Mapping Determinants of Gene Expression Plasticity by Genetical Genomics in C. elegans

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Recent genetical genomics studies have provided intimate views on gene regulatory networks. Gene expression variations between genetically different individuals have been mapped to the causal regulatory regions, termed expression quantitative trait loci. Whether the environment-induced plastic response of gene expression also shows heritable difference has not yet been studied. Here we show that differential expression induced by temperatures of 16 °C and 24 °C has a strong genetic component in Caenorhabditis elegans recombinant inbred strains derived from a cross between strains CB4856 (Hawaii) and N2 (Bristol). No less than 59% of 308 trans-acting genes showed a significant eQTL-by-environment interaction, here termed plasticity quantitative trait loci. In contrast, only 8% of an estimated 188 cis-acting genes showed such interaction. This indicates that heritable differences in plastic responses of gene expression are largely regulated in trans. This regulation is spread over many different regulators. However, for one group of trans-genes we found prominent evidence for a common master regulator: a transband of 66 coregulated genes appeared at 24 °C. Our results suggest widespread genetic variation of differential expression responses to environmental impacts and demonstrate the potential of genetical genomics for mapping the molecular determinants of phenotypic plasticity.


Introduction

Expression quantitative trait loci (eQTLs) are polymorphic genetic loci that cause heritable differences in mRNA concentration. eQTLs have been used in recent genetical genomics studies [1] to infer the structure of genome-wide gene regulatory networks [2–4]. The definition of eQTLs in these studies is essentially static and does not consider the highly dynamic nature of gene expression. However, mRNA levels respond rapidly to variable ambient conditions such as temperature change. This has been shown for yeast [5], bacteria [6], and C. elegans [7] after exposure to heat shock.

In contrast to these short-term exposures to extreme temperatures, populations under natural conditions are often exposed to longer periods of less extreme temperature changes. The ability to respond to these temperature changes (so-called phenotypic plasticity) differs among genotypes. Phenotypic plasticity to temperature plays an important role in the evolution of life histories in a variable climate [8] and is widespread among species. Typical examples are temperature-induced sex determination in reptiles [9] and seasonal polyphenism in butterflies [10]. The detection of temperature-specific proteins was reported by Madi et al. [11], who analyzed proteome temperature plasticity in wild-type C. elegans.

Insight into the genetic control of plasticity is a key issue for understanding evolutionary trajectories. Recently, we detected specific QTLs underlying plasticity to temperature in C. elegans life-history traits such as growth and fertility [12].

In this paper we focus on the plasticity of gene expression in C. elegans juveniles that have been exposed for their entire life to (different) constant temperatures. We used a genetical genomics approach for detecting loci controlling such gene expression plasticity (plasticity quantitative trait loci [pQTL]). It has been shown that intraspecific evolution of variations in gene expression is to a large extent dominated by intense stabilizing selection [13]. This implies that any beneficial mutation affecting gene expression levels should show its favorable effects selectively in certain environments without disrupting the existing adaptation to other conditions. This is much more likely the case for pQTLs than for nonplastic eQTLs. The “genotype-by-environment” interaction characterizing a pQTL is the prerequisite for adaptive evolution in a fluctuating environment [14]. In fact, it has been shown that more than half of the regulatory connections in a gene expression network are unique for specific conditions such as cell cycle, sporulation, DNA damage, and stress response [15]. Recently, genotype-by-environment interaction was found for genome-wide gene expression among yeast strains [16].

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Abbreviations: eQTL, expression quantitative trait locus; pQTL, plasticity quantitative trait loci; RI, recombinant inbred; RIL, recombinant inbred line; SL, single locus; TB, transband; TL, two loci

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Synopsis

It is widely documented that environmental changes will induce differential expression of genes, yet it is unknown how these patterns of environment-induced expression plasticity are inherited and how they differ between genetically divergent individuals of a biological species. In this paper the authors used recombinant inbred lines of the nematode worm *C. elegans* that were derived from parental lines originally collected in Bristol (United Kingdom) and Hawaii, and measured genome-wide gene expression at two different temperatures. Using statistical analysis tools developed for quantitative trait locus mapping, they found genes with genetically determined differences in their plastic response to temperature changes. A majority of them were found to be regulated by genes at a different genome position (regulated in *trans*). A striking observation was a group of 66 genes that share a common potential regulator and may be related to differences in fertility plasticity. These results show that differential responses of different genotypes to environmental changes are widespread. Because all species are subjected to environmental change, both at individual and evolutionary time scales, the authors’ work calls for studying the heritable component of plasticity of gene regulation in other organisms to enhance understanding of the environmental forces that drive evolutionary adaptation.

