Phylogenetic and Evolutionary Concepts in Nematodes

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Keeping the medical, ecological, and economical importance of nematode phylum in mind, it is remarkable to see that nematode systematics is far from established. It has a long history of constant revision, and there may be as many classifications as there are nematode taxonomists. Ferris and Ferris (1987) anticipated about the growing sense of excitement pervading systematics as new techniques make it possible a depth of understanding of phylogenetic relationships and affinities never before thought possible. They further stated that Darwin's "genealogical taxonomy," based on the concepts of descent with modification, is linked directly with two approaches to phylogenetic inference, viz., phenetics and cladistics. In both of these, patterns of descent take precedence over processes, and in classifications based on these procedures, "grades" and "gaps" beloved by the evolutionary systematics are ignored and categories are usually of lesser importance (Dupuis 1884). The phenetic approach deals with "natural classification" based on overall similarity and the belief that the more characters a classification is based on, the more reliable it will be.

A phylogeny allows the reconstruction of the historical changes that have led to current variation and provides a way to test how often convergent changes have occurred. Species phylogenies are also crucial for bioinformatic analyses of genomes; they provide a basis for selecting species for comparative genomic sequencing and for testing orthologous and paralogous relationships in gene phylogenies, an important foundation for genome annotation and prediction of gene function (Eisen 1998; Eisen and Fraser 2003). Although molecular biologists have long appreciated the value of sequence comparisons to identify conserved regions as indicators of function, arguably the most interesting aspects of evolution are changes in molecular functions, domains of expression, and developmental roles. Elucidating how such changes have shaped functional diversity at a variety of levels also has potential for augmenting our understanding of genome function and developmental mechanisms. Reciprocally, information from this model system facilitates studies of evolutionary pattern and process. The success of such comparative approaches to enhance the understanding depends upon the availability of material and information from multiple related species, as well as different wild populations of *C. elegans*. Knowing the phylogenetic relationships between *C. elegans* and other nematodes or animals is important for comparative analyses of behavior, morphology, development, molecular mechanisms, and genomics.

A quick tour of nematode diversity and the backbone of nematode phylogeny provide a view of general nematode diversity and phylogeny. The phylogenetic relationship of *C. elegans* and other rhabditids reviews what is known so far about the closer relationships within the rhabditids and within genus *Caenorhabditis* in particular. There is substantial variation among rhabditids at genetic and developmental levels.

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Reconstructing how this variation arose is likely to illuminate how developmentally robust systems can nevertheless be modified by evolutionary change, one of the most intriguing and fundamental questions in evolutionary biology today. Nematode genome evolution reviews work on the evolution of genome organization and chromosome architecture. Evolution of development in nematodes related to *C. elegans* provides an overview of comparative developmental biology using *C. elegans* and other satellite model organisms, such as *Pristionchus pacificus*.

3.1 Nematode Relationships to Other Animals

Nematodes were once classified with a very large and heterogeneous cluster of animals grouped together on the basis of their overall wormlike appearance, simple structure of an internal body cavity called a pseudocoelom, and the lack of features such as cilia and a well-defined head that are found in most animals. This group, variously known as Aschelminthes or Pseudocoelomata, is today no longer recognized as a natural one. It is quite likely that the simple body plan of these organisms has resulted from reduction and simplification from more than one group of ancestral organisms and so the pseudocoelom is neither a uniquely derived nor useful character (Wallace et al. 1996) The simplicity is thus a result of secondary simplification from a more complex body design and not necessarily an indication of primitive or simple origins. Current studies indicate that nematodes are actually related to the arthropods and priapulids in a newly recognized group, the Ecdysozoa. Nematode fossils are hard to find because the organisms are microscopic and lack hard structures. However, fossils have been found dating from the Cambrian period, and it is very likely that nematodes have been around since then (Waggoner and Brain 2004). As rather small and primitive organisms, nematodes display mostly simple evolutionary developments. The important steps in evolution follow a pattern similar to this:

- No symmetry (e.g., unicellular organisms) to radial symmetry (e.g., jellyfish) to bilateral symmetry (e.g., vertebrates, worms, crustaceans)
- Segmentation (e.g., earthworms)
- No coelom or body cavity (e.g., unicellular organisms) to with coelom (e.g., vertebrates, annelids)
- Vertebrae (e.g., mammals, fish, birds)

The following animal phylogeny illustrates many of the important relationships between nematodes and other phyla (Fig. 3.1).

The phylum Nematoda or roundworms obviously do not contain vertebrae. They are bilaterally symmetrical but lack segmentation. This characteristic distinguishes nematodes from other common segmented worms such as those in phylum Annelida. The difference between other bilaterally symmetrical organisms and worms lies in the presence of an internal body cavity or coelom in those organisms. The pseudocoelomates represent the first organisms to have an internal body cavity. This is significant in that it promotes more sophisticated and efficient mobility (Raven and Johnson 1985). Again, the insufficiency of nematode study makes comprehensive classification very difficult. Because only a small percentage of the different species of nematodes have been classified, constructing true phylogenetic relationships is hard. Similarly, because nematodes are so uniform in structure, classifying them is tough. It is widely believed that the shared ancestor of present-day nematodes had the same basic characteristics that we see in all species of roundworms. Thus, the differences between the most primitive and the most evolved nematode species are fairly small. Even where evolution is seen from primitive to advanced specimens, it is almost uniformly present in every branch (Malakhov 1994). This idea of parallelism presents further difficulty in classifying nematodes. Nonetheless, nematodes are all classified as pseudocoelomates because they have a primitive body cavity.

The division of nematodes into two classes in effect distinguishes between the more advanced in Secernentea and the more primitive in

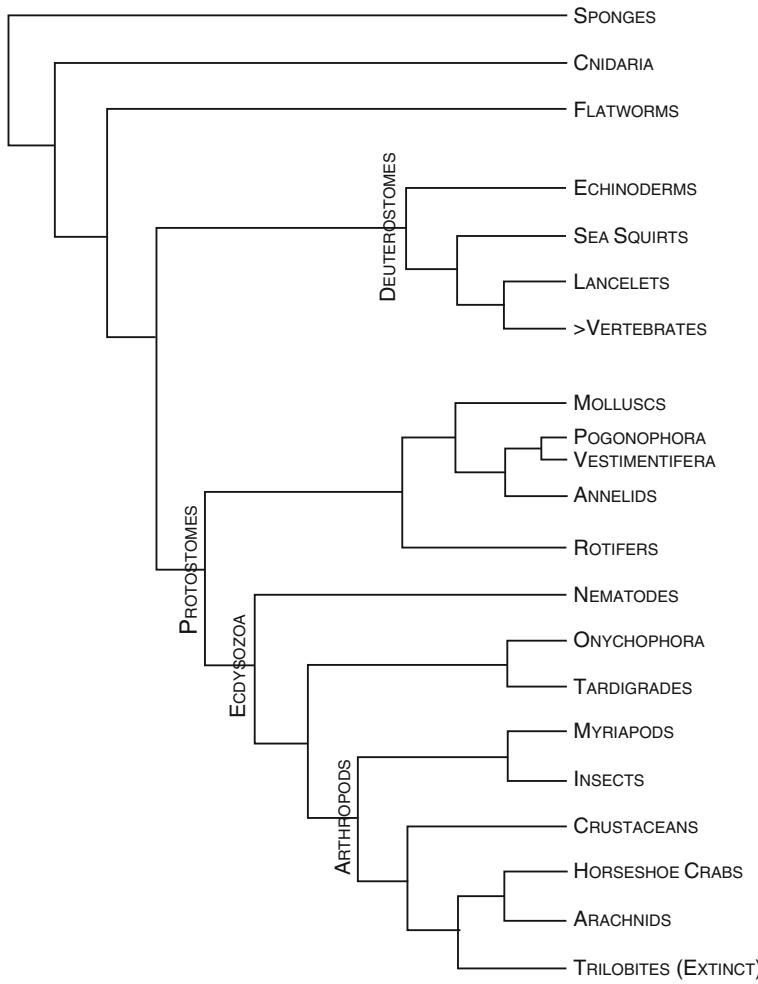

Fig. 3.1 Phylogeny depicting relationships between nematodes and other phyla

Adenophorea (Poinar 1983). As technology and taxonomy have become more advanced, the classification of nematodes has changed significantly. However, when considering the phylogenetic tree for nematodes, it is imperative to keep in mind that nematologists have not reached a consensus. There is no single comprehensive tree that all scientists agree on for nematodes.

3.2 Phylogenetic Concept

Molecular phylogenetic methods allow comparison of disparate taxa using the same metric, the evolution of a single conserved molecule. This approach sidesteps some of the problems of the definition of homology and is synergistically compatible with morphological systematics. The use of molecular markers certainly brings its own problems, but in general, the mode of evolution of DNA sequences is better understood than that of morphological traits and can be modeled with some confidence. This allows alternative analytical tools to be used and permits calculation of statistical support for the phylogenies produced. An important consideration is that the rates of phylesis (the generation of taxa; speciation) and fixation of molecular change must be of the same order. Thus, a rapidly evolving DNA segment should be used to examine the relationships

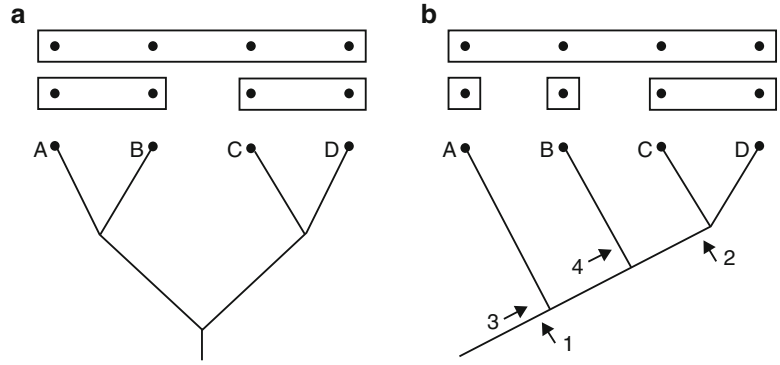

Fig. 3.2 Phylogenetic tree of a monophyletic species, (**a**) is a lumper which unites species in a genera and (**b**) is a splitter that divides into genera

between species in a genus and a very conserved segment for interordinal or interphylum relationships. It should be borne in mind that the phylogeny derived from a single molecule might not faithfully reflect the history of all species studied and that information from multiple unlinked genetic loci will give more robust estimates (Mortiz and Brown 1986).

The basic principle of phylogeny is that all are related and evolved from a common ancestor; phylogenetic systematics differs from the traditional approach as it is based on genealogy (common ancestry) and the diagram obtained is a cladogram (Dorris et al. 1999; Subbotin et al. 2004). Some of the basic principles to be assumed for phylogeny include that evolution has occurred; new taxa can be characterized by new features and these characters are derived (apomorphies) from previously existing ones (plesimorphies). The monophyletic group with four species (a) with two subgroups and (b) with three subgroups has been depicted in Fig. 3.2.

3.3 Criteria for Inferring Phylogenetic Tree

Different criteria can be used to infer phylogenetic trees from morphological or molecular data (Stone 1983). All methods are based on two processes: an algorithm for finding trees and a criterion for selecting the best ones. It is expedient to apply all the criteria to each data set and to test the derived trees for consistency and statistical support. The three major criteria used are as follows:

(a) *Neighbor Joining*: Neighbor joining analysis yields a point estimate of a minimum evolution tree based on data transformed into a pairwise distance matrix. In this method, the algorithm for finding the tree and the criteria for assessing its quality are combined.

(b) *Maximum Likelihood*: Maximum likelihood analysis uses an explicit model of evolution (direction and probability of character change) to derive the tree most likely to have occurred given the data. Many different trees can be built and tested.

(c) *Maximum Parsimony*: Maximum parsimony is a criterion for selecting an optimal tree based on the principle that the tree requiring the least number of changes in character states is more favored. Many different trees can be built and tested. Among the methods for testing the internal statistical support for the inferred tree is the bootstrap. Bootstrap resampling rebuilds a number (usually >100) of model data sets based on the test set (by sampling with replacement) and reanalyzes them with the chosen criteria. The percent retention of nodes in the set of bootstrap trees is a strong indication of their robustness. Bootstrap values >65 % are considered robust.

The 18S rDNA of 19 populations of *Meloidogyne* spp. has been amplified and directly sequenced. The region of mitochondrial

DNA, located in the 3′ portion of the gene that codes for cytochrome c oxidase subunit II (*COII*) through a portion of the 16S rRNA (*lRNA*) gene, from 16 of these populations was cloned and sequenced. Heteroplasmic sequences were identified from both rDNA and mtDNA regions for several taxa. Several sequences sampled from nominal taxa differed from previously published accounts. Phylogenetic trees based on alignments of these sequences were constructed using distance, parsimony, and likelihood optimality criteria. For 18S rDNA data, three main clades were identified. One well-supported clade (86–91 % bootstrap) included the most common and widely disseminated species, e.g., *M. arenaria*, *M. javanica*, and *M. incognita*, and some recently described or redescribed species (*M. floridensis*, *M. paranaensis*, and *M. ethiopica*) plus numerous unidentified isolates. All mitotic parthenogenetic species, except for *M. oryzae*, were included in this clade. Other, less well-supported clades included the amphimictic and facultative meiotic species *M. hapla*, *M. microtyla*, *M. maritima*, and *M. duytsi*. One such clade comprised three meiotic parthenogens (*M. exigua*, *M. graminicola*, and *M. chitwoodi*) and *M. oryzae*. This clade was moderately supported (77 % bootstrap) but the relationships within this clade were poor. For mitochondrial DNA data, only the species in clade 1 from rDNA analysis and *M. hapla* were analyzed. These species formed a well-supported clade (100 % bootstrap) to the exclusion of *M. mayaguensis* and *M. hapla*. The addition of taxa and mtDNA data to publicly available records improved the discrimination sensitivity of species and atypical, non-identified isolates.

Current accepted classification of the phylum Nematoda is based on morphological and ecological traits, primarily in the context of free-living or parasitic phenotypes (Dorris et al. 1999). The deceptively uniform basic anatomy of nematodes masks a complex pattern of diversity, and estimates of species number within the phylum range from 40,000 to 100 million. The reliability of nematode morphology in producing a coherent phylogeny has been called into question for several reasons. Not least is the disagreement in resolution at the highest levels evident in systematic studies based on morphology. In five major phylogenetic representations of the phylum, two classes are recognized: Adenophorea and Secernentea. In two of these analyses, 2 and 3, both classes are monophyletic, arising from the same ancestor. The other three phylogenies 4–6 suggest that the Adenophorea are paraphyletic and give rise to the Secernentea. The broad ecology of nematodes within each class supports the latter view. Adenophorea include a wide range of marine, freshwater, and soil nematodes but relatively few parasites of animals and plants, whereas Secernentea occur mostly in terrestrial habitats and include a plethora of parasitic and free-living groups. This sort of disagreement is echoed by competing analyses at all taxonomic levels within the phylum.

