

Worm variation made accessible

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In *Caenorhabditis elegans*, the recent advances in high-throughput quantitative analyses of natural genetic and phenotypic variation have led to a wealth of data on genotype-phenotype relations. This data has resulted in the discovery of genes with major allelic effects and insights in the effect of natural genetic variation on a whole range of complex traits as well as how this variation is distributed across the genome. Regardless of the advances presented in specific studies, the majority of the data generated in these studies had yet to be made easily accessible, allowing for meta-analysis. Not only data in figures or tables but meta-data should be accessible for further investigation and comparison between studies. A platform was created where all the data, phenotypic measurements, genotypes, and mappings can be stored, compared, and new linkages within and between published studies can be discovered. WormQTL focuses on quantitative genetics in *Caenorhabditis* and other nematode species, whereas WormQTL^{HD} quantitatively links gene expression quantitative trait loci (eQTL) in *C. elegans* to gene-disease associations in humans.

The nematode worm *C. elegans* is widely known for its research in forward and reverse genetic approaches. Many mutants have been investigated for unraveling genetic pathways together with RNAi screens. These studies have been invaluable for understanding the genetics of many different traits ranging from apoptosis, development to lifespan, and

behavior (www.wormbook.org). With regard to analyzing natural genetic variation, compared with other model species like *Arabidopsis*, mice, and yeast,¹⁻³ the worm is lagging behind.⁴ Yet, the worm is catching up rapidly. Over the past decade the number of quantitative genetic papers has strongly increased and efforts have been made to explore genotype-phenotype relations.⁵⁻²⁶ Quantitative genetics in *C. elegans* started in 1980s with a paper by Johnson and Wood²⁷ on genetic variation and lifespan in a Bristol N2 and Bergerac cross. Currently, most studies have focused on recombinant inbred lines (RILs)⁶⁻¹² or recombinant inbred advanced intercross line (RIAIL)^{14-19,21-24} populations derived from a cross between the most genetically diverse strains, Bristol N2 and Hawaii CB4856. Their genomes differ in about one single nucleotide polymorphism per 873 base pairs.²⁸ Independently from each other, the Kammenga lab and Kruglyak lab have developed populations by crossing these two divergent strains. The genetic variation between these lines, CB4856 and N2, has provided the basis for a great number of studies.⁵⁻²⁵ The CB4856 x N2 RIL and RIAIL populations displays large genetic and phenotypic differences for different traits such as reproduction, growth,^{8,29} gene expression,^{10,11,13,21,25} copulatory plug formation,¹⁹ heat stress tolerance,¹² and RNAi sensitivity.^{6,16,30} Next to CB4856 and N2, DR1350 x N2 RILs have been developed.^{31,32} For a more complete review, we refer to reference 33.

In addition to the RILs, a genome-wide introgression line (IL) population

Keywords: QTL, eQTL, *C. elegans*, WormQTL, WormQTL^{HD}, database, quantitative genetics, human disease, genotype-phenotype

