Comparative Genetics and Genomics of Nematodes: Genome Structure, Development, and Lifestyle

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Abstract

Nematodes are found in virtually all habitats on earth. Many of them are parasites of plants and animals, including humans. The free-living nematode, *Caenorhabditis elegans*, is one of the genetically best-studied model organisms and was the first metazoan whose genome was fully sequenced. In recent years, the draft genome sequences of another six nematodes representing four of the five major clades of nematodes were published. Compared to mammalian genomes, all these genomes are very small. Nevertheless, they contain almost the same number of genes as the human genome. Nematodes are therefore a very attractive system for comparative genetic and genomic studies, with *C. elegans* as an excellent baseline. Here, we review the efforts that were made to extend genetic analysis to nematodes other than *C. elegans*, and we compare the seven available nematode genomes. One of the most striking findings is the unexpectedly high incidence of gene acquisition through horizontal gene transfer (HGT).

INTRODUCTION

Nematodes, or roundworms, are small animals that are often difficult to see without a microscope. Given their size in the range of only millimeters, they are largely unknown to the general public. Nonetheless, nematodes represent the largest animal phylum, with an estimated number in the range of one to ten million species (58). The highest diversity is found in marine environments and in terrestrial settings, often in association with arthropods or other invertebrates. Not surprisingly, therefore, the majority of this enormous biodiversity is unexplored, as only ~25,000 species are described in the literature to date. Besides species richness, numerical abundance and ecological omnipresence are the two other key features that specify nematodes and point to their importance (59). Although free-living species are found in nearly all habitats—marine, freshwater, and soil—nematodes are also important parasites of plants, livestock, and humans. In many of these ecological niches, nematodes can occur in very high density, e.g., in excess of one million individuals per square meter in some soil samples (30). Molecular phylogenetics has resulted in a comprehensive understanding of the relationships among nematodes (10, 48, 49). Throughout the text we use the terminology and phylogenetic groupings according to Blaxter and colleagues (10), who distinguish five major nematode clades (**Figure 1**).

Biologists have used several nematode species as model organisms for both basic and applied research. *Caenorhabditis elegans* is one of the best-studied model species and has played a pivotal role in fundamental areas of research, such as genetics and genomics, covered in this review. One reason for the success of *C. elegans* as a model is the ease with which this species can be propagated in the laboratory. With a life cycle of only three days (20°C), *Escherichia coli* as food source, and self-fertilization as the typical mode of reproduction, *C. elegans* can be cultured indefinitely in large numbers. Starting from this simple regime, *C. elegans* researchers have championed many research areas in modern biology. The complete cell lineage of "the worm" was determined in the 1970s, providing the basis for detailed investigations of embryonic and postembryonic development (93, 94). Genetic screens for genes controlling basic processes in cell, developmental, and neurobiology have been performed, often reaching saturation level (16). In 1998, *C. elegans* was the first metazoan to have its genome fully

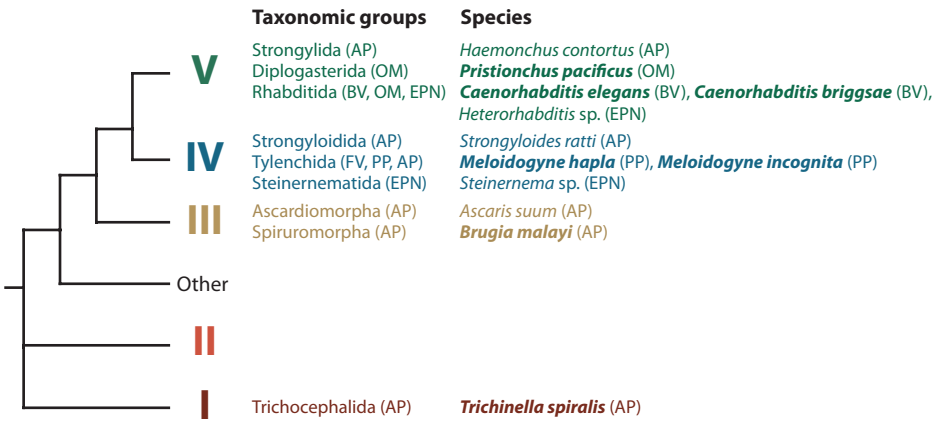

Figure 1

Phylogenetic relationship of nematodes with a particular emphasis on species mentioned in the text. Species with a published genome sequence are indicated in bold. Roman numerals indicate the clade according to Reference 10. AP, animal parasite; BV, bacteriovore; EPN, enthomopathogenic nematode; OM, omnivore; PP, plant parasite.

sequenced (13), followed by the sophisticated application of other "-omics" technologies.

The enormous advancements of *C. elegans* biology over the past three decades have also paved the way for the analysis of various parasitic nematodes that are of importance in human and veterinary medicine or for pest control. Both genetic methodology and various genomic sequencing platforms provide important tools for the analysis of parasites. In addition, the enormous knowledge about *C. elegans* was used as a starting point for studies in evolutionary biology (89). One common complication of working with nematodes other than *C. elegans* is the usually more complicated lifestyle of such species. In particular, human and animal parasites have complex life cycles, and only a few species can be cultured in the laboratory. Here, we review comparative genetics and genomics in nematodes with a special emphasis on nematode lifestyles. The application of several high-throughput methodologies provides for new research avenues on nonmodel nematodes.

LIFECYCLES

The typical nematode undergoes embryonic development within an eggshell. Postembryonic development consists of four stages, interchangeably called larval (L1–L4) or juvenile (J1–J4), which are separated by molts (59). The last molt results in the adult worm. This general life cycle exists in numerous variations. In some cases, alternative forms of certain developmental stages are possible and some or all stages can be parasitic. The minimum time required to complete a generation varies greatly between species, ranging from days to months or even years. Similarly, the duration of the reproductive life in some species is only a few days but several years in others. To illustrate the variety of nematode lifestyles, we summarize the life cycles of a few nematodes for which genome sequencing is complete or in progress (**Figure 2**). Nematode life cycles are described in numerous textbooks. To compile the paragraphs below we used references 2, 16, 27, 32, and 82 as sources.