Current taxonomy relies largely on the nematode morphological traits. Traits most commonly used are buccal and pharyngeal structure, but other anatomical features such as the cuticle, lip region, intestine, reproductive system, sense organs, and tail are also used, as well as life history traits such as parasitic host. Problems can arise when using morphological traits for phylogeny inference, and these become crucial when the paucity of applicable nematode characters is considered. In addition to observational bias and error, nematodes provide a limited number of characters that can be observed across taxa in relation to the known diversity of species.

The first major classification to incorporate both morphological and molecular phylogenetic information is that of De Ley and Blaxter (2002) (Fig. 3.3a, b). Till 1963 the double division was accepted by all the taxonomists with altering the name of two divisions, i.e., Phasmida (= Secernentea) and Aphasmidia (= Adenophorea). In 1963, Goodey rejected this double division as he found a connecting link (Teratocephalidae) between these two classes showing intermediate characters between the two. Later, that system was thoroughly reviewed by Kaestner (1965). Since nematodes form an incomparably more uniform group of Secernentea than Adenophorea

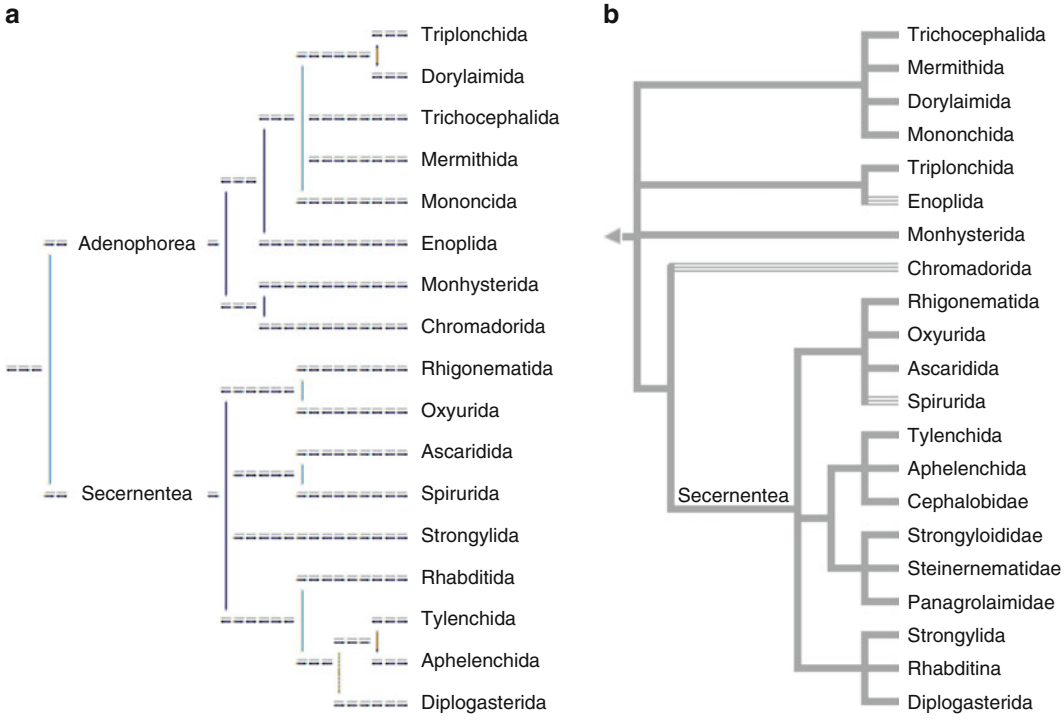

Fig. 3.3 (**a**, **b**) Relationship based on SSU rDNA sequences

as the proportion of uniform characters is 17:4 (Secernentea/Adenophorea), a three-line hypothesis was proposed by keeping Secernentea unaltered and splitting Adenophorea into Chromadorida and Enoplida.

The proportion of differential/identical characters emerged after the pairwise analysis, viz., Secernentea–Chromadorida, 10/6; Secernentea–Enoplida, 12/1; Chromadorida–Enoplida, 7/4; and Secernentea–Chromadorida–Enoplida, 10/7/3; it was thought that this could be the order or trend of evolution. Kaestner gave three nomenclatures as Chromadorida (spiral amphids) (= Torquentia), Enoplida (pocket-like) (=Penetrentia), and Secernentea (to separate or secrete off) based on the amphid arrangements and morphology.

Molecular data have also clarified the position of Nematoda in relation to other animals. Before the late 1990s, nematodes, along with a ragbag of other soft-bodied, "wormy" phyla, had been placed in a group termed the Pseudocoelomata (describing the nature of the body cavity in these taxa). However, the morphological arguments supporting this superphylum were never strong, and despite the absolute certainty expressed in textbook treatments of the phylogeny of the animals, leaders in the field, such as Libby Hyman, always expressed grave doubts as to the biological reality of this grouping (Hyman 1951). Analysis of ribosomal RNA sequence data from a range of nematodes, however, suggested instead a radical rearrangement of the animal part of the tree of life (Aguinaldo et al. 1997) (Fig. 3.4). In this new model, which has strong support from several genes and some support from morphological data, Nematoda is part of a superphylum of molting animals, the Ecdysozoa, that includes arthropods (and thus *D. melanogaster*, the other major non-vertebrate model), Nematomorpha (horsehair *worms*), Onychophora (velvet *worms*), Tardigrada (water

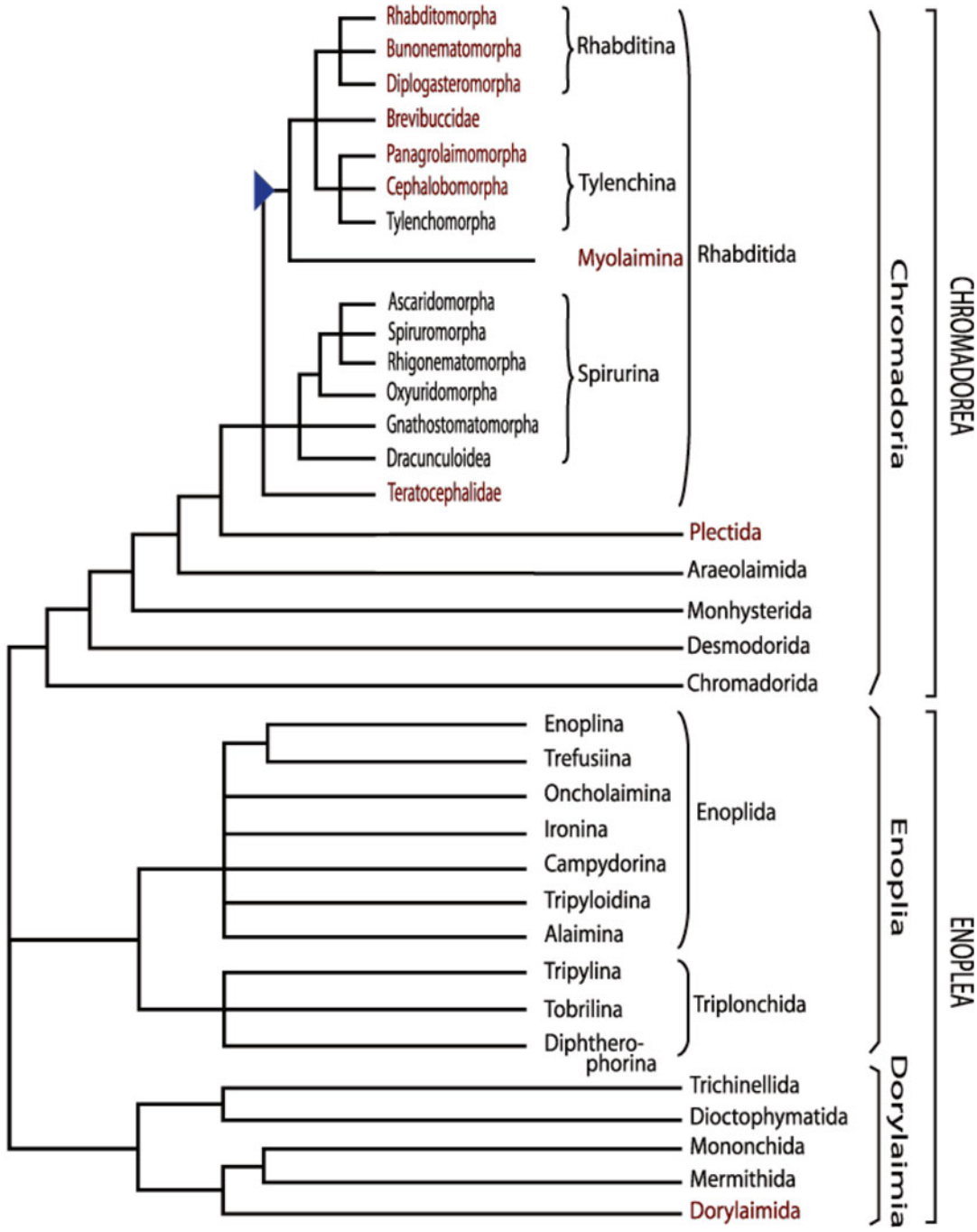

Fig. 3.4 Phylogenetic outline of Nematoda, derived primarily from small-subunit rDNA sequence analysis

bears), Priapulida (penis *worms*), and other minor phyla. The rest of the "pseudocoelomates" are now placed in the Lophotrochozoa (Halanych 1995; Philip et al. 2005), a group that includes Mollusca (snails and clams), Annelida (rag *worms* and earth*worms*), and Platyhelminthes (flat*worms*), among others.

3.4 Common Terminologies Used

Apomorphy: A derived character state.
Branch: The segment linking one node with another, or a node with a terminal taxon.
Characters: Variable features that can assume one of a number of different states.
Clade: A (monophyletic) group of organisms related by descent from a common ancestor.
Cladistics: A phylogenetic approach that only admits to bifurcations in lineages (no polytomies or reticulate evolution) and has explicit rules for their derivation.
Cladogram: A cladistic representation of a phylogeny, whereby only the branching order is displayed.
Homology: Common ancestry of two genes (characters, genes, positions).
Homoplasy, parallelism, or convergence: Independent derivation of a character state in two lineages.
Ingroup: The taxa under analysis.
Monophyletic: A clade where all the taxa derive from a single common ancestor and which includes all the descendents of that ancestor.
Node: A branch point in a tree (a presumed ancestral taxon).
Orthology (true homologues): Homologues that have arisen through speciation of their host genomes rather than by gene duplication within a genome.
Outgroup: A group of taxa assumed a priori to lie outside the monophyly of the taxa under analysis; used to give direction to determination of character change.
Paralogy: Homology having arisen through gene duplication.
Paraphyletic: A taxonomic group which does not include all the descendants of an ancestral taxon.
Phylogram: A representation of a phylogeny where evolutionary relatedness is shown by both branching order and a distance measure.
Phylogeny: A hypothesis of the relationships of organisms.
Plesiomorphy: The ancestral character state.
Polyphyletic: A taxonomic group which derives from >1 ancestral taxon.
Polytomy (unresolved node): A node that gives rise to >2 descendent taxa.
Resolved phylogeny: A phylogeny in which all relationships are represented by bifurcations.
Reversal: Change of a character from an apomorphic to a plesiomorphic state.
Rooted phylogeny: A phylogeny in which, by use of an outgroup, the last common ancestor of the clade of taxa under consideration can be placed.
Synapomorphy: A shared derived character state (in reference to a phylogenetic hypothesis).
Unrooted phylogeny: A phylogeny where no outgroup is specified.

3.5 Features Shared by Nematoda with Related Groups

Nematodes share a basic wormlike appearance with most other worms of different phyla. Segmentation is what differentiates Annelida from Nematoda. The difference between phylum Platyhelminthes and Nematoda is evident in their respective names – flatworms and roundworms. Nematodes share the pseudocoelom with rotifers and horsehair worms of the phyla Rotifera and Nematomorpha, respectively. Horsehair worms and rotifers are very common aquatic animals distinguishable by their cilia-crowned head and "wheel-like" appearance during cilia motion (Raven and Johnson 1985). The feature of a pseudocoelom is important in that it represents an intermediate step between total absence of a body cavity in unicellular organisms and a true coelom in more complex organisms. Recently, however, features like the pseudocoelom have been questioned when used to group organisms together. The nematode had previously been placed in the group Aschelminthes, which included the phyla Rotifera, Gastrotricha, Kinorhyncha, Priapulida, and Nematomorpha (Poinar 1983).

Similarly, the cuticle is a structure that is present in arthropods and other ecdysozoans, a group in which nematodes are now generally placed. The cuticle of the nematode is a rigid structure

that must be shed before further growth can occur. This process of molting is considered to be a link between nematodes and arthropods (Waggoner and Brain 2004). Moreover, the cuticle is often used to resolve phylogenetic issues within the phylum Nematoda. However, the cuticle may have arisen independently within the phylum and cannot necessarily be used to determine relationships between very closely linked nematodes (Decraemer et al. 2003). Finally, along with rotifers and tardigrades, nematodes are able to undergo a process known as cryptobiosis where normal life processes and functions are suspended during periods of environmental instability and inhospitality (Waggoner and Brain 2004).

Phylum Nematoda is found across the globe almost anywhere there is organic matter. Roundworm habitats include but are not limited to seas, freshwater, soil, and almost every species of plant and animal. Around 20,000 species of roundworms are known and have been classified (Malakhov 1994). Because there are very few scientists looking for new roundworm species, the discovery of new species can be rather slow, especially in regard to free-living nematodes. Moreover, nematodes share basic morphologies and are difficult to distinguish between each other. Roundworms are either parasitic or free living. Parasitic roundworms are much more likely to be discovered and classified because they are of more concern to humans. In fact, for some time only parasitic roundworms were known, and today they are much more likely to be studied. This provides skewed knowledge of nematodes because there are more free-living species than parasitic ones; around 65 % of classified nematode species are free living.

All nematodes, however, show incredible ability to reproduce. There are certain species that can carry more than 27 million eggs at once. These species can lay up to 200,000 eggs in one day (Waggoner and Brain 2004). So, needless to say, nematodes are extremely abundant in the world. Unfortunately, the amount of classified nematode species can be no more than 20 % of the total number of existing nematode species. Some scientists, taking into account the problems with finding and classifying roundworms and their relative abundance, have estimated the amount of undiscovered nematodes to be anywhere from 100,000 to 1 million (Malakhov 1994). If this statement has any merit, then nematodes would be second only to arthropods as the most diverse group of animals (Waggoner and Brain 2004).

