



LJMU Research Online

Rae, RG and Andrus, P

Natural variation in chemoattraction in the gastropod parasitic nematodes *Phasmarhabditis hermaphrodita*, *P. neopapillosa* and *P. californica* exposed to slug mucus

<http://researchonline.ljmu.ac.uk/id/eprint/9890/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Rae, RG and Andrus, P (2018) Natural variation in chemoattraction in the gastropod parasitic nematodes *Phasmarhabditis hermaphrodita*, *P. neopapillosa* and *P. californica* exposed to slug mucus. *Nematology*. ISSN 1568-5411

LJMU has developed [LJMU Research Online](#) for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>

1

2 **Natural variation in chemoattraction in the gastropod**
3 **parasitic nematodes *Phasmarhabditis hermaphrodita*,**
4 ***Phasmarhabditis neopapillosa* and *Phasmarhabditis***
5 ***californica* exposed to slug mucus**

6

7

Andrus, P. and Rae, R.*

8

9 Liverpool John Moores University, School of Natural Sciences and Psychology,
10 Byrom Street, L33AF.

11

12 Tel: 01512312486

13 *E-mail: r.g.rae@ljmu.ac.uk

14 Running Head: *Phasmarhabditis* attraction to slug mucus

15

16

1 **Abstract**

2

3 *Phasmarhabditis hermaphrodita* is a lethal parasite of several slug species and
4 has been formulated into a biological control agent for farmers and gardeners. *P.*
5 *hermaphrodita* responds to slug faeces, mucus and volatile cues to find hosts in soil.
6 However, these results have only focused on one strain of *P. hermaphrodita*
7 (DMG0001). We exposed four strains of *P. hermaphrodita* (and DMG0001), three
8 strains of *P. neopapillosa* and two strains of *P. californica* to mucus from seven
9 common slug species. Furthermore, we investigated whether there was a relationship
10 between chemoattraction and the numbers of offspring that were produced on each
11 host species. Natural isolates of *P. hermaphrodita* differed in their preference of slug
12 species whereas *P. neopapillosa* tended to prefer *Arion* sp. and strains of *P.*
13 *californica* displayed striking differences in their responses. The reasons for positive
14 chemoattraction to mucus were not due to higher numbers of offspring produced on
15 these hosts.

16

17 **Keywords:** *Phasmarhabditis*, behaviour, host finding, slugs

18

19

20

21

22

23

24

1 Parasitic nematodes have evolved to recognise an array of cues to detect and
2 infect hosts (Lee, 2002). For example, the human parasite *Strongyloides stercoralis* is
3 attracted to human skin and sweat odorants of mammalian hosts (Castelletto et al.,
4 2014). Entomopathogenic nematodes from the genera *Steinernema* and
5 *Heterorhabditis*, which have a symbiotic relationship with the bacteria *Xenorhabdus*
6 and *Photorhabdus* that they vector into insect hosts and kill them in 24-48 hours (Forst
7 et al., 1997) use host odorants, faeces and CO₂ to locate insects (Dillman et al., 2012).
8 Free-living nematodes such as *Caenorhabditis elegans* that feed on bacteria respond
9 to metabolites to find their food (Bargmann et al., 1993) and can even discriminate
10 between pathogenic and non-pathogenic *Serratia marcescens* bacteria (Zhang et al.,
11 2005). The scarab beetle associated nematode *Pristionchus pacificus* responds to
12 insect pheromones to find hosts to latch onto (Hong et al., 2008a). The terrestrial
13 gastropod parasitic nematode *Phasmarhabditis hermaphrodita* is a lethal parasite of
14 several pestiferous slug species (Wilson et al., 1993) and has been formulated into a
15 successful biological control agent by BASF Agricultural Specialities called
16 Nemaslug® (Rae et al., 2007). In order to find slugs in soil *P. hermaphrodita* is
17 attracted to slug mucus, faeces and volatile cues (Hapca et al., 2007a, Nermut et al.,
18 2012, Rae et al., 2009, Rae et al., 2006, Small & Bradford, 2008). These experiments
19 have not only been carried out on agar plates but in more realistic ecological
20 conditions in soil and sand (Hapca et al., 2007b, Nermut et al., 2012). Once a slug has
21 been found the nematodes enter through a pore at the back of the mantle and can kill
22 the host in 4-21 days (Tan & Grewal, 2001, Wilson et al., 1993). *P. hermaphrodita* is
23 strongly attracted to slugs such as *Arion subfuscus* but finds *Limax marginatus* and
24 the snail *Cepaea hortensis* less attractive for reasons unknown (Rae et al., 2009).
25 Previous research (Rae et al., 2009) investigated reasons for these differences in

1 attraction by focussing on examining whether more attractive slug species were better
2 hosts for producing dauer juveniles. Only weak correlations were found and therefore
3 the exact biological reason for these differences in chemoattraction remain unknown.