Free-Living Rhabditides and Diplogastrids

Saprobiotic, bacterial-feeding nematodes are common in the family Rhabditidae and Diplogastridae, which belong to Clade V and represent one of the best-characterized groups of nematodes (**Figure 1**). Under favorable food conditions in the laboratory, the development of *C. elegans* takes as little as three days. The total adult reproductive life span is also on the order of several days. If the conditions are unfavorable, these worms form a nonfeeding but motile alternative J3 called a dauer juvenile, which can survive for months and resume development once conditions improve (**Figure 2*a***). In some rhabditid and diplogastrid species, hermaphrodites, which in addition to mating with males can self-fertilize a portion of their eggs, replace females in the populations. These androdioecious mating systems have evolved multiple times independently. In both families, the best-studied representatives, i.e., *C. elegans* (Rhabditidae) and *Pristionchus pacificus* (Diplogastridae), are hermaphroditic.

Entomopathogenic Nematodes

The genera *Steinernema* (Clade IV) and *Heterorhabditis* (Clade V) are two phylogenetically unrelated groups of entomopathogenic nematodes (EPNs) (**Figures 1**, **2*c***). They have convergently evolved a very similar life cycle in which infective larvae carry a single individual of a symbiontic bacterium, *Xenorhabdus* spp. in the case of *Steinernema* spp. and *Photorhabdus* spp. in that of *Heterorhabditis* spp. Worms invade insect larvae through body openings (*Steinernema* sp.) or by penetrating the cuticle (*Heterorhabditis* sp.). In the insect, they release the bacterium, which reproduces very rapidly and kills the insect by the secretion of a number of toxins (24). The worms then feed on the bacteria and undergo a rapid succession of reproductive cycles. Although *Steinernema* spp. is gonochoristic, *Heterorhabditis* spp. infective juveniles develop into hermaphrodites, which then can give rise to hermaphrodites, females,

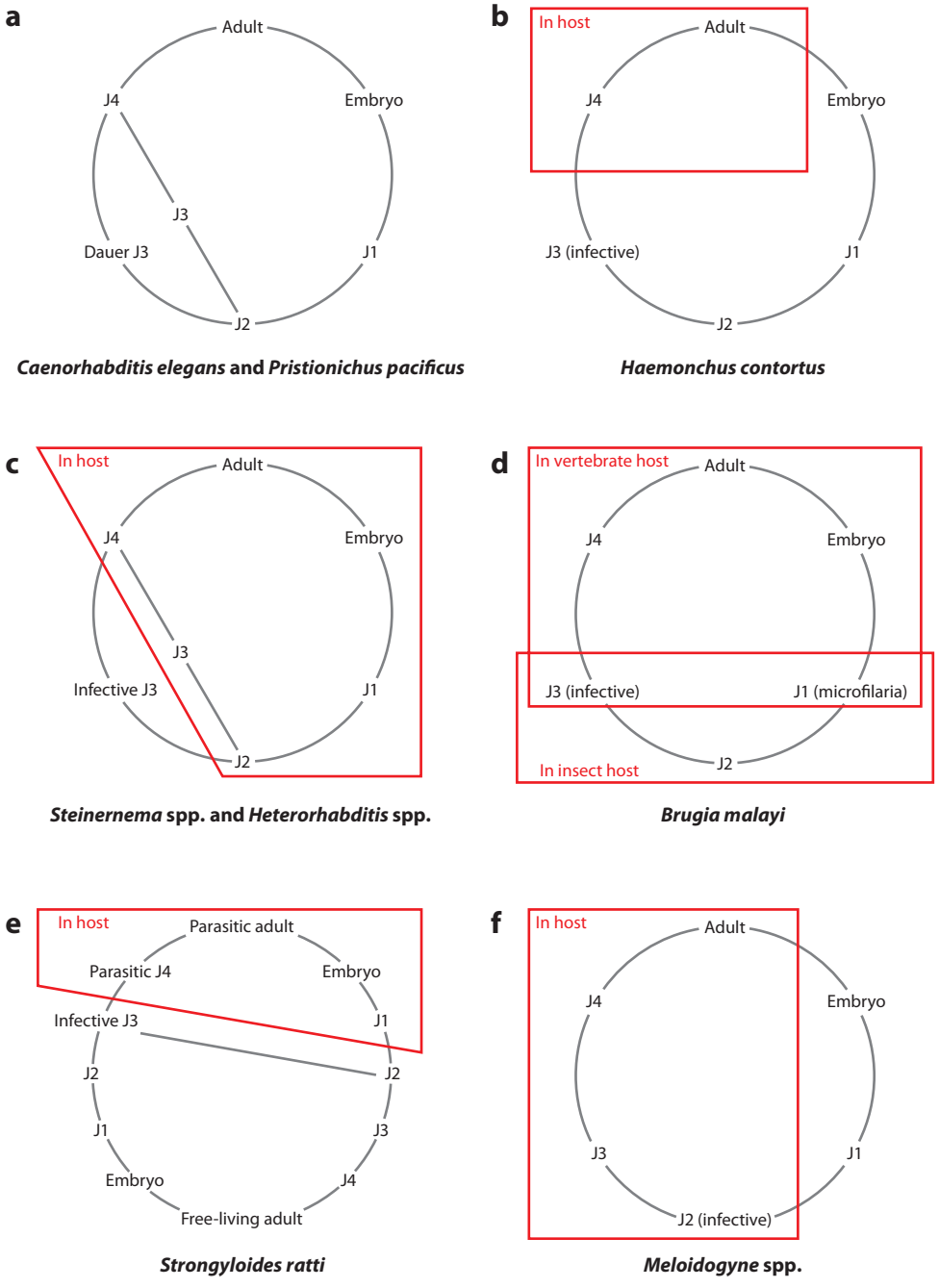

Figure 2

Examples of nematode life cycles.

and males. Once the food is exhausted, the juvenile worms develop into infective larvae, which search for new prey. Although some of these nematodes are generalists with respect to insects they attack, the nematode-bacterium association is highly species-specific.

Gastrointestinal Animal Parasites

Many nematodes are gastrointestinal parasites of vertebrates. Below, we summarize the life cycles of four medically and/or economically important representatives of this type. In spite of sharing a habitat, they are only very distantly related to each other and belong to different clades (**Figure 1**).

