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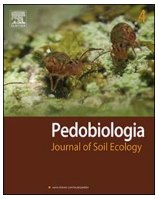

Track the snack – olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida)

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ABSTRACT

Olfactory cues are important for many heterotrophic organisms to find and identify food. In soil ecosystems, however, olfactory food selection has only been fragmentarily explored and it remains to be uncovered whether olfactory signals are involved in finding suitable food sources in microarthropods. We addressed this basic question in laboratory food-choice bioassays with two oribatid mite species [*Archegozetes longisetosus* (opportunistic feeder) and *Scheloribates* sp. (myco-/phytophagous feeder)] and bacteria, fungi, lichen and litter as resources. We found that both oribatid mite species used olfactory cues to find and differentiate among food resources. While *A. longisetosus* preferred bacterial diet, *Scheloribates* sp. mainly fed on fungal-based food sources (fungi and lichen). We analysed volatiles and nutrients of the resources and used modified food, synthetic compounds and amino/fatty acid mixtures in a second food-choice experiment. *A. longisetosus* preferred fatty acids (and their preferred bacterial diet was also rich in fatty acids), while *Scheloribates* was highly attracted by mushroom-alcohol (1-octen-3-ol). Our experiments showed that olfactory signals contribute to the trophic ecology of oribatid mites in soil ecosystems.

1. Introduction

Foraging processes of arthropods in complex habitats like soils rely on different tactile perception mechanisms, but also chemical cues could be involved (Bengtsson et al., 1991; Hedlund et al., 1995; Hall and Hedlund, 1999; Zirbes et al., 2011). Such stimuli enable soil organisms to localize and distinguish food sources in an opaque and a highly heterogeneous environment with patchy resource distribution (Anderson, 1975a; Burnett et al., 1998; Cain et al., 1999; Farley and Fitter, 1999; Hodge, 2006; Nielsen et al., 2010). Due to this patchiness, resource and habitat specialization appear to be rare in detritivorous soil arthropods such as springtails or oribatid mites, and it is assumed that most species predominantly feed on a broad food spectrum (Luxton, 1972; Anderson, 1975b; Behan-Pelletier and Hill, 1983; Walter, 1987; Vegter et al., 1988; Ettema and Wardle, 2002; Wehner et al., 2016). However, in contrast to this generally low degree of resource partitioning, stable isotope analyses indicate a more distinct feeding niche (Schneider et al., 2004a; Chahartaghi et al., 2005; Pollierer et al., 2009). Hence, soil detritivores have been termed “selective generalist feeders” or “choosy generalists” (Klironomos et al., 1992; Schneider and Maraun, 2005). Accordingly, it seems advantageous to have a general, olfactory based foraging strategy to recognize and find food in the first place, while gustatory properties or structure/morphology may facilitate the final choice to consume a resource. For “choosy generalist” feeders, it should be beneficial to use a broad spectrum of volatiles to locate food, instead of solely depending on one specific signal (Hedlund et al., 1995; Ferry et al., 2007; Pfeffer and Filser, 2010).

However, little is known about olfactory mediated foraging and food selection by soil microarthropods and soil-dwelling predatory larvae (Thomas et al., 2008). In pioneer studies Bengtsson et al. (1988, 1991) and Hedlund et al. (1995) have shown that springtails (Collembola) use fungal volatiles to perceive and choose among fungal resources, but are also attracted to odorants of other animals (e.g., Salmon and Ponge, 2001; Nilsson and Bengtsson, 2004). Thus, springtails are also able to sense and respond to fungal species with different levels of toxicity (i.e. secondary metabolites) using olfactory signals (Staaden et al., 2011; Stötefeld et al., 2012). Furthermore, springtails are differently attracted to sex-specific volatiles of mosses, can locate soil patches with high microbial activity and seem to move towards CO_2

