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Learning individual signatures: rove beetle males discriminate unreceptive females by cuticular hydrocarbon patterns

Jerry Schlechter-Helas*, Thomas Schmitt, Klaus Peschke

Department of Evolutionary Biology and Animal Ecology, Faculty of Biology, University of Freiburg, Freiburg, Germany

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Pheromones elicit a species-wide, class-specific and stereotyped reaction. By learning class- or individual-specific cues in association with pheromones and subsequently using the cues alone for discrimination, a receiver may react more effectively to its social environment than if its only reaction to pheromones was stereotyped. In the rove beetle *Aleochara curtula*, recently mated females are inappropriate mating partners; nevertheless they elicit male sexual reactions owing to olfactory excitation by the female sex pheromone. During physical contact, mated females can be discerned by gustatory perception of an antiaphrodisiac pheromone via the clasper-like parameres of the male genitalia. Males transfer the antiaphrodisiac pheromone during copulation, together with the spermatophore, onto their mate. Our experiments showed that in association with antiaphrodisiac pheromone perception, males learned the scent of a mated female and relied on this chemical signature to refrain from sexual grasping responses in subsequent encounters. Habituation–dishabituation experiments further showed that the learned patterns were individual specific. The application of cuticular extracts to alter the scent of females, and the resulting inability of males to discriminate between similar-smelling females, demonstrated that males relied on cuticular hydrocarbons for individual discrimination. Chemical analysis of the cuticular profiles revealed higher variation in female than in male patterns. Learning the scent of mated females in association with the antiaphrodisiac pheromone allows males to reduce the time and energy spent on sexual reactions towards inappropriate mating partners and hence gives them more time to search for a receptive female at the mating site.

Recognizing an opposite-sex conspecific is a prerequisite for an individual in any sexually reproducing species. In many insects, females produce a sex pheromone, which attracts males over a distance or induces sexual behaviour at close range (Jacobson 1972; Wyatt 2003). In general, such pheromones are species wide and class specific for all females (Wyatt 2010), but in a few species, females cease their sex pheromone emission after copulation to avoid detection and sexual harassment (Kingan et al. 1995; Ramaswamy et al. 1996). Although all females emitting the sex pheromone elicit a stereotyped reaction by males, their appropriateness as a mating partner may vary considerably depending, for instance, on their mating status or their recent mating history (Bonduriansky 2001; Simmons 2001; Gillott 2003; Takami et al. 2008; Thomas 2011). In the majority of cases, unreceptive females represent inappropriate mating partners, for example because they are reluctant to mate and repel male courtship, or because of a postcopulatory mate-guarding device from the last mating partner. An antiaphrodisiac pheromone may inform on the unsuitability of a recently mated female and effectively prevent males from copulating with the individual (Scott & Jackson 1988; Andersson et al. 2000, 2003; Schulz et al. 2008; Brent & Byers 2011; Estrada et al. 2011; Schlechter-Helas et al. 2011). On the one hand, the antiaphrodisiac pheromone may reduce male mating effort towards inappropriate females, but on the other the two-step system of being attracted by a sex pheromone and subsequently repelled by an antiaphrodisiac pheromone may still impose time and energy costs on males.

The costs of gathering information about several pheromones at each new encounter can be reduced by linking the pheromonal signals associatively with class- or individual-specific information at a first contact and using this cue alone for discrimination in subsequent encounters (Hurst & Beynon 2004; Ejima et al. 2007; Ramm et al. 2008; Roberts et al. 2010). The unpredictable combination of signals that is learned and subsequently used for discrimination is generally provided by scent cues (Hurst & Beynon 2004; Ejima et al. 2007; Roberts et al. 2010; Wyatt 2010), and in insects this chemically perceived signature is mostly made up by cuticular hydrocarbons (D'Ettorre & Heinze 2005; Widemo &

* Correspondence: J. Schlechter-Helas, Department of Evolutionary Biology and Animal Ecology, Faculty of Biology, University of Freiburg, Haupstraße 1, 79104 Freiburg, Germany.
E-mail address: jerry.schlechter@biologie.uni-freiburg.de (J. Schlechter-Helas).

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Johansson 2006; Steiger et al. 2008; Blomquist & Bagnères 2010; Steiger & Müller 2010). One behavioural context of learning individually distinctive signatures is represented by the Coolidge effect, defined as the progressive decline in the propensity of a male to copulate repeatedly with the same female combined with an increased sexual interest in novel females (Wilson et al. 1963; Dewsbury 1981; Steiger et al. 2008). However, learning the scent of an inappropriate female to avoid further sexual engagements with her may not be limited to former mating partners, but may also occur with females mated by rival males.