Haemonchus contortus

The adult worms are up to 3 cm long and live in the abomasum of sheep and goats. Embryonated eggs are passed with the feces. Like most gastrointestinal nematode parasites, *Haemonchus contortus* (**Figure 2*b***) undergoes a portion of its development outside of the host, where it forms infective third-stage juveniles, which are taken up by new hosts with the food. The larvae migrate to the abomasum where they molt to the J4 and penetrate the lining and feed on blood. After the final molt, the adult worms return to the lumen of the abomasum. *H. contortus* is a representative of the Strongylida (Clade V). This group contains the closest relatives of *C. elegans* among the economically and medically important parasites, e.g., the human hookworms.

Ascaris suum

Adult *Ascaris suum* are up to 30 cm long and 5 mm in diameter and live in the small intestine of pigs. The eggs are passed with the feces. Development to the infective third-stage juvenile occurs within the eggshell, and the J3 hatch only after being taken up orally by a new host. The J3s invade the intestinal veins and undergo a multistep migration through the liver (where they molt to J4s), the lungs, the mouth cavity, and the digestive tract, which leads them back to the small intestine, where the final molt takes place.

Strongyloides ratti

The parasitic adults of *Strongyloides ratti* are 2–3 mm long and live in the small intestine of rats. They are females with parthenogentic reproduction that give rise to parasitic and free-living progeny, the two of which differ morphologically and in many other aspects of their biology (**Figure 2*e***). Late-embryonated eggs or first-stage juvenile worms are passed with the feces. A portion of the female progeny develops into infective juveniles, which infect a new host by skin penetration followed by migration through the circulatory and respiratory systems. Finally, the worms are swallowed and establish themselves in the small intestine, completing a nonsexual reproductive cycle. The remaining females and all males develop into sexually reproducing free-living adults. In contrast to parasitic females, which can reproduce for several weeks, the free-living adults live for only a few days. The entire progeny of a free-living generation develops into infective juveniles, which are all females.

Trichinella spiralis

In contrast to most other nematode parasites, *Trichinella spiralis* is an intracellular parasite, and unlike most gastrointestinal nematodes it does not form any nonparasitic developmental stages. The adults can be more than 3 cm long and live in syncytia of the small intestine of the host. The viviparous females release first-stage juveniles, which migrate to the striated musculature, invade muscle fibers, and induce the formation of so-called nurse cells. They can remain dormant for years and wait for a predator or scavenger to take them up along with their host's meat. In this new host, they invade intestinal epithelial cells and resume development to close the cycle.

Brugia malayi: A Parasite with Multiple Hosts

Although the four parasites introduced above have only one host species, several nematode parasites have more complex life cycles with a host switch. Among them are the filarial nematodes. The adults of *Brugia malayi*, which can be up to 10 cm long, inhabit the lymphatic system of their hosts, including humans (**Figure 2*d***). First-stage juveniles (microfilariae) hatch within the uterus of the mother and translocate to the blood of the host, where they are taken up by their vector, mosquitoes of the genus *Mansonia*. In the vector, they develop to J3s, which are capable of infecting a new host during the next blood meal of the mosquito. Adult worms can survive and reproduce in the host for several years.

Meloidogyne sp. As an Example for Plant Parasitic Nematodes

Nematodes are equally successful parasites of animals and plants. *Meloidogyne* spp. are among the most important agricultural pests. Although males do exist in all known species, the most economically important species reproduce parthenogenetically. For some species (e.g., *M. incognita*), parthenogenesis is obligatory, whereas for others (e.g., *M. hapla*) occasional outcrossing is possible. Second-stage juveniles invade the roots of plants at the growing tip (**Figure 2*f***). They migrate between the cells and establish a permanent feeding site close to the developing vascular cylinder. There they molt three times, without feeding between molts, to become adults. At the feeding site they induce the formation of multinucleated "giant cells," off which they feed. Eggs are pushed to the surface of the root. The first molt takes place within the eggshell, and the J2s hatch and disperse in the soil to search for a host.

Evolution of Parasitism

It is unlikely that a species switches from a fully free-living to a parasitic lifestyle in one step, and it is generally accepted that prior to the transition to parasitism preadaptations must exist. Preadaptations are features that evolved for different reasons but facilitated the step towards parasitism (23, 75, 79). Indeed, many free-living nematodes can be found in association with other organisms without being parasitic (59). These associations range from short-term, rather unspecific, phoretic interactions to long-term associations that can be highly species-specific. For example, dauer juveniles of different species of the genus *Pristionchus* associate in a species-specific manner with scarab beetles (43, 104). The worms seem not to harm the beetle, but instead wait until the beetle dies to resume development on the rich microbial fauna that emerges on the carcass. There is empirical evidence that at least for some parasitic nematodes the infective stages, which are crucial for entering the host, are homologous to the dauer juveniles in free-living nematodes (73, 102). Therefore, dauer juveniles and phoretic and necromenic interactions are likely candidates for the preadaptations that facilitated the evolution of parasitism by allowing a stepwise formation of tight and specific interactions (23). The fact that we find closely related species with very different ecologies, and very distantly related species with similar ecologies, makes nematodes an interesting system with which to investigate how genomes are shaped by the environment and by evolutionary descent.

COMPARATIVE GENETICS

With *C. elegans*, the phylum Nematoda contains one of the best-studied model organisms for genetics and with this an excellent baseline for comparative genetic studies. Genetic work requires methods to induce, isolate, cross, and characterize mutants and, as probably the most challenging element, ways of physically identifying the genes that carry the mutations isolated based on their phenotypes. In *C. elegans*, this has been traditionally achieved by a process called positional cloning. This approach requires a dense, high-quality genetic map for accurate genetic mapping and a physical map (ideally a

full genome sequence) that is highly interlinked with the genetic map. In addition, transgenic technology is required to narrow down genomic regions during mapping and for gene verification after final identification (28, 47).

