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To complete their life cycle, pathogenic nematode–bacteria complexes deter scavengers from feeding on their host cadaver

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ABSTRACT

The life cycle of commercially used molluscicidal rhabditid nematodes *Phasmarhabditis hermaphrodita* and entomopathogenic steinernematid nematodes is similar: infective stages carry symbiotic bacteria, which kill their host. Nematodes complete their life cycle feeding on the proliferating symbiont and the host tissue. After 1–2 weeks, new infective stages carrying the bacteria leave the host cadaver in search of new hosts. The removal of invertebrate cadavers by scavengers is extremely fast and represents a severe threat to the developing nematodes.Two-choice trials were used to assess prey choice of the generalist predator/scavenger *Pterostichus melanarius* (Coleoptera: Carabidae) between *Deroceras reticulatum* (Mollusca: Agriolimacidae) slugs or wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) larvae killed by infection of *P. hermaphrodita*/*Steinernema affine* and control killed by freezing. We demonstrate that the presence of either of the two nematodes tested deters the beetles from consuming infected cadavers. As *P. hermaprodita* cannot infect an insect host, we hypothesise the deterrent effect being an evolutionary adaptation of the nematode/bacteria complex rather than the ability of the beetles to avoid potentially infective cadavers.

1. Introduction

Entomopathogenic nematodes from the Steinernematidae and Heterorhabditidae families are lethal parasites of various soil-dwelling insects and are used commercially in biological control (Mracek, 2002; Shapiro-Ilan et al., 2002; Arthurs et al., 2004; Georgis et al., 2006). Similarly the rhabditid nematode *Phasmarhabditis hermaphrodita*, is a lethal parasite of terrestrial molluscs (predominantly slugs) and has recently been commercialised in the EU as a biological molluscicide against slug pest.

Both nematodes appear to adopt a similar life cycle including development and reproduction inside a host and a free-living infective stage (IJ) carrying symbiotic bacteria, which kills their host. Nematodes subsequently finish their life cycle feeding on the proliferating symbiont and the host tissue, which takes typically 7–15 days for entomopathogenic nematodes (Poinar, 1979; Adams and Nguyen, 2002) and between 7 and 21 days for *P. hermaphrodita*, respectively. As food resources in the cadaver are depleted, new IJs, carrying symbiotic bacteria, are formed that leave the cadaver in search of new hosts (Poinar, 1979; Wilson et al., 1993; Tan and Grewal, 2001, 2002).

As is evident from the above, the critical factor for successful reproduction of both pesticidal nematodes is that the infected host and the subsequent cadaver remains intact while nematodes complete their life cycle. Dying hosts are vulnerable to predation as their defence efficiency declines (Pakarinen, 1993; Winder et al., 1994; Symondson, 1997; Foltan, 2004). The removal of invertebrate cadavers by scavenging invertebrates is typically rapid, taking only minutes or hours (Seastedt et al., 1981; Fellers and Fellers, 1982; Young, 1984; Retana et al., 1991; Bestelmeyer and Wiens, 2003; Foltan et al., 2005). However, nematodes need days to complete their life cycle.

There is evidence to suggest that nematode/bacteria infestation can reduce cadaver attractiveness to ant scavengers. Baur et al. (1998) placed 2-days and 8-days old cadavers (4 and 10 days after infection) of *Galleria mellonella* (L.)(Lepidoptera: Pyralidae) larvae infected by different heterorhabditid and steinernematid nematodes on the ground and 2 cm under the ground in a horticultultural habitat in California. Different ant species removed more steinernematid-killed (60–80%) than heterorhabditid-killed (10–20%) insects within 24 hours.

In another study, Zhou et al. (2002) placed 4-days old *G. mellonella* larvae killed by different strains of *Xenorhabdus*

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nematophilus and *Photorhabdus luminescens* or by freezing, into ant colonies or near to ant foraging trails. Significantly more non-infected cadavers were removed by the ants.

The critical period for "survival" of invertebrate cadavers in the field is the first 24 h after death as cadavers are typically scavenged during this period (Seastedt et al., 1981; Fellers and Fellers, 1982; Young, 1984; Retana et al., 1991; Bestelmeyer and Wiens, 2003; Foltan et al., 2005). Thus, estimating removal rates of several-days old cadavers infected by pathogenic nematodes, even when their repellent effect is evident (Baur et al., 1998; Zhou et al., 2002), does not fully answer the question as to how nematodes survive the extreme scavenging pressure on inhabited cadavers. Moreover, as the attractiveness of invertebrate cadavers declines with time (Foltan et al., 2005), comparing removal rates of rapidly decaying nematode infected cadavers with freshly killed controls (Baur et al., 1998; Zhou et al., 2002) does not demonstrate the deterrent effect of the infection.

This study is the first to address the significant difference between the fast removal rates of invertebrate cadavers from the field and the relatively long period which is necessary for successful reproduction of molluscicidal and entomopathogenic nematodes within the cadavers. We demonstrate for the first time the strong antifeedant effect of rhabditid nematode infected slug cadavers as well as the significant anti-feeding effect of steinernematid infected insect cadavers on invertebrate non-ant scavengers.