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sources (Moursi, 1962; Hassall et al., 1986; Rosenstiel et al., 2012). For (soil) mites, however, the olfactory base of food selection and responses to resource derived volatiles are poorly understood. For example, only few studies showed that predatory mites seem to respond to herbi-/fungivore induced volatiles of their prey (Hall and Hedlund, 1999; Aratchige et al., 2004; Pfeffer and Filser, 2010), and stored-product mites (Actinotrichida, Astigmata) are attracted by olfactory stimuli, mostly mixtures of semi-volatile amino- and fatty acids (Pankiewicz-Nowicka et al., 1986, 1987). Compared to above-ground invertebrates, the movement patterns of soil-dwellers are less directional and olfactory signals are presumably only detected over a few centimetres (Auclerc et al., 2010). Also the rather enigmatic nature of the belowground-system, i.e. the heterogeneity and patchiness of the habitat, the multi-layered structure of soil, litter and root system (e.g., Anderson 1975a; Hodge, 2006; Nielsen et al., 2010), but also the less developed receptor systems (Wu et al., 2017) may render soil arthropods less directional compared to their above-ground relatives. For example, collembolans exhibit irregular, non-directional walks and also systematic loop-searching behaviour at higher distances, but switch to directional movement if an olfactory source is nearby (Bengtsson et al., 2004; Auclerc et al., 2010).

Here we focus on oribatid mites – highly diverse soil-dwelling mites abundant in various ecosystems all over the world (Schatz, 2004; Schatz et al., 2011). Niche partitioning and especially food preferences have been intensively studied in oribatid mites (Schneider et al., 2004b; Maraun et al., 2011). Based on available data on stable isotope composition ($\delta^{15}N/\delta^{13}C$), oribatid mites can be classified as lichen feeders, as primary as well as secondary decomposers and predators/scavengers (Schneider et al., 2004a; Maraun et al., 2011). Whereas primary decomposers mainly feed on plant material, secondary decomposers are considered as microbivores, i.e. feeding on algae, bacteria and fungi (Schuster, 1956; Luxton, 1972). Also gut boli and fecal pellet analyses (Anderson, 1975b; Labandeira et al., 1997), cheliceral morphology (Kaneko, 1988; Perdomo et al., 2012), enzymology (Siepel and de Ruiter-Dijkman, 1993; Hubert et al., 2001), molecular gut bar-coding (Heidemann et al., 2011; Eitzinger et al., 2013) and especially laboratory preference tests demonstrated a broad resource spectrum with distinct preferences for certain leaf-litter material, algae or fungi (Pande and Berthet, 1973; Hubert and Lukesova, 2001; Schneider et al., 2005; Koukol et al., 2009). However, the actual mechanism enabling food selection is unknown. On numerous occasions we observed that mites in our laboratory cultures, although being blind, often quickly and directionally head towards their food. Thus, we asked ourselves how olfactory senses could be involved in this behaviour.

Generally, responses to volatile cues in oribatid mites are known in the context of chemical communication (Shimano et al., 2002; Raspotnig, 2006; Heethoff and Raspotnig, 2012). For example the giant moss mite (*Collohmannia gigantea*) uses a multicomponent gland secretion for chemical defense, which has evolved a secondary function as alarm pheromone (Raspotnig, 2006). Olfactory associated sensory organs, the solenidia, i.e. setea with wall-porous structures and branched dendrites, are found on the tarsi of oribatid mites (Baker, 1985; Alberti, 1998; De Lillo et al., 2004). Furthermore, most soil-dwelling oribatid mites do not possess structures to perceive and process visual information. Some kind of light-sensitive organs (i.e., lenticuli) are, however, present in some Enarthronota and Brachypylina, but absent from *Archegozetes* and *Scheloribates* (e.g. Alberti and Fernandez, 1988, 1990). Hence, it seems most likely that soil dwelling oribatid mites also use their olfactory sense to localize chemicals associated with food.

