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Short Communication

Avoidance and attraction behaviour of slugs exposed to parasitic nematodes

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ABSTRACT

Avoidance of pathogens and parasites is the first line of defense to survive. Several slug species avoid the parasitic nematode *Phasmarhabditis hermaphrodita* to reduce infection however, there is nothing known about whether slugs avoid other members of the *Phasmarhabditis* genus. I exposed two slug species (*Deroceras invadens* and *Limax maculatus*) to *Phasmarhabditis californica* and *P. neopapillosa*. *D. invadens* avoided *P. californica* but was strangely attracted to *P. neopapillosa*. *L. maculatus* did not avoid *P. californica*, but on day 1 and 3 significantly more slugs were found with *P. neopapillosa.* Reasons for host attraction to *P. neopapillosa* are discussed.

1. Introduction

In order to combat infection by parasites or pathogens and to avoid predators, many animals will escape from areas where they are present. For example, rainbow trout avoid flukes responsible for eye infections (Karvonen et al., 2004) and bumblebees avoid flowers with the bacterium *Escherichia coli* present (Fouks and Lattorff, 2011). We have recently shown slugs avoid the parasitic nematode *Phasmarhabditis hermaphrodita* (Wynne et al., 2016; Morris et al., 2018; Cutler et al., 2019), which has been formulated as a biological control agent (Nemaslug®) to control slugs on farms and gardens since 1994 (Rae et al., 2007). Nematodes are applied to soil, where they hunt for slugs following cues such as mucus and faeces. On discovery of a slug they enter through the back of the mantle and reproduce prolifically killing the slug in 4–21 days. Once the slug dies the nematodes reproduce on the cadaver and once the food is finished they then develop into infective juveniles and search for more slugs in the soil. Not all slugs are killed by infection by *P. hermaphrodita*, larger slugs such as *Arion ater* and *Arion vulgaris* are not killed even though they can be heavily parasitised by the nematode. Nevertheless, the efficacy of *P. hermaphrodita* in field trials to control slug damage has been demonstrated in many studies (see Rae et al., 2007).

In order for slugs to reduce infection by *P. hermaphrodita* they avoid areas where it has been applied. Wilson et al. (1999) showed two common pest species *Deroceras reticulatum* and *A. ater* avoided areas where *P. hermaphrodita* was present. Wynne et al. (2016) found *Arion hortensis*, *Deroceras invadens* and *Lehmannia valentiana* would also avoid *P. hermaphrodita* but slugs such as *Limax maculatus* and snails (*Cornu aspersum*) did not. Infection of these two latter species by *P. hermaphrodita* does not affect their survival or feeding, hence, it is presumed if these hosts are not affected by the nematode, the host does not need to avoid it.

Interestingly, Morris et al. (2018) showed slugs (*D. invadens* and *A. hortensis*) infected with *P. hermaphrodita* moved towards areas where the nematode has been applied – the opposite of the slug's natural avoidance response. The reasons for this are unknown, but it is speculated the nematodes can manipulate the behaviour of the slugs making them move towards *P. hermaphrodita* to ensure more nematodes could infect the slugs and kill them, ensuring the nematodes have a food source and can ultimately reproduce. Morris et al. (2018) found slugs fed the serotonin re-uptake inhibitor fluoxetine, would move towards *P. hermaphrodita* even when not infected. Also, Cutler et al. (2019) showed as well as serotonin signalling, dopamine pathways could also be implicated in avoidance and attraction behaviour used by the nematode to change slug behaviour.

