment interaction for lifespan QTL in *C. elegans*. Yet, this does not necessarily imply that such interactions do not exist, but certainly indicates that much larger population sizes or more dense genetic maps would be necessary to detect them.

We mapped phenotypic plasticity as a separate trait and found that plasticity QTLs were exhibiting QTL–environment interactions. This may indicate that the plasticity QTL harbored environment-specific genomic regions, suggesting that candidate genes underlying phenotypic plasticity are differentially expressed depending on the environmental conditions. This was also found for *Arabidopsis* by Ungerer *et al*. (2003), who reported the identification of QTL showing allelic sensitivity through the colocalization of sensitivity and main effect QTL. Similar results were obtained by Hausmann *et al*. (2005), also for *Arabidopsis*. Following the work of Remold and Lenski (2001), although obtained for *E. coli*, we expect that these environment specific regions likely harbor many genes affecting GEI rather than a few single so-called 'plasticity' genes.

The position of fertility QTL on chromosome *IV* was in agreement with the few studies which have reported on QTL mapping of life-history traits in *C. elegans*, all of which were conducted at 20°C. Shook and Johnson (1999) also found a QTL for fertility on the same chromosome. Yet, these authors also found QTL on other

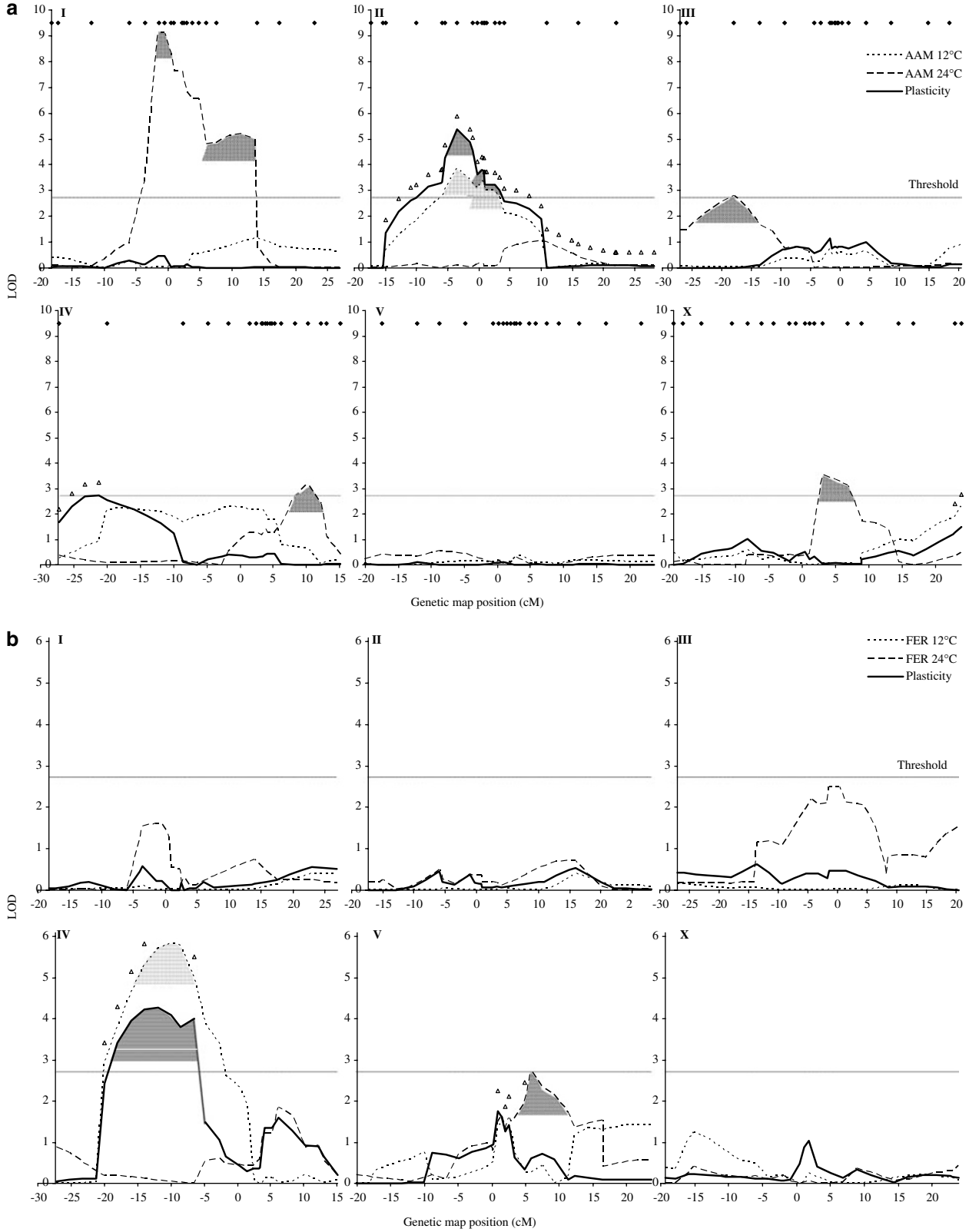

Figure 2 Genetic maps showing QTL-mapping results for age at maturity (**a**), fertility (**b**), growth rate (**c**) and egg size (**d**) at 12 and 24°C and for plasticity in each trait. Diamonds in (**a**) show the positions of the markers. Open triangles in each figure show the regions that display QTL × environment interaction ($P<0.05$). Ninety-five percent confidence intervals of significant QTL (maximum width of hatched regions) are based on decrement of 1 LOD. Threshold values in each figure are the highest 95% threshold score for one of the three sub-analyses (both temperatures and plasticity): (**a**) at 2.77 LOD for plasticity in age at maturity; (**b**) at 2.76 LOD for fertility at 12°C; (**c**) at 2.76 LOD for plasticity in growth rate; and (**d**) at 2.72 LOD for EGG at 12°C. Threshold values for the other conditions at maximum varied 0.1 LOD. *Y* axis scale from the subfigure. (**a**) varies from other subfigures.

