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Molecular detection of nematode predation and scavenging in oribatid mites: Laboratory and field experiments

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ABSTRACT

Recent stable isotope analyses indicate that a number of putative detritivorous soil microarthropods is not typical detritivores but rather live as predators or scavengers. Using molecular gut content analyses the present study investigates if nematodes indeed form part of the diet of oribatid mites. First, in a no-choice laboratory feeding experiment two nematode species (*Phasmarhabditis hermaphrodita* and *Steinernema feltiae*) were offered to eight species of oribatid mites and one gamasid mite. Second, after feeding for 4 and 48 h on each nematode species the detection time of prey DNA in the oribatid mite species *Steganacarus magnus* was investigated. Third, in a field experiment nematode prey (*P. hermaphrodita* and *S. feltiae*) in the diet of microarthropods was investigated distinguishing between scavenging and predation. In the no-choice laboratory experiment not only the gamasid mite but also several of the studied oribatid mite species consumed nematodes. After feeding on nematodes for 4 h prey DNA was detectable in *S. magnus* for only 4 h, but after feeding for 48 h prey DNA was detectable for 128 h, indicating that the duration of feeding on prey is an important determinant for prey DNA detection. The field experiment confirmed that oribatid mite species including *Liacarus subterraneus*, *Platynothrus peltifer* and *S. magnus* intensively prey on nematodes. Interestingly, DNA of dead *P. hermaphrodita* was detectable to a similar degree as that of living individuals indicating that scavenging is of significant importance in decomposer food webs. Results of our study indicate that predation and scavenging on nematodes by "detritivorous" microarthropods in soil food webs need to be reconsidered.

1. Introduction

Predator–prey interactions are an important component of terrestrial food webs influencing population densities and energy fluxes through the system (Terborgh and Estes, 2010). Knowledge on predator–prey interactions is of prime importance for understanding aboveground as well as belowground food webs and for evaluating the relative importance of top-down forces in decomposer systems (Milton and Kaspari, 2007; Tylianakis et al., 2008). In contrast to aboveground systems, predator–prey interactions in belowground food webs are difficult to study since (1) trophic links are difficult to observe without disturbing the system, (2) most soil animals are small (<1 mm) and therefore direct observation is difficult, and (3) liquid-feeding hampers tracing the food in the gut (Symondson, 2002; Read et al., 2006; Juen and Traugott, 2007).

Molecular methods may help to overcome these limitations (King et al., 2008). First, extraorally digested prey can be detected in the predator gut (Kuusk et al., 2008). Second, the investigation of the gut content of soil-living predators using specific primers allows distinguishing the prey at the level of species (Read et al., 2006; Juen and Traugott, 2007). Specific primers amplifying short DNA fragments allow detection of prey DNA in the gut of predators even days after prey consumption (Agusti et al., 1999; Zaidi et al., 1999; Hoogendorn and Heimpel, 2001). Using specific primers allows identifying the spectrum of predators of single species and thereby investigating the level of specialism *vs.* generalism in soil food webs. Molecular gut content analyses, however, also have limitations. One particular weakness is that predation, i.e. feeding on living prey, cannot be separated from scavenging (Juen and Traugott, 2005) and from secondary predation, i.e. the feeding of a predator by another predator (Sheppard et al. 2005). One of the first important studies using molecular markers for the detection of nematode DNA in predator guts was carried out by Read et al. (2006) who developed specific primers for three nematode species (*Phasmarhabditis hermaphrodita*, *Steinernema feltiae*, *Heterorhabditis megidis*).

Nematodes hold a key position in soil food webs (Yeates et al., 1993; Bongers and Ferris, 1999; Moore et al., 2003), little is known

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on their role as prey for other soil animals and to what extent their density is controlled by predators. They are among the most diverse and abundant animals on earth (Baxter, 2003). Nematodes live not only as parasites in animals and plants, but also freely in aquatic and soil systems, where they can reach densities of up to several million individuals and up to 100 species per square meter (Yeates et al., 2000). In soil they feed on bacteria, fungi, algae, invertebrates, plants or are omnivorous, and, due to their generally high density, they form an important food source for other soil animals (Yeates et al., 1993; Read et al. 2006).

Mites (Acari) also reach high diversity and density in soil systems (Maraun and Scheu, 2000) comprising not only "classical detritivores", such as Oribatida, but also predators, such as Gamasina and Uropodina. Laboratory studies (Muraoka and Ishibashi, 1976) as well as stable isotope analyses (Schneider et al., 2004) indicate that several species of oribatid mites can live also predatory, most likely on nematodes. Gamasid mites are free-living, motile, liquid-feeding predatory mites (Koehler, 1997). They are assumed to be generalist predators that feed on a variety of prey including nematodes. Uropodid mites live in soil where they mainly consume not only nematodes, but also slugs, insect larvae and dead animals (Karg, 1989; Raut and Panigrahi, 1991; Scheu and Falca, 2000).