We discuss whether such an effect enables the nematodes to survive in cadavers which would otherwise be removed through the extreme pressure from scavenging.

2. Materials and methods

Two-choice laboratory experiments were used to assess the prey choice of the common generalist predator/scavenger *Pterostichus melanarius* (Coleoptera: Carabidae) between slugs *Deroceras reticulatum* killed by infection with *P. hermaphrodit* or wax moth *G. mellonella* larvae killed by infection with *Steinernema affine* and non-infested controls.

D. reticulatum slugs and *P. melanarius* beetles were collected from arable fields around Ceske Budejovice, Czech Republic. The beetles were kept individually in plastic containers (9.5 cm × 7 cm × 5 cm) with 80 g moist sphagnum peat (Agro CS a.s.) and maintained in a controlled environment (16 h light:8 h dark photoperiod; 20 ± 1 °C). They were maintained on a diet of mixed pork and beef. Prior to the experiment, the beetles were fed *ad libitum* and then starved for 7 days, to ensure the same nutritional state in all individuals.

G. mellonella larvae were reared in multiclonal cultures according to Haydak (1936). Forty slugs and forty moth larvae were killed by freezing at −80 °C and weighed. Another forty slugs/larvae were killed by the infection with *P. hermaphrodita*/*S. affine* nematodes. The slugs were infected by exposure to a superabundance of *P. hermaphrodita* in a plastic container (approximately 10 million IJ's per 30 cm × 20 cm × 20 cm container) filled with moist filter paper. Moth larvae were infected in Petri dishes (7 cm diameter) with moist filter paper on the bottom by adding 2000 IJ's of *S. affine* in 1 ml of tap water per 10 moth larvae. The weight of freshly killed nematode infected slugs/larvae and the weight of the control slugs/larvae was recorded before storage at −80 °C (avg. w. slugs: 167.8 mg, SD 39.8; avg. w. larvae: 163.1 mg, SD 39.4).

A single frozen dead slug or larva, killed by freezing (=non-infected), was placed in a Petri dish (7 cm diameter) lined with moist filter paper, together with a frozen slug/larva killed by the nematodes (=infected). The prey items were placed opposite to each other approximately 1.5 cm from the edge of the Petri dish. Every following Petri dish was turned through 90° to prevent any effect of prey preference due to spatial orientation of the prey items. The slugs/larvae were let to thaw for several minutes. Single *P. melanarius* beetles (equal sex proportions) were placed in the middle of each dish and observed continuously for 30 min. The first attack by the beetle on the infected or non-infected cadaver, and first feeding on the infected or non-infected cadaver, were recorded as separate parameters. An attack was recorded when the beetle bit the prey. Feeding was recorded when biting occurred continuously for more than 30 s on one prey. After 30 min, any non-feeding beetles were discarded and the experiment was repeated with a fresh beetle, until the final number of 40 replicate trials for both the slugs and larvae was reached.

To assess the effect of beetle weight, beetle sex, weight of infected and non-infected cadaver and difference in weight between infected/non-infected cadaver on beetle choice, binomial regressions with preference as the dependent variable (and remaining variables as potential predictors) were used, separately for response variables (attack and consumption) and for different types of prey (slugs, larvae); using GLM (link binomial) in S-plus 2000 (1999).

3. Results

Separate effects of all potential predictors were compared with null models (that containing only the response variable) for potential decrease of deviance, measured by Cp criterion (Table 1). The only predictor that exhibited a decrease of deviance was beetle

Table 1
Effect of beetle sex, cadaver weight and difference in weight between infected/non-infected cadavers on the choice of the predatory beetle *P. melanarius* between slugs killed by *P. hermaphrodita*, *G. mellonella* larvae killed by *S. affine* and control slugs/larvae killed by freezing. Binomial regressions with preference as dependent variable (and remaining variables as potential predictors) were used, separately for attack and consumption and for different types of prey; using GLM (link binomial).

Term	Df	Sum of Sq	RSS	Cp	Df	Sum of Sq	RSS	Cp
	Attack on moth larva cadaver				Consumption of moth larva cadaver			
Null			40.00	42.05			40.00	42.05
Beetle sex	1	0.10	39.80	44.00	1	0.17	39.83	43.93
Beetle weight	1	0.25	39.75	43.85	1	0.57	39.43	43.53
Infected larvae weight	1	0.07	39.93	44.03	1	0.00	39.99	44.10
Non-infected larvae weight	1	0.11	39.89	43.99	1	0.16	39.84	43.94
Difference in larvae weights		0.68	39.32	43.42	1	0.00	39.99	44.10
	Attack on slug cadaver				Consumption of slug cadaver			
Null			40.00	42.05			40.00	42.05
Beetle sex	1	0.48	39.52	43.63	1	0.00	40.00	44.10
Beetle weight	1	0.00	39.99	44.10	1	2.32	37.68	41.79
Infected slug weight	1	0.62	39.38	43.48	1	0.01	39.99	44.09
Non-infected slug weight	1	0.68	39.32	43.42	1	1.07	38.93	43.04
Difference in slug weights	1	0.27	39.73	43.83	1	1.01	39.99	44.09