3.6 Unique Features to Nematoda

Nematodes are often confused with other closely related types of worms. These are often part of the phylum Platyhelminthes and are known as flatworms because they lack a body cavity. Similarly, annelids can sometimes be confused with nematodes but are distinguishable because they have a true coelom. Generally, nematodes are cylindrical, unsegmented, bilaterally symmetrical pseudocoelomates (Raven and Johnson 1985). Roundworms have a thick cuticle that covers their bodies and is shed in order to allow growth. Located between the cuticle and the pseudocoel are muscles that run the length of the nematode. These muscles push on both the cuticle and the pseudocoel and create a kind of hydrostatic skeleton. In contrast with most animals whose nerve cells branch out to each individual muscle cell, nematode's muscle cells branch toward the nerve cells. Nematoda is the only phylum of pseudocoelomates that includes a large amount of species. The function of this pseudocoel is very important in that it allows nematodes to gain or lose rigidity by way of fluid pressure. This rigidity allows resistance to muscle contraction, which in turn provides for more efficient motion. Nematodes do not possess a defined circulatory system as their pseudocoel fluids accomplish circulation (Raven and Johnson 1985). The nervous system of roundworms is comprised of anterior nervous tissue surrounding the pharynx that forms dorsal and ventral nerve cords that go from end to end (Waggoner and Brain 2004).

All nematodes do have a simple but defined digestive tract. A roundworm's mouth usually has 16 protruding sensory organs, and phytoparasites display piercing structures, "stylets." Food goes straight through the conveyor belt-like tract and is

broken down, diluted with water, absorbed, and excreted. Unlike most animals, nematodes do not depend on cilia or flagella for excretion. Rather they utilize cells that work as glands or systems of canals in order to get rid of waste (Raven and Johnson 1985).

Nematodes are sexual animals and the male is generally slightly smaller than the female, which usually displays a bent tail. Nematode reproduction in free-living specimens is a very interesting process involving six stages including an egg stage, four juvenile stages, and an adult stage. Males are dioecious in that they can have one or two testes and can have a variety of accessory sex organs depending on the species. Females give rise to eggs that are then fertilized and laid. Once the embryos in these eggs are mature, they will hatch into the first juvenile stage. The juvenile will then undergo four molts before it becomes an adult and is capable of reproduction. During molting, a nematode will shed its skin in order to facilitate growth. The third juvenile stage is normally the infectious stage for parasitic nematodes. Parasitic nematode life cycles vary more than those of free-living specimens. Often parasitic roundworms will have multiple stages and alternate between hosts and regions in their hosts' bodies. Finally, nematodes have much less cell multiplication than most other organisms as they achieve growth mainly through cell enlargement. The juvenile specimens, for the most part, have the same number of cells as adults (Poinar 1983).

In *C. elegans*, three possible hypotheses were outlined for relationships between three major model systems, the arthropod *Drosophila melanogaster*, the vertebrate *Mus musculus*, and the nematode *C. elegans* (Fitch and Thomas 1997). They emphasized that elucidating these relationships was important for making inferences and predictions about which components, mechanisms, and functions might be unique and derived or ancestrally shared by these or other related model and non-model species, such as humans. Of course, additional representatives of animals (including other nematodes) are needed in the phylogenetic framework for greater accuracy of such predictions. After we wrote that review, several interesting studies addressed relationships among the major animal phyla and particularly the relationship of nematodes to other animals. There is still considerable (even polemical) debate, but additional data and increased analytical sophistication may provide answers in the not-distant future.

On the basis of complete 18S ribosomal RNA (rRNA) sequences, Aguinaldo et al. (1997) proposed that nematodes were related to arthropods in a clade of molting animals they called "Ecdysozoa," to the exclusion of deuterostomes (represented by an echinoderm in their study), and some other protostome groups, such as mollusks and annelids. This hypothesis differed substantially from the more traditional "Coelomata" hypothesis that placed nematodes on a branch diverging before coelomates diverged from one another (i.e., before the divergence of lineages leading to mice and flies). Support for Ecdysozoa depended on excluding all but one nematode, *Trichinella spiralis* (which unfortunately possesses an rRNA sequence with an odd nucleotide composition compared to other nematodes characterized so far).

However, when other nematodes were included, the nematodes clustered together near the bottom of the tree, consistent with Coelomata and consistent with data from RNA polymerase II (Sidow and Thomas 1994). Use of *Trichinella* as a representative nematode was justified on the basis that its rRNA sequence evolved more slowly than that of other nematodes, such as *C. elegans*. A phenomenon called "long-branch attraction" (LBA) can cause taxa with long branches (representing many evolutionary changes) to artifactually group with other long branches, particularly those of the outgroup taxa near the root of the tree (Felsenstein 1978). Of course, the other possible reason that nematodes have long branches is simply that they diverged early from the other taxa, as predicted by the Coelomata hypothesis. The putative effect of LBA to provide artifactual support to Coelomata has been central to the debate, along with issues of taxon and character sampling. Thus, in all of the studies described above, inappropriate use of a potential ingroup taxon to measure relative rates could have mistakenly biased the conclusions in favor of Ecdysozoa. It has been claimed that a

phenomenon called "long-branch attraction" (LBA) results in an artifactual placement of nematodes near the base of the bilaterian phylogeny, thus appearing to be consistent with the "Coelomata" hypothesis and obscuring the phylogenetic signal for "Ecdysozoa." Recent studies using genome-scale numbers of genes have generally supported Coelomata and rejected Ecdysozoa (e.g., Brown et al. 2001; Blair et al. 2002; Wolf et al. 2004). Some of these studies tested for effects of LBA and found no significant effect.

A major criticism of these studies is that the sampling of taxa is small, as might be expected for whole-genome comparisons. To determine the effect of both taxon and character sampling, Philip et al. (2005) reviewed by Jones and Blaxter (2005) used data from 146 genes and a fairly diverse taxonomic sample of 35 species. In this case, the authors identified the fastest-evolving genes by appropriate comparison to the outgroup species and found strong support for Ecdysozoa when these genes were excluded. By including or removing taxa, the authors also demonstrated a clear effect of taxon selection. For example, adding hydra to the outgroup, which otherwise had only fungi and choanoflagellates, caused *C. elegans* to jump from a position consistent with Coelomata to one consistent with Ecdysozoa. The authors conclude that both accounting for LBA effects and including a denser sampling of taxa are required to uncover the phylogenetic signal for Ecdysozoa. This effect of taxon addition is explained by the ability of added taxa to "break" long branches and apportion changes more appropriately into different lineages, thus providing better phylogenetic information about which states are primitive and which are derived (Kim 1998).

Even when only one or a few genes are employed (such as for 18S rRNA), including more taxa has apparently aided resolution, generally resulting support for Ecdysozoa (Giribet and Ribera 1998; Giribet and Wheeler 1999; Peterson and Eernisse 2001). Adding more taxa, however, means that statistically testing the robustness of relationships becomes computationally much more time-consuming and most of these taxon-dense analyses do not have such tests.

Balavoine et al. (2002) focused on contributions from recent work using multigene data along with the insight provided by a few molecular characters which nevertheless have phylogenetic signatures complex enough to have arisen only once, such as insertions, deletions, and organization of gene clusters. For example, Hox gene organization may be one such complex and therefore informative feature. The *Abd-B* gene appears to be specific for Ecdysozoa. However, orthology of the *C. elegans php-3* to *Abd-B* is only very weakly supported (de Rosa et al. 1999). One problem with *C. elegans* as a representative nematode is that it clearly has a highly derived organization of the *Hox* gene cluster relative to other nematodes; genes have been lost and rearrangements have occurred in lineages leading to *C. elegans* (Aboobaker and Blaxter 2003).

3.7 Evolutionary Trends of Nematoda

Nematodes are suitable objects to study evolution of development because species from all branches of the phylogenetic tree can be analyzed; embryos develop outside the mothers and most of them are transparent enough to perform cellular analysis in vivo. Nematodes can be subdivided into basal Enoplea (clades 1 and 2) and more derived Chromadorea (clades 3–12). Embryogenesis of *Caenorhabditis elegans* (clade 9) has been analyzed in most detail. Their establishment of polarity and asymmetric cleavage requires the differential localization of PAR proteins. Earlier studies on selected other nematodes revealed that embryonic development of nematodes is more diverse than the essentially invariant development of *C. elegans* and the classic study object *Ascaris* had suggested. Studies conducted by Schulze and Schierenberg (2011) revealed that early embryogenesis varied considerably among species indicated that constraints are high on the preservation of crucial developmental steps but not on cellular behavior leading to these. The direction of evolution went from indeterminate

early cleavage without initial polarity to invariant development with establishment of polarity before division of the zygote. The observed action of primary polarity organizing centers gave a clue how polarity in certain nematodes and other related taxa like tardigrades can be established in a way that differs from *C. elegans*, that is, independent of the sperm entry point.

Nematodes are wholly unsuitable for fossilization, and as a result, the study of their evolution is a difficult task as there is no proof of when, where, and how they evolved, their primitive forms and advanced forms. So dependence is on the indirect means of evaluating most important morphological and anatomical characters and evaluating them phylogenetically, by studying primary (which are present in ancient nematodes) and secondary (advanced characters or derived characters in advanced forms) characters. Chitwood was the first to make efforts that these animals be grouped in such natural unit as would reflect the trends of phylogeny. He was the first to recognize (Maggenti 1983). Nematoda phylogenetically and morphologically do not comprise a uniform class as was thought but that they represent two well-delimited evolutionary trends. The two groups were named after the presence or absence of a peculiar little organ, the phasmids, as Phasmidia and Aphasmidia in 1933, containing two subclasses, Rhabditida and Spirurida and Chromadorida and Enoplida, respectively. Of course he did not mean it as an important organ of the group but he simply chose them from among many to provide a name for the two classes.

3.8 Morphological Characters of Nematode in the Light of Evolution

Several important morphological and anatomical characters were used for arriving the trend of nematode phylogeny and evolution, which are as follows:

- *Appearance*: Spindle or filiform shape represents primitive character and all other diverging characters are specialized.
- *Symmetry*: Radial symmetry is primitive and any deviation from this could be due to evolution.
- *Head*: Most of the species even today possess 3 or 6 lips, though evolved forms may show reduced lateral lips, still reduced to 2 lips in highly parasitic forms indicating they are evolved.
- *Setae*: Presence of six setae is the primitive character; out of 6, 1–1 lateral and 2–2 subventral and four or three setae is a derived character. The presence of setae is the most primitive character as advanced forms are without setae.
- *Amphids*: These are particular characteristic of nematode that are always present on each side of the proximal end, and their position and form are an indication of evolution as shape and structure vary in three subclasses indicating three routes of evolution, and they are evolved out of lateral lips (papillae origin).
- *Esophagus*: It is a muscular tube which is either cylindrical or may bear one or more swellings (bulb); a simple tube-like structure is a primitive character and bulb-like is an advanced character. The presence or absence of a bulb is of evolutionary importance (Secernentea have a bulb while Penetrentia do not have).
- *Esophageal glands*: The number of esophageal glands of evolutionary importance is 3, 5, or more, and as far as tri-radial symmetry is concerned, 3 is primitive and 5 gland systems may be an evolved or derived character.
- *Female genital organ*: It exists in two forms as paired or unpaired and branched or unbranched. Unbranched or single organ is a primitive form and paired and branched organs are evolved characters similar to male genital organs.
- *Caudal glands*: Three unicellular glands situated in the hollow of the tail (meant for hold fast) seen in aquatic nematodes are the primary characters as they are absent in evolved forms (Secernentea).

In the light of above characters, it was concluded that nematode development might have taken place in the phase of geohistory. As most primitive forms are found in marine species, subclass Torquentia (Chromadorida) comprising the highest number of marine species is the only evolved form among the three subclasses excluding parasitic forms and the parasites of plants and animals.

3.9 Evolutionary Concepts

The complete genomes of three animals have been sequenced by global research efforts: a nematode worm (*Caenorhabditis elegans*), an insect (*Drosophila melanogaster*), and a vertebrate (*Homo sapiens*) (Blair et al. 2002). Remarkably, their relationships have yet to be clarified. The authors feel that the confusion concerns the enigmatic position of nematodes. Traditionally, nematodes have occupied a basal position, in part because they lack a true body cavity. However, the leading hypothesis now joins nematodes with arthropods in a molting clade, Ecdysozoa, based on data from several genes.

Traditionally, the animal body cavity has played a major role in interpretations of metazoan evolution, from groups (e.g., flatworms) lacking a coelom to those (e.g., nematodes) with a false coelom and finally to the bulk of animal phyla having a true coelom (Coelomata) (Jenner 2000). There has never been complete agreement on animal phylogeny and classification, but most researchers have divided living coelomate animals into deuterostomes (echinoderms, hemichordates, urochordates, cephalochordates, and vertebrates) and protostomes (arthropods, annelids, mollusks, and other phyla) based on differences in early embryonic development. An analysis of small-subunit ribosomal RNA (18S rRNA) sequences challenged this arrangement by placing acoelomate and pseudocoelomate phyla in more derived positions among the protostomes and in further defining a clade (Ecdysozoa) of molting animals that includes arthropods and nematodes. This "Ecdysozoa" hypothesis has influenced diverse fields and interpretations of developmental evolution in animals (Carroll et al. 2001). Since its publication, evidence has appeared both for and against this hypothesis. Knowing the branching order of the major animal lineages, especially those three with fully sequenced genomes, is of importance to diverse fields such as medical genetics, physiology, neurobiology, paleontology, and astrobiology. With a genealogy of animals, it will be easier to determine the origins and inheritance of mutations, genes, gene functions, and structures.

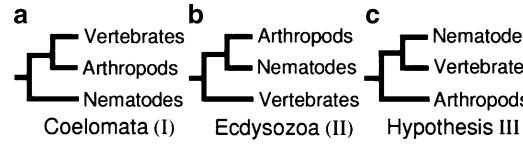

Fig. 3.5 The three possible relationships of vertebrates, arthropods, and nematodes

The three possible relationships of these animal phyla are as follows: (I) arthropods + vertebrates, (II) arthropods + nematodes, and (III) nematodes + vertebrates. The first hypothesis corresponds to the traditional grouping Coelomata and the second corresponds to Ecdysozoa (Aguinaldo, et al. 1997). For convenience, we will use these names in reference to the two hypotheses while recognizing that this study, by necessity, involves only a subset of all animal phyla. The third hypothesis will be referred to as "hypothesis III" (Fig. 3.5). To test each hypothesis, sequence alignments of more than 100 nuclear proteins were assembled and subjected to a series of analyses designed to reveal biases that could result in an incorrect phylogeny.

Blair et al. (2002) proposed that although it is possible that a basal position of nematodes is the result of some unknown and widespread bias not yet identified, a simpler explanation is that the grouping of nematodes with arthropods is an artifact that arose from the analysis of a single gene, 18S rRNA. The results obtained by them suggested caution in revising animal phylogeny from analyses of one or a few genes or sequence signatures. Although many other aspects of animal phylogeny remain unresolved, their findings indicated that insects (arthropods) are genetically and evolutionarily closer to humans (vertebrates) than to nematodes. Given the task of recovering and representing evolutionary history, nematode taxonomists can choose from among several species concepts (Adams 1998). All species concepts have theoretical and/or operational inconsistencies that can result in failure to accurately recover and represent species. This failure not only obfuscates nematode taxonomy but hinders other research programs in hematology that are dependent upon a phylogenetically correct taxonomy, such as biodiversity, biogeography, cospeciation, coevolution, and

adaptation. Three types of systematic errors inherent in different species concepts and their potential effects on these research programs are presented. These errors include overestimating and underestimating the number of species (type I and II error, respectively) and misrepresenting their phylogenetic relationships (type III error). For research programs in hematology that utilize recovered evolutionary history, type II and III errors are the most serious. Linnean, biological, evolutionary, and phylogenetic species concepts are evaluated based on their sensitivity to systematic error. Linnean and biological species concepts are more prone to serious systematic error than evolutionary or phylogenetic concepts. As an alternative to the current paradigm, an amalgamation of evolutionary and phylogenetic species concepts is advocated, along with a set of discovery operations designed to minimize the risk of making systematic errors.