4 Experiments examining how *P. hermaphrodita* responds to host cues (Hapca
5 et al., 2007a, Nermut et al., 2012, Rae et al., 2009, Small & Bradford, 2008) have all
6 focussed on using the commercial strain designated DMG0001 (Hooper et al., 1999),
7 which has been in culture for over 20 years (Wilson et al., 1993). There is no
8 information on how other strains of *P. hermaphrodita* or *Phasmarhabditis* species
9 respond to slug mucus. There are 11 *Phasmarhabditis* species (Rae, 2017) that have
10 been described, yet there are few experiments investigating their biology. Therefore,
11 we sought to understand whether there was natural variation in chemotaxis towards
12 slug mucus by utilising a collection of *Phasmarhabditis* species (*P. hermaphrodita*, *P.*
13 *neopapillosa* and *P. californica*) that were collected from around the U.K. and are
14 currently maintained in culture at Liverpool John Moores University (LJMU) (Andrus
15 & Rae, 2018).

16 Several studies investigating the chemotactic response using *P. hermaphrodita*
17 have focused on studying exposure to mucus from single slug species (*Deroceras*
18 *reticulatum*) (Hapca et al., 2007a, Hapca et al., 2007b, Rae et al., 2006) however, in
19 nature slugs can be found in mixed populations in close contact with different species
20 crowded together (Cook, 1981, South, 1992). These mixed populations contain
21 individuals that often vary in infection levels of *Phasmarhabditis* nematodes (Andrus
22 & Rae, 2018). How do *Phasmarhabditis* nematodes discriminate between mucus from
23 different slug species in mixed populations? We decided to test this by exposing the
24 nematodes to mucus from several slug species simultaneously. Furthermore, we also
25 examined why these *P. hermaphrodita* strains and *Phasmarhabditis* species

1 responded differently to slug mucus by investigating whether greater numbers of
2 offspring would be produced on the more attractive slug species. A previous study
3 (Rae et al., 2009) investigated whether increased chemoattraction of *P.*
4 *hermaphrodita* towards slugs was due to the higher number of dauer juveniles
5 produced on attractive hosts but found no relationship, however we decided to
6 understand whether the total number of offspring produced (rather than just number of
7 dauers) could be the factor for attraction.

8

9 **Materials and methods**

10

11 **Source of invertebrates**

12

13 Slugs (*Deroceras invadens* (mean weight 0.21 ± 0.02 g), *D. reticulatum*
14 (mean weight 0.26 ± 0.02 g), *Arion ater* (mean weight 1.27 ± 0.14 g), *A. hortensis*
15 (mean weight 0.47 ± 0.16 g), *Lehmannia valentiana* (mean weight 0.39 ± 0.1 g),
16 *Limax flavus* (mean weight 3.05 ± 0.35 g) and *Milax sowerbyi* (mean weight $1.56 \pm$
17 0.08 g) were collected from greenhouses at LJMU and stored in non-airtight plastic
18 boxes lined with moist paper and fed lettuce *ad libitum*. Mean weight of each slug
19 species were used from Rae et al (2009) apart from *A. hortensis* and *L. valentiana*
20 where 10 slugs were weighed. The commercial strain of *P. hermaphrodita*
21 (DMG0001) was purchased from Becker Underwood-BASF Agricultural Specialities
22 and stored at 15°C until use. Natural strains of *P. hermaphrodita* and species of
23 *Phasmarhabditis* were collected previously (Andrus & Rae, 2018). *P. hermaphrodita*
24 (DMG0002, DMG0007, DMG0009 and DMG0010), *P. neopapillosa* (DMG0012,
25 DMG0015 and DMG0016) and *P. californica* (DMG0018 and DMG0019) were

1 grown on White traps with rotting cellar slug (*L. flavus*) for 21-28 days until they
2 reached the dauer stage (White, 1927).

3

4 **Chemotaxis of *Phasmarhabditis* species towards slug mucus**

5

6 In previous experiments investigating the attraction of *P. hermaphrodita* to
7 slug associated cues a standard agar plate assay has been used (Hapca et al., 2007a,
8 Rae et al., 2009, Rae et al., 2006), based on *C. elegans* assays for examining
9 chemoattraction towards chemical odorants (Bargmann et al., 1993). This allows the
10 chemotactic response of the nematodes to be quantified when exposed to just one slug
11 species (compared to a water based control). We sought to understand whether the
12 nematodes would show a preference towards certain species when exposed to mucus
13 from all slug species simultaneously. We developed a set up where we could expose
14 the nematodes to mucus from seven species of slug at the same time (Fig. 1). We
15 swabbed 0.01g of foot mucus with separate pieces of 1 cm² piece of Whatman filter
16 paper number 1 from the foot of the seven slug species, and placed each 0.5 cm from
17 the side of a 10 cm Petri dish filled with 1.2% technical agar in a circle. The paper
18 with the mucus from each species was equidistant from the opposite piece. To another
19 piece of filter paper 10 µl of distilled water was added and acted as the control. Dauer
20 stage nematodes of five strains of *P. hermaphrodita* (DMG0001, DMG0002,
21 DMG0007, DMG0009 and DMG0010), two strains of *P. californica* (DMG0018 and
22 DMG0019) and three strains of *P. neopapillosa* (DMG0012, DMG0015 and
23 DMG0016) were added to the middle of each Petri dish and left for 10-20 mins so the
24 water evaporated and then sealed with Parafilm® and incubated at 20°C overnight.
25 The following day the numbers of nematodes that had migrated to each piece of filter

1 paper were then quantified. Three replicate plates were used for each nematode
2 species/strain and the whole experiment was repeated three times.