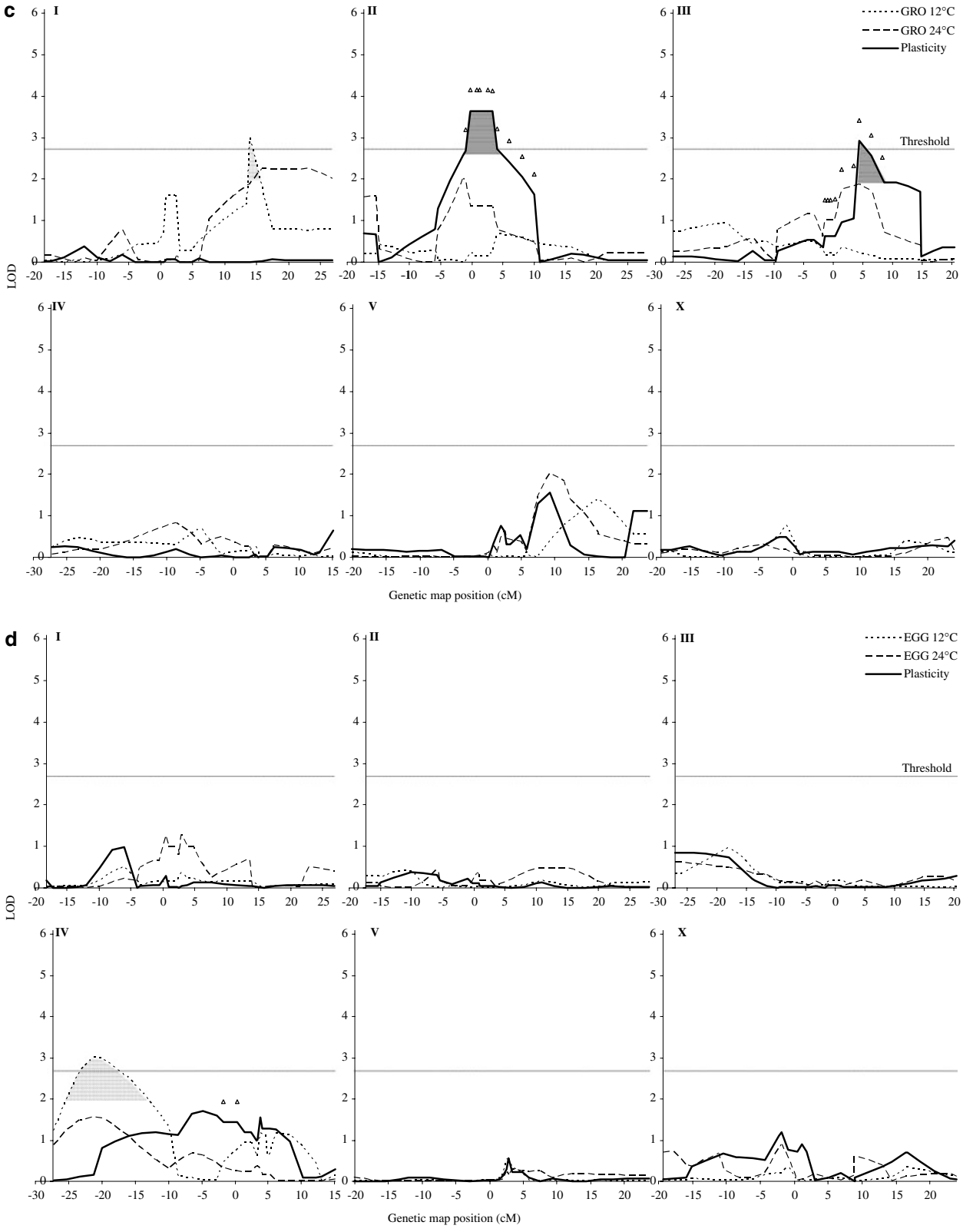

Figure 2 Continued.

chromosomes. A plausible reason for this discrepancy is that their mapping studies were based on 40 (Shook and Johnson, 1999) Tc1 transposable elements, which, in contrast to our SNP map, did not cover the full genome. Moreover, they used RI strains derived from an N2 × BO cross and were performed at a different temperature.

When comparing the QTL patterns for one trait at two temperatures, the general pattern that emerges is that

QTL mapping results for one trait vary across environments. Also in *Drosophila melanogaster* (Vieira *et al*., 2000) and *Arabidopsis thaliana* (Stratton, 1998; Rauh *et al*., 2002), different QTLs for single traits were discovered in response to varying environmental conditions.

As mentioned by Weinig *et al*. (2003b), QTL mapping also provides a means to test for environment-specific phenotypic expression at specific loci. We found temperature-specific QTL for various traits. Zimmerman *et al*. (2000) mapped QTL affecting components of wing shape in *Drosophila* at different temperatures. About half of the QTL were found at different temperatures, suggesting that temperature-specific genetic factors may exist, underlining the genetic complexity of the life-history traits. Such environment-specific expression underlies differences in heritability estimates that are commonly observed for the same trait measured in different environments (Falconer and Mackay, 1997; Lynch and Walsh, 1998; Weinig *et al*., 2003a). Using a mapping population of *Arabidopsis* in natural environments, Weinig *et al*. (2003a) reported that variation at specific loci determined resistance to rabbit herbivory in either the autumn and spring seasonal cohorts, but not both. The QTL–environment interactions imply that variation in certain loci may be masked from selection in some environments and that different QTL will be exposed to selection in different natural environments (Weinig *et al*., 2003b).

Molecular mechanisms of plasticity

