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1 **My favourite nematode - *Phasmarhabditis hermaphrodita***

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8 My favourite nematode is *Phasmarhabditis hermaphrodita*, a parasite of
9 terrestrial gastropods. So much so it has become the sole focus of my lab over the last
10 few years. The reasons for this are fourfold. First, *P. hermaphrodita* is the only
11 nematode to be developed as a weapon for slug and snail control. Research by David
12 Glen and Mike Wilson et al. (with nematological expertise from David Hooper) in the
13 late 80's and early 90's demonstrated that *P. hermaphrodita* can kill a selection of
14 pestiferous slug species within 4-21 days (Wilson et al., 1993a); it could be grown en
15 masse (Wilson et al., 1993b) and could provide protection against slug damage in
16 mini-plot trials (Wilson et al., 1994) and in field trials (Wilson et al., 1995a) using
17 many important agricultural, horticultural and floricultural crops (Rae et al., 2007).
18 Further research focused on optimising mass production of this nematode and the
19 influence that bacteria used as a food source could have on yield (Wilson et al.,
20 1995b) and infectivity (Wilson et al., 1995c). *P. hermaphrodita* has been sold as
21 Nemaslug[®] across Europe since 1994 and is routinely used by farmers and gardeners
22 to control slugs. How is *P. hermaphrodita* able to kill slugs and snails? This is
23 complicated but it has been suggested that *P. hermaphrodita* is like an
24 entomopathogenic nematode, in that it vectors a bacterium (*Moraxella osloensis*) into
25 slugs (Tan and Grewal, 2001), where it proliferates and causes septicaemia. Indeed it
26 has been shown that high doses of *M. osloensis* injected directly into slugs will kill
27 quickly and the purified lipopolysaccharide acts as an endotoxin (Tan and Grewal,
28 2002). However, this bacterium is not vertically transmitted and passed down to
29 following generations and yet these nematodes, without *M. osloensis*, are still
30 pathogenic (Rae et al., 2010). Therefore, the exact mode of action of *P.*
31 *hermaphrodita* is still uncertain but the co-evolution of all these players including
32 bacteria, nematodes and slugs warrants further attention.

33 Second, *P. hermaphrodita* has an interesting history and an exciting future. *P.*
34 *hermaphrodita* was discovered in 1859 by Schneider and studied briefly by both

35 Maupas (1900) and Mengert (1953). However, what I find fascinating is that it could
36 have easily been *C. elegans* a.k.a “the worm” as Sydney Brenner collected more
37 strains of *P. hermaphrodita* than *C. elegans* in the early 1960’s. Imagine a world
38 where the discoveries of RNAi, the genetic regulation of apoptosis and the
39 development of GFP (green fluorescent protein) that resulted in 3 Nobel prizes were
40 due to studying the slug parasite *P. hermaphrodita* rather than *C. elegans*! Recently,
41 *P. hermaphrodita* has been proposed as a model system of its own - specifically to
42 study the evolution of parasitism (Wilson et al., 2015; Rae, 2017a; Andrus and Rae,
43 2018). Several attributes make it a perfect system and recently protocols have been
44 developed that show how to culture it under lab conditions, mutagenize, mate and
45 make isogenic lines of *P. hermaphrodita* just like *C. elegans* (Andrus and Rae, 2018).
46 It is the only nematode from the 25,000 described species that has evolved to
47 parasitise and kill slugs and snails. This is unusual as from the 108 nematode species
48 associated with slugs and snails they are generally used as hosts for food, transport or
49 to find a mate (Grewal et al., 2003). One of the fundamental questions in my lab is
50 what are the genetic changes that lead to the emergence of parasitism? I think *P.*
51 *hermaphrodita* is the perfect nematode to study this. As well as being able to be kept
52 easily under lab conditions, it is in clade 9 and closely related to mammalian and
53 insect parasites (van Megen et al., 2009). The ease of culture and collection, as well as
54 the efforts of the 959 genome project will allow genomic comparison of *P.*
55 *hermaphrodita* with not just *C. elegans* but many of the other fascinating rhabditid
56 parasites that are free living, necromenic or phoretic as well as parasitic species.
57 Although there is this potential for interesting discoveries, there is a severe lack of
58 research into *P. hermaphrodita*. The majority of research is taxonomic or details the
59 results of conducting surveys of slugs describing their associated nematode fauna with
60 regular identification of *P. hermaphrodita* (or other *Phasmarhabditis* species) present.
61 A noble endeavour but *Phasmarhabditis* warrants more than this approach.
62 Specifically, what could these *P. hermaphrodita* isolates or *Phasmarhabditis* species
63 tell us at the genetic level? By taking a natural variation approach, that has worked
64 well in unravelling genes involved with traits such as phoretic behaviour (Lee et al.,
65 2017) and social behaviour (de Bono and Bargmann, 1998) (to name but a few) in *C.*
66 *elegans* and *Pristionchus pacificus* (see Sommer, 2015), the underlying genomic
67 architecture of numerous genes involved with parasitism e.g. infection, pathogenicity
68 or chemoattraction could be unravelled in *P. hermaphrodita*.