3 The results of chemotaxis assays using nematodes e.g. *C. elegans* tend to be
4 reported as a chemotaxis index (Bargmann et al., 1993), which records the number of
5 nematodes that graduated to the treated and control side. This does not take into
6 account the number of nematodes that remained at the point of application (which can
7 sometimes be large) and the calculation of chemotaxis index can sometimes rely on
8 small number of nematodes which can lead to misleading results. Therefore, we
9 recorded and presented our data as the number of nematodes found on and in the
10 mucus, in the water control and the numbers of nematodes found at the point of
11 application to give a clearer idea of where the nematodes could be found. In addition,
12 chemotaxis experiments with *C. elegans* and *P. pacificus* (Bargmann et al., 1993,
13 Hong & Sommer, 2006) use 1M sodium azide to stop nematode movement
14 immediately when they encounter the control or attractant. We do not use this as
15 when *P. hermaphrodita* dauers encounter mucus they stay there (Hapca et al., 2007a,
16 Rae et al., 2009, Rae et al., 2006).

17

18 **Examination of the numbers of offspring produced on each host species**

19

20 The numbers of offspring that were produced on each slug species by each
21 *Phasmarhabditis* species was quantified by using White traps (White, 1927). Briefly,
22 0.1 g of each slug was added to a 5 cm Petri dish that was lined with a 5 cm piece of
23 pre-moistened Whatman filter paper. Slugs of the seven species were placed into a -
24 20°C freezer for 20 mins to kill any resident nematodes that would interfere with the
25 experiment. The 5 cm Petri dish was then added to a 10 cm Petri dish, which was half

1 filled with distilled water. A single J2 stage hermaphrodite of each *Phasmarhabditis*
2 species was added to each piece of slug and the plates were sealed with Parafilm®
3 and left for 10 days. After which the numbers of offspring produced was quantified.
4 Five plates were made for each slug species and the whole experiment was repeated
5 twice. We did not test the numbers of offspring produced by *P. neopapillosa* as it is a
6 gonochoristic species and was difficult to mate and grow from two individuals on
7 pieces of rotting slug compared to single hermaphrodites.

8

9 **Data analysis**

10

11 The numbers of nematodes found in each piece of filter paper with different
12 slug mucus and the numbers of nematodes that were produced on 0.1 g of each slug
13 species were compared using a One Way ANOVA with Tukey's post hoc test.
14 Fisher's Least Significant Difference (LSD) at 5% ($p < 0.05$) was calculated and
15 presented in figures for chemotaxis data for each *Phasmarhabditis* strain. To test if
16 there was a relationship between chemoattraction and the numbers of nematodes
17 produced on a) 0.1 g of each slug species and b) adult slugs (calculated by using mean
18 weights of each species) regression analysis was used.

19

20 **Results**

21

22 **Natural variation in the ability of *Phasmarhabditis* species to detect slug mucus**

23

24 There was no significant difference between the numbers of *P. hermaphrodita*
25 (DMG0001) found in the foot mucus of the seven slug species and the control ($F(6,$

1 62) = 1.22, $p = 0.30$) (Fig 2A). Low numbers of nematodes were found in each piece
2 of filter paper (range 2-8 nematodes per slug species) and the majority of nematodes
3 (47 ± 3.8) remained in the centre of the plate where they were applied. In contrast to
4 the commercial strain the recently isolated wild isolate of *P. hermaphrodita*
5 (DMG0002) was significantly attracted to mucus from all seven species compared to
6 the control ($p < 0.001$). Specifically, *P. hermaphrodita* (DMG0002) was significantly
7 more attracted to mucus from *D. reticulatum*, *L. valentiana*, *A. hortensis* and *A. ater*
8 than mucus from *M. sowerbyi* and *L. flavus* ($p < 0.05$) (Fig 2B). *P. hermaphrodita*
9 DMG0007 (Fig 2C) was significantly more attracted to mucus from all seven slug
10 species compared to the control ($p < 0.001$). It had no preference of mucus from *D.*
11 *invadens*, *D. reticulatum*, *M. sowerbyi*, *L. flavus*, *A. hortensis* or *A. ater* ($p > 0.05$) but
12 significantly less nematodes were found in the mucus of *L. valentiana* than *D.*
13 *invadens* and *A. hortensis* ($p < 0.05$). Similarly, *P. hermaphrodita* DMG0009 (Fig
14 2D) found the mucus of all seven slug species significantly more attractive than the
15 control ($p < 0.001$). There was no significant difference between the numbers of *P.*
16 *hermaphrodita* DMG0009 (Fig 2D) found in mucus of the seven slug species ($p >$
17 0.05) and only the numbers of nematodes found in mucus from *M. sowerbyi* differed
18 with *A. hortensis* ($p < 0.05$). In contrast to the four other isolates of *P. hermaphrodita*
19 there was no significant difference between the numbers of *P. hermaphrodita*
20 DMG0010 (Fig 2E) found in mucus from *D. reticulatum*, *L. flavus*, *L. valentiana*, *A.*
21 *hortensis*, *A. ater* and the control ($p > 0.05$). However, there were significantly more
22 *P. hermaphrodita* DMG0010 found in the mucus from *D. invadens* and *M. sowerbyi*
23 compared to the control ($p < 0.001$).