Full tool sets for the isolation and systematic study of mutations in known genes generated by forward and reverse genetics are currently available only for two nematode species other than *C. elegans*, namely *Caenorhabditis briggsae* and *P. pacificus*. For both species, relatively dense genetic maps, which are well anchored in the genome, allow the positional cloning of genes (22, 56, 90). Mutations in molecularly defined genes have been isolated by polymerase chain reaction (PCR) based screening for small deletions (5, 77), and very recently zinc finger nucleases were used for targeted mutagenesis in *C. briggsae* (B. Meyer, personal communication). Finally, transgenic techniques are available for both species (5, 86). Comparative genetic work in these species has concentrated on developmental processes that are very well understood in *C. elegans*. These studies have offered interesting insights into how the genetic control of development can change during evolution. A few of these studies are discussed briefly below.

C. elegans, *C. briggsae*, and *P. pacificus* form self-fertile hermaphrodites instead of females, in which the germ line produces a limited number of sperm before switching to oogenesis. In all three species, the function of the transcription factor TRA-1 in somatic sex determination appears conserved (53, 78). Also mutations in genes that are components of the two regulatory steps upstream of *tra-1* (i.e., *fem-2/fem-3 and tra-1/tra-3*) have identical somatic phenotypes in *C. elegans* and *C. briggsae* (45, 53). Interestingly, contrary to *C. elegans*, in *C. briggsae*, *fem-2* and *fem-3* turned out not to be required for spermatogenesis. *fem-2* and *fem-3* mutants were self-fertile hermaphrodites and not females like their *C. elegans* counterparts (45, 46). These findings illustrated how the two species, in a case of convergent evolution, acquired different mechanisms to transiently repress the feminizing activity in the germ line, leading to the temporary production of sperm in otherwise female individuals.

The induction of the vulva in *C. elegans* is one of the best-studied genetic processes in animal development. In *C. elegans*, three vulval precursor cells are induced to form vulval tissue by an epidermal growth factor (EGF)-type signal (92). In *P. pacificus*, the same three cells form the vulva, and orthologs of the genes that are responsible for vulval induction in *C. elegans* exist in the genome. Nevertheless, the inductive signal in *P. pacificus* is a Wingless (Wnt)-type signal (95). The switch of the signaling system involves a novel regulatory linkage of Wnt signaling, which is unknown from other organisms (101a). In *C. elegans*–vulva development, Wnt signaling is also required but for different processes. It acts prior to induction to maintain the competence of vulval precursor cells to respond to the inductive signal, and it is used again after the induction for the correct specification of tissue polarity (39, 69). These findings illustrate how the genetic control of the development of an organ can change, essentially without changing the organ itself.

Inoue and coworkers (50) isolated a number of dauer formation defective *C. briggsae* mutants and cloned several of the corresponding genes. From this work, it appears that the genetic control of the dauer switch is conserved between *C. elegans* and *C. briggsae*. In *P. pacificus*, the downstream components of the genetic cascade, namely an endocrine regulatory module consisting of the nuclear hormone receptor DAF-12 and its ligand dafachronic acid, as well as a fork-head transcription factor encoded by the gene *daf-16*, are also involved in dauer formation (72, 73). In addition to its function in dauer formation, the *P. pacificus daf-12*, but not *daf-16*, module of the dauer control mechanism was recruited for the regulation of a mouth dimorphism and the development of teeth-like denticles (8). This polyphenism is an evolutionary novelty in *P. pacificus* and related taxa, and does not exist in *C. elegans*.

In addition to mutational analysis, RNA interference (RNAi), a technique to knock down the activity of genes that was pioneered in

RNAi: double-stranded RNA interference

C. elegans (29), has been used for comparative studies in *C. briggsae* and other species of *Caenorhabditis*. For example, knocking down the activity of *pop-1*, which encodes the transcription factor at the bottom of the Wnt signaling cascade, had opposite effects on an early cell fate specification event in *C. elegans* and *C. briggsae*. In both species, one blastomere, called EMS, in the four-cell embryo undergoes an asymmetric cell division to form one mesodermal daughter (MS) and one daughter (E) that is the sole endodermal precursor and gives rise to the intestine. *pop-1*(RNAi) led MS to take on the E cell fate in *C. elegans* but resulted in an E to MS transformation in *C. briggsae*. Of the two other species tested, *Caenorhabditis remanei* behaved like *C. elegans* and *Caenorhabditis* sp. 9 behaved like *C. briggsae* (60). This is an example of how the regulatory logic can change although the players and the developmental output remain the same.

As outlined above, hermaphroditism has evolved multiple times within the genus *Caneorhabditis*. Baldi and coworkers (6) showed that reducing the activity of only two genes is required to transform *C. remanei* females into self-fertile hermaphrodites. Lowering (not eliminating) the activity of *tra-2*, a key component of somatic and germline sex determination in *C. elegans*, was sufficient to allow spermatogenesis to occur in addition to oogenesis in *C. remanei*. However, the sperm formed was not activated. Sperm activation and subsequent self-fertilization was achieved by reducing the activity of *swm-1*, a gene known to prevent premature sperm activation in *C. elegans*.

Overall, however, RNAi as a method has not entirely fulfilled the high hopes it sparked originally for postgenomic nematode research. It works reliably only for a very limited number of nematode species, among them several of the different *Caenorhabditis* species and some plant parasitic nematodes. At the same time, it has failed to produce specific, reproducible results in many other nematodes, in particular all animal parasitic nematodes, in which it was tried (100). Even in species where it does work, RNAi has been shown to be prone to artifacts due to off-target effects (18, 84). Efforts to make RNAi work in more species and to improve its specificity are in progress and are likely to have some success. For example, in the plant-parasitic nematodes *Globodera pallida* and *M. incognita* the use of short interfering RNAs rather than long dsRNA molecules was recently reported to increase specificity (17). Nevertheless, RNAi as replacement for mutations should only be used with great care. The study of true mutants, be they isolated by forward or reverse genetic strategies, remains highly desirable.

