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Behavioural switch in the sex pheromone response of *Nasonia vitripennis* females is linked to receptivity signalling

Joachim Ruther [a,*], Kathleen Thal [b,1], Birgit Blaul [a], Sven Steiner [b,1]

[a] *Institute of Zoology, University of Regensburg*
[b] *Institute of Biology, Free University of Berlin*

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The benefits for insects using chemical information may depend on their physiological state and the behavioural context in which the chemical stimuli are perceived. Hence, insect behaviour is often characterized by olfactory plasticity. Mechanisms triggering changes in the postmating behaviour of insect females are typically mediated by constituents of the male ejaculate. Here we show that the behavioural switch displayed by mated females of the jewel wasp *Nasonia vitripennis* in response to a male abdominal sex pheromone is independent of the transfer of a male ejaculate. Rather, our results suggest a pheromone interaction in that prior exposure of a female to one pheromone and her subsequent receptivity signal modulated her response to a second one. We tested the pheromone response of females that had been experimentally prevented from receiving sperm or from experiencing certain elements of the male courtship behaviour. We show that the behavioural switch in *N. vitripennis* females is linked to the receptivity signal shown by females in response to a male-derived oral aphrodisiac during precopulatory courtship.

The chemical sense is of crucial importance for the orientation of insects in complex environments. Many pivotal competences of insects such as the ability to locate mates, food sources and oviposition sites, as well as to avoid natural enemies and suboptimal living conditions, are mediated by chemical messengers (Wyatt 2003; Schoonhoven et al. 2005). In many cases, however, benefits resulting from the exploitation of these so-called semiochemicals depend on the physiological state of the responder, the behavioural context in which they are perceived, and a number of environmental parameters (Anton et al. 2007). In fact, responding to chemical cues and signals at the wrong time might be costly rather than beneficial. In the context of pheromone-mediated sexual communication, for instance, the ability of the responding gender to detect a sex attractant should coincide with its own fertility and the receptivity of potential mating partners (Anton et al. 2007). On the other hand, it might be detrimental for individuals that mate only once (typically the females) to orient towards a sex attractant once mating has occurred, since this might cause fitness costs as a result of the waste of energy or sexual harassment (Gay et al. 2009). Therefore, sex pheromone-guided behaviour of insects has to be plastic, that is, adapted to conditions under which the benefits of a behavioural response are likely to outweigh possible costs. Most insect species studied so far with respect to olfactory plasticity in sex pheromone communication belong to the Lepidoptera and Diptera (reviewed in Anton et al. 2007). Apart from environmental parameters such as light (e.g. Kanno 1981), diel periodicity (e.g. Cardé et al. 1974) and temperature (e.g. Cardé & Roelofs 1973; Linn et al. 1988), a number of intrinsic factors such as age (Turgeon et al. 1983), hormone titres (Gadenne et al. 1993; Anton & Gadenne 1999) and mating status (e.g. Jang 1995; Gadenne et al. 2001) modulate sex pheromone responses in these taxa. Males of the black cutworm moth, *Agrotis ipsilon*, for instance, do not respond to the female sex pheromone until sexual maturity is reached (Gadenne et al. 1993). Then, they can mate only once per night and therefore do not respond to the female sex pheromone once a spermatophore has been transferred. After having replenished the sperm and accessory gland fluid supplies, males become responsive again (Gadenne et al. 2001). Female Mediterranean fruit flies, *Ceratitis capitata*, show a behavioural switch after mating with a shift of their olfactory preference from the male sex attractant to host odours (Jang 1995). This effect is mediated by proteinaceous molecules from the male accessory gland being transferred to the female together with sperm during copulation (Jang 2002). In *Drosophila*, sex peptides from

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[*] Correspondence: J. Ruther, Department of Zoology, Chemical Ecology Group, University of Regensburg, Universitätsstraße 31, D-93051 Regensburg, Germany.
E-mail address: joachim.ruther@biologie.uni-regensburg.de (J. Ruther).

[1] S. Steiner and K. Thal are at the Department of Biology, Free University of Berlin, Haderslebener Straße 9, D-12163 Berlin, Germany.

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male accessory gland secretions have been shown to trigger remating refractoriness and affect female reproductive activity in many other ways (Gillott 2003; Chapman et al. 2003; Yapici et al. 2008). The sex peptide/receptor system is thought to be highly conserved in insects (Werren et al. 2010) but appears to be missing in *Apis mellifera* and *Nasonia vitripennis*, the only two hymenopteran insects with sequenced genomes so far (Werren et al. 2010). This raises the question how mating-dependent behavioural changes, which have been reported at least for *N. vitripennis*, are mediated in these insects.

