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Love at first sniff: a spermatophore-associated pheromone mediates partner attraction in a collembolan species

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Mate choice is essential in most animals, as a good choice of mating partner largely determines reproductive success. Much evidence shows that olfactory cues play an important role in mate choice. However, the integration of chemical, visual and acoustic cues, often used when both partners meet, makes it hard to test whether olfaction alone can mediate reproductive decisions. Interestingly, several invertebrates have adopted a mating system where males deposit their sperm (packed in spermatophores) in the environment for females to pick up with no visual contact between the sexes. In this case the male cue is conveyed by the spermatophore only. Earlier studies on a species with indirect sperm transfer, the soil arthropod *Orchesella cincta*, showed that, even in these animals, female choice exists. In this study, we tested whether chemical cues provided by the spermatophores mediate this female choice. Chemical analysis of spermatophore extracts revealed that (*Z*)-14-tricosenol is the main compound in the male spermatophores and this compound attracted females in olfactometer bioassays. Our finding suggests that (*Z*)-14-tricosenol is thus a pheromone component, which is sufficient for female attraction. This is the first report of a spermatophore-associated sex pheromone in a species performing indirect sperm transfer.

The ability to rapidly detect a mating partner is crucial for an animal's fitness (Andersson, 1994; Andersson & Simmons, 2006; Weddle, Hunt, & Sakaluk, 2013). Usually, males and females use a range of sensory systems for sexual communication, including visual, acoustic and chemical cues to detect information about the potential mate (Candolin, 2003; Leonard & Hedrick, 2009). Most species in the animal kingdom rely to some extent on chemical cues for mating decisions (Wyatt, 2003); however, chemical cues are more difficult to observe than other signals (Penn, 2006). Moreover, in most mating systems with internal fertilization, reproduction involves pair formation, making it hard to test the hypothesis that a chemical cue alone mediates reproductive decisions. Even though there is growing evidence that olfactory cues provide information on the individual quality of a potential partner (see e.g. Chemnitz, Jentschke, Ayasse, & Steiger, 2015; Johansson & Jones, 2007; Rantala, Kortet, Kotiaho, Vainikka, & Suhonen, 2003; Ruther, Matschke, Garbe, & Steiner, 2009; Thomas, 2011), the functional integration of multiple sensory modalities during mating contact makes it hard to disentangle the relative contribution of chemical attractants.

Interestingly though, throughout the animal kingdom, the degree of contact during mating is highly variable and shows a continuum from full bodily contact to no contact at all (Zizzari, Jessen, & Koene, 2016; Zizzari, Smolders, & Koene, 2014) and can broadly be categorized as follows: (1) direct transfer of sperm involving intimate contact (i.e. copulation); (2) paired indirect transfer, in which a male courts a particular female before, during or after deposition of sperm in the environment (e.g. salamanders: Arnold, 1976; soil arthropods: Proctor, 1998; Schaller, 1971); (3) dissociated indirect sperm transfer, where males deposit their

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sperm in the environment for females to pick up and use for internal fertilization without meeting the male (e.g. soil and aquatic arthropods: Barazandeh, Davis, Neufeld, Coltman, & Palmer, 2013; Proctor, 1998; Zizzari, van Straalen, & Ellers, 2013; sessile marine invertebrates: Bishop & Pemberton, 2006).

In the latter case, there is no physical or visual contact between the sexes and, most likely, a female relies solely on chemical cues for reproductive decisions. Animals performing dissociated sperm transfer represent a common and widespread group, but are neglected in behavioural studies. Yet, the study of organisms that rely solely on sperm-associated chemical cues for reproductive decisions could deepen our insights in the evolution of chemical signals in general and shed light on their role as sperm competitive traits.