So far, the genetic analysis of parasitic nematodes is rather rudimentary because of technical constrains. Usually, the sexually reproducing worms are within their hosts, rendering them difficult to access and manipulate [except for a few cases where in addition to parasitic adults, free-living adults also occur, e.g., *Strongyloides* spp. (38, 98)]. Nevertheless, efforts to make parasitic nematodes amenable to genetic analysis have been made and some groundwork laid out. Strategies to perform controlled crosses between defined isolates or even individuals have been developed for a few parasitic nematodes and molecular genetic markers were isolated (4, 14, 26, 38, 80, 81, 83). Genetic maps were published for three plant parasitic [*Meloidogyne hapla* (74), *Heterodera glycines* (4), *Globodera rostochiensis* (83)] and one animal parasitic nematode (*S. ratti*) (71). These methods and tools are not yet sufficient for mutational analysis and positional cloning of genes but have already been used successfully to elucidate modes of inheritance and to characterize reproductive strategies. In *H. contortus*, a fairly large set of microsatellite markers that are polymorphic between different isolates has been developed and used to show genetically that *H. contortus* employs an XX/X0 sex determination system (females have two X chromosomes, males only one) and that females mate with multiple males (80). In *M. hapla*, almost 300 amplified fragment length polymorphism markers that are polymorphic between two isolates are present on the genetic linkage map (74). Analyzing the pattern with which these markers are inherited was used to characterize

the form of facultative meiotic parthenogenesis in this species (61). This form of reproduction was shown to lead to a rapid homozygotization of the genome that is only counteracted by occasional outcrossing. In *S. ratti* and in *S. papillosus*, minisatellites and SNP markers were used to demonstrate that reproduction is mitotically parthenogenetic in the parasitic generation and sexual in the free-living generation (26, 70, 71, 96, 99). Comparative analysis of SNP markers in homologous genes in *S. ratti* and *S. papillosus* showed that, although *S. ratti* employs XX/XO sex determination (41), in *S. papillosus* the X chromsome appears fused with one of the autosomes and in males only this portion of the fused chromosome undergoes sex specific chromatin diminution, a process in which a portion of a chromosome is puposefully eliminated and that has been found in relatively few animal species, among them several parasitic nematodes (70).

Protocols for the experimental induction of mutations have been reported for very few parasitic or parasitoid nematodes [e.g., *S. ratti* (97) and *H. bacteriophora* (105)]. In addition, spontaneous mutants were found and characterized genetically in several parasitic nematodes, e.g., worms that are virulent for otherwise resistant hosts or resistant against certain nematicidal drugs and pose an enormous economical and medical problem (14, 36). The main reason why more effort has not so far been made to isolate mutants in parasitic nematodes is probably because there was no straightforward way to identify the gene in which the mutation occurred. So far the only strategy that has led to the identification of mutated genes in nematodes other than *C. elegans*, *C. briggsae*, and *P. pacificus* is the candidate gene approach, based on knowledge from other systems, in particular *C. elegans*. This approach has, for example, been successful in identifying mutations that render *H. contortus* resistant to some classes of nematicidal compounds (e.g., benzimidazoles and amino-acetonitrile derivatives) but has failed for others (e.g., tetrahydropyrimidines/imidazothiazoles and macrocyclic lactones) (36, 52). However, there is reason for optimism. With full genome sequences becoming available and with rapid progress in sequencing technology, it will become feasible to identify mutated genes by whole genome sequencing of mutant and nonmutant siblings, as has been shown to work in *C. elegans* (85).

Transgenic techniques have been described for a few parasitic and parasitoid nematodes [e.g., *Parastrongyloides trichosuri* (37), *Strongyloides stercoralis* (51), and *H. bacteriophora* (42)]. Undoubtedly, this technical progress will dramatically enhance the usefulness of genetics in several additional nematode species and open the way for much more extensive comparative mutational studies that extend beyond *Caenorhabditis* and *Pristionchus*.

EST: expressed sequence tag

GENOMES

In recent years, nematode genomics has seen several finished and ongoing full-genome projects and a high number of expressed sequence tag (EST) sequencing projects. By March 2011, the Nembase4 database (**http://www.nematodes.org/nembase4/**) offered more than 600,000 ESTs representing over 250,000 genes derived from 63 different nematode species for download. These EST sequences sparked a number of comparative analyses of gene content and expression. ESTs also play a crucial role for the annotation of the increasing number of nematode genomes. However, given the space restriction of this review, we refer the reader to the databases provided in **Table 1** for further exploration of EST projects, and we restrict ourselves to the discussion of the seven finished and published genome sequence projects of members of five different nematode genera representing four of the five clades (1, 13, 21, 35, 67, 74, 91).

In comparison with other organisms, in particular with the human genome, all seven published nematode genomes are very compact (**Table 2**). Although they are only 1.7% to 4.2% the size of the human genome, they are predicted to contain roughly the same number of genes resulting in a much higher average gene density. While the size of an average exon in

Table 1 Selected web resources for nematode sequencing efforts

Web site	Title	Description
http://www.wormbase.org/	WormBase	Information about the *Caenorhabditis elegans* and *Caenorhabditis briggsae* genomes. Other genomes are being incorporated.
http://www.pngg.org/cbnp/	Plant Nematode Genomics Group	Information about the *Meloidogyne hapla* genome.
http://www.inra.fr/meloidogyne_incognita	*Meloidogyne incognita* resources	Information about the *M. incognita* genome.
http://www.pristionchus.org	**www.pristionchus.org**	Information about the *Pristionchus pacificus* genome.
http://www.nematodes.org/nematodegenomes/index.php/959_Nematode_Genomes	959 nematode genomes	Overview of and links to the various complete, ongoing, and planned nematode genome and expressed sequence tag (EST) sequencing efforts.
http://www.nematode.net/NN3_frontpage.cgi	Nematode.net	Home page of the parasitic nematode EST project at the Genome Center at Washington University
http://www.nematodes.org/nembase4/	NEMBASE4	A nematode transcriptome database offering access to ESTs from more than 60 species.
http://www.sanger.ac.uk/resources/downloads/helminths/	Helminth genomes - data download	Provides information about the nematode sequencing projects at the Wellcome Trust Sanger Institute.
http://genome.wustl.edu/genomes/list/invertebrates	Invertebrates	Provides information about the nematode sequencing projects at the Genome Center at Washington University. Go to this web site for more information about the *Trichinella spiralis* genome.

