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RHABDITID NEMATODES AS BIOLOGICAL MODELS: INSIGHTS AND IMPLICATIONS

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Abstract – Nematodes, with their diverse species and wide-ranging characteristics, have emerged as indispensable biological models across various scientific disciplines. Especially, the rhabditid clade of nematodes which exhibit diverse trophic lifestyles and ecological adaptations have been explored as research models. *Caenorhabditis* species, in particular, have provided fundamental insights into developmental biology, neurobiology, aging, and disease mechanisms. Other rhabditids such as *Pristionchus pacificus*, with its complex mouth morphology and interaction with beetles, offer insights into evolutionary biology and symbiosis. *Panagrellus redivivus* and *Turbatrix aceti* have significance in the food industry and as a complementary model to *C. elegans*. Entomopathogenic nematodes (EPNs), such as *Steinernema* and *Heterorhabditis* species, provide a model system for understanding host-pathogen interactions, and mutualism as well as have practical applications in biocontrol. Several animal-parasitic rhabditids facilitate parasitology and pharmacology studies. Overall, nematodes serve as powerful models for elucidating biological processes, offering valuable insights and implications for research advancements in genetics, development, ecology, and evolution.

INTRODUCTION

A model organism is a non-human species that is studied extensively to gain insights into biological processes, with the expectation that these findings will illuminate the biology of other species. Most model organisms possess traits that make them suitable for laboratory research. Examples include fruit fly (*Drosophila melanogaster*), yeast (*Saccharomyces cerevisiae*), mouse (*Mus musculus*), western clawed frog (*Xenopus tropicalis*), and zebrafish (*Danio rerio*). The nematodes being the most abundant and small multicellular organisms, serve as exceptional research models. The 'Rhabditid' order of the phylum Nematoda consists of more than 20 families. The rhabditid group consists of nematodes which exhibit a variety of lifestyles, including that of free-living bacterivores, predators, and parasites of insects and animals. Nematodes, particularly *Caenorhabditis elegans*, *Pristionchus pacificus*, *Panagrellus redivivus* and entomopathogenic nematodes (EPNs) *Heterorhabditis* and *Steinernema*, have become crucial model organisms, transforming our understanding of genetics, evolutionary developmental biology, neurobiology, biological phenomena such as parasitism, mutualism, and ecological adaptations. *Tokorhabditis*, a newly described rhabditid *from* arsenic-rich, hypersaline environment has been proposed as a comparative system for the study of evolutionary and extremophilic traits in multicellular organisms (Kanzaki *et al.,* 2021). Thus, a variety of rhabditid species are utilized as models and research implications suggest the importance of these microscopic multicellular organisms in obtaining insights into diverse biological processes. Rhabditid nematodes which are well-established biological models are briefly discussed in this review (Figure 1).

Caenorhabditis elegans: Sharing a lineage dating back to the pre-Cambrian era, approximately 500-600 million years ago, *Caenorhabditis elegans* traces its ancestry to the urbilaterian ancestor. This ancient precursor, common to bilaterally symmetric,

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multicellular organisms, serves as a relative to both invertebrates and vertebrates. Many of the genes and genetic mechanisms crucial for modern organismal development, including those relevant to human development and diseases originated in the urbilaterian ancestor and persist across various living species, encompassing both humans and nematodes (Meneely *et al*., 2019). Approximately 30-60% of *C. elegans* genes have orthologs or strong homologs in mammals, implying that gene function studies in these microscopic nematodes can be implied to mammals/human development and diseases (Apfeld and Alper, 2018).

C. elegans belongs to the family Rhabditidae, Order Rhabditida, Class Chromodorea, Phylum Nematoda. It is a free-living nematode found in organic-rich environments like rotting fruits and stems worldwide. Small body size, simple anatomy (fewer than 1,000 cells), ease of culture, brief life cycle, large progeny size, low maintenance cost, transparent body, consistent cell lineage, compact genome, and lack of biohazard are among the key features of *C. elegans* which greatly facilitate use of this nematode in biological research (Tejeda-Benitez and Olivero-Verbel, 2016).

Application of *C. elegans*

Genetics and developmental biology research: *C. elegans* was the first multi cellular organism to have its genome completely sequenced, providing a comprehensive map for genetic studies (*C. elegans* sequencing consortium, 1998). It's simple genome allows for easy manipulation and observation of gene functions and interactions.Various tools and techniques developed from *C. elegans* research have been the foundation for further genetic investigations in other species. The transparency and invariant cell lineage of *C. elegans* make it ideal for studying development. Researchers can observe cell division and differentiation in live organisms from a single fertilized egg to a fully formed adult. **Neuroscience**: *C. elegans* has a simple nervous system with precisely 302 neurons and serve as a powerful model for nervous system mapping and behavioral studies. This simplicity allows for detailed mapping of neural circuits and understanding of basic neuronal functions. This nematode is also used as a pharmacological model for studying neurotransmitter and their receptor systems, inter and intracellular signalling cascades (Walker *et al*., 2022). Studies on *C. elegans* have provided insights into the genetic and molecular bases of behavior. Researchers can link specific genes to behavioral phenotypes, such as feeding, mating, and locomotion.