In this study, we used the soil arthropod *Orchesella cincta* (Collembola: Entomobryidae) to investigate the role of olfactory communication when males and females never have contact during reproductive decisions. *Orchesella cincta* displays a mating system widespread among soil arthropods (Proctor, 1998; Walter & Proctor, 2013), i.e. males deposit their sperm (packed in spermatophores) in the environment irrespective of the presence of a female, and females pick up a spermatophore for internal fertilization without the presence of the male. Earlier work has provided evidence that female choice for spermatophores does exist in *O. cincta* (Zizzari, Braakhuis, van Straalen, & Ellers, 2009; Zizzari & Ellers, 2011; Zizzari et al., 2013), suggesting that chemical cues related to the spermatophore are involved in mate assessment. Here, we tested whether olfactory cues associated with spermatophores produced by *O. cincta* males attract conspecific females from a distance.

By performing gas chromatography coupled to high-resolution mass spectrometry (GC–MS) analyses of spermatophore extracts, we first identified the chemical compounds from the spermatophores. Subsequently, in olfactometer bioassays we measured the response of females *O. cincta* to the identified compounds. To our knowledge, this study is the first report of female attraction to a male spermatophore-associated pheromone.

METHODS

Study Species and Care

The *O. cincta* strain used in this study was obtained from a large outbred laboratory population that originated from a pine forest in The Netherlands (Roggebotzand; 52°34.40N, 05°47.90E). This stock population had been maintained at 20 °C in a climate-controlled room (70% relative humidity, 12:12 h light:dark) in the laboratory for over 30 years at the time of the experiments. In the experiments, animals were kept in Perspex vials with a moistened bottom of plaster of Paris, which kept humidity inside the vials at nearly 100%. Pine tree twigs naturally covered with green algae (*Desmococcus* sp.) were provided for food.

Natural History

Adult *O. cincta* (Fig. 1) alternate reproductive and nonreproductive periods (instars) separated by moults (Gols, Ernsting, & van Straalen, 2004; Stam, Isaaks, & Ernsting, 2002; Zizzari et al., 2009). Each instar lasts 4–5 days. Spermatophore deposition (Fig. 2) occurs only during the reproductive instar and males usually deposit dozens of spermatophores within a reproductive instar (mean = 58; Zizzari, van Straalen, & Ellers, 2016). In this species, as in several soil arthropods, the reproductive success of males depends on placing a large number of spermatophores, to increase the probability of detection by females and counterbalance spermatophore destruction by rivals (Dallai, Zizzari, & Fanciulli, 2009; Proctor, 1998; Stam et al., 2002; Zizzari, Jessen, et al., 2016). Female receptivity lasts 48 h after the onset of a reproductive instar (Gols et al., 2004; Zizzari et al., 2009), during which receptive females locate the spermatophores, and take up only one with the genital opening to fertilize all their eggs (Dallai, Zizzari, & Fanciulli, 2008; Gols et al., 2004). A female's reproductive instar can only be assessed by offering her a spermatophore and subsequently checking for eggs (Zizzari et al., 2009; Zizzari & Ellers, 2014; Zizzari, van Straalen, et al., 2016).

Figure 1. Image of *Orchesella cincta*. Photo: Z.V. Zizzari.

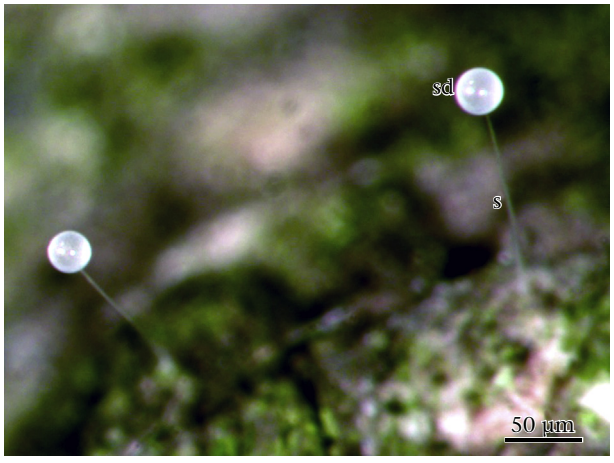

Figure 2. Light microscope view of *O. cincta* spermatophores: sd: apical sperm droplet; s: stalk. Photo: Z.V. Zizzari.