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Submitted: 01/21/2014

Revised: 02/17/2014

Accepted: 02/25/2014

<http://dx.doi.org/10.4161/worm.28357>

Table 1. Data sets featured in WormQTL and WormQTLHD

Phenotypes	Type of array	Sample size	Parental strains	References	PubMed link	Growing temperature	Stage	Food	Medium
Gene expression	Washington State University	2x40 RILs	CB4856; N2	Li et al. 2006; Mapping determinants of gene expression plasticity by genetical genomics in <i>C. elegans</i> .	17196041	16 °C and 24 °C	(72 h at 16 and 40 h at 24); L4	OP50	NGM Plate
Gene expression	Affymatrix tiling array	60 RILs	CB4856; N2	Li et al. 2010; Global genetic robustness of the alternative splicing machinery in <i>Caenorhabditis elegans</i> .	20610403	24 °C	(40 h) L4	OP50	NGM Plate
Gene expression	Washington State University	36x3 RILs	CB4856; N2	Vinuela and Snoek et al. 2010; Genome-wide gene expression regulation as a function of genotype and age in <i>C. elegans</i> .	20488933	24 °C	(40 h, 96 h, and 214 h) L4, Adult, Old	OP50	NGM Plate
Gene expression	Agilent 4x44k microarrays	208 RIALs	CB4856; N2	Rockman et al. 2010; Selection at linked sites shapes heritable phenotypic variation in <i>C. elegans</i> .	20947766	20 °C	YA	OP50	NGM Plate
Feeding curves RNAi exposure	n/a	56 RILs * 12 RNAi	CB4856; N2	Elvin and Snoek et al. 2011; A fitness assay for comparing RNAi effects across multiple <i>C. elegans</i> genotypes.	22004469	20 °C	Multi-generational	n/a	Liquid S-medium
Life-history traits	n/a	80 RILs	CB4856; N2	Gutteling et al. 2007; Mapping phenotypic plasticity and genotype-environment interactions affecting life-history traits in <i>Caenorhabditis elegans</i> .	16955112	12 °C and 24 °C	Egg, L4, YA	OP50	NGM Plate
Lifespan and pharyngeal-pumping	n/a	90 NILs	CB4856; N2	Doroszuk et al. 2009; A genome-wide library of CB4856/N2 introgression lines of <i>Caenorhabditis elegans</i> .	19542186	20 °C	All; synchronised	OP50	NGM Plate
Lifespan, Recovery and reproduction after heat-shock	n/a	58 RILs	CB4856; N2	Rodriguez et al. 2012; Genetic variation for stress-response hormesis in <i>C. elegans</i> lifespan.	22613270	20 °C and 35 °C heat-shock	L4 and Adult	OP50	NGM Plate
Gene expression	Washington State University	CB4856 and N2	CB4856; N2	Vinuela and Snoek et al. 2012; Aging Uncouples Heritability and Expression-QTL in <i>Caenorhabditis elegans</i> .	22670229	24 °C	(40 h, 96 h, and 214 h) L4, Adult, Old	OP50	NGM Plate

Table 1. Data sets featured in WormQTL and WormQTLHD (continued)

Phenotypes	Type of array	Sample size	Parental strains	References	PubMed link	Growing temperature	Stage	Food	Medium
Dauer formation	n/a	90 NILs and 20 Wild-Isolates	CB4856; N2	Green et al. 2013; Genetic mapping of variation in dauer larvae development in growing populations of <i>Caenorhabditis elegans</i> .	23715016	20 °C	Multi-generational	OP50	Dauer agar Plate
NEMADAPT	4 x 44K Agilent AGIWUR	48 Wild-Isolates	JU1511; JU1516; JU1522; JU1523; JU1545; JU1581; JU1582; JU1807; JU1937; JU1938; JU1939; JU1940; JU1941; JU1942; JU1943; JU1944; JU1945; JU1946; JU1947; JU1948; JU1949; JU1918; JU1919; JU1793; JU1920; JU1921; JU1922; JU1923; JU1924; JU1925; JU1926; JU1927; JU1928; JU1929; JU1930; JU1931; JU1932; JU1933; JU1934; JU1935; JU1936; JU314; JU396; WN2001; WN2002; WN2003	Volkers and Snoek et al. 2013; Gene-environment and protein degradation signatures characterize genomic and phenotypic diversity in wild <i>Caenorhabditis elegans</i> populations.	23957880	20 °C	L4	OP50	NGM Plate
Gene expression	4 x 44K Agilent AGIWUR	N2, CB4856 and 6 wild-isolates	N2; CB4856; JU1581; JU1921; JU1930; JU1932; JU1944; and JU1949	Snoek et al. 2014; A rapid and massive gene expression shift marking adolescent transition in <i>C. elegans</i> .	24468752	20 °C	(48 h) L4	OP50 and E. rhapontici	NGM Plate