24 There was no significant difference between the numbers of *P. californica*
25 DMG0018 exposed to mucus from *D. invadens* and *L. flavus* and the control (Fig 2F;

1 $p > 0.05$) but significantly more nematodes were found in mucus from *D. reticulatum*,
2 *M. sowerbyi*, *L. valentiana*, *A. ater*, *A. hortensis* than the control ($p < 0.05$).
3 Significantly more *P. californica* DMG0018 were found in the mucus from *D.*
4 *reticulatum* and *M. sowerbyi* compared to *L. valentiana*, *L. flavus* and *A. ater* ($p <$
5 0.05). In contrast to *P. californica* DMG0018, strain DMG0019 (Fig 2G) behaved
6 differently and there was no significant difference between the numbers of *P.*
7 *californica* DMG0019 found in mucus from *D. invadens*, *D. reticulatum*, *L. flavus*, *L.*
8 *valentiana*, *A. hortensis* and the control. However, significantly more *P. californica*
9 DMG0019 were found in the mucus of *M. sowerbyi* and *A. ater* compared to the
10 control ($p < 0.001$).

11 There was no significant difference between the numbers of *P. neopapillosa*
12 DMG0016 found in mucus from *D. invadens*, *D. reticulatum*, *L. flavus*, *L. valentiana*,
13 *A. hortensis*, *A. ater* and the control (Fig 2H) ($p > 0.05$). However, there were
14 significantly more *P. neopapillosa* DMG0016 found in mucus from *M. sowerbyi*
15 compared to the control ($p < 0.05$). There were significantly more *P. neopapillosa*
16 DMG0015 (Fig 2I) found in the mucus from all seven slug species compared to the
17 control ($p < 0.05$). *P. neopapillosa* DMG0015 showed a clear preference for *A.*
18 *hortensis* with significantly more nematodes found in the mucus from this species
19 compared to the other species ($p < 0.05$). Similarly, there were significantly more *P.*
20 *neopapillosa* DMG0012 (Fig 2J) found in the mucus of six slug species (*D. invadens*,
21 *D. reticulatum*, *L. flavus*, *M. sowerbyi*, *A. hortensis* and *A. ater*) and the control ($p <$
22 0.05), but there was no difference between the numbers of nematodes in the control
23 and in the mucus of *L. valentiana* ($p > 0.05$). Of these six slugs species *A. hortensis*
24 and *A. ater* were the most attractive with significantly more *P. neopapillosa*
25 DMG0012 found in their mucus compared to the other four slug species ($p < 0.05$).

1

2 **The fecundity of *Phasmarhabditis* species fed on each slug species**

3

4 There was a significant difference between the numbers of offspring produced
5 on the seven slug species by *P. hermaphrodita* DMG0001 ($F(6, 66) = 4.195, p =$
6 0.001). The highest numbers of offspring were produced on *L. flavus*, which was
7 significantly greater than *A. hortensis* and *L. valentiana* ($p < 0.05$) (Fig 3A). *P.*
8 *hermaphrodita* DMG0007 also grew well on all slug species but produced the most
9 amount of offspring on *L. flavus* and *A. ater* which was significantly different than the
10 other species ($p < 0.05$) (Fig 3C). *P. hermaphrodita* DMG0010 also grew best on *L.*
11 *flavus*, *A. ater* and *M. sowerbyi* which were significantly greater than all slug species
12 ($p < 0.05$) (Fig 3E). There was no difference between the numbers of offspring that
13 were produced by *P. hermaphrodita* DMG0002 or DMG0009 on each slug species
14 (Fig 3B, D) ($p > 0.05$).

15 The numbers of offspring that were produced by *P. californica* DMG0018 was
16 significantly different depending on slug species ($F(6, 64) = 3.349, p = 0.007$) (Fig
17 3F). The largest numbers of offspring were produced on *L. flavus*, which was
18 significantly different from *D. invadens*, *A. hortensis* and *A. ater* ($p < 0.05$). In
19 contrast, there were no significant differences between the numbers of offspring that
20 were produced on any of the slug species when a single *P. californica* DMG0019 was
21 added to the seven slug species ($F(6, 63) = 1.218, p = 0.310$) (Fig 3G).

22

23 **The relationship between chemotaxis and number of offspring produced on each**
24 **slug species**

