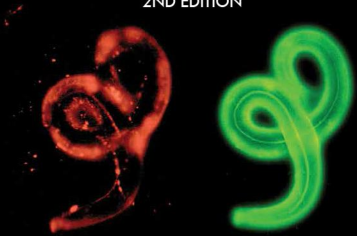
PARASITIC NEMATODES

MOLECULAR BIOLOGY, BIOCHEMISTRY AND IMMUNOLOGY

2ND EDITION



Edited by
Malcolm W. Kennedy and William Harnett

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Parasitic Nematodes

Molecular Biology, Biochemistry and Immunology 2nd Edition

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This book is dedicated to

Huw Smith and Gerry Schad

Parasitic Nematodes

Molecular Biology, Biochemistry and Immunology
2nd Edition

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Preface

The pace of advances in research into parasitic nematodes continues to accelerate, and there is a degree of interplay of ideas, techniques and analytical approaches amongst researchers working on parasites of humans, animals, plants, and free-living species that is equalled in few if any fields of infection biology. The potential benefits from such interactions have never been in doubt, but these benefits are now being fulfilled at an increasing pace, allowing new unifying principles to develop. For example, it is entirely possible that nematodes infecting humans and those infecting plants could be using similar or related means by which to modify the tissues and immunological defences of their hosts. In this, the breadth and depth of what has been achieved with the free-living *Caenorhabditis elegans* continues to provide a strong link between all branches of nematode parasitology, but it is becoming clear that this is not merely a one-way process. The vast amount of information now arising from genomics, transcriptomics and proteomics of nematodes is opening up new worlds, especially when one considers how diverse are the lifestyles of nematodes, and that there are arguably more species of nematode than in any other metazoan phylum. Moreover, the integration of these data with the immunology of infection is proceeding at a remarkable pace, revealing the common and distinctive ways in which nematodes control their tissue and immune system environments, and the molecules they use to do so. The effects of nematode infection on the immune system continue to bring forth new surprises including understanding the functions and behaviours of previously somewhat mysterious cell types and tissue responses - the understanding of the functions of Th2 immune responses and alternatively activated macrophages are cases in point.

In compiling this book we set out to illustrate the scope and dynamism of the subject area, knowing that it would be impossible to represent it all. So, we invited authors scattered widely across nematode parasitology and biology, selecting those who were strongly research active, of international standing, and with vision beyond their immediate subject areas. We asked them to choose a subject close to them, to introduce and develop it in their writing, but then also to extend their thinking into speculative areas that might be struck out by reviewers and editors of journals publishing primary research. Scientists can become conservative in their writing, but without extended and over-the-horizon thinking, new approaches and ideas will not make an appearance.

At about the time we were invited to produce this book, one of the most inspiring parasitologists of the last half-century died, Gerry Schad. He gained worldwide recognition as an authority on the population biology of helminth parasites and their behavioural neurobiology, and latterly had focused on the sensory biology of parasites. While fully able to engage in discussions on nematode genomics, immunoparasitology and mathematical epidemiology, Gerry was still able to hold an audience with what can be learned with a Petri dish, a culture of nematode larvae, and an attractant chemical or physical stimulus under test. When one of this book's editors was an undergraduate zoologist, Gerry published a paper in *Science* (1973; 180, 502-504), reporting a discovery that still enthrals today. The summary read "Contrary to general belief, larvae of *Ancylostoma duodenale* do not always develop directly to adulthood upon invasion of man. In West Bengal, India, arrested development appears to be a seasonal phenomenon which results in (i) reduction of egg output wasted in seeding an inhospitable environment and (ii) a marked increase in eggs entering the environment just before the monsoon begins." A remarkable phenomenon. How the worms know how to respond to outside influences like that remains as intriguing now as it did then, as with how microscopic infective larvae of nematodes find their partners in a whole human body (or, for that matter, that of a blue whale), or the precise selective advantage of periodicity patterns in microfilariae, all still mysterious.

