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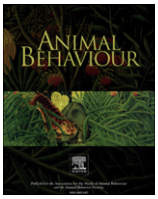

Jail baits: how and why nymphs mimic adult females of the German cockroach, *Blattella germanica*

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The male German cockroach performs a characteristic courtship behaviour upon contacting a sexually receptive female: he turns away from the female and raises his wings, thereby exposing tergal glands whose reservoirs contain phagostimulatory substances. The female then mounts the male and feeds upon these nuptial secretions; this behaviour places her in the appropriate precopulatory position. The contact sex pheromone on the cuticular surface of the female, responsible for eliciting courtship behaviour in males, consists of a blend of six components that share a common biosynthetic pathway. An excised female antenna can elicit the full courtship display in males. We found that antennae taken from either male or female nymphs of various ages also could elicit the full courtship response in adult males. We extracted lipids from the cuticular surface of nymphs and, guided by behavioural assays, we fractionated the extracts using various chromatography procedures, including flash (column) chromatography, high-performance liquid chromatography and gas chromatography. Mass spectrometry analysis of behaviourally active fractions revealed two classes of courtship-eliciting compounds: all nymphs possessed a novel, still unidentified compound that elicited courtship in adult males. In addition, in last-instar females, we isolated four of the six adult female-specific contact sex pheromone components, consistent with differentiation of the sexes at this stage, and the onset of sexual maturation of the pheromone biosynthetic machinery. Our results support the interpretation that nymphs engage in sexual mimicry to gain access to male-produced nuptial tergal secretions that are exposed and can be secured only during courtship.

Courtship behaviour is often a resource-exhausting activity, yet it is crucial for sexual reproductive success. Species- and sex-recognition signals are important in directing courtship towards potentially suitable mates, thereby allocating resources efficiently, while minimizing risks of predation and parasitism. Qualitatively and quantitatively unique sex pheromone blends are exceptionally effective species-specific signals for discriminating between the sexes and in most cases they unambiguously specify the reproductive state of a potential mate, thus facilitating recognition of receptive conspecifics of the opposite sex (Roelofs 1995; Ringo 1996; Rafaeli 2002; Symonds & Eigar 2008). Perhaps because of their 'privacy' and high signal value, sex pheromones have been exploited by highly specialist predators and parasitoids that use them to locate specific prey or hosts, or emit mimetic pheromone analogues to attract specific prey (Haynes & Yeargan 1999). Many plants, too, engage in sensory exploitation of male pollinators, some by mimicking sex pheromones of female insects. Orchids represent an especially spectacular adaptive radiation of this strategy (Raguso 2008). Moreover, sex pheromones and other sexual traits, especially of females, are commonly used by males of many species to deceive rival males and thus gain an advantage in sexual competition (Mason & Crews 1985; Saetre & Slagsvold 1996; Muller & Wrangham 2002; Hanlon et al. 2005). Much less understood are the mechanisms used by immature stages to elicit sexual courtship in conspecific adults, and whether such behaviour is adaptive (Peschke 1985; Haynes et al. 1992; Steiner et al. 2005; Ruther & Steiner 2008).

In the German cockroach, a complex courtship repertoire is vital for mating. The sexes are brought together by means of a volatile sex pheromone emitted only by receptive females that display a typical calling behaviour (Liang & Schal 1993; Nojima et al. 2005). Upon contact of the male antennae with the female's cuticle, the

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male displays a characteristic courtship: he rotates 180° away from the female and raises his wings, thereby exposing specialized glands that serve as nutrient reservoirs on the seventh and eighth tergites, and directs these glands towards the female's antennae and gustatory organs. The secretions of these glands (i.e. proteins, lipids and especially sugars) serve as attractants and phagostimulants. When the female mounts the male to feed on the secretion, she is placed in an appropriate position for copulation (Roth & Willis 1952; Nojima et al. 1999).

Courtship behaviour is elicited by a blend of six contact sex pheromone components identified as oxidation derivatives of cuticular long-chain methyl-branched alkanes. The most abundant component is the dimethyl ketone 3,11-dimethylnonacosan-2-one (compound 1, Fig. 1), and less abundant components include its derivatives 29-hydroxy-3,11-dimethylnonacosan-2-one (compound 2), and 29-oxo-3,11-dimethylnonacosan-2-one (compound 3). Additional components are the homologous C_{27} compounds, including 3,11-dimethylheptacosan-2-one (compound 4) and its oxidation derivatives 27-hydroxy-3,11-dimethylheptacosan-2-one (compound 5) and 27-oxo-3,11-dimethylheptacosan-2-one (compound 6) (Nishida & Fukami 1983; Schal et al. 1990b; Eliyahu et al. 2008b).