25

1 There was no significant relationship between the chemotactic response of *P.*
2 *hermaphrodita* DMG0001 ($r^2 = 0.006$, $p = 0.43$), *P. hermaphrodita* DMG0002 ($r^2 =$
3 0.001 , $p = 0.47$), *P. hermaphrodita* DMG0007 ($r^2 = 0.014$, $p = 0.39$), *P.*
4 *hermaphrodita* DMG0009 ($r^2 = 0.402$, $p = 0.06$), *P. hermaphrodita* DMG0010 ($r^2 =$
5 0.151 , $p = 0.19$), *P. californica* DMG0018 ($r^2 = 0.008$, $p = 0.42$) or *P. californica*
6 DMG0019 ($r^2 = 0.057$, $p = 0.30$) responding to slug mucus and numbers of offspring
7 that were produced on 0.1 g each slug species. There was also no significant
8 relationship between the chemotactic response of *P. hermaphrodita* DMG0001 ($r^2 =$
9 0.01 , $p = 0.42$), *P. hermaphrodita* DMG0002 ($r^2 = 0.247$, $p = 0.13$), *P. hermaphrodita*
10 DMG0007 ($r^2 = 0.025$, $p = 0.37$), *P. hermaphrodita* DMG0009 ($r^2 = 0.17$, $p = 0.18$),
11 *P. hermaphrodita* DMG0010 ($r^2 = 0.058$, $p = 0.3$), *P. californica* DMG0018 ($r^2 =$
12 0.103 , $p = 0.24$) or *P. californica* DMG0019 ($r^2 = 0.027$, $p = 0.36$) and overall
13 numbers of offspring that were calculated to be produced on each slug species at their
14 adult mean weight.

15

16 **Discussion**

17

18 Natural variation in behaviour towards host cues or food has been shown in
19 many nematodes. In *C. elegans* there is natural variation in avoidance behaviour of
20 many strains exposed to pathogenic *Bacillus thuringiensis* (Schulenburg & Muller,
21 2004) and the ability to cope with pathogenic *Serratia marcescens* (Schulenburg &
22 Ewbank, 2004). The scarab beetle associated nematode *P. pacificus* displays natural
23 variation in chemoattraction towards beetle pheromones (Hong et al., 2008b) and
24 pheromones produced to initiate dauer formation (Mayer & Sommer, 2011). We
25 found that *P. hermaphrodita* DMG0010 preferred mucus from *D. invadens* and *M.*

1 *sowerbyi* compared to the other five species. Whereas *P. hermaphrodita* DMG0002
2 disliked *M. sowerbyi* and *L. flavus* and *P. hermaphrodita* DMG0007 disliked mucus
3 from *L. valentiana*. However, some *P. hermaphrodita* strains such as DMG0007 and
4 DMG0009 (and the commercial strain DMG0001) had very little preference for any
5 of the seven slug species. Interestingly, the commercial strain of *P. hermaphrodita*
6 (DMG0001) responded poorly to mucus from all slug species with the majority of
7 nematodes staying at the point of application. This strain has been in industrial
8 production since 1994 fed on a monoxenic diet of *Moraxella osloensis* - a bacterium
9 that was initially chosen as it produced the greatest number of virulent nematodes
10 (Wilson et al., 1994, Wilson et al., 1995). Research using this same strain 6 to 10
11 years ago reported strong chemoattraction towards *D. reticulatum*, *A. subfuscus* and
12 *D. invadens* (Hapca et al., 2007a, Nermut et al., 2012, Rae et al., 2009, Small &
13 Bradford, 2008), which was not observed in this study. In fact the majority of
14 nematodes remained at the point of application. Perhaps there is in house lab
15 evolution due to the constant culturing conditions that have not changed for decades?
16 Culturing at constant temperatures with the same food source and no interaction with
17 the environment has been shown to increase deleterious mutations in other nematode
18 species (Huey & Rosenzweig, 2009). For example, continued lab cultivation (since
19 1944) of the Dougherty strain of *Caenorhabditis briggsae* fed on the bacterium
20 *Escherichia coli* and kept at constant temperatures resulted in defects in movement,
21 chemotaxis and the ability to respond to dauer pheromones (Fodor et al., 1983) due to
22 mutations in the G-protein coupled receptor genes *srg-36* and *srg-37* (McGrath et al.,
23 2011). However, it must be noted that even if there was some sort of within lab
24 evolution ongoing with the continued culturing conditions of *P. hermaphrodita*
25 DMG0001 it remains as virulent as 10 years ago (Williams & Rae, 2015).

1 The commercial strain of *P. hermaphrodita* (DMG0001) can kill *D. invadens*,
2 *D. reticulatum* and *M. sowerbyi* (Rae et al., 2009, Wilson et al., 1993) but it cannot
3 kill *L. valentiana*, *A. hortensis*, *L. flavus* or large *A. ater* (Dankowska, 2006, Grewal et
4 al., 2003, Iglesias & Speiser, 2001, Rae et al., 2008). Those that it cannot kill
5 represent necromenic hosts where they penetrate inside and wait for the hosts to die
6 when they can then reproduce (Schulte, 1989). Necromenic hosts offer the advantage
7 of allowing the nematode protection from the environment and to be transported to
8 new ecological niches. The reasons for chemotactic preferences of the nematodes
9 towards parasitic or necromenic hosts remains elusive and does not seem to be due to
10 the numbers of offspring that can be produced on each species; which has been
11 reported previously (Rae et al., 2009) (although they monitored numbers of dauer
12 juveniles). However, we did not test the fitness of these offspring. Perhaps they were
13 more pathogenic to slugs; had increased longevity or an increased fat content aiding
14 survival in soil?