Although this research has clearly shown slugs do not like *P. hermaphrodita*, all this work has concentrated on just one nematode species and specifically-one strain - *P. hermaphrodita* DMG0001 which has been used in commercial production for > 25 years. It is unknown if slugs avoid different members of the *Phasmarhabditis* genus (which contains 18 species). Therefore, I investigated whether two species of slugs (*D. invadens* and *L. maculatus*) would avoid two other species of *Phasmarhabditis* (*P. californica* and *P. neopapillosa*). These two nematode species were collected in the U.K. and have been kept in culture since 2014. Their complete host range and pathogenicity towards gastropods is still being investigated but both nematode species are lethal to slugs (*D. invadens*) and *P. californica* does not negatively affect snail health (*Cornu aspersum*) (Sheehy et al., 2022). Ultimately, this research will

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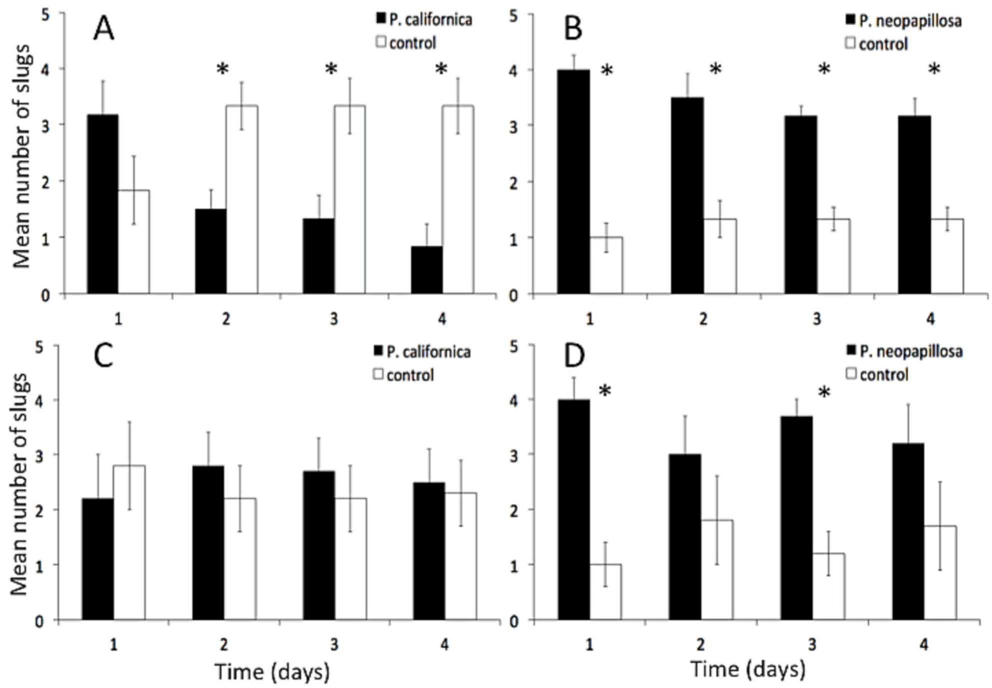

Fig. 1. Mean number of *D. invadens* (A, B) and *L. maculatus* (C, D) found on the side with nematodes (*P. californica* or *P. neopapillosa*) or control (water). Bars represent ± one standard error. A significant difference (P < 0.05) between the mean number of slugs found on the nematode or water side is represented by *.

show if slugs can also detect and avoid areas where two other *Phasmarhabditis* species are present or if this ability is confined to just *P. hermaphrodita*.

2. Materials and methods

P. californica or *P. neopapillosa* were supplied by Becker Underwood BASF Agricultural Specialities as infective juveniles and stored at 15 °C until use. Slugs (*D. invadens* and *L. maculatus*) were collected from gardens and stored in non-airtight containers and fed lettuce and carrot *ad libitum* until use. Slugs were checked 7 days after collection and examined for any signs of *Phasmarhabditis* infection e.g. swollen mantle. Any slugs that did exhibit infection symptoms were not used.

I used a similar set-up as Wynne et al. (2016); Morris et al. (2018); Cutler et al. (2019), which is based on Wilson et al. (1999). Briefly, non-airtight plastic boxes (24 × 9 × 6 cm), were filled with 95 ± 3 g of re-

