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# The genome of *Brugia malayi – all worms are not created equal*

**Alan Scott**[*,1] and **Elodie Ghedin**[2,a]

1*Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, 615 North Wolfe, Baltimore, MA 21205, USA*

2*The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850*

## Abstract

Filarial nematode parasites, the causative agents of elephantiasis and river blindness, undermine the livelihoods of over one hundred millions people in the developing world. Recently, the Filarial Genome Project reported the draft sequence of the ~95 Mb genome of the human filarial parasite *Brugia malayi* - the first parasitic nematode genome to be sequenced. Comparative genome analysis with the prevailing model nematode *Caenorhabditis elegans* revealed similarities and differences in genome structure and organization that will prove useful as additional nematode genomes are completed. The *Brugia* genome provides the first opportunity to comprehensively compare the full gene repertoire of a free-living nematode species and one that has evolved as a human pathogen. The *Brugia* genome also provides an opportunity to gain insight into genetic basis for mutualism, as *Brugia*, like a majority of filarial species, harbors an endosybiotic bacterium (*Wolbachia*). The goal of this review is to provide an overview of the results of genomic analysis and how these observations provide new insights into the biology of filarial species.

### Keywords

Nematode; filarial; genome; *Brugia*; *Caenorhabditis*

## INTRODUCTION

At any given time, over one third of humankind, mainly in developing regions, is infected with a parasitic nematode. Despite the staggering morbidity that these infections inflict, we know comparatively little about the details of the cell biology, biochemistry and molecular biology that will allow us to develop effective strategies to diminish pathology and control transmission of parasitic nematodes. Although useful information has been garnered from the study of free-living and animal nematode species, we are still woefully limited in our direct knowledge of the cellular and molecular basis for key host-parasite interactions for important parasitic species such as the intestinal roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichirua*, the hookworms *Necator americanus* and *Ancylostoma* and the filarial parasites in the genera *Wuchereria*, *Onchocerca* and *Brugia*.

*To whom correspondence should be addressed. E-mail: ascott@jhsph.edu.
[a]Current address: Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

In humans, parasitic nematodes typically cause long-term, chronic infections that are difficult to treat. A minimum of 150 million people are infected with, and over 1 billon are at risk of infection from, three major species of filarial nematodes: *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* (1). These vector-borne parasites cause significant morbidity and result in some of the most debilitating disease states recorded: chronic lymphatic blockage leading to elephantiasis, ocular pathology leading to river blindness, and severe dermatitis manifesting as sowda. While there are drugs that are effective against the parasite, there is no vaccine to prevent filariasis.

A recent reclassification of the Phylum Nematoda recognizes five clades (2). Most current knowledge of the molecular genetics and developmental biology of nematodes are based on the extensive studies carried out with the free-living, non-parasitic clade V species *Caenorhabditis elegans*. From its beginnings as a tractable tool for genetic and developmental studies, *C. elegans* has evolved into a powerful and flexible model system that has contributed to a variety of important fields including medical genetics, ageing, cancer and infectious diseases (reviewed in (3-5)). *C. elegans* was the first multicellular organism to be fully sequenced (6) and the results of genetic, RNA interference (RNAi), 2-hybrid and other screens have contributed to the extensive structural and functional annotation of the genome. Since a vast majority of nematode species have the same basic body plan and undergo a very similar developmental progression, the presumption of many has been that the structure, organization, gene content and regulation of the *C. elegans* genome would serve as accurate model for most nematodes. This supposition was initially undermined when the genome sequence of a closely related species, *C. briggsae*, revealed an unexpectedly high degree of intra-genus variance in genomic content (7). The notion was further dispelled with the publication of the draft genome from the first parasitic nematode *Brugia malayi* (8). Presumably, just as the similarities in genome structure and content between *Brugia* and *Caenorhabditis* are likely to represent core elements important for a functioning nematode, the dissimilarities reflect important fundamental differences in cell and molecular biology that have evolved in part due to the pressures of free-living and parasitic life strategies. The goal of this review is to provide a synopsis of this first comparative genome analysis between a free-living and a parasitic nematode species with emphasis on observations that give new direction for understanding the genetic and molecular basis for the host-parasite relationship as well as observations that may have potential for translation into new control strategies.

## History

The Filarial Genome Project (FGP) was organized and initiated under the auspice of funding from World Health Organization (WHO/TDR/UNDP/World Bank) in 1994. The FGP was founded as an international consortium of investigators with a common goal of generating genomic data and resources for the filarial community. *Brugia malayi* was selected as a representative filarial genome by virtue of a number of logistical advantages not the least of which was the fact that it was the only filarial pathogen of humans for which an inbred strain was available. The original objectives of the FGP included gene discovery through EST analysis of cDNA libraries representing the major life cycle stages, genome mapping and establishing an accessible database and resource center to serve the interests of the filarial community (9). The initial efforts of the FGP laid the groundwork that facilitated the whole genome sequencing and annotation effort that began in 2002 and resulted in the current build of the *B. malayi* genome (8).