We used two oribatid mite species (the desmonomatan *Archegozetes longisetosus* and the brachypyline *Scheloribates* sp.) to investigate whether oribatid mites can use olfactory cues for food localization and discrimination, and if so, which chemical substances may serve as candidate olfactory signals responsible for food choice.

2. Materials and methods

2.1. Mites

Archegozetes longisetosus Aoki (a middle-derivative desmonomatan species) is a common parthenogenetic (= all-female), widely distributed pantropical mite and an opportunistic feeder (Heethoff and Scheu, 2016). The laboratory strain *A. longisetosus* ran (Heethoff et al., 2007) has been used as a model organism for more than 20 years (Heethoff et al., 2013). *Scheloribates* Berlese (a highly derived brachypyline genus) is a sexually reproducing myco- and phytophagous feeder (Siepel, 1996; Hubert and Lukesova, 2001). The unidentified species used in this study is probably tropical, since we obtained it as a contamination of a tropical springtail culture send in coconut debris from Zoo Zajac (Duisburg, Germany). Both oribatid mite species were raised in polypropylene boxes, where the bottom was covered with a mixture of plaster of Paris: activated charcoal (9:1). The stock cultures were kept humid at 28 °C in darkness. *A. longisetosus* cultures were fed with lupine flour (Govinda Natur GmbH, Neuhofen, Germany), while *Scheloribates* sp. was fed with yeast (Rapunzel Naturkost GmbH, Legau, Germany).

2.2. Food and nutrient mixtures

To test olfactory food choice of the two oribatid mite species we prepared different semi-natural and artificial foods/mixtures. As semi-natural resources we chose the gram-negative soil bacterium *Pseudomonas fluorescens* CHA19, the mesophilic soil fungus *Chaetomium globosum*, the fruticose lichen *Cladonia rangiformis* and fallen leaf litter of *Tilia cordata*. *Pseudomonas fluorescens* CHA19 (hereafter: bacteria) was cultivated in liquid modified arabinose-gluconate medium (Cole and Elkan, 1973; van Berkum, 1990) without amino acids. *Chaetomium globosum* (hereafter: fungi) was grown on cellophane over Czapek- Dox agar (Warcup, 1950) plates. *Cladonia rangiformis* (hereafter: lichen) was collected in November 2016 on a dry, sandy meadow in the botanical garden in Darmstadt (49°52′10.4″N 8°40′48.1″E). *Tilia cordata* (hereafter: litter) leaf litter was collected in autumn 2013 in an old lime forest with mainly *Tilia cordata*, interspersed with some common oak (*Quercus robur*) and bordered by sycamore (*Acer pseudoplatanus*) and ash (*Fraxinus excelsior*) near Duderstadt (51°30′21.1″N 10°12′27.5″E). Bacteria, fungi and lichen material was freeze-dried over a period of 72 h, whereas litter was dried at 50 °C for two days. Subsequently, all four food sources were ground and stored dry until the experiment started. We also prepared five artificial nutrient mixtures consisting of only essential amino acids (mixture of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine in equal proportions), non-essential amino acids (mixture of alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine and tyrosine in equal proportions), fatty acids (C16:0, C18:0, C18:1Δ9, C18:2Δ6,9; mixing ratio (mass based) 1:1:0.5:0.1), pure D-glucose (99.9%) and D-glucose mixed with mushroom alcohol (1-octen-3-ol; 1 µl/1 g glucose). We used these mixtures because other sarcoptiform mites (Astigmata) appear to react and discriminate among these compounds (Pankiewicz-Nowicka et al., 1986, 1987). All nutrients/chemicals were analytical grade and purchased from Sigma-Aldrich, Darmstadt, Germany.

2.3. Experimental setup

Experiments were performed in plastic Petri dishes (4.5 × 1.5 cm or 9 × 1.5 cm, for the olfactory sensing test or choice experiment I–III, respectively) grounded with moist analytical filter paper (both, Hartenstein GmbH, Würzburg, Germany). Four different powders (either semi-natural food or nutrient mixtures, except for the olfactory