Attempts for unraveling the molecular genetic basis of plasticity generally assume two different mechanisms, allelic sensitivity and gene regulation as presented by Via *et al*. (1995). The latter implies that regulatory loci alter gene expression across environments, and the first refers to loci displaying environmentally based allelic sensitivity (pleiotropic model and correlated responses (Scheiner, 1993). We found that plasticity QTL colocalized with the single trait QTL and these QTL exhibited QTL × environment interaction. These findings support the allelic sensitivity model. Ungerer *et al*. (2003) studied GEI at QTL affecting inflorescence development in *Arabidopsis* and reported QTL exhibiting allelic sensitivity through the colocalization of sensitivity and main effect QTL. Similar results were obtained by Hausmann *et al*. (2005). However, we believe that conclusions considering allelic sensitivity drawn from QTL studies should be approached with great caution. Firstly, there are very good reasons to think that one simply cannot infer gene or allele function from population-level statistical patterns such as QTL analyses. Houle (1991) shows that genetic redundancy and gene–gene interactions (at the molecular level) can translate in any given pattern of gene–gene or gene–environment interaction (at the statistical level). Gromko (1995) reported that some combinations of pleiotropic gene effects constrain correlated responses to a narrow range, whereas other combinations allow a wide range. Given the large number of genes present within each QTL, the work of Gromko suggests the pleiotropic effects to be unpredictable and can yield any correlated responses. Secondly, it should be made explicit that colocalization of QTLs for different traits is not strong evidence for the hypothesis that the same allele is actually influencing the two traits, because the confidence intervals of each estimated position likely contain hundreds of genes (see also Knight *et al*., 2001). High-resolution mapping will be required in conjunction with complementation analyses of candidate gene mutants and gene silencing in order to identify the genes underlying plasticity.

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