Tahera Sultana et al. (2013) reported that among tylenchomorph plant parasites, members of the superfamily Tylenchoidea, such as root-knot nematodes, have great impact on agriculture. Of the five superfamilies within Tylenchomorpha, one (Aphelenchoidea) includes mainly fungal-feeding species but also some damaging plant pathogens, including certain *Bursaphelenchus* spp. The evolutionary relationships of tylenchoid and aphelenchoid nematodes have been disputed based on classical morphological features and molecular data. For example, similarities in the structure of the stomatostylet suggested a common evolutionary origin. In contrast, phylogenetic hypotheses based on nuclear SSU ribosomal DNA sequences have revealed paraphyly of Aphelenchoidea, with, for example, fungal-feeding *Aphelenchus* spp. within Tylenchomorpha, but *Bursaphelenchus* and *Aphelenchoides* spp. more closely related to infraorder Panagrolaimomorpha. They investigated phylogenetic relationships of plant-parasitic tylenchoid and aphelenchoid species in the context of other chromadorean nematodes based on comparative analysis of complete mitochondrial genome data, including two newly sequenced genomes from *Bursaphelenchus xylophilus* (Aphelenchoidea) and *Pratylenchus vulnus* (Tylenchoidea).

3.10 Evolutionary Relationships of Root-Knot Nematodes

To elucidate the biological relationships and to suggest positive pathways of evolution of one of the potential phytonematode group, root-knot nematodes, cytogenetic information has been very useful (Triantaphyllou 1985). The obligatory amphimictic species, viz., *M. megatyla, M. carolinensis*, and *M. microtyla*, are the closest relatives of the assumed ancestral root-knot nematode. Facultatively parthenogenetic species like *M. exigua, M. naasi*, and *M. graminis,* with $n = 18$, have evolved from an amphimictic ancestor with the same chromosome number, following evolution toward meiotic parthenogenesis. Some forms within this group, including *M. hapla* (race A) and *M. chitwoodi,* have evolved further by additional modifications of their chromosomal complement that resulted in the reduction of the haploid chromosome number from 18 to 17, 16, 14, and 13. Triantaphyllou (1985) opines that all the mitotic parthenogenetic forms are evolved from meiotic parthenogenetic ancestors or less likely from amphimictic ones during maturation of the oocytes. The variation in chromosome numbers noticed among the mitotic parthenogenetic forms indicates the existence of many pathways of derivation. Species with about 26 chromosomes apparently are diploid and may have evolved from diploid meiotic forms without any change in the degree of ploidy. Species with about 54 chromosomes could be considered as triploids and most likely they have been derived following hybridization of meiotic parthenogenetic forms involving, for example, fertilization of an unrelated egg with 36 (18 + 18) chromosomes. However, species with intermediate numbers of chromosomes, i.e., hypotriploid, may have been derived from the triploid forms through actual loss or fusions of a number of chromosomes. They may have derived from meiotic diploid forms with reduced chromosome numbers following fusion of unequal gametes. Thus, a hypotriploid form with 45 chromosomes may have been derived from the fusion of an unreduced egg with 30 chromosomes with a normal sperm with 15 chromosomes.

However, Triantaphyllou (1984), after analyzing the behavior of tetraploid forms of *M. hapla* and further consideration of the chromosomal complement of nematodes in general, offered alternative explanations about the possible pathways of evolution of root-knot nematodes. Since all nematodes, with the exception of some ascarids and a few other polyploidy forms, possess small chromosomal numbers ranging from 5 to 9 (n), the $n=18$ chromosomes of the genus *Meloidogyne* may represent a state of polyploidy/tetraploidy. The existence of *Hypsoperine spartinae*, a species very closely related to root-knot nematodes, with only seven chromosomes as the haploid number, provides additional support to this assumption.

Hampering in the inference of evolutionary relationships between nematodes was observed by Martijn Holterman et al. (2006) by their conserved morphology, the high frequency of homoplasy, and the scarcity of phylum-wide molecular data. To study the origin of nematode radiation and to unravel the phylogenetic relationships between distantly related species, they analyzed 339 nearly full-length small-subunit rDNA sequences from a diverse range of nematodes. Bayesian inference revealed a backbone comprising 12 consecutive dichotomies that subdivided the phylum Nematoda into 12 clades. The most basal clade is dominated by the subclass Enoplia, and members of the order Triplonchida occupy positions most close to the common ancestor of the nematodes. Crown clades 8–12, a group formerly indicated as "Secernentea" that includes *C. elegans* and virtually all major plant and animal parasites, show significantly higher nucleotide substitution rates than the more basal clades 1–7. Accelerated substitution rates are associated with parasitic lifestyles (clades 8 and 12) or short generation times (clades 9–11). The relatively high substitution rates in the distal clades resulted in numerous autapomorphies that allow in most cases DNA barcode-based species identification. *Teratocephalus*, a genus comprising terrestrial bacterivores, was shown to be most close to the starting point of Secernentean radiation. Notably, fungal-feeding nematodes were exclusively found basal to or as sister taxon next to the three groups of plant-parasitic nematodes, namely, Trichodoridae, Longidoridae, and Tylenchomorpha. The exclusive common presence of fungivorous and plant-parasitic nematodes supports a long-standing hypothesis that states that plant-parasitic nematodes arose from fungivorous ancestors.

Philippe Castagnone-Sereno et al. (2013) studied the diversity and evolution of root-knot nematodes (*Meloidogyne*) and observed that these worms exhibited a wide continuum of variation in their reproductive strategies, ranging from amphimixis to obligatory mitotic parthenogenesis. Molecular phylogenetic studies highlighted the divergence between mitotic and meiotic parthenogenetic root-knot nematode species and probable interspecific hybridization as critical steps in their speciation and diversification process. The recent completion of the genomes of *Meloidogyne hapla* and *M. incognita* that exhibit striking differences in their mode of reproduction (with and without sex, respectively), their geographical distribution, and their host range has opened the way for deciphering the evolutionary significance of (a)sexual reproduction in these parasites. Further, the accumulating evidence suggested that whole-genome duplication (in *M. incognita*) and horizontal gene transfers (HGTs) represent major forces that have shaped the genome of current root-knot nematode species and may account for the extreme adaptive capacities and parasitic success of these nematodes.

Root-knot nematodes are known to reproduce either by cross-fertilization (amphimixis), facultative meiotic parthenogenesis, or obligatory mitotic parthenogenesis (Castagnone-Sereno et al. 1993). Among them, *M. incognita, M. arenaria*, and *M. javanica* are obligatory mitotic parthenogenetic species, while *M. hapla* can reproduce by both cross-fertilization and meiotic parthenogenesis. Phylogenetic relationships in this genus have been investigated by hybridization of *Bam*HI-digested genomic DNAs of 18 geographical isolates belonging to six species with three homologous repeated DNA probes cloned at random from a genomic library of one population of *M. incognita*. Due to the repetitive

nature of the probes, the autoradiograms exhibited extensive restriction fragment length polymorphisms (RFLPs) both between and within nematode species. Genetic distance values estimated from hybridization patterns were analyzed by two phylogenetic tree-building distance methods, respectively, based on constant (UPGMA) and varying (FITCH) rates of nucleotide substitution, and the resulting dendrograms showed a very similar clustering of species and populations. Comparison of these results with the other sources of phylogenetic data available for this genus, i.e., cytogenetic, isoenzymatic, and mitochondrial DNA (mtDNA) data, revealed consistency with all but the mtDNA phylogeny. Due to the maternal inheritance of mtDNA and the parthenogenetic reproductive mode of these organisms, which excludes any possibility of horizontal transfer, they concluded that nuclear DNA phylogeny should represent a more likely evolutionary history of this particular genus and that interspecific hybridizations between sexual ancestors may account for the results with mtDNA. Thus, the early split-off of the mitotically parthenogenetic species cluster and *M. hapla* confirms the amphimictic ancestral mode of reproduction of root-knot nematodes. The authors also discussed the existence of polymorphism within each species at the repeated DNA level in relation to the adaptive evolution of these parthenogenetic species.

3.11 Nematode Genome Evolution

Nematodes are the largest animal phylum. But, out of the estimated number of 1–10 million species, only approximately 25,000 are formally described (Lambshead 1993). Next to their species richness, their ecological omnipresence in virtually all terrestrial and aquatic habitats and also their high number of individuals contribute to the importance of nematodes (Floyd et al. 2002).

One of the best studied model organisms, the free-living worm *Caenorhabditis elegans* belongs to the nematode community (Rödelsperger et al. 2013). With the extensive knowledge about *C. elegans* as an excellent baseline, nematodes are becoming increasingly popular for evolutionary studies. *C. elegans* was the first multicellular organism that had its genome sequenced in 1998. It is important to note that until today, *C. elegans* is the only metazoan with a fully sequenced genome in the sense that there are no sequence gaps left. Recently, draft genome sequences of multiple other free-living and parasitic nematodes were published and their number is increasing rapidly. These genome sequences are a yielding source for the investigation of the structure and evolution of genomes. Among nematodes, examples of phylogenetically very closely related species that have completely different ecologies and species with very similar ecologies that are, however, only very distantly related are found. This makes nematodes an attractive system to study how genomes are shaped by the environment and evolutionary descent.

In terms of the numbers of individuals, nematodes are the most abundant type of animal on earth. So far 25,000 species have been classified, and there could be 100 million species (Blaxter 2003). This abundance results from their ability to adapt, as well as their small size, resistant cuticle, and simple body plan. Small changes to their body plan have allowed invasion of many different habitats. Nematodes live in hot springs, polar ice, soil, and fresh- and saltwater and as parasites of plants, vertebrates, insects, and other nematodes. This evolutionary plasticity, which hints at an underlying genetic plasticity, has long fascinated biologists. In 1965, the German zoologist Alfred Kaestner wrote, “our knowledge concerning the evolution of nematodes is next to nothing.” Happily, with the genome sequences of the nematodes *Caenorhabditis elegans* and *C. briggsae* in hand and those of *C. remanei*, *C. japonica*, *C.* sp. PB2801, *Pristionchus pacificus*, *Haemonchus contortus*, *Meloidogyne hapla*, *Brugia malayi*, and *Trichinella spiralis* soon to follow, our knowledge is now growing fast (Avril Coghlan 2005).

In comparison with genomes of many other metazoans, in particular vertebrates which have genome sizes between 300 Mb and 3.3 Gb

(Rödelsperger and Dieterich 2010), all published nematode genomes are very small and compact. Variation in the gene composition of nematode genomes is attributed to extensive gain and loss of genes. Nematodes acquire their genes through the processes of de novo formation, gene duplication, and horizontal gene transfer, among others. The process of horizontal gene transfer allows nematodes to acquire new physiological features. In other words, after the transfer of genes, the nematodes appear different from what they were originally. Nematodes lose their genes through the processes of gene deletion and evolutionary changes. The genes are lost to a point where they cannot be recognized as homologous to genes in other species. Only a few nematode genomes have been sequenced so far. The sequenced nematodes contain multigene transcription units and operons, which give rise to a single pre-mRNA. The pre-mRNA is then broken up into single protein-coding mRNAs through the processes of trans-splicing and polyadenylation.

Rapid evolution, in particular after gene duplication events, seems to be a plausible explanation for the apparent lack of homologues of some genes. Duplications have been proposed to allow for the generation of novel protein functions in one of the two copies, whereas the original function is still retained by the other duplicate (Katju and Lynch 2006). Indeed, many orphan genes belong to larger gene families of which other members do have homologues in other nematode species. This suggests that evolution within gene families is highly dynamic and some members might have diverged to the extent that they are classified as orphan genes, whereas other members have recognizable homologues in other species.

In *C. elegans*, in a process called trans-splicing, a 22-nucleotide-long ribonucleic acid (RNA) fragment (spliced leader, SL) is added posttranscriptionally to the 5′ ends of the messenger RNAs (mRNAs) of approximately 70 % of all genes (Blumenthal 2005). Trans-splicing, along with polyadenylation, is also used to break up polycistronic pre-mRNAs into multiple mRNAs coding for a single protein each. In *C. elegans*, approximately 25 % of all genes are organized in such polycistronic transcription units called "operons." Although the same term is used, operons in nematodes are neither evolutionarily related nor functionally equivalent to bacterial operons, which combine multiple functionally related genes and give rise to a single polycistronic mRNA (Rödelsperger et al. 2013). Trans-splicing and operons were shown to exist in all nematode species with a sequenced genome, and the process appears widespread among nematodes of clades 3–5.

Nematode genomes emerge as an excellent test case for the study of the evolutionary dynamics of genomes (Rödelsperger et al. 2013). Although the genomes currently available are only able to detect the most obvious features of nematode genomes, the small size and low abundance of repetitive sequences will facilitate the sequencing of many more species and different isolates of the same species with manageable effort. In the future, within- and cross-species comparisons over the full range of evolutionary distances will facilitate dating the formation of novel genes and detecting signatures of selective constraints or rapid evolution.

3.12 The Range of Genome Size Across the Nematoda

Most nematodes have genomes ranging from 50 to 250 Mb. Among the nematodes being sequenced, sizes vary from 53 Mb for *Haemonchus contortus* to 240 Mb for *Trichinella spiralis* (Avril Coghlan 2005). A few nematodes even have genomes as large as those of mammals, such as the ~2,100 Mb genome of *Parascaris univalens*. Other nematode genomes are tiny, such as the ~30 Mb *Bursaphelenchus mucronatus* genome. The varying size in the genomes of the nematodes has only been estimated for about 50 species of nematodes, which is a small number as compared to the number or nematode species that exist today. Also, notable about nematode genomes is that they are compact and, therefore, make for a good study of the structure and evolution of genomes. Research has shown that the size of nematode genomes is

similar to that of flatworms, insects, and annelids. However, the genomes are smaller than those of invertebrates like echinoderms and mollusks. The causes for the variations in size of the nematode genomes are not known, but they have been linked to spontaneous deletions.

Most nematodes contain the haploid chromosome numbers of $n = 4–12$. Over 300 species of nematodes have been studied and studies indicate that nematodes display a lot of karyotypic variations. Additionally, it has been found that a third of the genes in the sequenced nematode genomes have no recognizable homologues outside their genus. Also noticeable is the fact that there are high rates of gene losses and gains among the nematode genomes. There are numerous examples of gene acquisitions that have been observed through gene transfers.