Table 2 Genome properties of the seven fully sequenced nematode genomes and outgroup comparison

	C. el[a]	*C. br*[a]	*P. pa*[a]	*B. ma*[a]	*M. ha*[a]	*M. in*[a]	*T. sp*[b]	*D. me*[c]	*H. sa*[c]
Genome size (Mb)	100	104	142	88	53,5	82	64	169	3137
Protein encoding genes	20,060	19,934	24231[d]	11,515	14,420	19,212	15,808	13,804	21,550
Gene density (genes per Mb)[e]	201	192	171	131	270	234	247	81,6	6,8
Mean number of exons per gene	6,4	6	9	7,3	6	6,6	n.a.	5,4	8,1
Median exon size (nt)	147	150	85	140	145	136	n.a.	247	126
Median intron size (nt)	68	54	110	219	55	82	n.a.	92	1492

[a]Numbers taken from Reference 23, which reanalyzed all six published nematode genomes with identical methods.
[b]Numbers taken from Reference 67.
[c]Numbers derived from Ensembl release 61 (**http://www.ensembl.org/index.html**).
[d]Number derived from Reference 12.
[e] Calculated as row 3 divided by row 2.
Abbreviations, *C. el*, *C. elegans; C. br; C. briggsae; P. pa*, *P. pacificus; M. ma*, *B. malayi; M. ha*, *M. hapla; M. in*, *M. incognita; T.* sp. *T. spiralis; D. me*, *Drosophila melanogaster*, *H. sa*, *Homo sapiens*.n.a., not available.

nematodes seems to be comparable to a human exon, the introns and intergenic regions tend to be considerably smaller.

It is striking that all seven sequenced nematode genomes contain a relatively high number of novel genes that have no recognizable orthologs in other nematodes or other organisms. Similar observations were also made by comparing EST sequences from many more species (103). This indicates that genes are born and lost relatively frequently in nematode evolution.

In *C. elegans*, a 22-nucleotide (nt)-long RNA fragment called a spliced leader (SL) is added post-transcriptionally to the 5′ ends of the mRNAs of about 70% of the genes (11). This process is called *trans*-splicing and in most cases occurs only a few nts upstream from the translation start site. Two kinds of SLs exist, namely SL1 and SL2. SL1 is uniform and is transcribed from 110 genes that occur in tandem repeats together with the genes for the 5S rRNA. Several variants of SL2 are transcribed from a total of 18 genes, which are dispersed in the genome. SL2 is specialized for the *trans*-splicing of downstream genes in so-called operons. These are multi-gene transcription units, which are transcribed into polycistronic premRNAs. The premRNAs are then broken up into individual mRNAs by means of polyadenylation and *trans*-splicing. Although *trans*-splicing and operons are widespread among nematodes of at least clades III–V, the presence of a specialized SL2 appears to be limited to members of Clade V (40). In other species, multiple similar variants of SL1, but no SL2, have been found. In representatives of Clade I, no canonical SL1 or SL2 has been found. *Trans*-splicing does occur in *T. spiralis*, but the 19 known SLs are highly variable and the fraction of mRNAs that are *trans*-spliced is only approximately 1% (67, 76). It is beyond the scope of this review to discuss the molecular mechanism of *trans*-splicing, which has been studied extensively in *C. elegans* and *A. suum* (11). However, we would like to point to two consequences of this process whose significance is not understood. First, because of the origin of the SL from a small nuclear ribonucleoprotein particle (snRNP), the 5′ cap at the messenger RNA is different from non*trans*-spliced pol II products (trimethylated as opposed to monomethylated). Second, many mRNAs share identical or very similar 5′ ends. This highly conserved SL nt sequence at the 5′ end might be required to allow efficient translation from 3m-G capped mRNAs (15). As a byproduct, the similarity of 5′ ends of mRNA sequences in nematodes has been a helpful tool in molecular studies of many species, primarily by simplifying PCR approaches.

SL: spliced leader

In the following section, we provide an overview of the finished genome sequencing projects and report them in chronological order of their publication.

***Caenorhabditis elegans* (http://www.wormbase.org).** In 1998, *C. elegans* was the first metazoan to have its genome fully sequenced (13) and since has been among the forerunners for genome-wide large-scale data generation. Gene expression profiling (44, 55), protein interaction maps (101), and RNA interference screens (31) were followed by many other high-throughput studies providing catalogs of the *C. elegans* proteome (87) and sets of noncoding RNAs (20). Over the years, the compactness of the 100.3 MB *C. elegans* genome has been a powerful test case for the improvement of genome annotations. The U.S. National Human Genome Research Institute (NHGRI) supports the *C. elegans* database WormBase and in 2007 initiated modENCODE (model organism Encyclopedia of DNA elements), which aims for systematic annotation of all functional genome elements. By the end of 2010, the modENCODE project provided an integrative analysis of the *C. elegans* genome (33). Based on more than one billion RNA-seq reads from 19 different nematode populations that included several developmental stages, pathogen-exposed animals, and selected mutants, modENCODE generated 64,824 transcripts from 21,733 genes. This resulted in 95% of transcripts in WormBase being supported by RNA-seq data with

HGT: horizontal gene transfer

accurate models of exon-intron boundaries, 5′ and 3′ untranslated regions, and *trans*-SLs.

C. elegans has also paved the way for the appreciation of genome dynamics. Comparative genomic hybridization of different *C. elegans* strains revealed natural gene content variation of more than 2% (62), which is now complemented by whole genome sequencing data for several natural isolates. The genome of one of the closest known relatives of *C. elegans*, *C. briggsae*, was sequenced in 2003 (91) and additional *Caenorhabditis* species are in the sequencing pipeline.