Nasonia vitripennis is a gregarious pupal parasitoid of numerous fly species (Whiting 1967). The stereotypic courtship behaviour of *N. vitripennis* males has been studied in detail (Barras 1960; van den Assem & Vernel 1979; van den Assem et al. 1980; van den Assem 1986; van den Assem & Werren 1994; Steiner et al. 2006). When encountering a female, the male immediately mounts her and shows vellicating wing movements together with a typical head-nodding behaviour (see Supplementary Video S1). Head nodding is typically accompanied by chewing movements of the mouthparts, which results in the release of a still unknown aphrodisiac pheromone from an oral gland eliciting female receptivity. Involvement of a volatile aphrodisiac was concluded from the observation that courting males whose mouthparts had been sealed by a drop of quick-drying superglue were unable to elicit receptivity in females. Exposure of these handicapped pairs to headspace volatiles from unconstrained pairs, however, restored receptivity in these females (van den Assem et al. 1980). Within the first 30 s of courtship, mostly even faster (van den Assem & Vernel 1979), the female signals receptivity by lowering her head and antennae and synchronously opening her genital orifice. After copulation, the male remounts the female and displays postcopulatory courtship, often resulting in another receptivity signal by the female. Postcopulatory courtship is crucial for making the female unreceptive for other males (van den Assem & Visser 1976) and, in fact, the majority of females mate only once under natural conditions (Grillenberger et al. 2008). Apart from remating refractoriness, *N. vitripennis* females also show a mating-dependent behavioural switch concerning their sex pheromone response. Only virgin females are attracted to the male sex pheromone consisting of a mixture of (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolides (HDL) and 4-methylquinazoline (MeQ; Ruther et al. 2007, 2008, 2009). The attractive chemicals are biosynthesized in the male rectal vesicle and released via the anal orifice (Abdel-latief et al. 2008; Steiner & Ruther 2009b). Within minutes after mating, however, females are no longer attracted to the male pheromone and prefer host odours instead (Ruther et al. 2007; Steiner & Ruther 2009a).

Supplementary video related to this article can be found at doi: 10.1016/j.anbehav.2010.09.008.

The present study was performed to investigate the mechanism involved in the behavioural switch of *N. vitripennis* females. We asked in particular whether the reception of a male ejaculate is essential for the modification of the female behaviour or whether the exposure of females to particular elements of the male courtship behaviour is sufficient. We also studied whether the behavioural switch is innate. To achieve this goal, we performed a series of olfactometer bioassays to test the pheromone response of females that had been experimentally prevented from receiving a male ejaculate or from experiencing particular details of the male courtship sequence.

METHODS

Insects

Nasonia vitripennis wasps were reared in petri dishes on freeze-killed puparia of the green bottle fly, *Lucilia caesar* (b.t.b.e. Insektenzucht, Schürpflingen, Germany) at 25 °C and 60% relative humidity with a daily light:dark cycle of 16:8 h. Unmated parasitoids for the experiments were obtained by excising pupae from host puparia 1–2 days prior to eclosion and keeping them singly in 1.5 ml microcentrifuge tubes until emergence. Females used in the olfactometer bioassays were 1–3 days old and unmated. Males used for pheromone extraction were unmated and 2 days old. Manipulations of parasitoids were done with a fine paint-brush.

Conditioning of Females at Different Levels of Courtship Experience

To study the olfactory responses of females subject to defined elements of the male courtship sequence, we allowed individual pairs to court in a round observation chamber (0.5 cm height, 1 cm diameter) but prevented females experimentally from receiving a male ejaculate or experiencing particular details of the male courtship behaviour (see below). Additionally, we tested unmated females as positive controls (referred to as ‘virgin’, $N = 20$; Table 1).

Experimental exclusion of postcopulatory courtship

Virgin pairs were allowed to court and mate but postcopulatory courtship was prevented by removing the male before he could remount the female (referred to as ‘no postcop’, $N = 20$).

Experimental exclusion of the copulation

Virgin pairs were allowed to court. However, after male head nodding and the female receptivity signal, pairs were separated with a fine paint-brush (referred to as ‘no cop’, $N = 20$).

Experimental exclusion of the male sex attractant

Nasonia vitripennis females are able to learn chemical stimuli associatively (Schurmann et al. 2009). They have been shown to prefer odours to which they were exposed during a single oviposition event and even learn odours that do not occur in the natural habitat. We therefore performed an experiment to address the question whether the opportunity to perceive the male sex

Table 1

Behavioural elements of the *Nasonia vitripennis* courtship sequence for females of the different experience levels before their response to the male sex pheromone was tested in the olfactometer bioassay

Experience level of females	Precopulatory courtship				Copulation
	Male mounting	Male head nodding	Male aphrodisiac	First female receptivity signal	
Virgin	−	−	−	−	−
‘No postcop’	+	+	+	+	+
‘No cop’	+	+	+	+	−
‘No HDL/4-MQ’*	+	+	+	+	−
‘No aphro’†	+	+	−	−	−

For an explanation of experience levels, see text and Fig. 1.

* Perception of the male sex pheromone during courtship was prevented by using newly emerged males and sealing their abdominal tip.

† Release of the aphrodisiac was prevented by sealing the mouthparts of the courting male.