15 As well as differences in *P. hermaphrodita* chemoattraction, *P. neopapillosa*
16 and *P. californica* also showed preferences for different slug species. In general, there
17 is little known about the basic biology of *P. neopapillosa* (Hooper et al., 1999) and
18 nothing known about *P. californica*, which has been recently described from north
19 America (Tandingan De Ley et al., 2016) and recently found in Ireland (Carnaghi et
20 al., 2017) and Wales (Andrus & Rae, 2018). We could show that there were striking
21 differences between the chemotaxis responses of two strains of *P. californica*. Both
22 strains were isolated from a single specimen of *Oxychilus draparnaudi* from Dale,
23 Wales yet *P. californica* DMG0018 preferred *M. sowerbyi* and *D. reticulatum*
24 whereas the majority of *P. californica* DMG0019 did not move and remained the

1 point of application. This shows that closely related strains of the same species can
2 have drastically divergent chemoattraction profiles and preferences for slug hosts.

3 Studies in *C. elegans* have managed to identify many genes, neurons and
4 neuropeptides that are essential for chemotaxis towards bacteria, associated
5 metabolites and alcohols (Bargmann, 2006). In addition, in more distantly related
6 species (*P. pacificus*) the protein kinase EGL-4 has been implicated in controlling
7 chemoattraction behaviour towards insect pheromones (Hong et al., 2008b). As *P.*
8 *hermaphrodita* has been proposed as a genetic model (Rae, 2017, Wilson et al., 2015)
9 currently being developed (Andrus & Rae, 2018) the molecular mechanisms of
10 ecologically relevant genes essential for locating hosts could be identified.

11

12

13

14 References

15

16 Andrus, P. & Rae, R. (2018). Development of *Phasmarhabditis hermaphrodita* (and
17 members of the *Phasmarhabditis* genus) as new genetic model nematodes to study the
18 genetic basis of parasitism. *Journal of Helminthology* 2, 1-13.
19 DOI:10.1017/S0022149X18000305

20 Bargmann, C. I. (2006). Chemosensation in *C. elegans*. *WormBook* 1-29.
21 DOI:10.1895/wormbook.1.123.1

22 Bargmann, C. I., Hartweg, E. & Horvitz, H. R. (1993). Odorant-selective genes and
23 neurons mediate olfaction in *C. elegans*. *Cell* 74, 515-527.

- 1 Carnaghi, M., Rae, R., Tandingan De Ley, I., Johnston, E., Kindermann, G.,
2 McDonnell, R., O'Hanlon, A., Reich, I., Sheahan, J., Williams, C. D. & Gormally, M.
3 J. (2017). Nematode associates and susceptibility of a protected slug (*Geomalacus*
4 *maculosus*) to four biocontrol nematodes. *Biocontrol Science and Technology* 27,
5 294-299. DOI:10.1080/09583157.2016.1277418
- 6 Castelletto, M. L., Gang, S. S., Okubo, R. P., Tselikova, A. A., Nolan, T. J., Platzer,
7 E. G., Lok, J. B. & Hallem, E. A. (2014). Diverse host-seeking behaviors of skin-
8 penetrating nematodes. *PLoS Pathogens* 10(8), e1004305.
9 DOI:10.1371/journal.ppat.1004305
- 10 Cook, A. (1981). A comparative study of aggregation of pulmonate slugs (genus
11 *Limax*). *Journal of Animal Ecology* 50, 703-713.
- 12 Dankowska, E. (2006). Laboratory studies on the use of a nematode *Phasmarhabditis*
13 *hermaphrodita* (Schneider) in slug control. *Folia Malacologica* 14, 61-62.
14 DOI:10.12657/folmal.014.009
- 15 Dillman, A. R., Guillermin, M. L., Lee, J. H., Kim, B., Sternberg, P. W. & Hallem, E.
16 A. (2012). Olfaction shapes host-parasite interactions in parasitic nematodes.
17 *Proceedings of the National Academy of Sciences of the United States of America*
18 109, E2324-E2333. DOI:10.1073/pnas.1211436109
- 19 Fodor, A., Riddle, D. L., Nelson, F. K. & Golden, J. W. (1983). Comparison of a new
20 wild-type *Caenorhabditis briggsae* with laboratory strains of *C. briggsae* and *C.*
21 *elegans*. *Nematologica* 29, 203–217.

- 1 Forst, S., Dowds, B., Boemare, N. & Stackebrandt, E. (1997). *Xenorhabdus* and
2 *Photorhabdus* spp.: bugs that kill bugs. *Annual Review of Microbiology* 51, 47-72.
3 DOI:10.1146/annurev.micro.51.1.47
- 4 Grewal, S. K., Grewal, P. S. & Hammond, R. B. (2003). Susceptibility of North
5 American native and non-native slugs (Mollusca : Gastropoda) to *Phasmarhabditis*
6 *hermaphrodita* (Nematoda : Rhabditidae). *Biocontrol Science and Technology* 13,
7 119-125. DOI:10.1080/0958315021000054449
- 8 Hapca, S., Crawford, J., Rae, R., Wilson, M. & Young, I. (2007a). Movement of the
9 parasitic nematode *Phasmarhabditis hermaphrodita* in the presence of mucus from
10 the host slug *Deroceras reticulatum*. *Biological Control* 41, 223-229.
11 DOI:10.1016/j.biocontrol.2007.01.005
- 12 Hapca, S. M., Budha, P., Crawford, J. W. & Young, I. M. (2007b). Movement of the
13 nematode, *Phasmarhabditis hermaphrodita*, in a structurally heterogeneous
14 environment. *Nematology* 9, 731-738. DOI:10.1163/156854107782024811
- 15 Hong, R. L. & Sommer, R. J. (2006). Chemoattraction in *Pristionchus* nematodes and
16 implications for insect recognition. *Curr Biol* 16, 2359-2365.
17 10.1016/j.cub.2006.10.031
- 18 Hong, R. L., Svatos, A., Herrmann, M. & Sommer, R. J. (2008a). Species-specific
19 recognition of beetle cues by the nematode *Pristionchus maupasi*. *Evolution and*
20 *Development* 10, 273-279. DOI:10.1111/j.1525-142X.2008.00236.x