Another loss to us was Huw Smith. Huw was a personal friend to both the editors and several of the authors in this book. We knew him from shortly after he arrived in Glasgow University to when he moved to create the Scottish Parasite Diagnostic Laboratory and bring it to international renown. He arrived working on nematodes, *Toxocara canis* in particular, was a positive influence on several of the scientific careers represented in this book, before moving on to parasitic protists. The expertise, reagents and technologies he developed in screening water supplies for contamination with parasite eggs and cysts led him to be in demand as an adviser across the globe, South East Asia in particular. Aside from being a famous and generous host and sometimes notorious, but lovable, merry-maker, Huw was able to come up with intriguing ideas drawn from unexpected directions. One that particularly stands out was an experiment he carried out with John Kusel in which he used human autoimmune antibody against the extracellular matrix of skin epidermal cells to show that the larvae of schistosomes traversing human skin take up host epidermal antigens (*Clinical and Experimental Immunolgy* (1979) 36, 430-435). Those experiments were not what Huw was being paid to do, but one can't and shouldn't ever keep a mind like Huw's down.

Both Huw and Gerry were inspirations to both established scientists and to those only just entering the field of nematode parasitology. We hope that this book will in some way contribute to that tradition.

Malcolm Kennedy
William Harnett
Glasgow, April 2013

Access to Colour Illustrations

In order to reduce the cost of this book, and thereby improve its accessibility, there are no colour reproductions. Many of the illustrations cannot be fully and properly appreciated without colour. All of the colour illustrations can therefore be viewed on the following internet site:

www.glasgow.ac.uk/nematodes

If you have any suggestions or problems relating to the illustrations appearing in the book, then please feel free to contact either malcolm.kennedy@glasgow.ac.uk or w.harnett@strath.ac.uk

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Cover Illustrations

The front cover shows two fluorescence images of a single larva of *Toxocara canis*. The larva had been double labelled with a green fluorescent lipophilic dye that associates with the epicuticle, and also with a red, rhodamine-labelled antibody against the excretory/secretory material of *T. canis* larvae that bound to the surface glycoprotein coat. The larva was then incubated for a short time before viewing under ultraviolet light with optical filters appropriate for the two dyes separately, and shows that the surface coat can be shed whilst leaving the underlying surface-labelled lipid layer intact. For full details, see Kennedy, M.W. (2006) The larval surface. In 'Toxocara – the Enigmatic Parasite', pp.32-41. Editors C.V. Holland and H.V. Smith. CABI Publishing, Wallingford, UK.

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1 The Genome of *Pristionchus pacificus* and the Evolution of Parasitism

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Introduction

From an evolutionary perspective, parasitism represents a derived character. That is, all parasitic life on earth derives from free-living ancestors. Molecular phylogenetics made the convincing argument that, within nematodes, parasitism has evolved multiple times independently. Based on the phylogenies of Blaxter *et al.* (1998) and Holterman *et al.* (2006; van Megen *et al.*, 2009), animal parasitism has evolved at least four times and plant parasitism at least three times within the nematodes. In contrast, many other parasitic taxa, such as the Trematoda or the Nematomorpha, are believed to represent fully parasitic taxa. This distinction makes the nematodes a superb phylum in which to study the evolution of parasitism. First, free-living relatives of present-day parasites are often available in nematode clades. Such species might share some characteristics with ancestral precursors of parasites and therefore might indicate evolutionary trends towards parasitism. Second, the parallel evolution of parasitism within nematodes provides the opportunity to compare different routes towards the parasitic life style and to identify shared characteristics.

For a long time, considerations about the evolution of nematode parasitism, in particular animal parasitism, were based on theoretical studies. However, recent advances in molecular biology, genomics and genetics in various nematode species have provided first experimental insight into the transitions towards parasitism and corresponding life-history adaptations. Here, we summarize recent experimental studies in the genetic model species *Pristionchus pacificus* that focus on genome evolution, the genetic regulation of dauer larvae and infective juvenile formation, and the evolution of predation.