***Brugia malayi* (http://www.wormbase.org/db/gb2/gbrowse/b_malayi/).** The genome of the filarial parasite *B. malayi* is one of two human-parasitic nematodes with a full genome draft (35). Several characters of the *B. malayi* genome have facilitated a better understanding of the nematode-host interactions. First, the assembled sequence of 88 Mb has currently 11,515 predicted protein-coding genes, indicating a substantial reduction in gene number. The high predictability of the host environment might result in a long-term evolutionary trend to reduce gene number and to rely on the host for certain functions. Second, a substantial amount of the *Brugia* gene predictions are *B. malayi*–specific, providing potential targets for drug design. Third, *B. malayi* harbors intracellular endosymbiontic *Wolbachia* bacteria that are essential for the development and the reproduction of the nematode. The mutualistic interaction between *Brugia* and *Wolbachia* might have strengthened the trend of gene loss in the nematode further. For example, *B. malayi* misses genes for purine, riboflavin, and heme de novo synthesis, all of which are present in the 1 Mb *Wolbachia* genome. At the same time, the intracellular *Wolbachia* might also be the source for novel genes in *Brugia*, as studies provide ample evidence for horizontal gene transfer (HGT) into the nematode genome (25). Finally, the dependency of the nematode on the endosymbiont might provide an easy genomic target to effectively fight the parasite. Interference of the filaria-*Wolbachia* interaction by antibiotic treatments as well, as the associated changes in gene expression in *B. malayi*, can prevent nematode growth and indicates essential factors for its development (34).

Meloidogyne (http://www.pngg.org/cbnp/; http://www.inra.fr/meloidogyne_incognita). Among the many nematode pests of plants, members of the genus *Meloidogyne* are of tremendous agricultural importance. In 2008, the genome of two species, the asexual *M. incognita* (1) and the diploid *M. hapla* (74), were sequenced, providing new opportunities to increase food production (66). With 82 and 53 Mb, respectively, the two *Meloidogyne* genomes are the smallest, fully-sequenced nematode genomes to date. HGT of genes encoding cell wall–degrading enzymes emerged as an overarching principle in nematode parasites of plants, and *Meloidogyne* spp. provide a genome-wide perspective of this phenomenon (see below).

The most promising attempt to block the nematode-plant interaction is interference with the mechanical penetration of the root by the juvenile nematode. Through the stylet, a specialized structure of the pharynx, the nematode secretes proteins that first degrade and then modify host tissue, resulting in the formation of the typical root galls that have given root-knot nematodes their name. Application of modern mass spectrometry has helped in the direct identification of some of the proteins secreted by the nematode. In a recent study, Bellafiore and colleagues (7) identified 486 proteins secreted by *M. incognita* and, most surprisingly, found that *M. incognita* secretes several proteins that mimic plant proteins. One current hypothesis is that such proteins play a crucial role in regulating the plant cell cycle and its growth, thereby reprogramming the development of the plant. Given this unexpected finding of molecular mimicry, the *Meloidogyne*-plant interaction might become an important model system to study developmental reprogramming as part of parasitic processes.

***Pristinchus pacificus* (http://www.pristionchus.org).** Nematodes provide clear evidence for tremendous variations in gene content among species with similar biological complexity. *P. pacificus* is a Clade V nematode similar to *C. elegans*, sharing a similar number of cells and identical tissues (**Figure 1**). Nonetheless, the *P. pacificus* genome is, at 142 Mb, substantially larger (21) and contains around 25,000 predicted protein coding genes (12). The difference in the number of predicted protein-coding genes between *P. pacificus* and *C. elegans* depends largely on two mechanisms. First, *P. pacificus* has acquired genes by HGT, a mechanism (discussed below) that is shared with parasitic nematodes. Second, *P. pacificus* has undergone a number of lineage-specific gene duplications that have resulted in a dramatic increases in gene copy numbers (21). One prominent example is of the cytochrome P450 enzyme-encoding genes, which have 198 copies in *P. pacificus* but only 67 in *C. elegans*. Other examples include ABC transporters, glycosyltransferases, and sulfotransferases (21). In this context, it is interesting to note that all of these genes encode for potential detoxification enzymes, which are of particular importance in the beetle ecosystem in which *P. pacificus* and related nematodes are found. These features of the *P. pacificus* genome support the preadaptation hypothesis for the evolution of complex parasitic traits because the expansion of the detoxification machinery provides hints at how genomic changes can be initiated as adaptations to a current environment (23, 79). Finally, a comparison of *P. pacificus* to *C. elegans* reveals that the ecology of a species can have a strong influence on its genomic composition independent of the morphological complexity of the organism.

***Trichinella spiralis* (http://genome.wustl.edu/genomes/list/invertebrates).** The publication of the *T. spiralis* genome draft represents the latest addition to the nematode genome projects (67). As a member of Clade I, the *T. spiralis* genome expands the phylogenetic coverage of nematode genomes by covering a Clade that diverged from the aforementioned species early in nematode evolution (**Figure 1**). The *T. spiralis* genome is 64 Mb in size and encodes an estimated 16,000 protein-coding genes. This finding supports the trend of reductive genome evolution in parasitic nematodes, although stronger support of this trend awaits future genome projects. Two features of the *T. spiralis* genome demonstrate the need for a broad phylogenetic coverage for the interpretation of genome sequence data. First, a comparison with *C. elegans*, *B. malayi*, *M. incognita*, *Drosophila melanogaster*, and humans revealed a group of 702 protein families at the node between nematodes and their outgroups; 274 of these families constitute a core group present in all nematode clades (67). In contrast, 88 families have been lost in nematodes with respect to insects and vertebrates. Interestingly, the trend of gene death is stronger in parasitic nematodes than in the free-living *C. elegans*. A second feature of the *T. spiralis* genome is that there is little support for shared molecular strategies among animal parasites. Mass-spectrometry comparisons between *T. spiralis* and *B. malayi* identified only two potentially shared secreted proteins, a serine peptidase and a cyanata hydratase (67).

Taken together, genomic approaches to nematode parasites are providing important inroads into the functional analysis of parasitic interactions. At the same time, an emerging principle of all these studies is the absence of general mechanisms of parasitism. The *Brugia-Wolbachia*-human interaction will be guided by specific worm and bacterial factors different from those regulating interactions in other filarial nematodes. Also, similarities between the *B. malayi* and *T. spiralis* genomes are very limited. The interaction of *Meloidogyne* spp. with plant roots will most likely not be able to serve as a guide for understanding *Globodera* spp. and other parasites and their interactions with plant hosts. Although the discoveries of HGT and molecular mimicry represent important intellectual principles, they do not provide the mechanistic power for anticipating other parasitic interactions. This is bad news for other plant and animal parasites among the

nematodes, but can be considered as a logical extension of the evolutionary independence of nematode parasitism.