- 1 Hong, R. L., Witte, H. & Sommer, R. J. (2008b). Natural variation in *Pristionchus*
2 *pacificus* insect pheromone attraction involves the protein kinase EGL-4. *Proceedings*
3 *of the National Academy of Sciences of the United States of America* 105, 7779-
4 77784. DOI:10.1073/pnas.0708406105
- 5 Hooper, D. J., Wilson, M. J., Rowe, J. A. & Glen, D. M. (1999). Some observations
6 on the morphology and protein profiles of the slug-parasitic nematodes
7 *Phasmarhabditis hermaphrodita* and *P. neopapillosa* (Nematoda : Rhabditidae).
8 *Nematology* 1, 173-182. DOI:10.1163/156854199508144
- 9 Huey, R. B. & Rosenzweig, F. (2009). Laboratory evolution meets catch-22. In: T.
10 Garland Jr. & M. R. Rose. (Eds). *Experimental evolution: concepts, methods and*
11 *applications of selection experiments*. Berkeley: University of California Press, Pp.
12 671-691
- 13 Iglesias, J. & Speiser, B. (2001). Consumption rate and susceptibility to parasitic
14 nematodes and chemical molluscicides of the pest slugs *Arion hortensis* s. s. and *A.*
15 *distinctus*. *Journal of Pest Science* 74, 159-166. DOI:10.1046/j.1439-
16 0280.2001.01037.x
- 17 Lee, D. L. (2002). Behaviour. In: D. L. Lee. (Eds). *The Biology of Nematodes*.
18 London: Taylor and Francis, Pp. 369-388.
- 19 Mayer, M. G. & Sommer, R. J. (2011). Natural variation in *Pristionchus pacificus*
20 dauer formation reveals cross-preference rather than self-preference of nematode

1 dauer pheromones. *Proceedings of the Royal Society of Biology B* 278, 2784-2790.
2 DOI:10.1098/rspb.2010.2760

3 McGrath, P. T., Xu, Y., Ailion, M., Garrison, J. L., Butcher, R. A. & Bargmann, C. I.
4 (2011). Parallel evolution of domesticated *Caenorhabditis* species targets pheromone
5 receptor genes. *Nature* 477, 321-325. DOI:10.1038/nature10378

6 Nermut, J., Puza, V. & Mracek, Z. (2012). The response of *Phasmarhabditis*
7 *hermaphrodita* (Nematoda: Rhabditidae) and *Steinernema feltiae* (Nematoda:
8 Steinernematidae) to different host-associated cues. *Biological Control* 61, 201-206.
9 DOI:10.1016/j.biocontrol.2012.02.009

10 Rae, R. (2017). *Phasmarhabditis hermaphrodita* - a new model to study the genetic
11 evolution of parasitism. *Nematology* 19, 375-387. DOI:10.1163/15685411-00003053

12 Rae, R., Verdun, C., Grewal, P., Robertson, J. F. & Wilson, M. J. (2007). Biological
13 control of terrestrial molluscs using *Phasmarhabditis hermaphrodita* - progress and
14 prospects. *Pest Management Science* 63, 1153-1164. DOI:10.1002/ps.1424

15 Rae, R. G., Robertson, J. F. & Wilson, M. J. (2006). The chemotactic response of
16 *Phasmarhabditis hermaphrodita* (Nematoda : Rhabditida) to cues of *Deroceras*
17 *reticulatum* (Mollusca : Gastropoda). *Nematology* 8, 197-200.
18 DOI:10.1163/156854106777998746

19 Rae, R. G., Robertson, J. F. & Wilson, M. J. (2008). Susceptibility and immune
20 response of *Deroceras reticulatum*, *Milax gagates* and *Limax pseudoflavus* exposed to