Theoretical Considerations

All parasitic life on earth derives from free-living ancestors and while parasitism has evolved many times, the transition towards parasitism itself is a very slow process. As a result, such transitions cannot easily be observed in the wild in present-day species. Also, it is impossible to predict whether a present-day free-living organism is on a clear ‘route’ to becoming a parasite. With these caveats of evolutionary processes, there have been only limited experimental attempts to study the evolutionary transition towards parasitism. In principle, there are two main obstacles: If one studies a present-day parasite, the major event for the evolution of parasitism – the transition itself – has already occurred. On the other hand, if one studies a free-living species there is no guarantee that the organism under consideration will ever evolve into a true parasite because evolution cannot be foreseen. It is this dilemma that has resulted in the near absence of experimental studies on the evolution of parasitism.

While experimental studies on the topic are still in their infancy, there have been several theoretical investigations that have resulted in a conceptual framework. Osche (1956) was the first author to consider the evolution of parasitism in nematodes and was followed by Poinar (1983), Anderson (1984), Weischer and Brown (2001) and most recently Poulin (2007). In the following, we briefly summarize the argument by Poulin, who has discussed the issue in the most comprehensive manner.

The transition towards parasitism requires a contact between a 'parasite-to-be' and its future host. However, this contact is not the only precondition that is important for becoming a parasite. Rather, the 'parasite-to-be' must possess certain characteristics that allow it to: (i) identify the host in a specific manner; (ii) survive in or on that host; (iii) obtain food; and (iv) successfully reproduce in this new environment. These characteristics have often been called 'pre-adaptations' to explain the fitness benefit that has to be assumed for the 'parasite-to-be' to start host exploitation (Rothschild and Clay, 1952; Osche, 1956; Poulin, 2007; Dieterich and Sommer, 2009).

These pre-adaptations are the true challenges for the understanding of the transition towards parasitism. The concept of pre-adaptation argues that transitions towards parasitism are facilitated by an organism's current environment and adaptations associated with such an environment (Dieterich and Sommer, 2009). Thus, pre-adaptations are adaptations to the current environment of the 'parasite-to-be' and to its life style. In the future, such adaptations might be co-opted to new functions to facilitate the transition to a new (parasitic) environment. Pre-adaptations might therefore be helpful for an organism to acquire a new niche.

Life-history Adaptations

Many free-living rhabditid nematodes live in a saprobionthic environment. Osche (1956) and most recently Sudhaus (2008) have argued that adaptations to the saprobionthic life were crucial pre-adaptations for the transition towards parasitism. Two innovations have been given special consideration in this context. First, rhabditid nematodes can form arrested dauer larvae as a long-term survival strategy. Second, these dauer larvae, which will be discussed in more detail below, allowed the acquisition of specific behavioural traits: dauer larvae can attach to insects or other invertebrates and the worms can use these 'hosts' for transportation and/or for shelter.

When looking at nematode–invertebrate associations in more detail, several different forms of association can be distinguished. While not all authors agree on exactly the same nomenclature, we want to briefly summarize some of these associations below. For that, we use the nomenclature created by Sudhaus (2008).

BACTERIAL FEEDING SAPROBIONT. As an initial state, one can assume bacterial-feeding nematodes that live in saprobionthic environments. Saprobionthic environments derive from decaying organic matter and they are short-lived and patchy in distribution. Some adaptations to this environment can already be seen as 'pre-adaptations' towards parasitism because organisms living in a saprobionthic environment have to deal with low oxygen concentrations and unpredictable conditions. Often, saprobionthic environments allow only survival, but not reproduction of a given organism. Species that introduce resting stages into their life cycles have a major fitness advantage under these conditions. Nematodes have evolved several strategies that involve developmentally arrested stages (Ogawa and Sommer, 2009). The most prominent and important one is the formation of the specialized dauer stage: most saprobionthic nematodes form dauer larvae.

PHORESY. The term phoresy describes the capability of an organism to use another organism (i.e. an insect) for transportation, i.e. between saprobionthic environments. Usually, the specialized stage in the nematode life cycle used for transportation is the dauer stage. Phoretic associations are known from many rhabditid and diplogastrid nematodes, but also others. Poinar (1983)