3.12.1 Genome Size and Gene Count

Nematode genomes are similar in size to those of flatworms, annelids, and insects (~60–100 Mb upward) but are smaller than those of some invertebrates such as mollusks and echinoderms (~400–500 Mb upward). The compact nature of nematode genomes may be due to a high rate of large, spontaneous deletions and perhaps to selection for deletions (Denver et al. 2004). The *C. briggsae* genome is slightly (~4 Mb) larger than the *C. elegans* genome, due to a larger amount of repetitive DNA in the *C. briggsae* genome (Stein et al. 2003). This must be due to proliferation of repeat families in the *C. briggsae* genome or loss of repetitive DNA from *C. elegans*. Comparison of the *C. elegans* and *C. briggsae* genomes to those of closely related nematodes will shed light on the relative importance of deletions (which will decrease the genome size) versus insertions and proliferation of repeats (which will both increase the genome size).

Species with smaller effective population sizes (a smaller number of individuals that contribute different alleles to the next generation) have larger genomes, because they tend to accumulate repetitive DNA and genomic duplications. In a study of two nuclear genes, the diversity in *C. elegans* and *C. briggsae* was just 6–13 % of the diversity seen in *C. remanei*. The effective population sizes of parasitic nematodes probably depend on those of their hosts, so parasites of herbivores may have larger effective population sizes than parasites of carnivores or omnivores. Thus, one could speculate that this explains why the sheep parasite *Haemonchus contortus* has such a small genome (53 Mb) compared to the human parasite *Brugia malayi* (85–95 Mb) or the pig parasite *Trichinella spiralis* (240 Mb). Since the size difference between the 104 Mb *C. briggsae* and 100 Mb *C. elegans* genomes is due to repetitive DNA, they both have ~19,500 genes. The *Brugia malayi* genome has a similar size to the *Caenorhabditis* genomes, ~85–95 Mb, and a similar number of genes, ~18,500 genes. The *Haemonchus contortus* genome is just 53 Mb, but it is not yet clear whether it contains half as many genes as *C. elegans* or rather has the same number of genes but half as much noncoding DNA (Whitton et al. 2004).

Meloidogyne hapla was established as a tractable model phytonematode amenable to forward and reverse genetics and presented a complete genome sequence (Opperman et al. 2008). It was observed that at 54 Mbp, *M. hapla* represented not only the smallest nematode genome yet completed but also the smallest metazoan and defined a platform to elucidate mechanisms of parasitism by what is known as the largest uncontrolled group of plant pathogens worldwide. The *M. hapla* genome encoded significantly fewer genes than *C. elegans,* most notably through a reduction of odorant receptors and other gene families, yet it acquired horizontally from other kingdoms numerous genes suspected to be involved in adaptations to parasitism. In some cases, amplification and tandem duplication had occurred with genes suspected of being acquired horizontally and involved in parasitism of plants. Although *M. hapla* and *C. elegans* diverged >500 million years ago, many developmental and biochemical pathways, including those for dauer formation and RNAi, were conserved. They concluded that although overall genome organization is not conserved, there are areas of microsynteny that may

suggest a primary biological function in nematodes for those genes in these areas.

Most nematodes have haploid chromosome numbers of $n = 4–12$. The karyotypes of just ~300 species have been studied, but nematodes display a lot of karyotypic variation. The lowest haploid number is $n = 1$ in *Parascaris univalens*, but very high counts are seen in polyploid species in the Tylenchomorpha. For example, the race of *Meloidogyne hapla* being sequenced is diploid and has $n = 14$, but another race of *M. hapla* is polyploid with $2n = 45–48$. Many tylenchomorphs including *M. hapla* are parthenogens, in which unfertilized eggs develop into new individuals. Animal species that reproduce in this way seem to be susceptible to polyploidization. The *M. hapla* race being sequenced has twice as many chromosomes as most rhabditines, so it could reveal traces of an ancient genome duplication in the Tylenchomorpha. In contrast to the tylenchomorphs, most rhabditines have $n = 5–6$ (Blaxter et al. 2000). Indeed, *C. elegans* and *C. briggsae* both have $n = 6$, even though their chromosomes have undergone ~4,000 rearrangements since they diverged. The lack of fissions or fusions suggests that there could be selection for a stable chromosome number in the Rhabditina.

3.13 Ancient Linkage Groups

The genome of *C. elegans* was compared to that of *C. briggsae*, and ~4,800 conserved segments, with an average size of 37 kb, were observed (Stein et al. 2003). They estimated that there have been 3.6 interchromosomal rearrangements per Mb in the *C. briggsae* genome. Thus, an average *C. briggsae* chromosome of ~10–20 Mb consists of a mosaic of ~35–70 chunks that match several *C. elegans* chromosomes. However, some of these segments are very small, so it may be possible to detect ancient *Caenorhabditis* linkage groups by considering just the largest conserved segments. A genetic map for *C. briggsae* is currently underway and should allow us to match each *C. briggsae* chromosome to the *C. elegans* chromosome(s) with which it shares common ancestry. Sequencing of random regions of the *Pristionchus pacificus* and *Brugia malayi* genomes suggests that despite the frequent occurrence of reciprocal translocations, ancient Secernentean linkage groups may still be detectable.

In an 11-gene region sequenced from *P. pacificus* chromosome III, 10/11 genes had orthologs on *C. elegans* chromosome III. This led Lee et al. to suggest that *P. pacificus* chromosome III and *C. elegans* chromosome III shared a common ancestor. If this is true, there must have been a lot of intrachromosomal rearrangement since just three pairs of the *P. pacificus* genes are closely linked in *C. elegans*, but these pairs are scattered over 12 Mb. An evidence was found suggesting that *B. malayi* chromosomes can be matched to their *C. elegans* homologues. They sequenced BAC ends containing 8 Mb of *Brugia malayi* sequence and found that 60 % of the BACs matched the same *C. elegans* chromosome at both ends. However, large rearrangements seem to have occurred within chromosomes, because the average distance between two matches was 4 Mb.

With respect to the evolutionary patterns in the arms and centers of nematode chromosome, each of *Caenorhabditis elegans*' chromosomes is divided into a repeat-poor "central cluster" that rarely undergoes meiotic exchange and two repeat-rich "arms" that have a ~7-fold higher recombination rate (*C. elegans* Sequencing Consortium 1998). Intriguingly, the arms are evolving far more rapidly than the centers of chromosomes, in terms of both substitutions and chromosomal rearrangements such as translocations, inversions, and duplications (*C. elegans* Sequencing Consortium 1998). This may reflect a lower tolerance to mutation in the central clusters, which contain most of the essential genes and operons. Alternatively, the arms may simply have a higher mutation rate, since the high recombination rate may provoke substitutions, while the abundance of repeats probably triggers chromosomal rearrangements (Coghlan and Wolfe 2002).

There are ~1,000 operons in the *C. elegans* genome, of which 96 % are conserved in *C. briggsae*, far more than expected if selection did not act to preserve operons (Stein et al. 2003).

Gene order in ~15 % of the genome is stabilized by selection against rearrangements of operons, since 15 % of *C. elegans* genes are part of operons. In fact, operons are concentrated in the central clusters of *C. elegans* chromosomes, so they probably contribute to the lower rearrangement rate in the centers compared to the arms. One *C. elegans* operon is conserved in the closely related rhabditine *Oscheius*, but at least one *C. elegans* operon has been broken in *Pristionchus pacificus.*

Operons probably exist in the Rhabditina, Tylenchina, and Spirurina, since *trans*-splicing has been observed in *Haemonchus contortus*, *Panagrellus redivivus*, *Ascaris suum*, *Anisakis* spp., and *Brugia malayi*. Two unresolved questions are whether *Trichinella spiralis* has *trans-splicing* and operons and whether nematode operons are related to those in flatworms. *C. elegans* chromosomes also contain small clusters of ~2–5 genes that are co-expressed in the muscle, even though they do not belong to operons, as well as clusters co-expressed in the germ line, intestine, and neurons (intestine = Mountain 08 and neurons = Mountain 06).

3.14 The Nematode HOX Gene Cluster

Hox genes are of much significance and their central role in anterior–posterior patterning provides a framework for molecular comparison of animal body plan evolution (Aboobaker and Blaxter 2003). The nematode *Caenorhabditis elegans* stands out as having a greatly reduced Hox gene complement. To address this, orthologs of *C. elegans* Hox genes were identified in six species from across the Nematoda, and they show that rapid homeodomain sequence evolution is a general feature of nematode Hox genes. Some nematodes express additional Hox genes belonging to orthology groups that are absent from *C. elegans* but present in other bilaterian animals. Analysis of the genomic environment of a newly identified *Brugia malayi* Hox6-8 ortholog (*Bm-ant-1*) revealed that it lay downstream of the *Bm-egl-5* Hox gene and that their homeodomain exons are alternately *cis*-spliced to the same 5′ exon. This organization may represent an intermediate state in Hox gene loss via redundancy. The Hox clusters of nematodes are the product of a dynamic mix of gene loss and rapid sequence evolution, with the most derived state observed in the model *C. elegans*.

Hox proteins have been intensively studied in insects and vertebrates, but little is known about how Hox proteins provide specificity to their many specific roles during nematode development (Gutierrez et al. 2003). Nematodes provide an interesting example, as studies in *C. elegans* have indicated that several aspects of Hox genes, including their organization in the cluster and their function and sequence, differ strongly from Hox genes in other phyla. Nematodes are renowned for sharing a conservative body plan. The model *C. elegans* has a strongly determinative, lineage-driven mode of development resulting in an invariant cell lineage and eutely (Voronov and Panchin 1998). Hox gene functions in *C. elegans* have been evolving within this deterministic developmental mode, and their expression is now cell lineage, and not cell position, dependent. They suggested a three-step process in which a lineage-dependent mechanism of development was first adopted, ultimately releasing some Hox genes from a core role in positional identity pathways, followed by recruitment of these genes to new function in the context of lineage. Once a gene is released from its essential role, it is free to be lost, possibly through the exon-sharing mechanism observed for *B. malayi ant-1* and *egl-5*, or to move rapidly through sequence space to assume novel functions. Current models of the modes of evolution of Hox gene function involve gene duplications, micro and macro changes in expression pattern, and changes in sequences outside the 60-amino-acid homeodomain (Averof and Patel 1997). In general, the homeodomains evolve slowly, but, when Hox genes are divorced from homeotic function, as has happened with *Hox3* and *ftz* genes in the Diptera, their homeodomains are observed to evolve more rapidly. The independently duplicated posterior-group Hox genes of deuterostomes also have elevated rates of substitution.

Aboobaker and Blaxter (2003) observed that all of the nematode ortholog groups show elevated substitution rates across the phylum when compared to genes from arthropods and other bilaterians. By analogy to other systems, the functions of all the nematode Hox genes may have changed rapidly across the phylum, as constraint on all the Hox homeodomains has been lost.

HOX genes are transcription factors that are closely clustered in the genomes of most animals (Bürglin 1994). They control the expression of anterior–posterior patterning along the body axis during early embryogenesis collinearly with their arrangement on the chromosome. The HOX cluster has been conserved in most animal phyla over hundreds of millions of years of evolution, but the nematode HOX cluster is surprisingly poorly preserved. The ancestral bilaterian probably had a cluster of nine HOX genes (nine ortholog groups), but all nematodes have lost at least three ortholog groups (Aboobaker and Blaxter 2003). A further two ortholog groups were lost in the lineage leading to *C. elegans*, after the Spirurina–Rhabditina–Tylenchina clade diverged from other nematodes. Aboobaker and Blaxter (2003) pointed out that these two HOX ortholog groups were lost around the time when *C. elegans*' ancestor switched from a regulative mode of development to a deterministic lineage-driven mode. They suggest that perhaps the transition freed the two HOX ortholog groups from their role in anterior–posterior patterning, making their loss tolerable. Interestingly, the HOX cluster has been broken up in *C. elegans*: its six HOX genes (belonging to four ortholog groups) are arranged in three pairs scattered over 5 Mb of chromosome III. *Trichinella spiralis* probably has a regulative mode of development, but it is not yet known whether its HOX genes are clustered. However, even though *Brugia malayi* has lineage-driven development, most of its HOX cluster seems to have been preserved intact.

Hox genes encode evolutionarily conserved transcription factors involved in morphological specification along the anterior–posterior body axis of animals (Arturo Gutierrez et al. 2003). The two most striking features of Hox genes are colinearity and the strong sequence conservation. Among all animals studied so far, the nematode Caenorhabditis elegans contains one of the most divergent Hox clusters. The core cluster contains only four members, which in part deviate from the colinearity rule. In addition, orthologous and paralogous nematode Hox sequences diverged substantially. They investigated the role of MAB-5 during ray formation and established an in vivo assay using Cel-mab-5 regulatory elements to express orthologous, paralogous, and chimeric cDNAs in a Cel-mab-5 mutant background. It was shown that the MAB-5 ortholog from *Pristionchus pacificus* but not the *C. elegans* paralogous Hox proteins can rescue Cel-mab-5. Experiments with chimeric, truncated, and mutagenized Hox proteins suggest the specificity to be conferred by the *N*-terminal arm and helix I, but not helix II of the homeodomain.

3.15 Evolution of X and Y Chromosomes in Nematodes

Nematodes were one of the first animals chosen for cytological studies which ultimately led to the discovery of the correlation between chromosomal makeup of an embryo and its future sexual development, male or female (McClung 1902). The study of the mechanisms and evolution of sex determination intersects several fundamental questions in biology such as why and how sex is maintained, what forces govern the evolution of genome structure, how ecological factors constrain or favor reproductive mode, and how genetic networks evolve.

Most nematodes have chromosomal sex determination, in which the female is XX and the male is heterogametic sex (XY) or XO (the O indicates the absence of Y) (Pires-daSilva 2007). It is likely that the XX:XO sex determination system is ancestral because it is found in most of the nematode clades so far studied. The few XY systems occur among parasitic nematodes of clades 1, 3, and 4. It is thus possible that the XY system is derived from the fusion of an autosome to the old X chromosome, although clear cytogenetic evidence is still lacking. In *C. elegans*, sex determination acts through an X-chromosome dosage

mechanism: animals with two X chromosomes develop as hermaphrodites, whereas XO animals develop as males. XX/XO sex determination is very common across the Nematoda, suggesting that the first nematode possibly had XX/XO sex determination. Even if the *C. elegans* and *Trichinella spiralis* XX/XO systems did share common ancestry, the traces will be hard to find, since sex determination pathways and genes are evolving very quickly both in terms of sequence change and gene regulation (Haag and Doty 2005). However, at least one key gene is conserved in the XX/XO sex determination pathways of *C. elegans* and *Pristionchus pacificus*, so it should be possible to determine whether the *P. pacificus*, *C. elegans*, and *Haemonchus contortus* XX/XO systems are orthologous.

