Short communication

Phoresy of the entomopathogenic nematode *Heterorhabditis marelatus* by a non-host organism, the isopod *Porcellio scaber*

Michael S. Eng[a], Evan L. Preisser[a,b,*], Donald R. Strong[a,c]

[a] *Center for Population Biology, University of California at Davis, Davis, CA 95616, USA*
[b] *Department of Plant, Soil, and Insect Sciences, University of Massachusetts at Amherst, Amherst, MA 01003, USA*
[c] *Bodega Marine Lab, P.O. Box 247, Bodega Bay, CA 94923, USA*

Abstract

Entomopathogenic nematodes are widespread in nature and commonly used in the biological control of insect pests. However, we understand little about how these organisms disperse. We show in a laboratory setting that the entomopathogenic nematode *Heterorhabditis marelatus* is phoretically dispersed by a non-host organism, the isopod *Porcellio scaber*. These species both inhabit tunnels excavated in the roots and lower stems of bush lupine (*Lupinus arboreus*) by the nematodes' primary prey, larvae of the ghost moth *Hepialus californicus*. Phoretic dispersal via *P. scaber* may play a role in the metapopulation dynamics of this nematode.
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1. Introduction

Entomopathogenic nematodes (EPNs) are of considerable economic importance as biological control agents for a range of insect species (Gaugler et al., 1997). How EPNs disperse over both short and long distances is an important issue to biological control efforts. Experimental research has focused largely on small-scale (1–10 cm) dispersal of nematodes from either infected cadavers or from the site of an initial application (Lewis, 2002; Moyle and Kaya, 1980; Westerman, 1995). In contrast, relatively little work has been done on longer-range dispersal occurring on the scale of one to tens of meters. The free-living infective juvenile (IJ) stage of EPNs relies on the thin film of moisture surrounding soil particles for movement and to prevent desiccation (Grant and

Villani, 2003). Given the heterogeneity in soil moisture in many terrestrial landscapes, IJs are unlikely to traverse such distances under their own power. Free-living EPN populations are often patchily distributed (reviewed in Lewis, 2002), however, organisms capable of phoretically dispersing EPNs could play an important role in the development and persistence of such metapopulations.

Most research into phoretic dispersal of EPNs has focused on hosts that survive long enough after infection to allow the infective juvenile nematodes to emerge somewhere other than site of the initial infection (Downes and Griffin, 1996). While the larvae of the Japanese beetle *Popillia japonica* are the primary host of the EPN *Steinernema glaseri*, adult beetles were also susceptible to the nematode and phoretically dispersed IJs before dying (Lacey et al., 1995). A study of EPN predation by arthropods revealed that *Steinernema feltiae* IJs clustered on the dorsal surfaces of several microarthropod species and remained infective for

several days (Epsky et al., 1988; Gouge and Hague, 1995). Because none of these microarthropods were potential hosts for *S. feltiae*, the IJs' clustering behavior seems useful primarily as a means of facilitating phoretic dispersal. *Heterorhabditis heliothidis* IJs did not cluster on non-host organisms, however, leaving open the question of whether Heterorhabditid nematodes can or do use non-host organisms as facultative dispersal vectors.

The EPN *Heterorhabditis marelatus* is patchily distributed throughout the sandy coastal prairie of the Bodega Marine Reserve (Bodega Bay, CA, USA). The most common habitat for this nematode is the rhizospheres of bush lupine (*Lupinus arboreus*) and its primary host is root-feeding larvae of the ghost moth *Hepialus californicus*. This insect feeds exclusively on bush lupine at BMR. Field surveys provide no evidence of *H. marelatus* infections in organisms other than *H. californicus* (Strong et al., 1996). The dense litter layer under bush lupines creates moist conditions that facilitate nematode persistence, but the dry soils of the coastal grassland are a much harsher environment. Long-term experiments show that nematode survival is greatly reduced in grassland versus lupine soils, and field surveys reveal that nematodes are an order of magnitude more abundant under lupines than in the surrounding grasslands (Preisser et al., in preparation; Strong et al., 1996). The patchy distribution of lupines mirrors the patchy distribution of *H. marelatus* across the landscape; how do existing nematode populations, separated from lupine habitat by inhospitable grasslands, generate new populations?

While the primary prey of *H. marelatus* is sessile larvae of *H. californicus*, the most common invertebrate in the coastal prairies is the isopod *Porcellio scaber*. These organisms make up approximately 50% of the cursorial invertebrates detected in pitfall trapping and are found throughout the reserve (Bastow, unpublished data). This omnivorous isopod is abundant within the galleries excavated in lupine roots and lower stems by ghost moth larvae (Bastow, unpublished data), and *P. scaber* crawls upon the cadavers of *H. californicus* killed by *H. marelatus* in these tunnels in nature. In other systems, isopods have often been found carrying the bactivorous nematode *Caenorhabditis vulgaris* on their outer surfaces (Baird et al., 1994). *Porcellio* collected from within ghost moth galleries at the reserve and rinsed with water were often found to be similarly carrying nematodes with no apparent ill effects. This observation, and the fact that isopods aggregate within ghost moth tunnels and move between lupine bushes, led us to test whether *H. marelatus* IJs can infect and kill *P. scaber* in soil, and whether the nematode can use this isopod as a facultative vector for phoretic dispersal.