Figure 1. Data exploration options in WormQTL. QTLs are genomic regions associated with phenotypic variation and provide the basis for studying the genetic architecture of traits and to detect potential phenotypic regulators. eQTL are loci associated with gene expression variation. WormQTL provides easy access to most of the (e)QTL studies published, by search, browse and plot functions.

to study single locus effects has also been developed.⁵ The genome of an IL consists of the N2-recipient genome and a short, homozygous segment of the CB4856 genome. In this way, the difference in the phenotype of the N2 strain and the IL can be precisely attributed to the introgressed CB4856 locus. Used in many studies to confirm and narrow down QTL, some studies take advantage of using the genome-wide set to discover new, mostly closely linked, QTLs.^{7,17}

A number of mapping studies in RILs have investigated loci and polymorphic genes associated with variation in gene expression (coined “genetical genomics”³⁴), yielding expression Quantitative Trait Loci (eQTL). eQTL methods take advantage of natural genetic variation for identifying the loci that underlie variation in gene expression between genotypes, treating gene expression phenotypes as quantitative traits on a genome-wide scale. The polymorphic regulatory loci causal to gene expression differences between different genotypes are pinpointed by eQTL. So far, a handful of studies have been published on eQTL analyses in *C. elegans*.^{10,11,13,21,25,35} Compared with QTL mapping phenotypic traits, eQTL studies are data heavy and can hardly be presented in all their detail in a single paper.

Data Availability

Most of the (e)QTL data sets are available as separate supplementary data in a

non-uniform format. Usually a genome-wide overview of eQTLs is presented in a paper. To gain access to gene-specific eQTLs not originally presented, they have to be re-calculated using the original data. To do this, genotypic information is required as well as the gene expression measurements, not always available from the same study. Even with the data retrieved it is a daunting task to find if a gene or locus of choice has an eQTL, because (re-)calculation of eQTLs is a specialized task. If one would like to compare QTLs and eQTL from different studies, this time-consuming process has to be repeated for each trait, gene, and spot (in case of microarray studies) several times.

This wealth of worm data, especially from eQTL studies,^{10,11,13,21,25} provides a treasure trove for the detection and functional analysis of loci, alleles, and genes. We collected resources, like genotypes, phenotypes, and gene expression data sets, as well as the QTL and eQTL mapping results. In case QTLs or eQTLs were not originally available, we re-calculated them to share with the community. To facilitate the comparison between different genes and studies, we have developed two database and analysis platforms, based on the MOLGENIS (MOlecular GENetics Information Systems) bio-software.^{36,37} The MOLGENIS toolkit provides a simple model for generating versatile web platforms for many different genomic, molecular, and phenotypic experiments.

The two developed web platforms comprise quantitative genetic data available for *C. elegans*, and in the future, other *Caenorhabditis* species. WormQTL (<http://www.wormqtl.org>)³⁸ and WormQTL^{HD} (HD for Human Disease [<http://www.wormqtl-hd.org>])³⁹ are easily accessible and enable search, comparative analysis, and meta-analysis of all data on variation in *C. elegans*. Moreover, both web-portals provide a workbench of analysis tools for genotype–phenotype linkage and association mapping. Data can be uploaded by contacting one of the curators of WormQTL to ensure proper integration with the rest of the data. Results can be accessed and downloaded per data matrix or a specific selection via a public web user interface. Retrieved data are stored in simple tab-delimited text or Excel formats, ensuring easy local access. In WormQTL^{HD}, the online user friendly tools can be used for revealing functionally coherent, evolutionary conserved gene networks, and for predicting novel gene-to-gene associations and the functions of genes underlying the disease of interest. To further simplify the access to quantitative genetic data, most genes in Wormbase (www.wormbase.org) have a link (which can be found in the “external link” section), which takes the user to the gene-specific WormQTL page upon clicking. Retrieving all the data for QTL mapping from all experiments stored in WormQTL (Table 1) or finding out if your favorite worm gene has an eQTL has never been this easy (Fig. 1).