Results/Discussion

We used a set of 80 recombinant inbred (RI) strains generated from a cross of N2 (Bristol) and CB4856 (Hawaii), representing two genetic and ecological extremes of *C. elegans* [17,18]. Their genetic distance amounts to about one polymorphism per 873 base pairs [19]. Both strains have contrasting behavioral phenotypes (solitary versus gregarious) [18] and differ strikingly in their response to a temperature change [12]. We have exposed the RI strains to 16 °C and 24 °C, temperatures that are known to strongly affect phenotypic characteristics such as body size, lifespan, and reproduction [12]. Gene expression patterns were assessed by oligonucleotide microarray hybridization (Genisphere) using a distant pair design, which pairs the RI strains with the largest genetic difference on the same array, to maximize the amount of useful signal for the QTL mapping [20]. The genetic architecture of the 80 RI strains and the description of a dense single nucleotide polymorphism (SNP) map can be found in Protocol S1 and Tables S1–S3.

Genome-Wide Detection of Expression and Plasticity QTLs

Schematic examples of eQTL, temperature, and eQTL-by-temperature interaction (pQTL) effects are shown in Figure 1A–1C, respectively. We used a two-step procedure to detect pQTLs. First, we applied a separate eQTL analysis for the expression data at either temperature (see Materials and Methods). With a genome-wide significance threshold of 4.25 (corresponding to an effective *p*-value of 0.001; throughout the paper, thresholds are in units of −log10(*p*)) there are 186 transcripts with significant eQTL effects at 16 °C and 279 at 24 °C, respectively (42 of these are common for both temperatures), suggesting eQTLs vary significantly between environmental conditions. To detect how much of this difference is due to pQTLs (plasticity regulators), we used the eQTL positions from the separate analyses. We postulated that interaction must happen at positions with eQTL effects and focused on these positions in a joint statistical analysis of data from both temperatures, thereby increasing the power of the method (see Materials and Methods for details).

Differential expression for a given gene can result from cis-regulation due to variation in the region of the gene itself or from trans-regulation by other genes. The criterion used in our analysis is that the putative cis-acting QTL peak is within 2 Mb of the transcript. It is worthwhile to notice that the cis-QTLs could actually be trans-QTLs, due to the limited resolution of the mapping. We found 308 transcripts showing significant trans-acting eQTL effects (effective *p* < 0.001) and 182 of these (59%) showed a significant pQTL effect (eQTL-by-temperature interaction) (Figure 2). This indicates that a large part of the observed gene expression dynamics differs consistently between the two parental alleles at plasticity-controlling loci.

That the temperature shift indeed leads to a drastic change in the gene regulation network is confirmed by the major differential gene expression observed between the two temperatures (Figure 3A). The amount of genes with a significant eQTL is relatively small (Figure 3B), while significant pQTLs are even less common, despite their...
relatively large effect size (Figure 3C). This justifies our use of the powerful two-stage statistical analysis outlined above.

Test for Genetic Assimilation

The parental lines of our RI strains originated from two very different thermal environments, and even though they have been maintained for many generations in controlled laboratory conditions, their highly divergent genomes are still expected to reflect the original allelic differences to a large extent. This gives us a unique opportunity to test our data for evidence of the controversial concept of “genetic assimilation,” whereby originally plastic traits become genetically fixed in a novel environment, e.g., because the original selective pressure favoring plasticity is no longer experienced [21]. In our case, we predict that genetic assimilation would be observed for temperature-related traits in the Hawaiian strain: genes that show strong differential expression in the highly seasonal conditions in Bristol lost this behavior in the more constant tropical oceanic climate of Hawaii. This behavior would be reflected in the alleles in our RI strains. However, we find no evidence that genetic assimilation plays a role in the observed expression patterns. Out of 182 genes with pQTL, equal numbers of genes show strong differential expression when the plasticity-controlling trans locus carries the Hawaiian allele as when it carries the Bristol allele, and the most extreme differential expression is seen for control by the Hawaiian allele (p = 0.002, one-sided t-test), exactly the opposite of the predicted pattern. This result may be due to a lack of adaptation of Hawaiian worm strains to their specific environment, possibly due to recent population dispersal.