2. Materials and methods

2.1. Experiment 1: can H. marelatus infect and kill P. scaber in soil?

We performed a laboratory experiment to determine whether *H. marelatus* could infect and kill *P. scaber* in soil. We collected *H. marelatus* from BMR using waxworms (larvae of the wax moth *Galleria mellonella*) as 'bait insects' for infective juvenile nematodes (Kaya and Stock, 1997). Waxworms killed by *H. marelatus* turn a distinctive orange–gold color and the cadavers are easily identifiable. The isopods (*P. scaber*) used in this experiment also came from BMR, where they were collected from galleries bored by ghost moth larvae in the lower branches and roots of *L. arboreus*. The isopods were kept without soil in a common laboratory environment for at least one week after collection to minimize the possibility of them transmitting pre-existing nematodes, fungal spores, or debris other soil predators. We rinsed the isopods twice with distilled water before using them in the experiment.

We prepared experimental arenas for testing whether *H. marelatus* infected and killed *P. scaber* by adding $50 \, cm^3$ of pasteurized BMR soil to each of 10 15 cm diameter plastic petri plates. We hydrated the soil to 10% moisture ($0.1 \, ml \, H_2O$/g soil) with distilled water and placed a single piece of pasteurized lupine wood in the center of each arena as an isopod refuge. We randomly assigned five of the soil-filled arenas to each of the two treatments, *H. marelatus* IJs present and *H. marelatus* IJs absent. We prepared a stock solution of *H. marelatus* IJs and pipetted 0.5 ml of solution (approximately 10,000 IJs) into each of the five nematode-present arenas; the five nematode-absent arenas each received 0.5 ml of distilled water.

Two hours after applying the nematode treatments, we added six *P. scaber* isopods to each arena. The plates were loosely taped shut after adding the isopods to slow moisture loss without preventing gas exchange. Plates from each treatment were interspersed randomly in a shaded box at 25 °C and placed on a shelf in the lab. We examined each plate every two days for isopod status (living/dead); any dead isopods were removed from the plate, incubated on white traps (Kaya and Stock, 1997), and microscopically examined after one week for the presence of offspring IJs. After 10 days, the experiment was terminated and the mean number of surviving isopods per treatment was analyzed using ANOVA in JMP v.4.03 (SAS Institute Inc, 2001).

2.2. Experiment 2: can H. marelatus use P. scabers as a vector for phoretic dispersal?

This experiment tested whether the isopod *P. scaber* promoted dispersal of *H. marelatus* between two
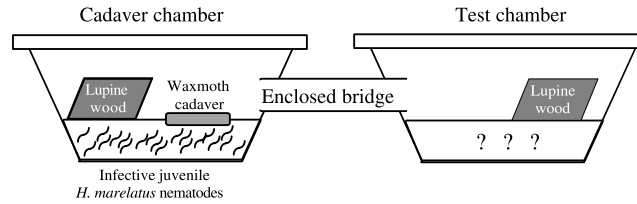
Fig. 1. Experimental design. Each experimental replicate consisted of two terraria (16 oz Superfoz deli tubs) connected by a 20 cm Falcon plastic centrifuge tube. The centrifuge tube was connected to each terrarium via a single 2.5 cm diameter hole in the side of each terrarium, cut 2.5 cm above the bottom of the terrarium. We added 150 g of pasteurized and wetted (0.1 ml H_2O/g soil) BMR soil and 20 g of pasteurized lupine wood (to create a sheltered refuge for the isopods) to each terrarium. We designated one of the two connected terraria as the 'cadaver' terrarium and added a single *H. marelatus*-killed waxworm to it; the non-cadaver terrarium was the 'test' terrarium.

connected terraria. The source populations of both the nematode and isopod, and their treatment pre-experiment, are detailed in Experiment 1 (above). The terraria were plastic deli containers partly filled with soil and connected by a plastic tube bridge (Fig. 1). Waxworm cadavers infected with *H. marelatus* were placed in one terrarium of each connected pair; each terrarium pair thus had one 'cadaver' terrarium and one no-cadaver, 'test' terrarium. The cadavers produced IJs within a week after being placed in the cadaver terrarium; our experiment tested the mechanism of IJ movement from the cadaver terrarium to the test terrarium.

Our experiment consisted of a single treatment: isopods present or isopods absent. One replicate of the isopod treatment combination was a pair of connected terraria with isopods (*P. scaber*) inside. The no-isopod treatment combination was an identical pair of terraria with no isopods. The isopod and no-isopod treatment combinations were each replicated 10 times. At the start of the experiment, we added 50–60 isopods to each terrarium in the isopod treatment combination. Isopods freely and frequently moved back and forth across the bridge between the cadaver and test terraria throughout the experiment.