It is well established that in other phyla the molecular and genetic mechanisms underlying sex determination can be completely distinct, even when comparisons of closely related species are undertaken (Pires-daSilva 2007). The most striking differences are in the more upstream events of the sex determination pathway. Intriguingly, the ortholog of the *C. elegans* X signal element fox- 1 is also in the X chromosome of the distantly related nematode *P. pacificus.* Functional analysis of this gene and other signal element gene orthologs will be necessary to test if the counting mechanism is the same between these nematodes. Nothing is known about the molecular switch mechanism present in XX:XY species. One possibility is that it is based on a genic balance mechanism, which depends on a balance between female-determining genes in the X chromosome and male-determining genes in autosomes, as with *C. elegans.* Another option is that the Y chromosome plays a dominant role in the sex determination.

Nematodes with environmental sex determination have a high rate of intersexes (Triantaphyllou 1960). Intersexes are usually functional females (i.e., individuals with well-developed reproductive system that produces oocytes) with some male somatic characters such as spicules. In *M. javanica* and *M. incognita,* female larvae can undergo sex reversal relatively late in development, which causes the appearance of males with two gonadal arms (typical of females) instead of one. Sex reversal and intersexes can be induced experimentally in crosses between two different species such as in the rhabditids *C. remanei* and *C. briggsae*. In this case, the effect has been attributed to dysgenic interactions among fast-evolving sex determination genes.

Phylogenetic analyses suggest that hermaphroditism in *C. elegans* and *C. briggsae* has occurred due to an evolutionary convergence rather than being homologous (Pires-daSilva 2007). This conclusion is supported by genetic data, which indicate that there are fundamental differences in spermatogenesis between these two species. fog-2, the most upstream germ line-specific regulator of spermatogenesis in *C. elegans* hermaphrodites, is not present in the *C. briggsae* genome. Other genes involved in *C. elegans* hermaphroditic spermatogenesis (e.g., gld-1, fem-2, and fem-3) do not have a function in sperm formation in *C. briggsae* hermaphrodites. Traditional forward genetic approaches will be necessary to unravel the genes involved in germ line sex determination in *C. briggsae.*

Sex is determined in *C. elegans* by an X-chromosome-counting mechanism that reliably distinguishes the twofold difference in X-chromosome dose between males (1X) and hermaphrodites (2X) (Nicoll et al. 1997). This small quantitative difference is translated into the "on/off" response of the target gene, xol-1, a switch that specifies the male fate when active and the hermaphrodite fate when inactive. Specific regions of X contain counted signal elements whose combined dose sets the activity of xol-1. They ascribed the dose effects of one region to a discrete, protein-encoding gene, fox-1, and demonstrated that the dose-sensitive signal elements on the X chromosome control xol-1 through two different molecular mechanisms. One involves the transcriptional repression of xol-1 in XX animals. The other uses the putative RNA-binding protein encoded by fox-1 to reduce the level of xol-1 protein. These two mechanisms of repression act together to ensure the fidelity of the X-chromosome counting process.

Random amplification of polymorphic DNA was used to analyze genomic DNA from virgin females and males of *Brugia malayi,* with a view to identifying sex-specific differences predicted by an XX/XY system of chromosomal sex determination (Underwood and Bianco 1999). A product of 2,338 bp, amplified with the arbitrary primer 5′ GTTGCGATCC 3′, was obtained exclusively from males. Primers based on the sequence of this product amplified a DNA fragment of the expected size from each of two independent isolates of *B. malayi* (from Malaysia and Indonesia) by PCR. No reaction product was obtained from the closely related species *Brugia pahangi.* In a genetic cross between *B. malayi* males and *B. pahangi* females, F1 hybrid microfilariae were PCR positive, indicating that the locus is paternally inherited. Southern blotting demonstrated that the target sequence resides in the high molecular weight fraction of genomic DNA, confirming that it is of chromosomal, rather than mitochondrial, origin. Sequencing of the locus revealed significant similarity with members of a family of reverse transcriptase-like genes in *Caenorhabditis elegans.* In-frame stops indicate that the gene is nonfunctional, but multiple bands of hybridization in Southern blots suggest that the RT sequence may be the relic of a transposable element. Multiple repeats of the dinucleotide AT occurred in another region of the sequence. These varied in number between the two isolates of *B. malayi* in the manner of a microsatellite, surprisingly the first to be described from the *B. malayi* genome. Because of its association with the Y chromosome, we have given the locus the acronym TOY (tag on Y). Identification of this chromosome-specific marker confirms the XX/XY heterogametic karyotype in *B. malayi* and opens the way to elucidation of the role of Y in sex determination.

To achieve X-chromosome dosage compensation, organisms must distinguish X chromosomes from autosomes (Csankovszki et al. 2004). They identified multiple, cis-acting regions that recruit the *C. elegans* dosage compensation complex (DCC) through a search for regions of X that bind the complex when detached from X. The DCC normally assembles along the entire X chromosome, but not all detached regions recruit the complex, despite having genes known to be dosage compensated on the native X. Thus, the DCC bound first to recruitment sites and then spread to neighboring X regions to accomplish chromosome-wide gene repression. From a large chromosomal domain, a 793-base-pair fragment was defined that functioned in vivo as an X-recognition element to recruit the DCC. Only a handful of nematodes have Y chromosomes: *Brugia malayi*, *Onchocerca volvulus, Baylisascaris transfuga*, *Contracaecum incurvum*, and *Trichuris muris*. Since Ys are only known in these few distantly related nematodes, it was suggested that they probably emerged recently. In papaya the sequence of the Y chromosome betrays its recent origin from autosomes, and it will be interesting to see if the *Brugia malayi* Y arose in a similar way.

The involvement of the *C. elegans* X chromosome in sex determination may have restrained its pace of structural evolution. Since *C. elegans* diverged from *C. briggsae*, its X chromosome has undergone about half as many rearrangements as its autosomes. Indeed, two of the three largest conserved segments between the two genomes are on *C. elegans* X. Furthermore, a genetic linkage map of *Pristionchus pacificus* suggests that the X chromosome may have been preserved largely intact since the divergence of *P. pacificus* from *C. elegans.*

Nematodes are very diverse in reproductive modes and in sex determination mechanisms. It has been suggested that gonochoristic nematodes evolved into hermaphrodites and that some of those became parthenogenetic. Autotokous reproduction seems to have evolved relatively often in nematodes, which makes an interesting case for studying which factors lead to the evolution of this reproductive mode (Castagnone-Sereno 2006). Although rare in other animals, nematodes that reproduce mainly asexually are one of the most ubiquitous plant parasites in the world. To understand the factors that are driving the evolution of sex determination and asexual reproduction, integration of many diverse fields such as ecology, genetics, and cell

biology will be required. Basic questions such as whether there is the occurrence of hermaphroditism in non-rhabditid nematodes are still unanswered.

The most common reproductive strategy among nematodes is sexual reproduction between males and females or amphimixis, which is seen in *Caenorhabditis remanei*, *Caenorhabditis* sp. PB2801, *Caenorhabditis japonica*, *Haemonchus contortus*, *Brugia malayi*, and *Trichinella spiralis* apart from several phytopathogens. However, alternative reproductive strategies have arisen in some nematode groups, including hermaphroditism, parthenogenesis, and haplodiploidy. For example, *C. elegans*, *C. briggsae*, and *Pristionchus pacificus* are hermaphroditic. The strain of *Meloidogyne hapla* sequenced is a facultative meiotic parthenogen. Because hermaphroditic species (and perhaps parthenogenetic species) have a smaller effective population size than amphimictic species, they will tend to accumulate deleterious mutations, resulting in a faster substitution rate and rate of chromosomal rearrangement (Archetti 2004). This may explain why substitution rates in *C. elegans* and *Meloidogyne* seem to be high compared to most nematodes (Blaxter et al. 1998).

Studies have been conducted on the effect of kinetochore organization on genome stability. Since *C. elegans* and *C. briggsae* diverged, their chromosomes have been splintered by ~250 reciprocal translocations, ~1,400 inversions, and ~2,700 transpositions. Intrachromosomal rearrangement is about four times more frequent than interchromosomal rearrangement. Even so, translocations are surprisingly common in *Caenorhabditis* compared to flies, in which translocations are extremely rare (Sharakhov et al. 2002). This may be because almost all dipterans have "monocentric" chromosomes, in which the kinetochores assemble on a localized region in each chromosome. In contrast, "holocentric" species such as *C. elegans* and *C. briggsae* have diffuse kinetochores that form along the length of their chromosomes during mitosis. Since the kinetochores are the primary chromosomal attachment site for spindle microtubules, they play a key role in ensuring high-fidelity chromosome transmission in both monocentric and holocentric species.

However, little is known of the relationship between the distribution of kinetic activity along chromosomes and the pattern of chromosomal rearrangement. In species with monocentric chromosomes, many translocations will be lethal because they will give rise to acentric or dicentric chromosomes, while species with holocentric chromosomes may be more tolerant of translocations. Most nematodes have holocentric chromosomes, but *Trichinella spiralis* and some other trichinellids have monocentric chromosomes. Thus, comparison of the *T. spiralis* genome to that of *C. elegans* may provide clues as to whether holocentric chromosomes are more susceptible to rearrangement and whether the first nematode had holocentric chromosomes.

3.16 Evolution of Gene Content

The Nematoda is one of the oldest among the animal phyla. Subclass divergence within the Secernentea is thought to have occurred over 550 million years ago, with separation of the class Adenophorea predating that event. It may be predicted that mtDNA sequence comparisons between nematode classes and subclasses would generate low similarity scores, as observed with any alignment involving *R. culicivorax* and *M. incognita* (Hyman and Beck Azevedol 1995). In their study, ten of 12 mitochondrial protein-coding genes and the large (16s) mitochondrial rRNA gene were identified and mapped within the *Romanomermis culicivorax* mitochondrial genome. This transcriptional map differs from other nematode mitochondrial DNAs (mtDNAs) with respect to gene order and transcriptional orientation of some genes. Several of these coding regions are components of a 3.0-kilobase mtDNA repeating unit, allowing a direct comparison of nucleotide and amino acid sequence composition for repeated and single-copy genes. Analysis of protein-coding regions representing repeated (ND3, ND6) and single-copy genes (ATPase 6, cyt.b, COI, COIII, NDl, ND4, ND5) and four repeat-associated open reading frames (ORFs)

with unassigned function have revealed striking similarities in nucleotide composition, amino acid frequencies, and codon biases. Although they anticipated that reiterated protein-coding regions might be evolving under relaxed selection, results indicated that both repeated and unique mitochondrial genes appear subject to similar functional constraints.

Mitochondrial genomes of metazoa are typically composed of a single, circular molecule that varies in size from approximately 14–39 kilobases (kb) (Brown 1985; Moritz et al. 1987; Snyder et al. 1987). These molecules reveal a nearly identical coding potential consisting of structural genes for an organelle-specific translation system (2 rRNAs and 22 tRNAs) and 12 or 13 protein-coding genes. The encoded polypeptides are components of the mitochondrial electron transport and oxidative phosphorylation systems: apo-cytochrome b (cyt.b); F-ATPase subunits 6 and 8 (ATPases 6 and 8); cytochrome c oxidase subunits I, II, and III (COI–COIII); and subunits L-6 and 4 L of the respiratory chain NADH dehydrogenase (NDl-ND6 and ND4L) (Chomyn and Attardi 1987). Therefore, the large size variation observed among animal mitochondrial DNAs (mtDNAs) is not usually a consequence of differential gene content. Rather, size polymorphism most frequently results from copy number variation of tandem repeats within noncoding mtDNA sequences, often residing in the vicinity of the control region.

Parkinson et al. (2004) sequenced ESTs from 30 different nematode species across the phylum and defined ~94,000 genes from ~60,000 families. Surprisingly, only about 15,000 (15 %) of the ~94,000 genes are found in all four clades of nematodes studied (Rhabditina, Tylenchina, Spirurina, Dorylaimia). These 15,000 genes are probably involved in core metabolic or structural pathways, since most of them (91 %) have sequence matches outside the Nematoda. In addition, they identified ~1,300 genes that are nematode specific but that are found in most nematodes. These ~1,300 genes probably have roles that are important for nematode body plan and life history and so may shed light on the early evolution of the phylum.

3.17 Proliferation and Loss of Gene Families

Since *C. elegans* has diverged from *C. briggsae*, chemoreceptors have proliferated in the *C. elegans* genome so that it now has almost twice as many as *C. briggsae* (718 versus 429) (Stein et al. 2003). Duplication and divergence of extra chemoreceptors may have allowed *C. elegans* to adopt a slightly different ecological niche than *C. briggsae*, since it uses chemoreceptors to find food and to avoid predators, pathogens, and toxins. On the other hand, *C. elegans* seems to have lost several genes (~30 genes) that are found in both *Pristionchus pacificus* and *Haemonchus contortus*. For example, *C. elegans* has lost a DNA methyltransferase gene that is found in *P. pacificus,* a loss that probably led to the abolition of DNA methylation in *C. elegans* (Gutierrez and Sommer 2004). Contrasting the gene families that have been duplicated or lost in each of the ten nematode genomes may reveal selection for different gene contents in different species.

The seven transmembrane receptor (*str*) and *srj* (renamed from *stl*) families of chemoreceptors have been updated, and the genes were formally named following completion of the *C. elegans* genome sequencing project (Hugh J. Robertson 2001). Analysis of gene locations revealed that 84 % of the 320 genes and pseudogenes in these two families reside on the large chromosome V. Movements to other chromosomes, especially chromosome IV, have nevertheless been relatively common, but only one has led to further gene family diversification. Comparisons with homologues in *C. briggsae* indicated that 22.5 % of these genes have been newly formed by gene duplication since the species split while also showing that four have been lost by large deletions. These patterns of gene evolution are similar to those revealed by analysis of the equally large *srh* family of chemoreceptors and are likely to reflect general features of nematode genome dynamics. Thus, large random deletions presumably balance the rapid proliferation of genes and their degeneration into pseudogenes, while gene movement within and between chromosomes keeps these nematode genomes in flux.

With completion of the sequencing of the *C. elegans* genome, it is now possible to provide a complete description and formal naming of the *str* family, as well as the related family previously called *stl* but here renamed *srj*. Phylogenetic analysis of these two gene families confirms several of the genome dynamics inferred from the *srh* family, including loss of *C. briggsae* orthologs, recent formation of many genes within *C. elegans*, and the frequent occurrence of movements of genes between chromosomes. In addition, preliminary analysis of gene location within chromosome V revealed frequent gene movement within it.

3.18 Species-Specific Genes

C. elegans has ~1,000 genes that are not found in *C. briggsae* and that lack any match in sequence databases (Coghlan 2005). Of these, ~200 have been confirmed by EST or cDNA data, so they are definitely not gene prediction errors. These genes may have diverged so rapidly that their *C. briggsae* homologue is unrecognizable or may have been assembled de novo via chromosomal rearrangements in the *C. elegans* genome. Duplications, chromosomal rearrangements, and transposable elements are known to play a role in the birth of novel genes. Thus, the abundance of species-specific genes in the arms of *C. elegans* chromosomes probably results from the arms' high rate of rearrangement. *C. elegans* is not alone in having so many species-specific genes.