Functional Assessment of Temperature-Specific Coregulated Genes

The most prominent case of pQTL in our dataset is found for a group of 66 genes that map to the same genomic region (Figure 4A) and in 63 out of 66 cases have a strong eQTL only at 24 °C (Figure 4B). Of these genes, 41 have a stronger differential expression for the Hawaii allele (p = 0.05, one-sided Wilcoxon test) (Figure 4C). Such a temperature-specific “transband” (TB) seems extremely unlikely, both statistically (p << 0.001, hypergeometric test) and biologically, because it has been demonstrated recently that natural selection leads to the elimination of mutations in loci that affect many downstream gene expression levels [13]. To test that the TB is not an artifact, we applied a permutation test (Materials and Methods). The results show that the TB does have a strong and significant genetic component (p << 0.0001). In addition to three miRNA genes in this region (cel-mir-48, cel-mir-241, and cel-mir-257), potential plasticity regulators for the transband genes are listed in Table S4. Additional analysis of the partial correlation coefficients between TB genes (Materials and Methods) shows that they are only partly controlled by the plasticity regulator at the cis position. This suggests that these genes are involved in the same pathway and controlled by a number of shared upstream factors. In fact, the TB genes form a conspicuous biological unit according to a gene ontology analysis [22], with enrichments in signal transduction (p = 0.03 after multiple testing correction) and cell communication (p = 0.04 after multiple testing correction).

The expression patterns of TB genes are also significantly correlated in an independent dataset (Kim dataset) [23] as compared with randomly selected genes (one-way Kolmogorov-Smirnov test, p << 0.001) and they are enriched in the “neuronal” functional group (coexpression mount 6, p << 7.9 e-14) [23]. It is particularly interesting to see that the group of 66 TB members contains one gene for an FMRFamide-related neuropeptide (flp-9) and four for G-protein coupled receptors (C17H11.1, C48C5.1, C24B5.1, and K10C8.2), all of them uncharacterized (Fisher’s exact test, p = 0.02). Expression variations of neuropeptides of the FMRFamide-related group (flp-1 [24], flp-18, and flp-21 [25]) as well as single amino acid mutations of their G-protein coupled receptor (npr-1) [13] underlie important ecological and behavioral differences among C. elegans strains [13,24,25]. It is therefore tempting to speculate that the TB regulator occurred in two different alleles in the pedigree of the two parental populations (N2 and CB4856) because it controls an adaptive phenotypic difference in response to particular thermal conditions.

Interestingly, we found, in our related study of genotype-
by-temperature interaction in classical phenotypic traits, that a fertility QTL maps to the immediate vicinity of our transband and shows the same interaction pattern. This suggests that our TB is possibly involved in fertility regulation or regulated by the same upstream factor(s).

Estimating the Rate of False-Positives in \textit{cis}-QTL Effects

In addition to the \textit{trans}-acting (p)eQTLs, which are the primary focus of the present paper, previous studies\cite{26,27} have also reported numerous \textit{cis}-acting eQTLs, i.e., QTLs that explain expression variation of genes that are physically located at the same position as the QTL. However, as shown in Figure 4B, in our data there is a surprisingly high proportion of \textit{cis}-acting QTLs that show a negative eQTL effect ($p = 1e-9$, Fisher’s exact test). One likely explanation is the confounding effect of SNPs on array hybridization. Under the assumption that true \textit{cis}-acting QTLs with positive and negative eQTL effects should occur in equal proportions, we estimate that there are about 226 false positives among the \textit{cis}-acting QTLs (402 total \textit{cis}-QTLs minus twice the number of 88 \textit{cis}-QTLs with positive eQTL effects). Following Hughes et al. \cite{28}, we estimate that, on average, a single mismatch or indel in the ten nucleotides most 5' in our 60-mer probes would result in a significant detectable hybridization difference (about 40% decreased signal). The parental strains, N2 (for which the arrays were designed) and CB4856, differ in their genome sequence by up to one per 873 bp of aligned sequence. Ignoring, for simplicity, the unequal distribution of SNPs in coding and noncoding sequences, we thus estimate the number of genes with one influential SNP to be 238, which corresponds closely to the 226 false positives estimated above. This indicates that \textit{cis} effects are not only less relevant for regulatory gene expression plasticity (topology of the gene regulatory network), but also very prone to hybridization artifacts.

Power Analysis for Plasticity QTLs

Our ability to detect numerous pQTLs is even more striking when we consider that our approach is likely to underestimate the extent of environment-specific genotype effects (pQTLs). This underestimation is due to the fact that such effects have been diluted by measuring the average abundance of transcripts from all cells of \textit{C. elegans} (Figure 1C); it is hard to detect a large pQTL effect if such an effect is actually cell-type specific.