Aging and Longevity

C. elegans has a short lifespan of about 2-3 weeks, making it an excellent model for studying molecular and cellular mechanisms of aging and longevity. Offering insights into processes such as genomic instability, epigenetic changes, proteostasis, mitochondrial function, and nutrient sensing. Various genetic pathways influencing lifespan, such as insulin/IGF-1 signaling, have been identified using this nematode. Age pigments in nematodes and mammals exhibit similarities, prompting the use of age-retarding chemicals against nematodes to save resources. Centrophenoxine, initially employed against nematodes, notably reduces lipofuscin pigmentation upto a certain percentage, providing insights that could be relevant to mammals as well. Research on *C. elegans* has elucidated mechanisms of stress responses and their implications for aging and diseases. For example, the role of heat shock proteins and oxidative stress in aging has been extensively studied in these nematodes (Mack *et al.*, 2018).

Disease Models

Many genes in *C. elegans* have homologs in humans, making it a valuable model for studying human diseases. Models for neurodegenerative diseases, such as Alzheimer's and Parkinson's, have been developed using transgenic *C. elegans* strains expressing human disease-related proteins. *C. elegans* have also been used as *in vivo* model in obesity research to study fat metabolism and major signalling pathways involved in this process (Apfeld and Alper, 2018; Yue *et al*., 2021). Additionally, *C. elegans* can be infected by various pathogens, providing insights into host-pathogen interactions and innate immunity. The use of *C. elegans* in research poses fewer ethical concerns compared to higher organisms. This allows for extensive genetic manipulation and high-throughput studies without the ethical issues associated with vertebrate models.

Biotechnology and Drug Discovery

C. elegans is used in high-throughput screening for drug discovery. Its small size and ease of cultivation allow for large-scale screening of chemical libraries to identify potential therapeutic compounds. Genes and pathways identified in *C. elegans* can be targeted for developing new drugs. The simplicity of its biological systems facilitates the validation of drug targets. Gene expression studies gained extensive benefits from using *C. elegans* as a research model. A number of molecular markers have been generated out of *C. elegans* research to monitor molecular mechanisms and biological pathways in multi cellular organisms.

Environmental monitoring and toxicology Studies: *C. elegans* is used to assess the toxicity of environmental pollutants. Its sensitivity to various chemicals and pollutants makes it a reliable indicator species for ecotoxicological studies. By studying the effects of pollutants on *C. elegans*, researchers can gain insights into the potential impacts of these substances on ecosystems and human health.Acute toxicity assessment and effect on biological parameters is relatively easy in these tiny worms where locomotion assessment considers criteria like head thrash and body bend frequency. Pharyngeal pumping movement indicates metabolic rate, while feeding behaviour is observed through bacterial food density in the intestine (Tejeda-Benitez and Olivero-Verbel, 2016).

Ecology and evolution: C. *elegans* worms are excellent models for exploring the benefits of sexual reproduction for progeny flexibility, inducing adult diapause under starvation stress, and studying modifications of foraging strategies. They have also been used to understand inter domain interactions, specifically animal-bacterial interaction using *Yersinia pestis* - C. *elegans, E. coli-C.elegans* models (Schulenburg and Félix, 2017). These applications highlight the versatility and relevance of *C. elegans* in ecological and evolutionary research.

Educational tool: *C. elegans* serves as an excellent educational tool in teaching genetics, development, and cell biology. Its ease of handling and the availability of extensive genetic resources make it ideal for educational purposes at various levels.

C. briggsae and *C. remanei*

Having more model species facilitate comparative studies. *C. briggsae* and *C. remanei* belongs to the same family as that of *C. elegans* and have strikingly similar morphological and behavioural characteristics to C. elegans. These worms have been used as model in comparative genetic and genomic studies with *C. elegans*. *C. briggsae* has a well-developed system for RNA interference (RNAi), which makes it useful for genetic research. In *C. remanei*, studying variations in nucleotide sequences and conducting genome sequencing provides valuable information for understanding evolutionary processes (Gupta *et al*., 2007).

Pristionchus pacificus

The nematode species *P. pacificus* has been established as a satellite model in evolutionary developmental biology. *P. pacificus* belongs to Diplogasteridae family under Rhabditida order. Though primarily dependent on bacterial diet, they also have stomas armed with a large dorsal tooth used for predation on other nematodes and arthropods. *P. pacificus* is frequently found in association with various species of scarab beetles, where it has developed specific relationships such as predation on beetle larvae and mutualistic interactions. Additionally, *P. pacificus* exhibits significant natural variation in traits like morphology, behaviour, and life history, offering valuable insights into phenotypic plasticity or polyphenism and multitrophic interactions (Bhat *et al*., 2018; Sommer and McGaughran, 2013).