## The genome – overview

The *B. malayi* nuclear genome, estimated to be 95 Mb, is organized on five chromosome pairs including an XY sex determination pair (10). A majority of filarial species, including *B.*

*malayi*, also harbor two additional genomes – a 14 Kb mitochondrial genome and a 1 Mb genome of a bacterial endosymbiont (*w*Bm) (11). As we will discuss later, the co-evolution of the worm and the endosymbiont appears to have had demonstrable impact on the nature of the *Brugia* genome.

Like most genome projects, the *Brugia* genome is a work in progress. Although the current ~9-fold coverage has resulted in ~90% assembly of the genome into scaffolds of that range in size from less than 2 Kb to more than 6.5 Mb there is still work ahead to fill sequence gaps and assemble the scaffolds into chromosomal units. It is anticipated that the application of "Next Generation Sequencing" techniques will overcome some of the technical obstacles encountered with conventional sequencing approaches and quickly provide the data that will bridge the gaps and allow for full genome assembly.

The vital statistics of the *B. malayi* genome are outlined in Table 1. Because major aspects of the *B. malayi* genome have been discussed in detail previously (8), we will only highlight key elements here.

- ***Nucleotide content*** - The overall A+T content of 69.5% is higher than that of *C. elegans* (64.6%) or *C. briggsae* (62.6%) (7).
- ***Repeated sequences*** – Repetitive elements, which make up ~14% of the genome, include the major A+T-rich 322 bp *HhaI* repeat family, the 62/53 bp *MboI* repeat family as well as large number of simple and low complexity repeats. The number and distribution of tandemly arrayed repeats continue to present a challenge to the full assembly of the *B. malayi* genome.
- ***Conservation of linkage but not gene order*** - In general, long-range gene linkage between *B. malayi* and *C. elegans* is conserved, but local gene order is not (Figure 1). These relative genome organizations support an evolutionary model where within-linkage group rearrangements are far more numerous than between-linkage group translocations - a archetype that may be common for nematode genomes (7,12).
- ***Operons*** – Like *C. elegans* (13,14), *B. malayi* appears to have evolved a genomic organization in which up to 15% of the genes are found in 'operons' of 2 to 5 genes per operon. While one definitive example of a polycistronic mRNA encoding for two proteins has been reported (15), the remaining potential *B. malayi* operons await conformation. Most of the putative *B. malayi* operons tend to have a greater intergenic spacing than seen in *Caenorhabditis* and most represent combinations of genes not found in *Caenorhabditis*. The significance of operons in *Brugia* is yet to be determined.
- ***Gene Structure*** – The median of 5 exons per gene in *B. malayi* is one less than that found for *C. elegans* or *C. briggsae*. In addition, the median size of an exon is smaller (140 bp vs. 147 bp) and the median length of an intron is larger (217 bp vs 68 bp) in *B. malayi* compared to the caenorhabditids (7).
- ***Number of genes*** –. It is estimated that the *B. malayi* genome contains between 14,500 and 17,800 protein-coding genes (16). Even the higher estimate is lower than the 19,762 (WormBase release WS133) and 19,507 (7) genes reported for *C. elegans* and *C. briggsae*, respectively. Further work will be necessary to discern whether this disparity in the number of genes can be accounted for by an expansion in certain gene families in the caenorhabditids or a selective contraction of gene families in *Brugia*.
- ***Orthology*** – Approximately 50% of the *B. malayi* genes have clear orthologues in *Caenorhabditis* with an average pair wise identity of 48%. Assuming that the rates of protein evolution are congruent between nematodes and other invertebrate species,

it is estimated that *Brugia* and *Caenorhabditis* last shared a common ancestor ~350 million years ago.

- ***B. malayi*-specific proteins** - About 20% of the predicted protein coding genes do not have identity to any sequences in the databases. Although a majority of these putative *B. malayi*-specific genes/proteins are hypothetical, the authenticity of around half of these putatively species-specific genes is supported by EST data. The number of putative *B. malayi*-specific genes/proteins will no doubt decline as additional nematode genomes are sequenced.

## Comparative analysis

What have we learned from comparative genomic analysis with *Caenorhabditis* and *Brugia*? One clear message is that the two genomes have a lot to tell each other. Genes long considered hypothetical in *C. elegans* have been confirmed by the discovery of conserved orthologues in *B. malayi*. Knowledge of the genomic organization of individual genes in *C. elegans* played a pivotal role in gene finding and annotation in the *Brugia* genome. The *C. elegans* genome was much less informative for efforts to assemble large scaffolds. The expectation that knowledge of gene order in *C. elegans* would be useful in assembling and annotating the *Brugia* genome was not fulfilled. Because of the limited level of short-range synteny (Figure 1), the gene order in *C. elegans* could not be used as a template for assembly of the *Brugia* genome. While disadvantageous for assembly, this divergence in gene order might prove to be an advantage when it comes to searching for common, conserved gene-proximal regulatory motifs. The understanding of the evolutionary dynamics of gene regulation will be further advanced by studies on conserved and novel operons.