To check that the number of pQTLs is not seriously underestimated due to our stringent statistical threshold, which might lead to false negatives, we estimated the
SNP markers with high because this chromosome is larger than the other chromosomes. We 20 evenly spaced markers, for Chromosome V we selected 21 markers data website, (http://www.genome.wustl.edu/ge nome/celegans/1
0.1 ml 70% ethanol and subsequently dissolved in 100
l
C. elegans
l
C. Total reaction volume was 10
C. Genotyping RILs. All markers were selected on the C. elegans SNP data website, (http://www.genome.wustl.edu/genome/ecelegans/celegans_snpcgi). For Chromosomes I, II, III, IV, and X, we selected 20 evenly spaced markers, for Chromosome V we selected 21 markers because this chromosome is larger than the other chromosomes. We selected easily detectable (i.e., with a common restriction enzyme) SNP markers with high 

**Materials and Methods**

**Genetical genomics experiment. Strain culturing.** Both N2 and CB parental strains were homozygous. Strains were grown in 9-cm petri dishes at 15°C or 20°C on standard nematode growth medium with Escherichia coli strain OP50 as a food source and transferred to new dishes by a clump of agar once a week. Recombinant inbred lines (RILs) were constructed by putting, on each of ten 6-cm dishes, one J1 hermaphrodite of strain N2 with five males of strain CB4856, and vice versa on each of ten other 6-cm dishes to avoid any maternal or paternal effects. Mating was considered to be successful if the ratio of males to hermaphrodites was approximately 1:1 in the F1 hybrids. Approximately 1,500 F1 hermaphrodites were transferred to individual dishes in 2,400-well multiplates and allowed to self-fertilize at 20°C. This was repeated until F9.

**DNA isolation.** For all lines, liquid cultures in S-basal (100 mM NaCl, 50 mM KCl, 10 mM MgCl2 (pH 7.0), 5 mg/ml cholesterol) were started and allowed to develop for one week in 50-ml tissue-culture flasks at 20°C. Cultures were transferred to 10-ml blue caps and centrifuged for 5 min at 4,000 rpm. Pelleted nematodes were transferred to a 1.5-ml Eppendorf tube, washed once with 1 ml M9 buffer, and centrifuged for 3 min at 8,000 rpm. After removal of the supernatant, 300 llysis buffer (20 mM Tris-HCl [pH 8.0], 2 mM EDTA, 2% Triton X-100) and 5 l proteinase K (10 mg/ml) were added, and samples were left for 3 h at 65°C in a rotary shaker. Samples were washed with 400 ml phenolchloroformisoamylalcohol (25:24:1) and centrifuged for 3 min at 14,000 rpm, after which the upper layer was transferred to a new tube. This step was repeated once. Next, 30 ml 3 M sodium acetate (pH 5.0) and 750 pl ice-cold isopropanol was added and samples were centrifuged for 3 min at 14,000 rpm. The DNA was washed once with 1 ml 70% ethanol and subsequently dissolved in 100 ml Milli-Q water. 1 pl RNase A was added and samples were incubated for 2-3 h at 37°C, after which they were stored at 4°C.

**Genotyping RILs.** All markers were selected on the C. elegans SNP data website, (http://www.genome.wustl.edu/genome/ecelegans/celegans_snpcgi). For Chromosomes I, II, III, IV, and X, we selected 20 evenly spaced markers, for Chromosome V we selected 21 markers because this chromosome is larger than the other chromosomes. We selected easily detectable (i.e., with a common restriction enzyme) SNP markers with high 

**Probe construction and hybridization.** The parental N2 and CB4856 strains differ in their genome sequence by up to one per 873 bp of aligned sequence [19]. Kobayashi et al. reported that 85% of the SNPs were found in noncoding DNA [31]. In an attempt to minimize hybridization differences based on SNPs, 60-mer oligonucleotide microarrays were used in this study. The frozen nematode samples were ground and DNA was extracted using the Trizol method, and cleaned up with the Qiagen RNeasy Micro kit where appropriate. The DNA concentration and quality was measured with a NanoDrop spectrophotometer (http://www.nanodrop.com). cDNA was obtained using Array 900 HS kit (Genisphere, http://www.genisphere.com) and Superscript II Invitrogen). The cDNA samples were hybridized to 60-mer oligonucleotide microarrays and subsequently scanned with a GenePix 4000B using Genomics Suite 3.1 (Axon Instruments). The intensity of each probe was calculated as a percentage of the whole array signal.
minimum of secondary structure potential. All microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) and are accessible through the GEO Series accession number listed under the Accession Numbers heading in Supporting Information.

Pairwise design. We adopted a novel distant-pair design for the microarray experiments, which was proposed especially for genetic studies on gene expression [20]. In this design, the 80 RILs are hybridized directly on 40 arrays, in pairs that are maximally genetically different.