Taxonomically restricted genes (TRGs) are genes that are restricted to a limited subset of phylogenetically related organisms and may be important in adaptation. In parasitic organisms, TRG-encoded proteins are possible determinants of the specificity of host–parasite interactions (Tomalova et al. 2012). In the root-knot nematode (RKN) *Meloidogyne incognita*, the *map-1* gene family encodes expansin-like proteins that are secreted into plant tissues during parasitism, thought to act as effectors to promote successful root infection. MAP-1 proteins exhibit a modular architecture, with variable number and arrangement of 58 and 13-aa domains in their central part. The evolutionary origins of this gene family were studied using a combination of bioinformatics and molecular biology approaches. *Map-1* genes were solely identified in one single member of the phylum Nematoda, i.e., the genus *Meloidogyne*, and not detected in any other nematode, thus indicating that the *map-1* gene family is indeed a TRG family. A phylogenetic analysis of the distribution of *map-1* genes in RKNs further showed that these genes were specifically present in species that reproduce by mitotic parthenogenesis, with the exception of *M. floridensis*, and could not be detected in RKNs reproducing by either meiotic parthenogenesis or amphimixis. These results highlighted the divergence between mitotic and meiotic RKN species as a critical transition in the evolutionary history of these parasites. Analysis of the sequence conservation and organization of repeated domains in *map-1* genes suggested that gene duplication(s) together with domain loss/duplication had contributed to the evolution of the *map-1* family and that some strong selection mechanism might be acting upon these genes to maintain their functional role(s) in the specificity of the plant–RKN interactions.

Long terminal repeat (LTR) retrotransposons may be important contributors to host gene evolution because they contain regulatory and coding signals (Ganko et al. 2003). In an effort to assess the possible contribution of LTR retrotransposons to *C. elegans* gene evolution, they searched upstream and downstream of LTR retrotransposon sequences for the presence of predicted genes. Sixty-three percent of LTR retrotransposon sequences (79/124) were located within 1 kb of a gene or within gene boundaries. Most gene–retrotransposon associations were located along the chromosome arms. The findings were consistent with the hypothesis that LTR retrotransposons have contributed to the structural and/or regulatory evolution of genes in *C. elegans*.

3.19 Horizontal Gene Transfer in the Nematoda

Horizontal gene transfer occurs frequently in prokaryotes, but seems to be rare in eukaryotes. For example, ~1 % of the gene repertoire in the nematode *Meloidogyne* probably originated by

horizontal transfer (Scholl et al. 2003), compared to 1–5 % of single-copy genes and at least 22 % of gene duplicates in Y-proteobacteria. *Meloidogyne hapla*, a plant-parasitic nematode, seems to have gained at least a dozen genes by horizontal gene transfer from bacteria that occupy similar niches in the soil and roots. Those genes gained are useful for the nematode's parasitic lifestyle, such as cellulases for digesting plant material and signaling molecules that induce morphological changes in the plant, facilitating invasion. A distantly related plant parasite, *Bursaphelenchus xylophilus*, seems to have independently acquired a cellulase gene from a fungus. Perhaps horizontal transfer can spur the transition to parasitism. Several groups of parasitic nematodes, including *Brugia malayi*, live in symbiosis with specific bacteria carried by the nematodes (Blaxter 2003). Some of these are extracellular symbionts, but others are intracellular, such as *Wolbachia* living in *B. malayi* and other filarial nematodes. The capture of the *Wolbachia* gene set seems to have been adaptive for filarial nematodes, since killing *Wolbachia* with antibiotics reduces the growth and fecundity of the nematodes.

3.20 Identifying Parasitism Genes

Parasitism of plants and animals has evolved independently at least nine times in the history of the nematodes (Dorris et al. 1999). Four of the nematodes whose genomes are being sequenced are parasites: *Haemonchus contortus*, *Meloidogyne hapla*, *Brugia malayi*, and *Trichinella spiralis*. The adoption of parasitism in nematodes probably required adaptation of genes present in their free-living ancestors (Blaxter 2003). For example, modification of nutrient-acquisition genes found in *C. elegans*, such as digestive enzymes or secreted hydrolases, are likely to have been important for the evolution of parasitism. The ability of parasitic nematodes to survive immunological attack, some living in an infected individual for years, has long been a puzzle. In viral, bacterial, and protozoan parasites, genes involved in host immune evasion or recognition are often under positive selection and hence show patterns of rapid amino acid substitution. Indeed, *B. malayi* GPX-1 shows signs of positive selection. By scanning for *Haemonchus contortus* genes that have diverged sharply in sequence from their *Pristionchus* and *Caenorhabditis* orthologs and that bear secretory signals (Harcus et al. 2004), it may be possible to identify *H. contortus* genes that have adapted for a parasitic lifestyle.

Some genes essential for parasitism in worms may be novel genes. One possible source is gene duplication, which allows one duplicate to keep the original role and the other duplicate to take on a parasitic role. For example, the *alt* gene family of filarial nematodes, which has been implicated in establishing infection, has a single *C. elegans* assembled de novo or has been gained by horizontal gene transfer: plant-parasitic nematodes seem to have acquired "parasitism genes" from bacteria in their environment.

When a comparison was made between 36 *C. elegans* and *Drosophila* protein orthologs to their yeast counterparts, many *C. elegans* genes were found to be evolved twice as fast as their *Drosophila* orthologs. Nematode rRNA genes also seem to have a substitution rate that is 2–3 times that of other animal phyla (Aguinaldo et al. 1997). For example, the rRNA gene divergence between *Caenorhabditis* species is comparable to that between vertebrate species. To accurately estimate the evolutionary rate in nematodes, ideally we would divide the number of mutations between two closely related species by their divergence date. Many mysteries remain in eukaryotic genome evolution. Information is available on data set of ten nematode genome sequences that will be ideal for investigating unresolved questions, such as what are the forces governing the evolution of chromosome number, size, and structure; how does sex chromosome evolution differ from that of autosomes; how do differences in life history traits and reproductive strategy affect genome evolution; and what are the major genomic changes that enable species to adapt to new ecological niches such as parasitism. Looking forward, it seems very possible that once again these tiny animals will be first in revealing some of nature's deepest secrets.

3.21 Phylogeny and Introns

A phylogeny of *Caenorhabditis r*eveals frequent loss of introns during nematode evolution. The most popular model to explain how introns are lost involves homologous recombination between the genomic copy of a gene and an intronless cDNA copy produced by reverse transcription. Because retrotransposons can reverse-transcribe the cell's own mRNA, the required cDNA templates are expected to be present in eukaryotic cells. Furthermore, a cDNA could recombine with its corresponding gene, resulting in intron loss. However, other researchers have suggested that the loss of introns most often occurs by a simple deletion, caused by imprecise recombination. The most common mechanisms proposed to explain intron gain are the insertion of mobile genetic elements that contain splicing signals into a gene, "reverse splicing," and recombination between homologous copies of a gene. Finally, recent work indicates that some introns might be created by the activation of new splice sites in a degenerate coding region.

It has been a topic of curiosity to know how changes in intron/exon structure occur and what role these changes play in evolution. Cho et al. (2004) studied gene structure in nematodes related to *Caenorhabditis elegans.* They cloned a set of five genes from six different *Caenorhabditis* species and used their amino acid sequences to construct the first detailed phylogeny of this genus. They observed that nematode introns are lost at a very high rate during evolution, almost 400-fold higher than in mammals. These losses do not occur randomly, but instead favor some introns and do not affect others. In contrast, intron gains are far less common than losses in these genes. On the basis of the sequences at each intron site, we suggest that several distinct mechanisms can cause introns to be lost. The small size of *C. elegans* introns should increase the rate at which each of these types of loss can occur and might account for the dramatic difference in loss rate between nematodes and mammals.

Kelchner (2002) reported that group II introns comprise the majority of noncoding DNA in many plant chloroplast genomes and include the commonly sequenced regions *trnK/matK*, the *rps16* intron, and the *rpl16* intron. As demand increases for nucleotide characters at lower taxonomic levels, chloroplast introns may come to provide the bulk of plastome sequence data for assessment of evolutionary relationships in infrageneric, intergeneric, and interfamilial studies. Group II introns have many attractive properties for the molecular systematist: They are confined to organellar genomes in eukaryotes and the majority are single copy; they share a well-defined and empirically tested secondary and tertiary structure; and many are easily amplified due to highly conserved sequence in flanking exons. However, structure-linked mutation patterns in group II intron sequences are more complex than generally supposed and have important implications for aligning nucleotides, assessing mutational biases in the data, and selecting appropriate models of character evolution for phylogenetic analysis. These unique features might allow these animals to develop some nematode-specific ways of constructing and altering genomes. To learn how intron/exon structure changes during evolution, genomic and cDNA sequences for *fog-3* and the CPEB genes *fog-1*, *cpb-1*, *cpb-2*, and *cpb-3* were compared from several species in the genus *Caenorhabditis*. These comparisons elucidate the recent history of each intron. Furthermore, because the four CPEB genes were formed by earlier duplication events, comparisons between them revealed information about ancient changes in intron structure.

The rate of intron loss is very high in nematodes. From an evolutionary perspective, changes in intron/exon structure might be an important force for generating differences in gene function. However, a recent study showed that such as it seems likely, then the rate at which introns are lost in worms exceeds that of mammals by more than 400-fold. It was observed that the deleted introns in mammals were probably much smaller than the average human size of 2,500 bp. In *C. elegans*, most introns were about 50 bp long, and small introns were found in each of the other nematode species examined. Thus, it seems possible that the frequency at which introns are lost

is inversely proportional to their size. Because nematodes also have some large introns, this hypothesis can be directly tested when additional genome sequences from *Caenorhabditis* are finished. The rate at which introns are gained might also be higher in worms. No insertions were found in any human, mouse, or rat genes, whereas they observed either two or three insertions in the nematode genes analyzed. However, in the mammalian study, 16 introns in coding regions of low amino acid sequence conservation were not considered and some of these excluded cases might have involved insertions. For comparison, the two recently inserted introns we detected in *fog-1* are both located in a poorly conserved region that would have been excluded from the mammalian study.

3.22 Intron Losses

The rate of intron loss is very high in nematodes (Cho et al. 2004). From an evolutionary perspective, changes in intron/exon structure might be an important force for generating differences in gene function. However, a recent study showed that such changes are rare in mammalian evolution. For example, of 10,020 introns considered in a comparison between humans and mice, only five were lost in the mouse lineage, and none were lost in humans. Gene structures change much more rapidly in nematodes. First, a direct comparison of the *C. elegans* and *C. briggsae* genomes showed that they have significant differences in intron/exon structure. In a study, of 60,275 introns that were examined, 4,379 were unique to *C. elegans* and 2,200 were unique to *C. briggsae.* Because no outgroups were considered, it was unclear if these differences were caused by losses or gains. Several studies have documented dramatic changes in intron/exon structure within large *C. elegans* gene families. These data showed that intron losses were more common than gains, but could not determine the rate of loss, as the dates of each gene duplication were unknown. Intron losses occur by the following manner.

Recombination with cDNA: The most common hypothesis for how introns are lost is by recombination with reversed-transcribed copies of a message, which should lack all introns (Fig. 3.6). In its simplest form, this model implies that adjacent introns have a high probability of being lost together in a single

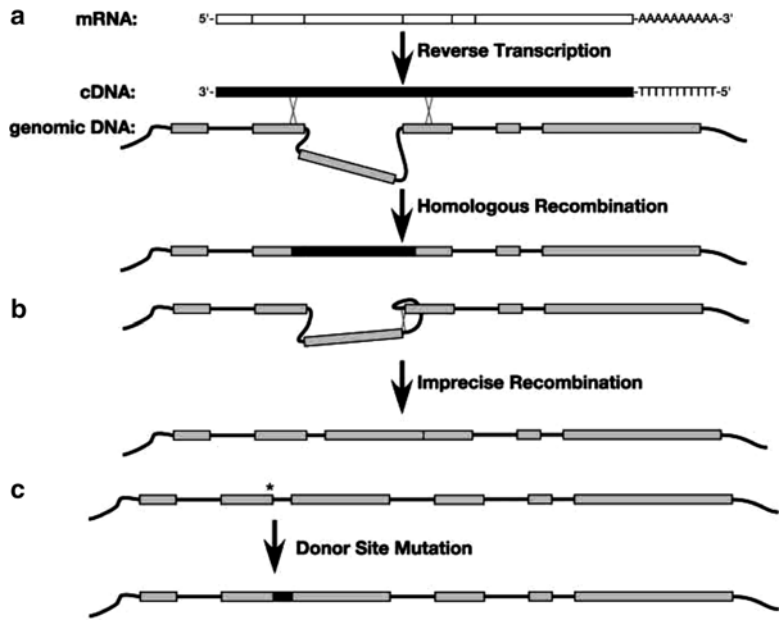

Fig. 3.6 Intron losses

event. Some data strongly support this model. For example, five adjacent introns seem to have been lost simultaneously during the evolution of the catalase 3 gene in *Zea mays*, and all of the introns in the *Oikopleura longicauda* EP-1 gene might have been lost in a single event (Wada et al. 2002). However, adjacent losses like these are rare in our data and in data from plants (Frugoli et al. 1998), insects, and deuterostomes. One potential explanation is that the cDNA templates that recombine with genomic DNA are usually small fragments rather than complete genes. For each species, the size of these fragments would determine which introns could be lost together. If so, in worms, these cDNA fragments are unlikely to be formed by partial reverse transcription starting from the 3_ end, as has been hypothesized for unicellular eukaryotes, as there is no bias toward loss of introns at the 3_ ends of genes.

Deletions: Introns could also be lost by spontaneous genomic deletions. In theory, these deletions could either be precise, which would yield a product indistinguishable from an intron lost by recombination, or imprecise. Such events are known to occur, as the *jingwei* gene of *Drosophila teissieri* has two alleles, one of which is an imprecise deletion that did not remove 12 nucleotides of the original intron. Similarly, intron #1 of the *C. elegans cpb-1* gene might have been formed by imprecise deletion, as it appears to have been lost along with some adjacent coding sequence. Because the probability of a 2,500-bp intron being exactly deleted is much lower than it is for a 50-bp intron, this mechanism should also favor the loss of short introns.

3.23 Changes in Splice Donor Sites

In regions that are tolerant of changes in amino acid sequence, one might also expect some introns to be lost by the mutation of a splice donor site. If this was to happen and no cryptic donor sites were activated, the associated intron would become part of the coding region. This mechanism could explain how intron #3 was lost from the *fog-1* gene of *C.* sp. CB5161. Because longer introns are more likely to contain in-frame stop codons, this mechanism should only work for very short introns. It was suspected that all of these mechanisms contribute to intron loss during evolution but that spontaneous genomic deletions are far more important than previously suspected. Once additional nematode genome sequences become available, a global comparison of the loss rate for introns was planned in germ line and somatic genes to test this hypothesis. Because of the high loss rate for nematode introns, such a comparison could also test the hypothesis that all mechanisms for intron loss favor the elimination of short introns over longer ones.