So, is *C. elegans* a good model for parasitic species such as *B. malayi*? The answer to this question demands nuance. *C. elegans* undoubtedly encodes the information to construct the nematode bauplan and for the basic elements of nematode biochemistry. However, *C. elegans*, like *B. malayi*, has had a unique evolutionary history - a record of which is imprinted in the genome. *B. malayi* has many genes that have apparently been lost in the caenorhabditid lineage, but are shared with other organisms. These genes may have been retained in *B. malayi* because of their relevance to parasitism or because *B. malayi* itself is ‘parasitized’ by a species of *Wolbachia*. In general, because *C. elegans* is likely to report poorly on adaptations specific to interactions with the tissue environments of arthropod or mammal hosts, one of the abiding values of comparative analysis may be as a guide to genes that are not shared between the free-living and parasitic species. A systematic comparison of the genes from a large number of free-living, plant-parasitic and animal-parasitic nematode species may identify the complement of genes and the physiological strategies that define parasitism for this diverse collection of multicellular organisms.

## Targets for intervention

### Exploiting the genome sequence for novel drugs

Treatment of lymphatic filariasis relies on a small group of drugs that have been in sustained use for nearly a half-century. While these drugs are successful in limiting parasite burden, pathology and transmission, they require repeated administration over years to be effective at a population level. In addition, there is recent evidence that drug resistant parasites have begun to emerge (17,18). Four classes of drugs are available for treatment of filarial diseases: (1) diethylcarbamazine, which is thought to interfere with fatty acid metabolism; (2) the benzimidazoles, which disrupt parasite microtubules; (3) the avermectins, which agonize glutamate-gated chloride channels (19); and (4) oxytetracycines and other antibacterials, which target the *Wolbachia* endosymbiont (1). The first three classes selectively target larval stages

in the blood (microfilariae or L3s), reducing the numbers and limiting transmission. These microfilarialcides are highly effective in the short term, but do not prevent recrudescence, because they have variable efficacy on adult parasites. A major objective is thus to identify compounds effective in killing or sterilizing adult parasites. From the genome sequence we can identify several systems in *Brugia* that are likely to be fruitful for drug design: nematode-centric physiologies such as molting, the nematode nervous system, and putative nematode-specific genes (many of which have lethal knockout phenotypes in *C. elegans*).

**Molting and Cuticle Formation—**Nematodes are members of the superphylum Ecdysozoa, a defining feature of which is molting. A genome-wide RNAi screen in *C. elegans* identified 159 genes important for successful molting (20). Over 98% of the 159 *C. elegans* molting genes have orthologues in *B. malayi*. For the 47 genes considered essential for molting (>90% reduction in molting by RNAi (20)), 46 were found in the *B. malayi* genome. In addition to proteases, protease inhibitors, peroxidases and a number of DNA-binding and signaling proteins, these 46 genes encode a number novel proteins that could serve as potent, pathogen-specific targets of inhibitors.

The bulk of the nematode cuticle is comprised of a nematode-specific class of small collagens. *B. malayi* has 67 genes that encode cuticle collagens. It has been shown previously that *B. malayi* cuticular collagens are differentially expressed during development resulting in a distinct cuticle structure for each stage (21). Enzymes of cuticle collagen processing (blisterase-like proteases, protease inhibitors, tyrosinases, mixed-function oxidases, and peptidyl-prolyl isomerase) identified in the proteome could also serve as targets to disrupt nematode growth.

Molting in filarial parasites appears to be under control of an ecdysone-like response system (22) where members of the nuclear hormone receptor (NHR) family of proteins play a key role (23). Twenty-seven members of the NHR family were identified in the *B. malayi* genome. Interestingly, while this number is similar to *D. melanogaster* (21 NHRs) (24), it is only a fraction of the ~280 NHR genes found in the *C. elegans* genome. *B. malayi* has orthologues of *Ecr* (not present in caenorhabditids) and other NRs acting in the *D. melanogaster* ecdysone response cascade. *B. malayi* also has an orthologue of the thyroid receptor (*Bm-nhr-1;* also present in a related filarial parasite, *O. volvulus* (25)). These receptors appear to represent additional novel targets for directed blocking of parasite development within the insect and mammalian host.

**The Nervous System—**Neuronal signaling controls essential functions in nematodes including muscle contraction and body movement, secretion, feeding, osmoregulation and mechanosensory activity (26-31). The *Brugia* genome provides a spectrum of neuronal receptors that could function as effective drug targets. Drug development for parasitic nematode neural targets could benefit from the large existing pharmacopoeia as well as from the analysis of neural mutants in *C. elegans* (reviewed in (32)).