The aims of this study were to investigate (1) if soil mite species (mainly from the taxa Oribatida, but also Gamasina and Uropodina) feed on nematodes, (2) if the nematode species are consumed to a different degree, (3) the detection time of prey DNA after different time periods of feeding by *Steganacarus magnus*, (4) the relevance of laboratory feeding experiments for understanding trophic interrelationships in the field, and (5) the relevance of predation and scavenging in soil food webs, i.e. if microarthropod predators discriminate between dead and living prey. Therefore, laboratory no-choice feeding experiments were established using the two model nematode species *S. feltiae* and *P. hermaphrodita*. In the laboratory, nematodes were offered separately to investigate if the studied mite species differentially feed on nematode species. To determine the detection time of prey DNA *S. magnus* was fed with each nematode species for 4 h and 48 h. To prove the validity of the results of the laboratory studies in the field the two nematode species were added to the soil of a beech forest. Each species was added dead and alive to separate the role of scavenging and predation for soil mite nutrition. Low natural abundance of the added model nematodes in field soil allowed separating them from the resident nematode community. We hypothesized that a large number of mite species, including putatively detritivorous taxa such as oribatid mites feed on nematodes in the field.

2. Material and methods

2.1. The organisms

Infective juveniles (dauerlarvae) of two pathogenic nematode species were used for this study. The infective non-feeding third-stage of the rhabditid *P. hermaphrodita* (Schneider, 1859) has a length of ~1 mm. *P. hermaphrodita* is a parasitic bacterivore that infects the mantle of slugs where it reproduces and kills the slug before the new larvae are spread to the soil. The entomopathogenic *S. feltiae* (Filipjev, 1934) dauerlarvae has a length of ~0.8 mm and parasitizes the hemocoel of sciarid and drosophilid larvae. Juveniles of both species search in soil for new hosts. *P. hermaphrodita* and *S. feltiae* are cosmopolitan species; however, their density in the field is low. At the studied beech forest they could not be detected by standard soil sampling and nematode extraction making them ideal model prey species as there are no background signals in nematode consumers (K. Heidemann, unpubl. data). Both nematode species were supplied by 'prime factory' (www.schneckenprofi.de, Hennstedt, Germany). They are able to survive in soil for several weeks which is more than sufficient for the purpose of our experiment (Kaya and Gaugler, 1993).

Three mite taxa, i.e. Oribatida, Gamasina and Uropodina, were studied as potential predators for the two nematode species. In the laboratory experiment the gamasid mite *Hypoaspis aculeifer* (Canestrini, 1883) and eight oribatid mite species were included: *Achipteria coleoptrata* (Linné, 1758), *Atropacarus striculus* (Koch, 1835), *Carabodes coriaceus* Koch, 1835, *Damaeus riparius* Nicolet, 1855, *Eupelops plicatus* (Koch, 1835), *Hypochthonius rufulus* Koch, 1835, *Steganacarus magnus* (Nicolet, 1855) and *Archegozetes longisetosus* Aoki, 1965. In the field experiment the gamasid mite *Pergamasus septentrionalis* (Oudemans, 1902), the uropodid mite *Uropoda cassidea* (Hermann, 1804) and seven species of oribatid mites were investigated: *Chamobates voigtsi* (Oudemans, 1902), *Liacarus subterraneus* (Koch, 1844), *Platynothrus peltifer* (Koch, 1839), *Nothrus silvestris* Nicolet, 1855 and *S. magnus*. All species are inhabitants of European forest soils except *A. longisetosus* which is tropical and was included as it is the most frequently cultured oribatid mite species.

2.2. The study site

The Kranichstein forest is an oak-beech forest near Darmstadt, Hesse, Germany. The annual temperature is 9.5 °C and the annual precipitation is about 700 mm. Soil pH is 3.6–4.3 and the humus form is moder. The tree layer is dominated by beech (*Fagus sylvatica*, L.); parent rock is Cisuralian (Rotliegendes, Early Permian) (Schneider et al., 2007).

2.3. Test of primer specificity

Both nematode primer pairs used were tested against 16 non-target species by Read et al. (2006). Additionally, we tested them against further 74 non-target species (Table 1, Electronic Appendix), including the 15 mite species used in this study. The animals were starved for seven days and stored separately at −80 °C in 180 µL ATL-Buffer of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). After DNA extraction the mesofauna taxa were tested with the D3 primers, and the macrofauna taxa with general COI primers (Folmer et al., 1994), to check success of the DNA extraction. The specific primers for *P. hermaphrodita* and *S. feltiae* were used in a PCR with the non-target taxa and with the respective nematode species as positive control to check for cross-reactions.

2.4. Detection time of prey in consumers

Detection time of prey DNA in consumers was investigated using the oribatid mite *S. magnus* which were extracted from soil of the Göttinger forest and the Hainich forest. All individuals were starved for seven days. The two nematode species (*S. feltiae*, *P. hermaphrodita*) were offered separately to 20 individuals of *S. magnus* for 4 and 48 h. Afterward mites were checked for attached nematodes and starved for 0, 2, 4, 8, 16, 32, 64 and 128 h. After these time periods we checked if mites were alive to avoid false negatives, and stored individual mites separately at −80 °C in 180 µL ATL-Buffer of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). During the experiment mites were kept in darkness at 13 °C.

2.5. Laboratory experiment

Seven oribatid mite species were extracted from soil of the Kranichstein forest whereas *A. longisetosus* (Oribatida) and *H. aculeifer* (Gamasid mites) were taken from laboratory cultures. All species