Panagrellus redivivus and *Turbatrix aceti*

Panagrellus redivivus and *Turbatrix aceti*belongs to Panagrolaimidae family of order Rhabditida. *P. redivivus* has significance as a food source in aquaculture of fish and crustaceans, popular as microworm and as a complementary model to *C. elegans*. This nematode has been used in aging studies, toxicological studies such as screening of Bt Cry proteins against nematodes, assessment of soil pollutants and influence of environmental conditions on genome architecture. *T. aceti* (vinegar eels) were earlier explored for aging research, recently also utilised for investigation of swarming and active particles behavior (Aryukarn *et al*., 2021; Peshkov *et al*., 2022)

Animal-parasitic and entomopathogenic rhabditids

Entomopathogenic nematodes (EPNs) are specialised insect parasitic rhabditids which exhibit mutualistic association with insect pathogenic bacteria. Nematodes from the family Steinernematidae and Hetero rhabditidae exhibit specific mutualistic association with *Xenorhabdus* and *Photorhabdus* bacteria respectively. Nematode-bacterial pair is exploited in biological insect pest management. Additionally, these nematodes are excellent model systems for studying biological

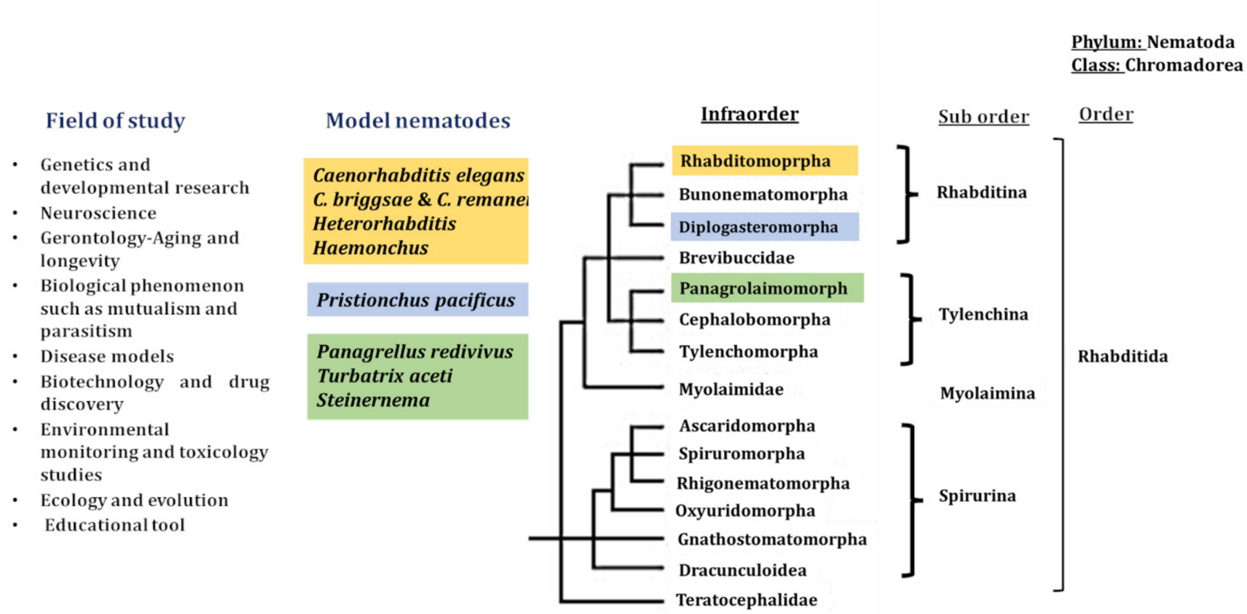

Fig. 1. Model nematodes from Rhabditida clade which are currently utilized to gain insights into various biological processes and phenomena (adapted from De Ley, 2006)

phenomenon such as parasitism, animal-bacteria symbiosis, and ecological adaptions. EPNs are considered evolutionary bridges between free-living nematodes and animal-parasitic nematodes. This makes them an excellent model to study host-parasite relationships as well as anthelmintic drugs (Bhat *et al.,* 2022; Campos–Herrera *et al*., 2012). Furthermore, animal parasitic nematodes, such as *Haemonchus contortus*is within the same clade as the rhabditid nematodes, and thus provide a very helpful bridge for bioinformatic and functional study between free-living and more distantly related animal-parasite species to understand parasite evolution and drug resistance (Gilleard, 2013).

Limitations in using nematodes as model organisms

Nematodes have a simpler anatomy, limiting extrapolation of findings to organisms with complex tissues and organs. Despite conserved biological processes, significant genetic differences exist between nematodes and higher organisms. Their behavioural repertoire is limited, restricting behavioural and neurological studies. Additionally, their small size and short lifespan hinder detailed anatomical or long-term physiological observations.

Conclusion and prospects

Nematodes are poised to remain at the forefront of biological research due to their genetic tractability, suitability for high-throughput screening, and contributions to understanding fundamental biological processes. Their applications are set to expand into drug discovery, neurobiology, aging, and environmental studies, offering critical insights into both basic science and human health. To fully realize their potential, it will be essential to address their limitations related to simplified anatomy, genetic differences, and behavioural constraints. As research progresses, more nematodes will continue to drive innovation and shape scientific inquiry across diverse fields.

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