HORIZONTAL GENE TRANSFER IN NEMATODES

One of the most unexpected findings from whole genome sequencing projects in nematodes is the widespread occurrence of HGT, which is the transmission of genes between organisms in a form other than vertical inheritance. Although HGT is frequent in prokaryotes, it was thought to be rare among eukaryotes with sexual reproduction (3). Recent genome and EST sequencing projects, however, provide strong evidence for HGT from bacteria, fungi, amoebozoa, or endosymbionts into various nematode genomes. Best characterized are examples of HGT in plant parasites of the *Meloidogyne*, *Heterodera*, *Globodera*, and *Pratylenchus* groups, the fungivorous *Bursaphelenchus*, necromenic *Pristionchus* species, and the filarial parasite *B. malayi* (23, 25). A second exciting aspect of these HGT events is the potential role of the transmitted genes in the receiver organism. Many genes acquired by HGT encode for cell wall–degrading enzymes of the glycosyl hydrolase (endo-1,4-beta-glucanases; cellulases), pectate lyase, and xylanase families (68, 88). It is very likely, therefore, that these genes play a crucial if not essential role in nematode parasitism. Several recent reviews have highlighted the patterns of HGT into nematodes and have discussed the role of HGT in the evolution of plant parasitism (66, 68, 88). Therefore, we will focus below on two other aspects of HGT that have been the subject of more recent studies.

Phylogenetic reconstruction of nematode cell wall–degrading enzymes strongly indicates the independent acquisition from distinct microbial donors (19, 23). For example, the characterized cellulases from plant parasitic Tylenchida are from glycoside hydrolase family 5 (GHF5). A GHF5 gene cassette consisting of the catalytic domain and the carbohydrate-binding module 2 (CBM2) was acquired as an intronless ancestral gene from putative bacterial donors (57). In contrast, the pine wood nematode *Bursaphelenchus xylophilus*, which is part of the same clade as the Tylenchida, has independently acquired a different family of cellulases (GHF45) from fungi (54). Similar findings have been made for other families of cell wall–degrading enzymes of plant parasitic nematodes by systematic investigations of the evolutionary history of the corresponding genes (19). These studies also suggest massive gene duplications after the ancestral acquisition by HGT, a finding that has several evolutionary implications.

Evolutionary theory predicts that the successful integration of HGT-acquired genes into the biology of the host requires gene activity and longevity (9), and therefore, such genes should be under positive selection. Testing for gene activity, longevity, and selection requires a detailed analysis of the evolutionary history of such genes that can only be achieved by working in organisms with a well-established phylogenetic framework at the (*a*) species and (*b*) family level and in a (*c*) number of natural isolates for intraspecies comparisons. One group of nematodes that provides such phylogenetic frameworks is the beetle-associated *Pristionchus*, with more than 400 strains of worldwide origin available for *P. pacificus*, more than 25 species within the genus *Pristionchus*, and a molecular phylogeny of the family Diplogastridae to which *Pristionchus* belongs (43, 63, 64).

A recent study by Mayer et al. (65), used transcriptomics of seven *Pristionchus* species and three additional diplogastrid species to study the evolution of cellulase genes and compared individual cellulase genes in 24 natural isolates of *P. pacificus*. This study revealed that all species with cellulase genes in their transcriptomes exhibited cellulase activity. Thus, gene activity seems to be strictly correlated with the presence of genes in the genome/transcriptome of a given species. Most interestingly, cellulase genes showed high turnover with significant birth and death rates. For example, cellulase genes were phylogenetically more closely related within one species than they were between species. This finding was confirmed by the

comparison of 24 natural isolates of *P. pacificus* from around the world, which indicated copy number variations and signs of positive selection (65). It has been suggested, therefore, that functional assimilation, high gene turnover, and selection might represent key features of HGT in nematodes. Given the functional importance of cell wall–degrading enzymes in plant parasitism, many additional studies are expected to be conducted in the near future, making HGT of microbial-derived genes encoding for cellulases and other cell wall–degrading enzymes a superb model system to study the evolution and function of HGT into eukaryotes. One of the most crucial challenges will be the establishment of gene-knockout technologies for the functional analysis during plant parasitism.

CONCLUSIONS

Several conclusions can be drawn from the comparative studies carried out in the past decade. Some of these will influence future research strategies, in particular for species of medical and agricultural importance. First, the comparison of genetic processes between *C. elegans*, *C. briggsae*, and *P. pacificus* reveals a high number of divergent control mechanisms. Although this finding might be considered to be counterintuitive to the often stressed conservation of developmental control genes and mechanisms between worms, flies, and mice, it highlights the power of genetics and should alert the research community that sequence conservation and gene function are, in part, unrelated issues. Second, all seven sequenced nematode genomes and various EST sequencing projects indicate a relatively high number of novel genes, which have no recognizable orthologs in other organisms. Genes are born and lost relatively frequently in nematode evolution, although nematode genomes are rather compact. Third, given the large ecological variety of nematodes, the total nematode gene space is largely unexplored and awaits future analysis by high-throughput genomic applications. It is very likely that such projects would identify genes of high potential interest for industry. Finally, an emerging principle of comparative genomics of nematode parasites is the absence of a general mechanism of parasitism. This finding seems to hold true for both animals and plant parasites, and most likely reflects the phylogenetic independence of nematode parasitism. The only logical solution for parasite and pest control is the development of genetic and genomic tools in all those species considered to be of applied interest.

SUMMARY POINTS

1. Full tool sets for the isolation and analysis of mutations in known genes exist for three nematodes only, namely *C. elegans*, its close relative *C. briggsae*, and the more distantly related *P. pacificus*.
2. Efforts to apply genetic analysis to other, in particular parasitic, nematodes are being made.
3. For seven nematodes species, representing four of the five major clades, draft genome sequences are published, namely for the free-living Clade V nematodes *C. elegans*, *C. briggsae*, and *P. pacificus*, the plant parasitic Clade IV nematodes *M. hapla* and *M. incognita*, and the human parasites *B. malayi* (Clade III) and *T. spiralis* (Clade I).
4. All seven published nematode genomes are small and compact.
5. In nematodes, genes are gained and lost frequently.
6. HGT is a central process in shaping nematode genomes.

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Errata

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