The biogenic amines (BA), serotonin (5-HT), dopamine (DA), tyramine (TA) and octopamine, modulate essential processes in nematodes. Stimulating BA receptors in filarial nematodes with exogenous 5-HT and DA results in paralysis (32). The *Brugia* genome encodes only seven putative G-protein coupled BA receptors (33). BA receptors have been pharmacologically characterized and individual roles identified using null mutants in *C. elegans* (reviewed in (32)).

Cys-loop receptors are a large family of ligand-gated ion channels that are found on post-synaptic nerve or muscle cells where they transduce signals from pre-synaptic neurons by binding acetylcholine, serotonin, GABA, glutamate, glycine and histamine. The cys-loop family includes nicotinic acetylcholine (nAChR) and 5-$HT_3$ receptors, which form cation

channels, and GABA and glycine receptors, which form chloride channels. Cys-loop receptors are of particular interest because they are the targets of some of the most important anti-nematode drugs (34,35). *B. malayi* possesses at least 44 cys-loop receptor genes, including 21 nAChR-like genes and 23 that code for chloride channel subunits. In contrast, *C. elegans* possesses more than twice as many cys-loop receptor genes including 52 nAChR genes and approximately 50 chloride channel genes. While the *B. malayi* genome contains fewer cys-loop receptor genes, it has at least one representative for most cys-loop receptor classes. Novel cys-loop receptors present in *B. malayi* may represent additional targets for rational drug development.

The *B. malayi* genome contains 36 potassium channel genes with representatives for each of the three major classes: 2-pass (1 gene) 4-pass (16 genes) and 6-pass (9 genes) transmembrane proteins. Voltage-gated potassium channels specifically regulates cell membrane potential and excitability in neurons and other cell types through diverse mechanisms that include regulated transcription, RNA splicing, posttranslational modifications and through association with interacting proteins and accessory subunits. Several *B. malayi* potassium channel genes are orthologues of *C. elegans* genes that give paralytic or uncoordinated phenotypes when deleted or mutated, suggesting that the products of these genes may be effective drug targets.

**Reproductive Biology—**Filarial parasites are dioecious and carry out the reproductive process within the human host. Many facets of the filarial reproductive biology are distinct from human reproduction (36) and thus represent a pathogen-specific therapeutic target. A seminal example of the level of distinction that exists between nematodes and humans can be found in sperm cell biology and biochemistry. Unlike the flagellated sperm cells found in humans, nematode sperm are crawling cells that have evolved a completely unique cytoskeletal protein – the major sperm protein (MSP) - for propulsion (37). Results of studies to define the biochemistry and structure of MSP in model and parasitic nematode species have identified new opportunities for intervention to block transmission (38). Preliminary analysis suggests that *Brugia* and *C. elegans* will have a similar number of genes (~900) associated with germ line development and gamete biology (39). It is likely that detailed studies on other gene products that work in a unique fashion within the male and female reproductive system will afford additional approaches to block the dissemination of filarial parasites.

**Parasite-Host Interactions—**Like all filarial species, *B. malayi* interfaces with the physiology and defense responses of two distinct hosts during its life cycle – the definitive human host and a mosquito vector. Doubtless selective pressures brought to bear by these complex interactions have sculpted the essential nature of the filarial genome. Understanding the specific genomic adaptations that promote parasite survival and fitness is a key step in developing targeted control approaches that minimizes detrimental side effects.

At the parasite-human interface, there is abundant evidence that chronic filarial infection results in a modulation in host immunity. The prevailing hypothesis is that parasite-derived molecules contribute to the regulation of the immune response modifying the nature and magnitude immune response against filarial-derived antigens in a manner that promotes parasite survival and chronicity (40) Parasite-mediated immune modulation also is purported to influence the nature of the immune responses against allergens, vaccines other infectious challenges (41, 42). While a number of studies have identified individual filarial genes whose products appear to function in immune modulation, it remains unclear how these molecules function in consort to effect immunosuppression. Presumably there are a number of additional gene products that are necessary for the parasite to effectively blunt the host defense responses. Assessment of the *Brugia* genome indicates that these additional molecules are not encoded by close orthologues to classical immune regulatory molecules such as cytokines, chemokines and their receptors. It is likely that it will take functional annotation of the over 5,000 hypothetical genes

found in the genome in order to understand the mechanism used by filarial parasites to modulate host immunity. It is possible that this same group of genes also encode molecules that promote survival in the mosquito vector.