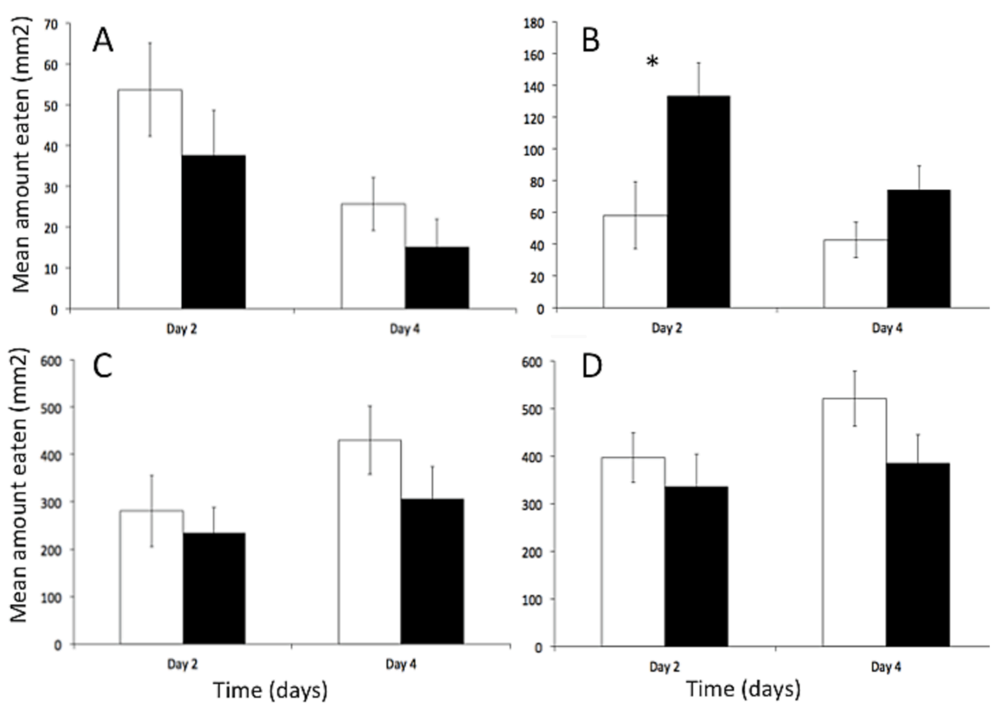

Fig. 2. Mean number of 1 × 1 mm^2 squares of cabbage eaten by *D. invadens* (A, B) and *L. maculatus* (C, D) found on the side with nematodes (black bars) (*P. californica*, A,C or *P. neopapillosa* B, D) or control (water) (white bars). Bars represent ± one standard error. A significant difference (P < 0.05) between the amount of cabbage eaten by slugs on the nematode or water side is represented by *.

moistened coconut husk. The tops of the boxes were lined with copper tape (2.5 cm wide) to keep the slugs from moving away from the soil and crawling up the sides of the boxes. Six millilitres of water were evenly spread on one side (12 × 9 × 6 cm) designated as the control side. On the other side (12 × 9 × 6 cm) 6 mls of water were also added but contained either *P. californica* or *P. neopapillosa* infective juveniles. The dose of nematodes applied was 120 nematodes per cm^2 – a dose used by the aforementioned studies that slugs have been shown to avoid (e.g. Wynne et al., 2016; Morris et al., 2018). Three discs of cabbage (diameter 3.5 cm) were added to each side to monitor feeding of the slugs. The amount of cabbage eaten was quantified on days 2 and 4 by removing the discs and tracing the area eaten onto 1 × 1 mm^2 graph paper. Fresh cabbage discs were added on day 2. Five *D. invadens* (mean weight = 0.38 ± 0.02 g; n = 60) or *L. maculatus* (mean weight = 5.66 ± 0.49 g; n = 60) were added to the middle of the box, then sealed and stored at 18 °C. Every morning for four days the number of slugs on the nematode side or the water side was counted and then placed back in the middle of the box. Three replicate boxes were used for each slug and nematode combination (1. *D. invadens* vs *P. californica*, 2. *D. invadens* vs *P. neopapillosa*, 3. *L. maculatus* vs *P. californica*, 4. *L. maculatus* vs *P. neopapillosa*) and the experiment was repeated twice (N = 6 replicate boxes; n = 30 slugs per slug/nematode combination).

The numbers of slugs of each species found on either the nematode side or the water side (as well as the amount of cabbage eaten on each side of the box) was compared using a Student's *t* test.