Contact sex pheromone production in the German cockroach is regulated by juvenile hormone (JH), which also paces and controls yolk protein synthesis and oocyte maturation. The adult female produces large quantities of the pheromone when she becomes sexually receptive approximately 4–6 days after eclosion. Production of the contact sex pheromone diminishes dramatically after the female oviposits, and it remains low during a 3-week gestation while she incubates an egg case (Schal et al. 1990a, 1991). Thus, female contact sex pheromone production in the German cockroach is related to differentiation of pheromone producing cells (oenocytes) and hormonal regulation of dimethyl ketone production (Fan et al. 2003).

Interestingly, male courtship can also be elicited by either male or female teneral (newly eclosed) adults (Roth & Willis 1952), some other cockroach species (Eliyahu et al. 2008a), and even by phylogenetically unrelated insect species (Nishida & Fukami 1983). The chemical cues that elicit *B. germanica* courtship towards *Blatta orientalis* (Oriental cockroach) share chain length and functional features with the native contact sex pheromone components of *B. germanica* (Eliyahu et al. 2008a). This suggests that the male courtship response is broadly tuned to a range of structurally related compounds. It is not known whether teneral adults use the same contact sex pheromone components that receptive females use to stimulate courtship in males. Although teneral females produce a small amount of the contact pheromone, teneral males do not (Schal et al. 1990a). Teneral nymphs also elicit sexual responses in males, and it was suggested that 'moulting fluids contain a stimulating substance' (Roth & Willis 1952), but the identity of these compounds remains unknown.

We observed that older nymphs, several days after the moult, retained the capacity to elicit courtship responses in adult males, not only in mixed populations in the laboratory, but also in German cockroach-infested barns on swine farms. We conducted experiments to confirm these observations by monitoring male sexual responses during the ontogenetic changes that occur in male and female nymphs throughout nymphal development. We then conducted qualitative and quantitative analyses of nymph extracts of both sexes to elucidate the mechanisms by which they elicit courtship. Finally, we hypothesized that sexual mimicry in nymphs functions to obtain access to the male nuptial tergal secretion and that nymphs might benefit from this interaction.

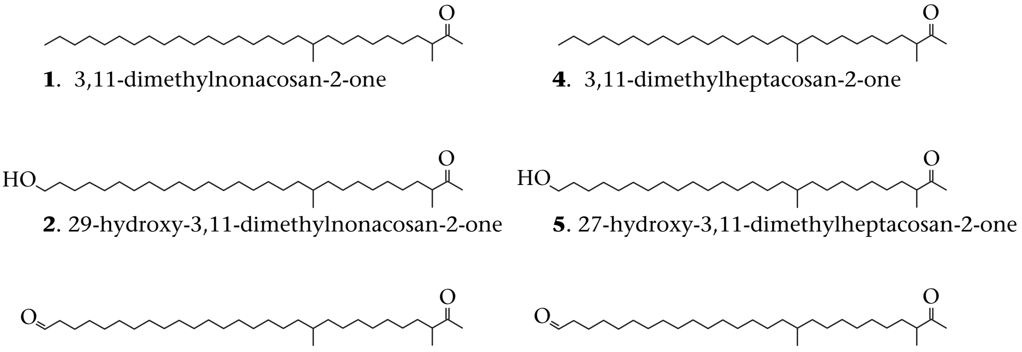
METHODS

Insects

Blattella germanica cockroaches were kept in groups at 27 °C under 12:12 h light:dark cycle and provisioned with dry Purina rat chow and water. First-instar nymphs were removed from adult cages into a collective nymph-rearing container. Third-instar nymphs were sexed and caged separately prior to behavioural assays.

Behavioural Assay

We tested male behavioural response using a modification of the assay developed by Roth & Willis (1952). For testing behavioural activity of chromatographic fractions from nymph and female cuticular extracts, we excised an antenna of an adult male *B. germanica* (14–21 days old), attached the antenna to a glass Pasteur pipette, and extracted it briefly in hexane to remove male cuticular lipids before application of the test sample; hexane-extracted male antennae do not elicit courtship in adult males. A hexane solution of a test sample (3 μl, equivalent to the cuticular extract of 0.5–1 nymph) was then applied to the distal 1 cm of the test antenna. The hexane was allowed to evaporate and the antenna was used immediately to test the responses of several groups of 10 males, 14–21 days old, which were housed individually in 9 × 9 × 7.5 cm plastic cages supplied with rat chow and water. A similar procedure was conducted using nontreated antennae excised from male and female individuals throughout nymphal and adult development, to test their capacity to stimulate courtship. We used antennae taken from nymphs at different developmental stages throughout each stadium, including newly moulted nymphs,



1. 3,11-dimethylnonacosan-2-one

4. 3,11-dimethylheptacosan-2-one

2. 29-hydroxy-3,11-dimethylnonacosan-2-one

5. 27-hydroxy-3,11-dimethylheptacosan-2-one

3. 29-oxo-3,11-dimethylnonacosan-2-one

6. 27-oxo-3,11-dimethylheptacosan-2-one

Figure 1. Components of the contact sex pheromone of female *B. germanica*.