**Parasite-Wolbachia Interactions—**As noted earlier, *B. malayi* harbors a bacterial symbiont, wBm. Current evidence indicates that the association between the parasite and wBm is ancient and has evolved to be mutualistic in nature (43). This mutualistic relationship is supported by the consequences of antibiotic treatment of *wBm*-infected larvae and adults where it is clear that filariae are dependent on the bacteria for a diverse range of biological and stage-specific processes (43). Access to the sequence of both genomes (11) has allowed us to ask questions regarding the adaptations that have taken place in order to establish this mutualistic relationship. For example, it appears that *B. malayi* cannot synthesize purines *de novo* as 9 out of the 10 enzymes required to produce inosine monophosphate from phosphoribosyl pyrophosphate are missing from the genome. While *B. malayi* might meet some of its requirements for nucleotide cofactors by active uptake from the host or vector (44), *w*Bm has an intact purine synthesis pathway and could be an important source of precursors for the worm (11). Other examples are in the areas of heme and riboflavin biosynthesis. *B. malayi* is missing 6 of the 7 genes required for heme biosynthesis and all 5 enzymes required for *de novo* riboflavin biosynthesis. The *w*Bm genome encodes a majority of the enzymes for both pathways (11). Heme-dependent enzymes are essential for energy metabolism and steroid synthesis and thus are important for process such as molting. Parasites that have been cleared of wBm by antibiotics no longer molt (45). A comprehensive systems biology approach to define the molecular basis for the *w*Bm-*Brugia* mutualistic relationship could point to additional biochemical and physiological dependencies that can be exploited for control.

## Where do we go from here?

Parasitic nematodes of plants and animals exact a shocking toll on human development and wellbeing (46). While *C. elegans* will continue to be an important model for understanding certain aspects of nematode biology, the *B. malayi* genome data highlights the fact that *C. elegans* does not come close to capturing the complexity and diversity that will be found in the phylum Nematoda (47). The fascinating differences in genome content and organization between *Caenorhabditis* and *B. malayi* underscore the importance of obtaining additional genome data from representative species from across the diversity of the Nematoda (48). The ability to carry out large scale comparative genomics within Nematoda will be key in defining molecules unique to nematode development and parasitism that can serve as the targets for the next generation of nematode control strategies.

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## References

1. Ottesen EA. Lymphatic filariasis: Treatment, control and elimination. Adv Parasitol 2006;61:395–441. [PubMed: 16735170]
2. Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK. A molecular evolutionary framework for the phylum Nematoda. Nature 1998;392:71–75. [PubMed: 9510248]

3. Olsen A, Vantipalli MC, Lithgow GJ. Using Caenorhabditis elegans as a model for aging and age-related diseases. Ann N Y Acad Sci 2006;1067:120–128. [PubMed: 16803977]
4. Poulin G, Nandakumar R, Ahringer J. Genome-wide RNAi screens in Caenorhabditis elegans: impact on cancer research. Oncogene 2004;23:8340–8345. [PubMed: 15517014]
5. Pradel E, Ewbank JJ. Genetic models in pathogenesis. Annu Rev Genet 2004;38:347–363. [PubMed: 15568980]
6. Consortium, C. e. S. Genome sequence of the nematode C. elegans: A platform for investigating biology. Science 1998;282:2021–2018.
7. Stein LD, Bao Z, Blasiar D, Blumenthal T, Brent MR, Chen N, Chinwalla A, Clarke L, Clee C, Coghlan A, Coulson A, D'Eustachio P, Fitch DH, Fulton LA, Fulton RE, Griffiths-Jones S, Harris TW, Hillier LW, Kamath R, Kuwabara PE, Mardis ER, Marra MA, Miner TL, Minx P, Mullikin JC, Plumb RW, Rogers J, Schein JE, Sohrmann M, Spieth J, Stajich JE, Wei C, Willey D, Wilson RK, Durbin R, Waterston RH. The genome sequence of Caenorhabditis briggsae: a platform for comparative genomics. PLoS Biol 2003;1:E45. [PubMed: 14624247]
8. Ghedin E, Wang S, Spiro D, Caler E, Zhao Q, Crabtree J, Allen JE, Delcher AL, Guiliano DB, Miranda-Saavedra D, Angiuoli SV, Creasy T, Amedeo P, Haas B, El-Sayed NM, Wortman JR, Feldblyum T, Tallon L, Schatz M, Shumway M, Koo H, Salzberg SL, Schobel S, Pertea M, Pop M, White O, Barton GJ, Carlow CK, Crawford MJ, Daub J, Dimmic MW, Estes CF, Foster JM, Ganatra M, Gregory WF, Johnson NM, Jin J, Komuniecki R, Korf I, Kumar S, Laney S, Li BW, Li W, Lindblom TH, Lustigman S, Ma D, Maina CV, Martin DM, McCarter JP, McReynolds L, Mitreva M, Nutman TB, Parkinson J, Peregrín-Alvarez JM, Poole C, Ren Q, Saunders L, Sluder AE, Smith K, Stanke M, Unnasch TR, Ware J, Wei AD, Weil G, Williams DJ, Zhang Y, Williams SA, Fraser-Liggett C, Slatko B, Blaxter ML, Scott AL. Draft genome of the filarial nematode parasite Brugia malayi. Science 2007;317:1756–1760. [PubMed: 17885136]
9. Blaxter M, Daub J, guiliano D, Parkinson J, Whitton C, Project FG. The Brugia malayi genome project: expressed sequence tags and gene discovery. Trans R Soc Trop Med Hyg 2002;96:7–17. [PubMed: 11925998]
10. Sakaguchi Y, Tada I, Ash LR, Aoki Y. Karyotypes of *Brugia pahangi* and *Brugia malayi* (Nematoda: Filarioidea). J Parasitol 1983;69:1090–1093. [PubMed: 6674460]
11. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J, Vincze T, Ingram J, Moran L, Lapidus A, Omelchenko M, Kyrpides N, Ghedin E, Wang S, Goltsman E, Joukov V, Ostrovskaya O, Tsukerman K, Mazur M, Comb D, Koonin E, Slatko B. The Wolbachia genome of Brugia malayi: endosymbiont evolution within a human pathogenic nematode. PLoS Biol 2005;3:e121. [PubMed: 15780005]
12. Lee KZ, Eizinger A, Nandakumar R, Schuster SC, Sommer RJ. Limited microsynteny between the genomes of Pristionchus pacificus and Caenorhabditis elegans. Nucleic Acids Res 2003;31:2553–2560. [PubMed: 12736304]
13. Blumenthal T, Evans D, Link CD, Guffanti A, Lawson D, Thierry-Mieg J, Thierry-Mieg D, Chiu WL, Duke K, Kiraly M, Kim SK. A global analysis of Caenorhabditis elegans operons. Nature 2002;417:851–854. [PubMed: 12075352]
14. Zorio DA, Cheng NN, Blumenthal T, Spieth J. Operons as a common form of chromosomal organization in C. elegans. Nature 1994;372:270–272. [PubMed: 7969472]
15. Guiliano DB, Blaxter ML. Operon conservation and the evolution of trans-splicing in the phylum Nematoda. PLoS Genet 2006;2:e198. [PubMed: 17121468]
16. Whitton C, Daub J, Thompson M, Blaxter M. Expressed sequence tags: medium-throughput protocols. Methods Mol Biol 2004;270:75–92. [PubMed: 15153623]
17. Awadzi K, Boakye DA, Edwards G, Opoku NO, Attah SK, Osei-Atweneboana MY, Lazdins-Helds JK, Ardrey AE, Addy ET, Quartey BT, Ahmed K, Boatin BA, Soumbey-Alley EW. An investigation of persistent microfilaridermias despite multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. Ann Trop Med Parasitol 2004;98:231–249. [PubMed: 15119969]
18. Schwab AE, Boakye DA, Kyelem D, Prichard RK. Detection of benzimidazole resistance-associated mutations in the filarial nematode Wuchereria bancrofti and evidence for selection by albendazole and ivermectin combination treatment. Am J Trop Med Hyg 2005;73:234–238. [PubMed: 16103581]
19. Vande Waa EA. Chemotherapy of filariases. Parasitol Today 1991;7:194–199. [PubMed: 15463492]