3. Results

On day 1 there was no significant difference between the numbers of *D. invadens* found on the side with *P. californica* or the control/water side ($P > 0.05$) (Fig. 1A). However, on days 2, 3 and 4 *D. invadens* was recorded significantly more on the control/water side away from *P. californica* (Fig. 1A) ($P < 0.05$). Strangely, when *D. invadens* was exposed to *P. neopapillosa,* the slugs were found significantly more on the nematode side compared to the water side on days 1, 2, 3 and 4 (Fig. 1B) ($P < 0.05$).

When *L. maculatus* was exposed to *P. californica* there was no significant difference between the numbers of slugs found on the nematode side or the control/water side on day 1, 2, 3 or 4 (Fig. 1C) ($P > 0.05$). In contrast, when exposed to *P. neopapillosa*, *L. maculatus* were found significantly more on the side with the nematodes on days 1 and 3 (Fig. 1D) ($P < 0.05$).

The amount of cabbage eaten by *D. invadens* on the side with *P. californica* or the control (water) side did not differ significantly on day 2 or day 4 ($P > 0.05$; Fig. 2A). However, when exposed to *P. neopapillosa* there was significantly more cabbage eaten by *D. invadens* on the side with the nematodes compared to the control side on day 2 ($P < 0.05$; Fig. 2B) but not on day 4 ($P > 0.05$).

The amount of cabbage eaten by *L. maculatus* on the side with nematodes (*P. californica* and *P. neopapillosa*) or the side with water did not differ significantly on day 2 or day 4 ($P > 0.05$; Fig. 2C,D).

4. Discussion

Results from this study show *D. invadens* strongly avoided *P. californica*, much like *P. hermaphrodita* (see Morris et al., 2018), but it was oddly attracted to *P. neopapillosa*. This is peculiar, as infection by *P. neopapillosa* can kill *D. invadens* rapidly (Sheehy et al., 2022). *L. maculatus* did not avoid *P. californica* but was attracted to *P. neopapillosa* on days 1 and 3. It is currently unknown if *P. californica* or *P. neopapillosa* kill *L. maculatus,* but it has been shown to be unaffected by *P. hermaphrodita*.

It is confusing why a host would be attracted to a parasite but there are a few examples of hosts/parasites showing similar behaviour. Kelly et al. (2015) showed the malaria protozoan parasite *Plasmodium falciparum* releases a volatile chemical profile of plant-like terpenes and derivatives to attract the mosquito vector *Anopheles gambiae*. It certainly makes sense for *P. neopapillosa* to be attractive to slugs, so it can penetrate inside, kill them and reproduce or use the host for transport. Whether it does this by releasing a chemical cue, such as pheromone or an ascaroside, to attract slugs, which are exuded from nematodes such as *Caenorhabditis elegans* and strongly influence nematode behaviour is currently unknown.

At the time of writing the *Phasmarhabditis* genus contains 18 species and new species are being discovered on an annual basis. Using a mitochondrial based phylogenetic approach Howe et al. (2020) demonstrated the *Phasmarhabditis* genus consists of two clades. *P. hermaphrodita* is more closely related to *P. neopapillosa*, in one clade, whilst in the other clade contains *P. californica* and *P. papillosa*. Thus, the ability to induce aversion in slugs seems to be evolutionary conserved across the genus, though it would be of interest to repeated experiments with *P. huizhouensis*, which has been recently discovered and described and is the most distantly related from the two other *Phasmarhabditis* clades.

In summary, slugs avoid *P. californica* but were curiously attracted to *P. neopapillosa.* Clearly, this co-evolutionary relationship between nematodes and terrestrial gastropods warrants further research, but from an applied perspective the use of different *Phasmarhabditis* species in slug control should be considered. Specifically, it has been suggested smaller doses of slug parasitic nematodes could be used in targeted areas e.g. under slug shelters, round the bases of plants to deter slugs, which would work well for *P. hermaphrodita* or *P. californica* but if *P. neopapillosa* was used, it could actually attract more slugs, potentially increasing slug damage to crops.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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