- 1 the slug parasitic nematode *Phasmarhabditis hermaphrodita*. *Journal of Invertebrate*
2 *Pathology* 97, 61-69. DOI:10.1016/j.jip.2007.07.004
- 3 Rae, R. G., Robertson, J. F. & Wilson, M. J. (2009). Chemoattraction and host
4 preference of the gastropod parasitic nematode *Phasmarhabditis hermaphrodita*.
5 *Journal of Parasitology* 95, 517-526. DOI:10.1645/GE-1637.1
- 6 Schulenburg, H. & Ewbank, J. J. (2004). Diversity and specificity in the interaction
7 between *Caenorhabditis elegans* and the pathogen *Serratia marcescens*. *BMC*
8 *Evolutionary Biology* 4, 49. DOI:10.1186/1471-2148-4-49
- 9 Schulenburg, H. & Muller, S. (2004). Natural variation in the response of
10 *Caenorhabditis elegans* towards *Bacillus thuringiensis*. *Parasitology* 128, 433-443.
- 11 Schulte, F. (1989). The association between *Rhabditis necromena* Sudhaus and
12 Schulte, 1989 (Nematoda: Rhabditidae) and native and introduced millipedes in South
13 Australia. *Nematologica* 35, 82-89.
- 14 Small, R. W. & Bradford, C. (2008). Behavioural responses of *Phasmarhabditis*
15 *hermaphrodita* (Nematoda : Rhabditida) to mucus from potential hosts. *Nematology*
16 10, 591-598. DOI:10.1163/156854108784513824
- 17 South, A. (1992). *Terrestrial slugs: Biology, Ecology and Control*. Springer.

- 1 Tan, L. & Grewal, P. S. (2001). Infection behavior of the rhabditid nematode
2 *Phasmarhabditis hermaphrodita* to the grey garden slug *Deroceras reticulatum*.
3 *Journal of Parasitology* 87, 1349-1354. DOI:10.1645/0022-3395
- 4 Tandingan De Ley, I., Holovachov, O., Mc Donnell, R. J., Bert, W., Paine, T. D. &
5 De Ley, P. (2016). Description of *Phasmarhabditis californica* n. sp. and first report
6 of *P. papillosa* (Nematoda: Rhabditidae) from invasive slugs in the USA. *Nematology*
7 18, 175-193. DOI:10.1163/15685411-00002952
- 8 White, G. F. (1927). A method for obtaining infective juvenile nematode larvae from
9 cultures. *Science* 66, 302-303. DOI:10.1126/science.66.1709.302-a
- 10 Williams, A. J. & Rae, R. (2015). Susceptibility of the Giant African snail (*Achatina*
11 *fulica*) exposed to the gastropod parasitic nematode *Phasmarhabditis hermaphrodita*.
12 *Journal of Invertebrate Pathology* 127, 122-126. DOI:10.1016/j.jip.2015.03.012
- 13 Wilson, M. J., Glen, D. M. & George, S. K. (1993). The rhabditid nematode
14 *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs.
15 *Biocontrol Science and Technology* 3, 503-511. DOI:10.1080/09583159309355306
- 16 Wilson, M. J., Glen, D. M., George, S. K. & Pearce, J. D. (1994). Selection of a
17 bacterium for the mass production of *Phasmarhabditis hermaphrodita*
18 (Nematoda:Rhabditidae) as a biocontrol agent for slugs. *Fundamental and Applied*
19 *Nematology* 18, 419-425.

1 Wilson, M. J., Glen, D. M., Pearce, J. D. & Rodgers, P. B. (1995). Monoxenic culture
2 of the slug parasite *Phasmarhabditis hermaphrodita* (Nematoda, Rhabditidae) with
3 different bacteria in liquid and solid-phase. *Fundamental and Applied Nematology* 18,
4 159-166.

5 Wilson, M. J., Ivanova, E. S. & Spiridonov, S. E. (2015). Born to be wild-don't forget
6 the invertebrates. *Trends in Parasitology* 31, 530-532. DOI:10.1016/j.pt.2015.09.002

7 Zhang, Y., Lu, H. & Bargmann, C. I. (2005). Pathogenic bacteria induce aversive
8 olfactory learning in *Caenorhabditis elegans*. *Nature* 438, 179-184.
9 DOI:10.1038/nature04216

10

11

1

2 **Figure Legends**

3

4 Fig 1. A diagram of the chemotaxis assay used to test the behaviour of
5 *Phasmarhabditis* nematodes exposed to mucus from seven species (and a control)
6 simultaneously. A 10 cm Petri dish was half filled with 1.2% technical agar and 0.01g
7 of foot mucus was swabbed from *Arion ater* (AA), *M. sowerbyi* (MS), *L. valentiana*
8 (LV), *L. flavus* (LF), *D. invadens* (DP), *D. reticulatum* (DR) and *A. hortensis* (AH)
9 using a 1 cm² piece of Whatman filter paper. A control (C) consisted of the filter
10 paper with 10 µl of distilled water. Nematodes were applied to the center of the plate
11 (marked “X”).

12

13 Fig 2. The mean number of *P. hermaphrodita* DMG0001 (A), DMG0002 (B),
14 DMG0007 (C), DMG0009 (D), DMG0010 (E); *P. californica* DMG0018 (F) and
15 DMG0019 (G), *P. neopapillosa* DMG0016 (H), DMG0015 (I) and DMG0012 (J)
16 exposed to mucus of seven species of slug. [Fisher’s Least Significant Difference](#)
17 [\(LSD\) at 5% \(\$p < 0.05\$ \) was calculated for each strain.](#)

18

19 Fig 3. The mean number of *P. hermaphrodita* DMG0001 (A), DMG0002 (B),
20 DMG0007 (C), DMG0009 (D), DMG0010 (E); *P. californica* DMG0018 (F) and
21 DMG0019 (G) [that were](#) produced on [0.1 g of](#) each of the seven slug species. Bars
22 represent \pm one standard error.

23