69 Third, *P. hermaphrodita* can manipulate the behaviour of slugs. Four hundred
70 million years of co-evolution with terrestrial gastropods has created an arms race that
71 at the present time has resulted in some striking effects that nematodes, specifically *P.*
72 *hermaphrodita* can have on gastropods. Slugs infected with *P. hermaphrodita* are
73 more likely to be found under refuge traps (Wilson et al., 1994), reduce feeding (Glen
74 et al. 2000), move deeper down into soil to die (Pechova and Foltan, 2008) and move
75 slower (Bailey et al., 2003). Whether these behaviours are because the slug is sick or
76 the nematode is actively manipulating slug behaviour is unknown. Slugs will avoid
77 areas where *P. hermaphrodita* is present (Wilson et al., 1999; Wynne et al., 2016)
78 however, it has recently been shown that slugs infected with *P. hermaphrodita* are
79 attracted to areas where *P. hermaphrodita* is present. This is presumably so more
80 nematodes can infect and kill slugs and they can proliferate and mate faster (Morris et
81 al., 2018). This study also showed (indirectly) that the potential mechanism of how
82 these nematodes may control the slug's behaviour is by affecting serotonergic
83 signalling, which is a similar method to other behaviour manipulating parasites
84 (Hughes et al., 2012). The use of *P. hermaphrodita* has real potential to unravel the
85 genetic mechanism of how parasites change the behaviour of hosts, which could be
86 applied to other parasite/host systems.

87 Fourth, co-evolution of nematodes and gastropods has produced some
88 fascinating abilities in the immune system of gastropods to provide protection against
89 nematodes such as *P. hermaphrodita*. To date, the susceptibility of 19 slug species
90 and 18 snail species have been tested by exposure to *P. hermaphrodita* under
91 laboratory conditions (see Rae, 2017a for a complete description of gastropod species
92 tested). Twelve slug species and eight snail species can be killed by *P. hermaphrodita*
93 but it is unknown why some species are resistant to high doses of *P. hermaphrodita*.
94 There is little information on the immune system of gastropods in general and
95 specifically to do with infection by *P. hermaphrodita*. However, recent research has
96 shown that the difference between slug and snail susceptibility is potentially due to an
97 intriguing ability that the snail's shell possesses. Snails such as *Achatina fulica*
98 (Williams and Rae, 2015) and *Cepaea nemoralis* (Williams and Rae, 2016) can
99 actively trap, encase and kill nematodes using their shell. This whole process is
100 remarkably efficient at killing nematodes, often hundreds at a time and is evolutionary
101 conserved across the phylogeny of the Stylommatophora (terrestrial slugs and snails)
102 (Rae, 2017b). An exciting opportunity has indirectly arisen from this research that

103 may allow us to actually track the evolution of nematodes at the molecular level over
104 time. Snail shells that are hundreds of years old present in museums have been shown
105 to have nematode encased in their shells (Rae, 2017b, 2018). As nematode DNA can
106 be extracted from shells this may facilitate genomic analysis over time using these
107 preserved ‘fossilised’ specimens.

108 Here I hope I have outlined why *P. hermaphrodita* is a nematode that is worth
109 studying. Unlike current model nematodes, it can be used to answer a suite of
110 evolutionary questions about parasitism. Protocols are now in place detailing how to
111 maintain *P. hermaphrodita* (Andrus and Rae, 2018) and the genome is currently being
112 sequenced. New species are being discovered regularly, as well as strains of *P.*
113 *hermaphrodita*, and the combination of using genomics and these natural strains could
114 unravel how this biocontrol agent evolved to kill slugs and how this information could
115 be translated to other species of nematodes or other organisms.

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