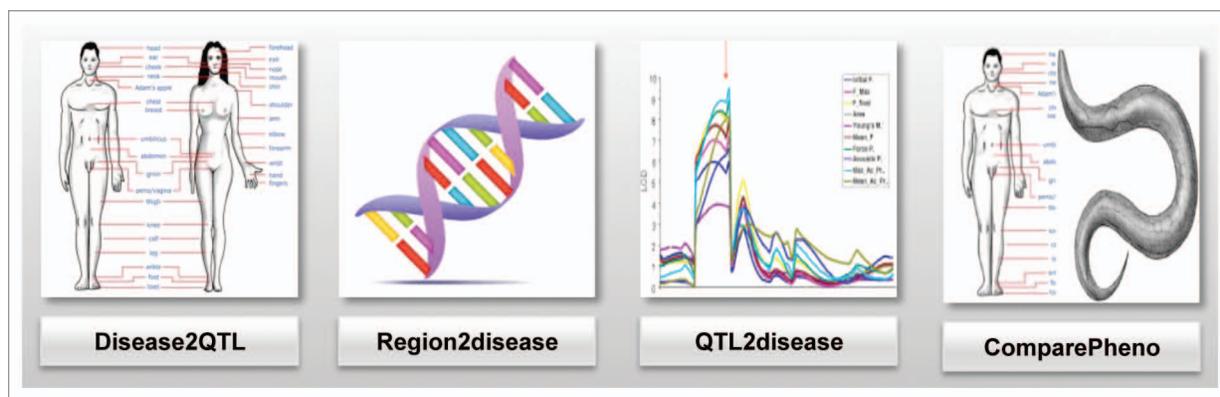


Figure 2. Tools of WormQTL^{HD} for combining human–disease and *C. elegans* genetics. One can select for disease or phenotype and search for associated loci (Disease2QTL). Select worm genomic regions and search for associated diseases (Region2disease), or interrogate the database for specific probes and thresholds (QTL2disease). Finally, worm phenotypes can be compared with human disease phenotypes (ComparePheno).

On WormQTL^{Human Disease}

C. elegans is an important model organism to study the genetics of human diseases.⁴⁰ To use the *C. elegans* eQTL data to the benefit of human disease research, WormQTL^{HD} links *C. elegans* genetics to human gene–disease associations. These links are based on orthologous genes between *C. elegans* and humans and can be associated with different phenotypes. Taken from different gene expression profiling platforms and a diverse range of experiments, the database provides a number of analysis tools to search and mine *C. elegans* phenotypes, including gene expression for human–worm InParanoid orthologs.⁴¹ In this way, data stored in the databases on human disease genetics like GWAS central,^{42,43} GWAS Catalog,⁴⁴ and OMIM⁴⁵ can be combined with data stored in WormQTL and WormBase. The results can be downloaded and visualized in a comprehensive and clear way, accessible via a public web user interface.

WormQTL^{HD} provides four tools to explore the database: “Disease2QTL,” “Region2disease,” “QTL2disease,” and “ComparePheno” (Fig. 2). “Disease2QTL” finds the *C. elegans* orthologs of human genes associated to a specific disease and visualizes the eQTL(s) of these genes. This enables one to find a genetic variant in *C. elegans* of a human disease-associated gene or pinpoints the locus of a possible modifier of this disease. “Region2disease” provides all the *C. elegans* genes, their human orthologs,

and disease associations in a user-specified genomic region, enabling fast candidate gene selection for QTLs found for *C. elegans* phenologs of human disease. “QTL2disease” enables one to select a phenotype of choice and show the genes underlying QTLs for that phenotype as in “Region2disease.” “ComparePheno” provides links between human diseases and classical worm phenotypes so “new” phenologs can be discovered and the genes/orthologs underlying both human and worm phenotypes. A more extensive explanation, help, tutorials, and case studies can be found on the WormQTL^{HD} website and original paper.³⁹