Data analysis. Full ANOVA model for pQTLs and eQTLs. The expression data of two temperatures were first analyzed separately by the following ANOVA model [20]

\[ y_{ij} = \mu + Q_i + T_j + e_{ij} \]

where \( y_{ij} \) is the log ratio at the \( i \)th microarray; \( \mu \) is the mean; \( Q_i \) is the effect of a single QTL that is specific for temperature \( j \); \( T_j \) is the temperature effect for \( j \)th temperature; \( e_{ij} \) is the residual error. To increase the power of detecting interaction, we not only did a genome-wide linkage analysis, but also reduced the multiple testing issue by focusing on those three marker positions that show a maximum eQTL in either the full model or one of the two single temperature models. Interaction was assessed at these three positions using significance thresholds determined by simulation. The same strategy was applied for detecting significant eQTL effects.

Two-stage search for pQTLs. To increase the power of detecting pQTLs, we not only did a genomewide linkage analysis, but also reduced the multiple testing issue by focusing on those three marker positions that show a maximum eQTL in either the full model or one of the two single temperature models. Interaction was assessed at these three positions using significance thresholds determined by simulation. The same strategy was applied for detecting significant eQTL effects.

Determination of genome-wide significance thresholds. To calculate the genome-wide threshold for separate analysis, we performed the following five steps. (1) We simulated trait data by randomly sampling from a standard normal distribution (with zero mean and unit variance) \( 1,000 \) times under the null hypothesis of no eQTL. We did this for \( 16^2 \) variances) and for the interaction effect (pQTL between the \( i \)th eQTL genotype and \( j \)th temperature, \( S_i \) is the random spot effect, \( D_i \) is the interaction effect of the \( i \)th eQTL genotype and \( j \)th temperature, and \( e_{ij} \) is the residual error. To increase the power of detecting interaction, we not only did a genome-wide linkage analysis, but also reduced the multiple testing issue by focusing on those three marker positions that show a maximum eQTL in either the full model or one of the two single temperature models. Interaction was assessed at these three positions using significance thresholds determined by simulation. The same strategy was applied for detecting significant eQTL effects.

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Genetic Genomics of Expression Plasticity

Author contributions. YL analyzed and interpreted data and wrote the manuscript. OAA performed the microarray experiment. EWG created RIL and genotyping. MT performed SNP mapping and RIL generation. JEK contributed to the statistical analysis and reviewed the manuscript.

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Competing interests. The authors have declared that no competing interests exist.


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15. Luscombe NM, Babu MM, Yu H, Snyder M, Teichmann SA, et al. (2004) Searching region. There are 1,180 potential candidates in total with a physical location in this region (819 potential candidates had a measured expression level in our dataset). We divided them into different groups according to their eQTL and pQTL effect and their annotation (see Table S4). The top candidates might be the genes that themselves have a significant pQTL effect (e.g., Y75B12B.3), and eQTL effect, (e.g., nhr-54 and nhr-116) involved in transcription factor activity, and map in cis; i.e., have a possible regulatory polymorphism in their promoter region.

Supporting Information
Protocol S1. Detailed Description of the RIL Population
Found at doi:10.1371/journal.pgen.0020222.s001 (115 KB DOC).
Table S1. Information on Cosmid SNP Location, Map Position, and Primers and Restriction Enzymes Used
Found at doi:10.1371/journal.pgen.0020222.s001 (92 KB PDF).
Table S2. N2 and CB Polymorphisms of the SNP Markers in the RILs and Marker Segregation Ratios
Found at doi:10.1371/journal.pgen.0020222.s002 (66 KB PDF).
Table S3. Marker-Association Frequencies between Markers on the Same and on Different Chromosomes
Found at doi:10.1371/journal.pgen.0020222.s003 (175 KB PDF).
Table S4. Potential Master Regulator Candidates for the Transband
Found at doi:10.1371/journal.pgen.0020222.s004 (17 KB PDF).

Accession Numbers
The National Center for Biotechnology Information (NCBI) Entrez Gene database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) accession numbers for the genes discussed in this paper are C15H11.1 (GeneID181075), C48S5.1 (GeneID183554), C24B5.1 (GeneID179301), flp-1 (GeneID177737), flp-9 (GeneID178229), flp-18 (GeneID 180587), flp-21 (GeneID182944), K10C8.2 (GeneID187257), nhr-116 (GeneID180129), nhr-54 (GeneID180106), spr-1 (GeneID180752), and 177B12.3 (GeneID190717).


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