20. Frand AR, Russel S, Ruvkun G. Functional genomic analysis of C. elegans molting. PLoS Biol 2005;3:e312. [PubMed: 16122351]
21. Merriweather A, Guenzler V, Brenner M, Unnasch TR. Characterization and expression of enzymatically active recombinant filarial prolyl 4-hydroxylase. Mol Biochem Parasitol 2001;116:185–197. [PubMed: 11522351]
22. Barker GC, Mercer JG, Rees HH, Howells RE. The effect of ecdysteroids on the microfilarial production of Brugia pahangi and the control of meiotic reinitiation in the oocytes of Dirofilaria immitis. Parasitol Res 1991;77:65–71. [PubMed: 1994372]
23. Antebi A. Nuclear hormone receptors in C elegans. 2006
24. Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabrielian A, Galle RF, Gelbart WM, George RA, Goldstein LS, Gong F, Guan P, Harris NL, Hay BA, Hoskins RA, Li J, Li Z, Hynes RO, Jones SJ, Kuehl PM, Lemaitre B, Littleton JT, Morrison DK, Mungall C, O'Farrell PH, Pickeral OK, Shue C, Vosshall LB, Zhang J, Zhao Q, Zheng XH, Lewis S. Comparative genomics of the eukaryotes. Science 2000;287:2204–2215. [PubMed: 10731134]
25. Yates RA, Tuan RS, Shepley KJ, Unnasch TR. Characterization of genes encoding members of the nuclear hormone receptor superfamily from Onchocerca volvulus. Mol Biochem Parasitol 1995;70:19–31. [PubMed: 7637701]
26. Hills T, Brockie PJ, Maricq AV. Dopamine and glutamate control area-restricted search behavior in Caenorhabditis elegans. J Neurosci 2004;24:1217–1225. [PubMed: 14762140]
27. Horvitz HR, Chalfie M, Trent C, Sulston JE, Evans PD. Serotonin and octopamine in the nematode Caenorhabditis elegans. Science 1982;216:1012–1014. [PubMed: 6805073]
28. Sanyal S, Wintle RF, Kindt KS, Nuttley WM, Arvan R, Fitzmaurice P, Bigras E, Merz DC, Hebert TE, van der Kooy D, Schafer WR, Culotti JG, Van Tol HH. Dopamine modulates the plasticity of mechanosensory responses in Caenorhabditis elegans. Embo J 2004;23:473–482. [PubMed: 14739932]
29. Sawin ER, Ranganathan R, Horvitz HR. C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron 2000;26:619–631. [PubMed: 10896158]
30. Schafer WR, Kenyon CJ. A calcium-channel homologue required for adaptation to dopamine and serotonin in Caenorhabditis elegans. Nature 1995;375:73–78. [PubMed: 7723846]
31. Trent C, Tsuing N, Horvitz HR. Egg-laying defective mutants of the nematode Caenorhabditis elegans. Genetics 1983;104:619–647. [PubMed: 11813735]
32. Komuniecki RW, Hobson RJ, Rex EB, Hapiak VM, Komuniecki PR. Biogenic amine receptors in parasitic nematodes: what can be learned from Caenorhabditis elegans? Mol Biochem Parasitol 2004;137:1–11. [PubMed: 15279946]
33. Smith KA, Komuniecki RW, Ghedin E, Spiro D, Gray J. Genes encoding putative biogenic amine receptors in the parasitic nematode Brugia malayi. Invert Neurosci 2007;7:227–244. [PubMed: 18027007]
34. Dent JA, Davis MW, Avery L. avr-15 encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in Caenorhabditis elegans. Embo J 1997;16:5867–5879. [PubMed: 9312045]
35. Martin RJ, Robertson AP, Bjorn H. Target sites of anthelmintics. Parasitology 1997;114 (Suppl):S111–124. [PubMed: 9309773]
36. Scott AL. Nematode sperm. Parasitology Today 1996;12:425–430. [PubMed: 15275275]
37. Roberts TM, Stewart M. Nematode sperm: amoeboid movement without actin. Trends Cell Biol 1997;7:368–373. [PubMed: 17708982]
38. Tarr DE, Scott AL. MSP domain proteins. Trends Parasitol 2005;21:224–231. [PubMed: 15837611]
39. Reinke V, Gil IS, Ward S, Kazmer K. Genome-wide germline-enriched and sex-biased expression profiles in Caenorhabditis elegans. Development 2004;131:311–323. [PubMed: 14668411]