Outlook

On data

The quantity of data on natural variation in *C. elegans* has strongly increased as measuring and genotyping technologies have become less expensive and technically improved over the past few years. Moreover, in these recent years, many more labs have embraced and included natural variation in their research, thereby generating more valuable data. Most of this data will be integrated in WormQTL and WormQTL^{HD}. The curators are committed to maintain data and software for years to come and invite the community to add and share new data and ideas. Curators will also contact authors to upload their data and authors can contact curators for assistance. Recently, almost

all of the SNPs between N2 and CB4856 have been published along with the SNPs in 40 other wild isolates.⁴⁶ Over 630 000 unique SNPs and over 220 000 unique INDELS are reported showing the high abundance of genetic variation between wild-isolates. Other studies also report high-density genetic differences between wild-isolates.^{47,48} Moreover, Volkers et al. also shows variation in many different phenotypes, including gene expression, between multiple different wild strains as well as N2 and CB4856. These efforts pave the way toward a widely available and usable resource for studies on natural genetic variation in *C. elegans*. This likely includes research on gene–environment interactions since *C. elegans* has been intensively used to study the effects of different stressors next to ambient environmental conditions.^{49–53}

The recently introduced Wiki on genetics and evolution of *Caenorhabditis* species (<http://evolution.wormbase.org>) illustrates that there are more worm population resources present than currently featured in WormQTL or WormQTL^{HD}. The Wiki not only summarizes *C. elegans* studies on variation in specific phenotypes, but also resources beyond the two most frequently used strains N2 and CB4856. Most of these populations are available or will be upon publication.^{54–56} As the Wiki has just started, the list of available or populations under construction is likely to grow rapidly. Even so it will be a challenge to link the data generated between different population let

alone the complex task of making quantitative genetics comparable between species. At the same time, our knowledge of the *C. elegans* N2 genome also increases. Many different genomic binding sites are available via the modENCODE project.⁵⁷ These binding-sites, as well as other genomic data, are very useful to evaluate the likeliness of causality for a genetic polymorphism underlying a QTL.

Upcoming experiments

Within the next few years a steady flow of new data are expected to come out by different groups. Data will appear on high-throughput phenotyping of large, genetically diverse populations, including N2 x CB4856 RIALs (Erik Andersen pers. comm.), mapping of loci associated with bacterial feeding (Hinrich Schulenburg pers. comm.), spindle formation (Daniel Needleman and Reza Farhadifar pers. comm.), genomic incompatibility (Simon Harvey, pers. comm.), behavioral assays (Bart Braeckman pers. comm.), and more. The curators of WormQTL or WormQTL^{HD} will keep in touch with authors and assist them with uploading their data. As a number of labs are working on or have developed high-throughput assays for RNAi,⁶ proteomics, phenotyping, epi-genetics, metabolomics, and other levels of variation, layered, and interacting sets of data become available. Already a challenge to study one by one, exploring them together in a structured accessible environment becomes a necessity for efficient and discovery focused exploring of large data sets where WormQTL and WormQTL^{HD} can be very helpful.

On tools

As stated in our previous papers, we constantly keep developing and updating visualization tools, QTL mapping tools, and candidate gene selection tools. These can be tools to test for functional enrichment of genes sharing an eQTL, finding SNPs on a specified locus, or the integration of R/QTL.³⁶ We are currently working on a new version of MOLGENIS, and thus, WormQTL to make data exploration more interactive and “clickable.” A genome browser type environment is being created to link information such as SNPs and other polymorphisms to QTLs and eQTLs. By focusing on community data accessibility we hope to strengthen

the foundations of worm quantitative genetics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

All authors were funded by the European Union Seventh Framework Programme (FP7/2007-2013) project PANACEA, contract nr. 222936, Snoek LB was funded by the ERASysbio-plus ZonMW project GRAPPLE (90201066) and Li Y was funded by NWO VENI grant (863.13.011). We would like to thank Wormbase for proving a gene-specific direct link to WormQTL.

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