Martijn Holterman et al. (2006) observed that inference of evolutionary relationships between nematodes was severely hampered by their conserved morphology, the high frequency of homoplasy, and the scarcity of phylum-wide molecular data. To study the origin of nematode radiation and to unravel the phylogenetic relationships between distantly related species, 339 nearly full-length small-subunit rDNA sequences were analyzed from a diverse range of nematodes. Bayesian inference revealed a backbone comprising 12 consecutive dichotomies that subdivided the phylum Nematoda into 12 clades. The most basal clade is dominated by the subclass Enoplia, and members of the order Triplonchida occupy positions most close to the common ancestor of the nematodes. Crown clades 8–12, a group formerly indicated as "Secernentea" that includes Caenorhabditis elegans and virtually all major plant and animal parasites, show significantly higher nucleotide substitution rates than the more basal clades 1–7. Accelerated substitution rates are associated with parasitic lifestyles (clades 8 and 12) or short generation times (clades 9–11). The relatively high substitution rates in the distal clades resulted in numerous autapomorphies that allow in most cases DNA barcode-based species identification. *Teratocephalus*, a genus comprising terrestrial bacterivores, was shown to be most close to the starting point of Secernentean radiation. Notably, fungal-feeding nematodes were exclusively

found basal to or as sister taxon next to the three groups of phytonematodes, viz., Trichodoridae, Longidoridae, and Tylenchomorpha. The exclusive common presence of fungivorous nematodes and phytonematodes supports a long-standing hypothesis that states that plant-parasitic nematodes arose from fungivorous ancestors.

Meloidogyne species are known to reproduce either by cross-fertilization (amphimixis), facultative meiotic parthenogenesis, or obligatory mitotic parthenogenesis (Castagnone-Sereno et al. 1993). Among them, *M. incognita, M. arenaria*, and *M. javanica* are obligatory mitotic parthenogenetic species, while *M. hapla* can reproduce by both cross-fertilization and meiotic parthenogenesis. Phylogenetic relationships in this genus have been investigated by hybridization of *Bam*HI-digested genomic DNAs of 18 geographical isolates belonging to six species with three homologous repeated DNA probes cloned at random from a genomic library of one population of *M. incognita*. Due to the repetitive nature of the probes, the autoradiograms exhibited extensive restriction fragment length polymorphisms (RFLPs) both between and within nematode species. Genetic distance values estimated from hybridization patterns were analyzed by two phylogenetic tree-building distance methods, respectively, based on constant (UPGMA) and varying (FITCH) rates of nucleotide substitution, and the resulting dendrograms showed a very similar clustering of species and populations. Comparison of these results with the other sources of phylogenetic data available for this genus, i.e., cytogenetic, isoenzymatic, and mitochondrial DNA (mtDNA) data, revealed consistency with all but the mtDNA phylogeny. Due to the maternal inheritance of mtDNA, and the parthenogenetic reproductive mode of these organisms, which excludes any possibility of horizontal transfer, it was concluded that nuclear DNA phylogeny should represent a more likely evolutionary history of this particular genus and that interspecific hybridizations between sexual ancestors may account for the results with mtDNA. Thus, the early split-off of the mitotically parthenogenetic species cluster and *M. hapla* confirms the amphimictic ancestral mode of reproduction of root-knot nematodes.

Kaplan et al. (2000) compared the nucleic acid sequences of rDNA ITS1 and the rDNA D2/D3 expansion segment for 57 burrowing nematode isolates collected from Australia, Cameroon, Central America, Cuba, Dominican Republic, Florida, Guadeloupe, Hawaii, Nigeria, Honduras, Indonesia, Ivory Coast, Puerto Rico, South Africa, and Uganda. Of the 57 isolates, 55 were morphologically similar to *Radopholus similis* and seven were citrus parasitic. The nucleic acid sequences for PCR-amplified ITS1 and for the D2/D3 expansion segment of the 28S rDNA gene were each identical for all putative *R. similis.* Sequence divergence for both the ITS1 and the D2/D3 was concordant with morphological differences that distinguish *R. similis* from other burrowing nematode species. This result substantiated previous observations that the *R. similis* genome is highly conserved across geographical regions. Autapomorphies that would delimit phylogenetic lineages of non-citrus-parasitic *R. similis* from those that parasitize citrus were not observed. The data supported the concept that *R. similis* is comprised of two pathotypes, one that parasitizes citrus and one that does not.

Internal transcribed spacer (ITS) sequences of rDNA from 53 populations and species of gall-forming nematodes of the subfamily Anguininae, along with five populations of the *D. dipsaci* species complex, were used for phylogenetic analyses (Subbotin et al. 2001). The molecular analyses support a concept of narrow specialization for seed-gall nematodes and reveal distinction of at least nine undescribed species of *Anguina* inducing seed galls, previously identified as *A. agrostis*, and two species within the *D. dipsaci* species complex. Both the maximum parsimony and maximum likelihood analyses of the ITS data strongly support monophyly of the genus *Anguina.* Also, non-monophyly of *Subanguina* in the broad sense of Brzeski (1981) and of *Mesoanguina* and *Heteroanguina* according to the classification by Chizhov and Subbotin (1985, 1990) was indicated. Morphological and biological characters are congruent with the anguinid groups supported by the ITS phylogeny. The test of topologies conducted by maximum likelihood analyses showed that the monophyletic origin of

anguinids parasitizing grasses and sedges could not be rejected. The main anguinid groups are generally associated with plant hosts belonging to the same or related systematic groups.

3.24 Evolution of Parasitism in Nematodes

Despite extraordinary diversity of free-living species, a comparatively small fraction of nematodes are parasites of plants. These parasites represent at least three disparate clades in the nematode tree of life, as inferred from rRNA sequences (Baldwin et al. 2004). Plant parasites share functional similarities regarding feeding, but many similarities in feeding structures result from convergent evolution and have fundamentally different developmental origins. Although Tylenchida rRNA phylogenies are not fully resolved, they strongly support convergent evolution of sedentary endoparasitism and plant nurse cells in cyst and root-knot nematodes. This result has critical implications for using model systems and genomics to identify and characterize parasitism genes for representatives of this clade. Phylogenetic studies reveal that plant parasites have rich and complex evolutionary histories that involve multiple transitions to plant parasitism and the possible use of genes obtained by horizontal transfer from prokaryotes. Developing a fuller understanding of plant parasitism will require integrating more comprehensive and resolved phylogenies with appropriate choices of model organisms and comparative evolutionary methods.

3.24.1 Mode and Tempo of the Evolution of Parasitism

Branch length data from molecular phylogenies can be related to evolutionary time if a model of molecular evolution is applied that assumes clocklike accumulation of genetic change (Dorris et al. 1999). This assumption of a molecular clock allows inferred branch lengths to be read as time intervals, i.e., a time axis can be placed on the tree. Fossils are required to calibrate and validate the clock, and their absence in nematodes invalidates clock assumptions for the SSU nematode phylogeny. In addition, extreme rate differences in inferred accumulation of changes are seen between 25 taxa. These rate differences are even more problematic in that they can cause significant artifacts in the building of trees. Of note here is that the distances between genera within the Rhabditina (in clade 5) are similar to those seen between tetrapod classes; the Nematoda appears to be old and diverse (Fitch et al. 1995). The genetic divergence between taxa in the Strongylida is remarkably low. This pattern suggests either a relative slowdown in molecular evolutionary rates, correlated with the adoption of parasitic mode of life, or an increase in the relative rate of molecular evolution or both. One possibility may be that the rate of molecular evolution is correlated with generation time.

The diversity of parasitic lifestyles displayed by nematodes, and the diversity of hosts used, reflects both a propensity toward parasitism in the phylum and an adaptability to new and challenging environments (Blaxter 2003). Parasitism of plants and animals has evolved many times independently within the Nematoda. Analysis of these origins of parasitism using a molecular phylogeny highlights the diversity underlying the parasitic mode of life. Many vertebrate parasites have arthropod-associated sister taxa, and most invade their hosts as third-stage larvae: These features co-occur across the tree and thus suggest that this may have been a shared route to parasitism. Analysis of nematode genes and genomes has been greatly facilitated by the Caenorhabditis elegans project. However, the availability of the whole-genome sequence from this free-living rhabditid does not simply permit definition of "parasitism" genes; each nematode genome is a mosaic of conserved features and evolutionary novelties. The rapid progress of parasitic nematode genome projects focusing on species from across the diversity of the phylum has defined sets of genes that have patterns of evolution that suggest their involvement with various facets of parasitism, in particular the problems of acquisition of nutrients in new hosts and the evasion of

host immune defenses. With the advent of functional genomics techniques in parasites and in particular the possibility of gene knockout using RNA interference, the roles of many putative parasitism genes can now be tested.

3.25 Nematode Mating Systems and Evolution

In animals, sexual traits evolve rapidly. Ronald E. Ellis and Soochin Cho (2003) studied nematode mating systems. *C. elegans* (Ce) and *C. briggsae* (Cb) have male and hermaphrodite sexes, whereas *C. remanei* (Cr) has male and female sexes. They were interested to know why XX animals become self-fertile hermaphrodites in some species but females in others and how these different systems arose. The essential difference between hermaphrodites and females is that the former produce sperm and oocytes, but the latter only make oocytes. In Ce, they demonstrated that FOG-3 and the CPEB protein FOG-1 were required for germ cells to initiate spermatogenesis. To study the control of germ cell fate, homologues of these genes were cloned from Cb and Cr, which revealed that each species had four CPEB genes, just like Ce. In all three species, dsRNAi against the fog-1 homologue caused germ cells to become oocytes rather than sperm. The requirement for cpb-1 in early spermatogenesis has been conserved as well. Thus, the divergence of these proteins predated the origin of this genus. All three species had one fog-3 gene, which is required for germ cells to become sperm rather than oocytes. Since the levels of fog-3 transcripts were correlated with spermatogenesis, the control of fog-3 expression could be responsible for determining if XX animals become females or hermaphrodites. Experiments with chimeric transgenes showed that the fog-3 promoters from all three species could drive expression of fog-3 in Ce XX larvae.

Furthermore, these promoters each contained multiple binding sites for the sex determination protein TRA-1A. It was proposed that fog-3 controls germ cell fate in all caenorhabditids and that the activity of TRA-1A was modulated in hermaphrodite species to allow fog-3 expression in XX larvae. To test this hypothesis, they cloned tra-1 from Cr. Surprisingly, in Cr, tra-1(RNAi) males only produced oocytes. Similar results were found in Cb. These results suggested that TRA-1 plays an important role promoting spermatogenesis in these species. In Ce, the activity of TRA-1A is modulated by upstream factors like FOG-2, to allow hermaphrodite spermatogenesis. They screened for Fog mutants in Cb and identified a mutation, v35, with a similar phenotype; XX animals are female and XO animals are male. They used sequence analysis of fog-1, fog-3, cpb-1, cpb-2, and cpb-3 to establish a phylogeny for all caenorhabditids. It was observed that Ce and Cb were not sister species and that mating systems must have switched multiple times during the evolution of this genus. They also observed rapid evolution of intron number in these genes.

Self-fertilizing species often harbor less genetic variation than cross-fertilizing species, and at least four different models have been proposed to explain this trend (Graustein et al. 2002). To investigate further the relationship between mating system and genetic variation, levels of DNA sequence polymorphism were compared among three closely related species in the genus *Caenorhabditis*: two self-fertilizing species, *C. elegans* and *C. briggsae*, and one cross-fertilizing species, *C. remanei.* As expected, estimates of silent site nucleotide diversity were lower in the two self-fertilizing species. For the mitochondrial genome, diversity in the selfing species averaged 42 % of diversity in *C. remanei.* Interestingly, the reduction in genetic variation was much greater for the nuclear than for the mitochondrial genome. For two nuclear genes, diversity in the selfing species averaged 6 and 13 % of diversity in *C. remanei*. They argued that either population bottlenecks or the repeated action of natural selection, coupled with high levels of selfing, is likely to explain the observed reductions in species-wide genetic diversity.

The mechanisms by which new modes of reproduction evolve remain important unsolved puzzles in evolutionary biology (Chaudhuri et al. 2011). Nematode worms are ideal for studying the evolu-

tion of mating systems because the phylum includes both a large range of reproductive modes and large numbers of evolutionarily independent switches. *Rhabditis* sp. SB347 is a nematode with sexual polymorphism, which produces males, females, and hermaphrodites. To understand how the transition between mating systems occurs, the mechanisms that regulate female versus hermaphrodite fate in *Rhabditis* sp. were characterized. Hermaphrodites develop through an obligatory nonfeeding juvenile stage, the dauer larva. It was shown that by suppressing dauer formation, *Rhabditis* sp. SB347 develops into females. Conversely, larvae that under optimal growth conditions develop into females can be respecified toward hermaphroditic development into females if submitted to dauer-inducing conditions. These findings are of significance to understanding the complex mating system evolution in phytonematodes.

To analyze changes in gene structure during nematode evolution, the first detailed phylogeny of the *C. elegans* group was prepared (Blaxter et al. 1998). This phylogeny also showed that mating systems have changed multiple times during the evolution of this small group within the genus *Caenorhabditis.* This result dramatically extends previous analyses of the entire phylum Nematoda which showed that mating systems had changed many times during the long evolutionary history of the nematodes.

The mechanisms by which new modes of reproduction evolve remain important unsolved puzzles in evolutionary biology (Chauduri et al. 2011). Nematode worms are ideal for studying the evolution of mating systems because the phylum includes both a large range of reproductive modes and large numbers of evolutionarily independent switches. *Rhabditis* sp. SB347, a nematode with sexual polymorphism, produces males, females, and hermaphrodites. To understand how the transition between mating systems occurs, they characterized the mechanisms that regulate female versus hermaphrodite fate in *Rhabditis* sp. SB347. Hermaphrodites develop through an obligatory nonfeeding juvenile stage, the dauer larva. They observed that by suppressing dauer formation, *Rhabditis* sp. SB347 develops into females. Conversely, juveniles that under optimal growth conditions develop into females can be respecified toward hermaphroditic development if submitted to dauer-inducing conditions. These results are of significance to a better understanding of the evolution of complex mating systems present in parasitic nematodes.

The fact that most species in this genus use male/female mating systems suggests that the ancestor of the elegans group was male/female. Thus, if our phylogeny is correct, the simplest model is that *C. elegans* and *C. briggsae* each evolved hermaphroditism separately. However, it remains possible that the common ancestor of *C. briggsae*, *C. remanei*, *C.* sp. *CB5161*, and *C. elegans* acquired a male/hermaphrodite mating system, and this system then reverted to a male/female one in *C. remanei* and *C.* sp. *CB5161.* Although the second scenario involves more steps than the first one, it might be equally probable, because we do not know the relative likelihood of switching from a male/female mating system to a male/hermaphrodite one, or vice versa. In the past few years, a major effort has been launched to determine the molecular changes that have influenced the control of sex determination during nematode evolution.

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