40. Maizels RM, Blaxter ML, Scott AL. Immunological genomics of *Brugia malayi*: filarial genes implicated in immune evasion and protective immunity. Parasite Immunol 2001;23:327–344. [PubMed: 11472553]

41. Hoerauf A, Satoguina J, Saeftel M, Specht S. Immunomodulation by filarial nematodes. Parasite Immunol 2005;27:417–429. [PubMed: 16179035]

42. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003;3:733–744. [PubMed: 12949497]

43. Fenn K, Blaxter M. Are filarial nematode *Wolbachia* obligate mutualist symbionts? Trends in Ecology and Evolution 2004;19:163–166. [PubMed: 16701248]

44. Chen SN, Howells RE. Brugia pahangi: uptake and incorporation of nuclei acid precursors by microfilariae and macrofilariae in vitro. Exp Parasitol 1981;51:296–306. [PubMed: 6970680]

45. Rao RU, Moussa H, Weil GJ. Brugia malayi: effects of antibacterial agents on larval viability and development in vitro. Exp Parasitol 2002;101:77–81. [PubMed: 12243742]

46. Mitreva M, Blaxter ML, Bird DM, McCarter JP. Comparative genomics of nematodes. Trends Genet 2005;21:573–581. [PubMed: 16099532]

47. Parkinson J, Mitreva M, Whitton C, Thomson M, Daub J, Martin J, Schmid R, Hall N, Barrell B, Waterston RH, McCarter JP, Blaxter ML. A transcriptomic analysis of the phylum Nematoda. Nat Genet 2004;36:1259–1267. [PubMed: 15543149]

48. Blaxter ML. Nematoda: genes, genomes and the evolution of parasitism. Adv Parasitol 2003;54:101–195. [PubMed: 14711085]