After five days we closed the bridge connecting the two terraria and added six live, bait waxworms to each individual terrarium to assay for *H. marelatus*. After three days, the bait waxworms were removed, incubated on filter paper in petri plates for two days, and their survival or infection status recorded. We then replaced any dead isopods with live individuals, re-opened the bridge between the replicates and repeated the above procedure over the next 10 days (a total of two sampling dates spread over 20 days). We repeated the above experiment using new isopods, soil, etc., for a total of 20 replicates per treatment combination. All data were analyzed using χ^2 likelihood-ratio tests in JMP v.4.03 (SAS Institute Inc, 2001).

3. Results and discussion

We found no evidence that *H. marelatus* infected and killed the isopod *P. scaber*. There was no statistically significant difference between the mean proportion of isopods surviving for 10 days in the nematode-present versus nematode-absent treatments (nematode-present [mean ± SE]: $0.867 ± 0.062$; nematode-absent: $0.90 ± 0.067$; $F_{1,8} = 0.133$, $p > 0.73$). Although examination of dead isopods from the nematode-present treatments revealed some nematodes crawling on and around the isopod's integument, we found neither mature *H. marelatus* nor developing infective juveniles within the isopod cadaver itself.

Heterorhabditis marelatus dispersed between the terrarium containers only in the presence of the isopod *P. scaber*. We infer that the isopods carried viable *H. marelatus* IJs from the cadaver terraria to the test terraria. After 20 days, 16 of the 20 test terraria with isopods tested positive for *H. marelatus*, while all of the test terraria without isopods were negative ($\chi^2 = 37.64$, df = 1,38, $p < 0.001$). Nematode presence in the cadaver chamber did not differ between the two treatments ($\chi^2 = 2.88$, df = 1,38, $p < 0.090$).

Our finding that *H. marelatus* does not infect and kill *P. scaber* seems to contradict an earlier study examining interactions between entomopathogenic nematodes and several isopod species. Poinar and Paff (1985) tested the ability of the nematodes *Steinernema carpocapsae*, *S. glaseri*, and *H. heliothidis* to infect and kill three species of *Porcellio* (*P. scaber americanus*, *Porcellio dilatatus*, and *Porcellio laevis*). They found species-specific differences in the ability of entomopathogenic nematodes to infect and kill isopods: *S. carpocapsae* and *H. heliothidis* infected and killed all three *Porcellio* after 12 days, while *S. glaseri* was ineffective at killing any of the three *Porcellio* species (Poinar and Paff, 1985). Even the 'successful' nematode species found isopods to be poor hosts for larval development: the host integument broke down shortly after death, allowing the cadaver to be colonized by generalist bacteria. As a consequence, only 30% of isopod cadavers showed evidence of nematode reproduction, and the resulting offspring numbered in the hundreds rather than the thousands typical of similarly sized insect hosts (Poinar and Paff, 1985). We cannot reject the possibility that *H. marelatus* might, under some conditions, infect and kill *P. scaber*; regardless,

however, such infections seem unlikely to produce nematode offspring sufficient for *P. scaber* to be considered an effective host.

The high rate of isopod movement between the connected terraria suggests that *P. scaber* can potentially carry *H. marelatus* greater distances than those tested in our experiment. Based on their speed when crossing the bridge, we estimate that even small isopods can cover 1–2 m/h. Research done at BMR on *P. scaber* found that they traveled extensively between experimentally created habitat patches, with 30% traveling 2.5 m and 10% traveling 10 m over eight days (Grosholz, 1993). While it is likely that other mobile arthropods could also serve as facultative vectors, both *H. marelatus* and *P. scaber* seek out tunnels made by *H. californicus* larvae; their natural proximity to each other further supports the hypothesis that isopods could be a major source of facultative phoretic dispersal for *H. marelatus*. Given that phoretically dispersing EPNs can resist desiccation for several days (Epsky et al., 1988), *P. scaber* may serve as a facultative vector for transmission of this insect 'pathogen' between isolated lupine microhabitats.

Heterorhabditis marelatus is capable of suppressing root herbivores and indirectly protecting bush lupines (Preisser, 2003; Strong et al., 1999); however, the driving forces behind its ecology and population dynamics are unclear. Mathematical models suggest that isolated EPN populations should quickly go extinct (Dugaw et al., 2004). Isopod phoresy provides a plausible mechanism for isolated lupine rhizospheres to become 'colonized' with EPNs, and a means by which the patchily distributed nematode persists on the landscape. That this facultative phoretic association occurs between *H. marelatus* and a non-host organism emphasizes the importance of considering interactions with the larger food web rather than focusing solely on EPNs and their hosts. It also suggests that the presence of 'non-target' organisms in soil food webs may enhance the long-term efficacy of EPN-based biological control efforts.

Acknowledgments

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