In the rove beetle *Aleochara curtula* Goeze (Coleoptera: Staphylinidae), females release a sex pheromone, *(Z)*-7- and *(Z)*-9-heneicosene as well as *(Z)*-7- and *(Z)*-9-tricosene, which males perceive at close range through their antennal olfactory receptors (Peschke 1978, 1987a; Peschke & Metzler 1987). The sex pheromone induces a grasping response: the male bends his abdomen over his head and protrudes his genitalia with the clasper-like parameres. Males show grasping responses towards unmated and mated females, as sex pheromone emission continues after copulation (Schlechter-Helas et al. 2011). However, recently mated females represent inappropriate mating partners for males, because they are first plugged by a spermatophore and, after removal of the spermatophore, they repulse male mating attempts by oscillating their abdomen (Peschke 1987b). Unsuitable females are discerned only after a grasping response, when the male's parameres seize the female's abdomen to couple with her genitalia and thereby perceive the antiaphrodisiac pheromone, resulting in the male aborting the mating attempt (Schlechter-Helas et al. 2011). Males transfer the antiaphrodisiac pheromone during copulation together, with the spermatophore, into their mate's genital chamber, from where it spreads onto the entire surface of the female. In contrast to the volatile sex pheromone, which is perceived over a short distance, the antiaphrodisiac pheromone is only detected through paramere contact (Schlechter-Helas et al. 2011). After the first mating attempt is aborted, olfactory-triggered grasping responses towards the same mated female are drastically reduced (Peschke 1987b). We hypothesized that, in association with gustatory perception of the antiaphrodisiac pheromone, males learn the scent of a mated female and can subsequently discriminate her by olfaction and avoid further sexual grasping responses. In a behavioural assay with repeatedly presented dummies, we investigated the male's sexual reaction under the conditions that caused the first mating attempt to be aborted, as shown in earlier experiments (Schlechter-Helas et al. 2011). We also carried out habituation–dishabituation experiments (Thom & Hurst 2004 and references therein) to investigate whether the scent of mated females is individual specific. We altered the chemical profiles of mated females by applying cuticular extracts of virgin females to examine further whether hydrocarbons present on virgin and mated females act as individual-specific cues. Finally, as variations in the proportions of different components may supply the cues for individual recognition (Singer 1998; Howard & Blomquist 2005; Johansson & Jones 2007; Steiger et al. 2008), we also investigated the individual variation in cuticular hydrocarbon composition in both sexes.

METHODS

Beetle culture

Aleochara curtula individuals were baited with rabbit carcasses in the surroundings of Freiburg i.Br., Germany and reared in the laboratory according to Fuldner (1968) and Peschke (1986, 1987a). Immediately after emergence, sexes were separated; males were kept in groups of 10 individuals (in plastic boxes 10 × 10 cm and 7 cm high) and females in groups of 30 individuals (in plastic boxes 20 × 10 cm and 7 cm high). All beetles were kept at 22 °C and 65% relative humidity and fed ad libitum with *Calliphora vicina* maggots.

Behavioural assays

All behavioural assays were conducted with dummies prepared as follows. A female was killed by freezing for 30 min at −20 °C and, with a droplet of hot-melt adhesive, glued at the pronotum to a piece of wire connected to a glass Pasteur pipette as a handhold. In a glass dish, 9 cm in diameter and laid out with moistened filter paper, each test male was presented 10 times in succession with a dummy. The dummies were presented for 10 s with an interval of 5 s between two presentations. To assess the sexual attractiveness of a female dummy, we recorded for each presentation whether the dummy induced a male grasping response, that is, the extrusion of the male parameres with the abdomen bent over the head. After a grasping response, paramere contact with the dummy occurred in most cases. We used either virgin males or males that had not mated for at least 2 days, to exclude refractory period effects on the grasping response.

Sexual Attractiveness of Females

We assessed whether aborting a mating attempt after paramere contact with a mated female has an influence on further grasping responses towards the same female by presenting a mated female dummy 10 times in succession to a test male. As a control for male fatigue or the influence of copulation failure, we presented a virgin female dummy 10 times in a row to a test male.

Reduced Attractiveness through Antiaphrodisiac Pheromone

The antiaphrodisiac is transferred approximately in the middle of copulation (normal copulation duration: 46 min), together with the spermatophore, and when, after copulation, a male perceives the pheromone on the female's body surface, he aborts the mating attempt (Schlechter-Helas et al. 2011). To differentiate whether perception of the antiaphrodisiac pheromone or that of another cue related to copulation influences the female's attractiveness in 10 consecutive encounters, we presented mated females that differed with respect to the antiaphrodisiac pheromone in the behavioural assay. We therefore paired individuals in small plastic boxes (5 × 3 cm and 2 cm high) and interrupted the copulation 20 min after the beginning by separating the pair with forceps. After the interrupted copulation, 19 females retained either amorphous secretions or a complete spermatophore and thus the antiaphrodisiac pheromone, while 21 females lacked any part of the spermatophore and thus also lacked the antiaphrodisiac pheromone.

In a second experiment, the sexual attractiveness in 10 consecutive presentations of virgin females that were treated with the antiaphrodisiac pheromone contained in a spermatophore extract was compared to that of virgin females lacking the antiaphrodisiac pheromone. The spermatophore extract was prepared as follows. We gently pressed the abdomen of a recently mated female, so that the spermatophore, which consists of an amorphous secretion and a sperm sac, protruded from the genital chamber. The amorphous secretion was removed with forceps, whereas the sperm sac was left in the female. The amorphous secretion was transferred into a vial and washed in approximately 300 µl of dichloromethane for 30 min to allow substances of different polarity to dissolve. Thereafter the solvent was reduced by a gentle stream of nitrogen. Female dummies were treated with the combined extracts of three amorphous secretions dissolved in 10 µl of solvent or with 10 µl of pure solvent. The extract or solvent was