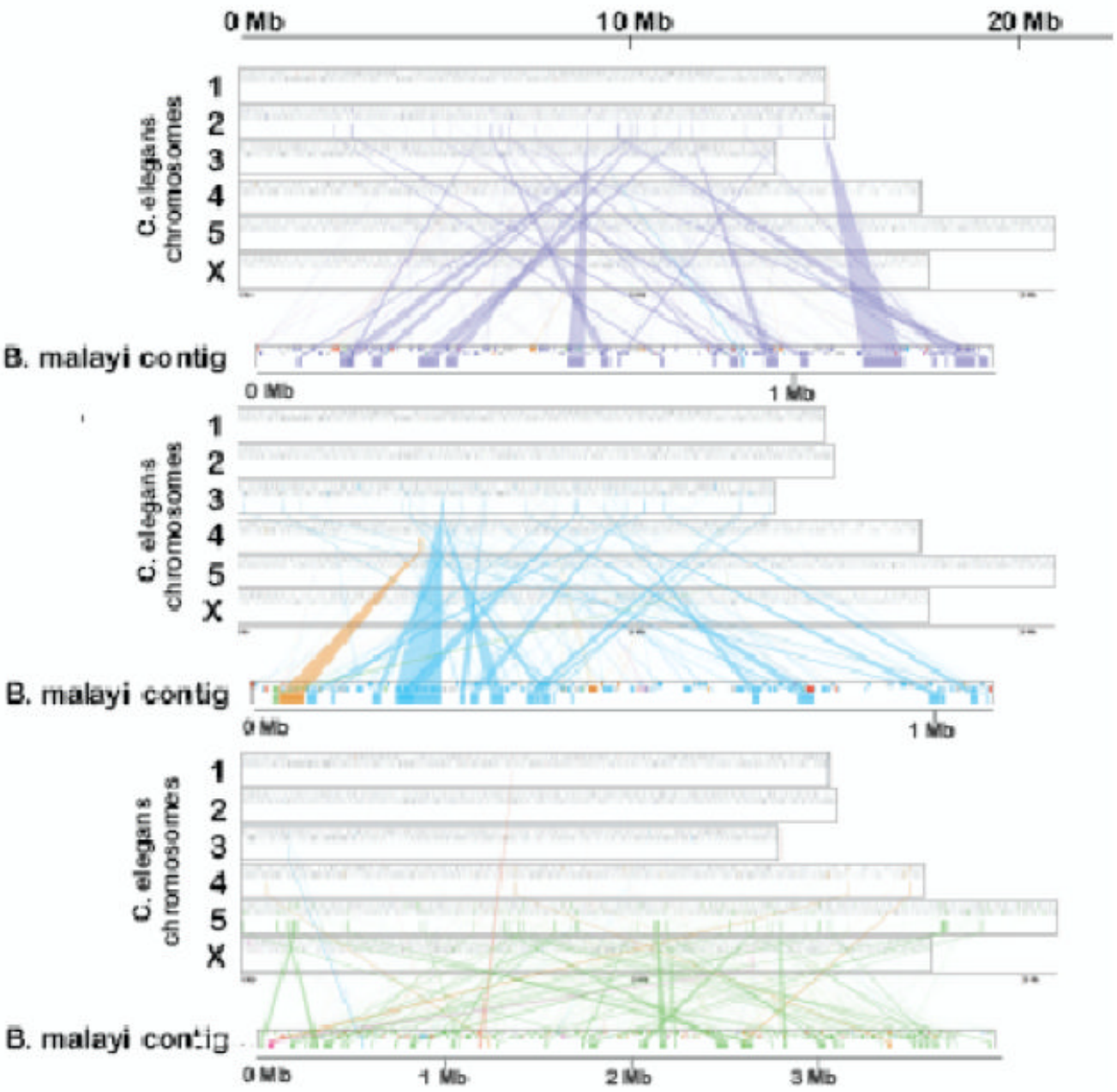


**Figure 1.**

Long-range synteny between *B. malayi* and *C. elegans* is conserved but short-range synteny is not. Examples of three *B. malayi* of contigs of ~1.25 Mb (top), ~1.1 Mb (middle) and ~3.9 Mb (bottom) demonstrating the genes on the *B. malayi* contigs are found predominantly on *C. elegans* chromosomes 2 (purple), 3 (aqua) and 5 (green), respectively. While association at the chromosome level is maintained the gene order between *B. malayi* and *C. elegans* is not conserved. The scale at the top of the figure is applied to the linear maps of *C. elegans* chromosomes 1 through 5 plus X. The scale for each *B. malayi* contig map is indicated beneath each map. Forward and reverse-strand genes are distinguished (forward on top, reverse below).

**Table 1**

Features of the *B. malayi* and C. elegans genomes

| | **B. malayi** | **C. elegans** |
|---|---|---|
| Genome size | ~ 95 Mb | 100 Mb |
| Percent protein-coding sequence | 17.8% | 30.2% |
| Number of proteins | 11,508* | 19,762** |
| Max/Avg protein length (aa) | 9,445 / 371 | 18,563 / 440 |
| Gene density | 161 per Mb | 228 per Mb |
| Number of exons | 83,672 | 146,027 |
| Mean/median exon size (bp) | 159 / 140 | 307 / 147 |
| Mean/median number of exons per gene | 7.27 / 5 | 6.38 / 6 |
| Mean/median intron size (bp) | 311 / 219 | 320 / 68 |
| Mean length of intergenic region (bp) | 3783 | 2218 |
| Overall G+C content | 30.5% | 35.4% |
| Exons G+C content | 39.6% | 42.9% |
| Introns G+C content | 27.6% | 29.1% |
| Intergenic regions G+C content | 30.9% | 32.5% |

* number of protein-coding genes identified in assembled contigs - estimates for fully assembled genome are 14,500 to 17,800

** WormBase release WS133