



COMMENTARY



Small worms, big ideas: evolutionary inferences from nematode DNA

There is a huge disparity across the phylum Nematoda in terms of our molecular knowledge. For some species, such as the parasitic *Brugia malayi*, we possess complete genome sequences (both nuclear and mitochondrial) as well as detailed information about life history, reproduction and relationships with symbionts. For countless other species (especially free-living species from marine environments) not even a single published gene sequence exists. For a phylum with an insurmountable taxonomic deficit, molecular methods offer cheap, quick and non-specialist ways to investigate biodiversity and speciation. Unsurprisingly, such methods are now revolutionizing our understanding of nematode evolution and biogeography.

In the study of nematodes, DNA sequences represent impartial and neutrally evolving markers for retracing evolutionary history. Inferences based on morphological characters, in comparison, can be quite subjective, depending on a given taxonomist's interpretation and level of experience. Over the past 10 years, molecular data have shed much light on deep phylogenetic relationships amongst nematode groups; several published phylogenies based on nuclear 18S ribosomal RNA gene sequences have completely restructured previous morphological frameworks (e.g. Blaxter *et al.*, 1998). Other studies focused on population genetics have shown that cryptic speciation is probably rife, even for well-known, 'cosmopolitan' species (Derycke *et al.*, 2005).

Nematodes are a diverse group: they occupy a huge range of ecological niches, pursue a wide variety of lifestyles and exist in practically every habitat on Earth (Lambshhead, 2004). Although we cannot currently explain how such diversity arose, a recent paper by Holterman *et al.* (2008) provides some intriguing insights into nematode evolution. The authors' robust molecular phylogeny of the chromadorid nematode clade suggests that it is relatively easy for nematodes to move into new habitats during speciation events – they suggest that transitions from marine to

limnoterrestrial habitats (and vice versa) have occurred a staggering 16 times within this group. The authors note that environmentally adaptive mechanisms in nematodes must be morphologically cryptic. There do not seem to be any obvious anatomical features that point to an existence in a marine or terrestrial habitat, and we do not yet have the capacity to fully explain the genetic mechanisms that facilitate transitions between these two physically distinct environments. However, if one labels such habitat transitions with the more general term of 'lifestyle change', this process becomes reminiscent of another transition in nematodes: the shift from free-living to parasitic lifestyles.

The first molecular framework, by Blaxter *et al.* (1998), indicated that the transition to parasitic lifestyles has arisen independently at least seven times within the phylum Nematoda. This suggests that changes required for the switch to parasitism – such as switches between marine and terrestrial habitats – may be relatively simple to acquire, given that such changes have occurred discretely so many times. Recent molecular investigations have focused on detecting 'pre-adaptations' to parasitism in closely related but non-parasitic nematode taxa (Dieterich & Sommer, 2009). This concept insinuates that niche specialization in current habitats can lead to bigger 'jumps' later on – such as a leap to parasitism, or alternatively a hop from a marine to a terrestrial environment. Current thinking suggests that pre-adaptations to parasitism may first manifest as associations with fungi (for plant parasitic nematodes) or invertebrates (for animal parasitic nematodes) before fully parasitic taxa evolve. The pre-adaptations needed for habitat transitions may instead appear as physiological differences within nematode populations. Holterman *et al.* (2008) cite an increased capacity for osmoregulation as a potential pre-adaptation for facilitating habitat transitions from marine to terrestrial environments. There is some evidence to suggest that terrestrial nematodes possess a

reduced permeability to water, as well as the ability to rapidly control glycerol synthesis and breakdown.

A more complicated depiction of nematode evolution is emerging from genome sequencing projects. The recently published genome of the human parasite *B. malayi* suggests that horizontal gene transfer (the exchange of genes between different species) and gene loss in nematode species represent key adaptations for parasitic lifestyles (Dieterich & Sommer, 2009). The genome of *B. malayi* seems to be missing genes that code for key enzymes in regulatory pathways. However, gene loss in the *B. malayi* genome is complemented by the retention of these necessary pathways in the genome of its α -proteobacterial symbiont, *Wolbachia* – these bacteria seem to be pivotal in providing many genes needed for *de novo* purine synthesis, haem biosynthesis and *de novo* riboflavin biosynthesis in *B. malayi*. Such tales of gene loss and gene transfer between hosts and symbionts are becoming more and more common in the animal kingdom; similar accounts have already been recorded in many insect taxa (Moran, 2007). Plant parasitic nematodes provide more evidence for horizontal gene transfer. *Meloidogyne* species possess specialized enzymes for degrading plant cell walls that are not present in their closest nematode ancestors; analysis of gene sequences reveals that they are most closely related to genes in bacteria and unicellular organisms, hinting at a past horizontal gene transfer event that facilitated development of a parasitic lifestyle in this group (Dieterich & Sommer, 2009). Little research has been conducted on symbionts of free-living nematodes, but it seems likely that non-parasitic species may also form close associations with fungi or bacteria; perhaps there will soon be evidence of horizontal gene transfer outside the parasitic realm.

Efforts to sequence whole nematode genomes are continuing to improve our understanding of nematode phylogenetics and speciation, while gene expression studies are offering powerful tools for linking

molecular processes to biological function. Genomic comparisons reveal that we are a long way from fully understanding the extent of gene diversity in nematodes; an average of 45% of genes per species were found to have no known homologues in other nematodes, with 23% representing putatively species-specific genes (Mitreva *et al.*, 2005). Transcriptome analyses between mutation-accumulation (MA) lines and natural isolates of *Caenorhabditis elegans* offer insight into natural selection; gene expression profiles were found to show the first signatures of mutational change long before gene sequences acquired telltale nucleotide differences (Denver *et al.*, 2005). Such analyses are revealing that nematodes are surprisingly complex and divergent at the molecular level – a stark contrast to the comparatively uniform morphology seen across the phylum.

With the advent of next-generation sequencing, it is likely that our knowledge of nematode diversity and biogeography will increase exponentially in the near future. The newest sequencing technologies can currently deliver 400,000 sequences (each up to 400 bp in length) within a few hours, at a very low cost per base. While past molecular studies have required the extraction and sequencing of single nematodes (a time-consuming affair), the newest ‘metagenomic’ methods offer a way to simultaneously sequence every nematode within a sediment sample. Most metagenomic work so far has focused on microbial communities. Initial use of this technology on nematodes has tested the amplification of an informative section (c. 250 bp) of the 18S rRNA gene from artificial community samples (Porazinska *et al.*, 2009). For nematodes, the results seem very positive – despite the short sequence length, metagenomic methods can distinguish most species in a given sample, although some methodological

optimization is currently needed to ensure accurate abundance counts. These quick genetic snapshots of nematode communities will be of crucial importance across many fields of biology: from fundamental investigations of biodiversity to projects requiring environmental surveys and monitoring.

The ever-increasing ease and ubiquity of molecular investigations will undoubtedly continue to transform our understanding of nematode evolution. With so many species still unknown, it seems inevitable that we will continue to uncover many novel and intriguing mechanisms within this phylum. With new molecular technologies on the horizon, nematodes look set to emerge as model organisms for investigating species distributions, community compositions and biogeography.

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