



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.

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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; Rutherford & 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 C₂₇ 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; Elyahu 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 (Elyahu 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* (Elyahu 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,

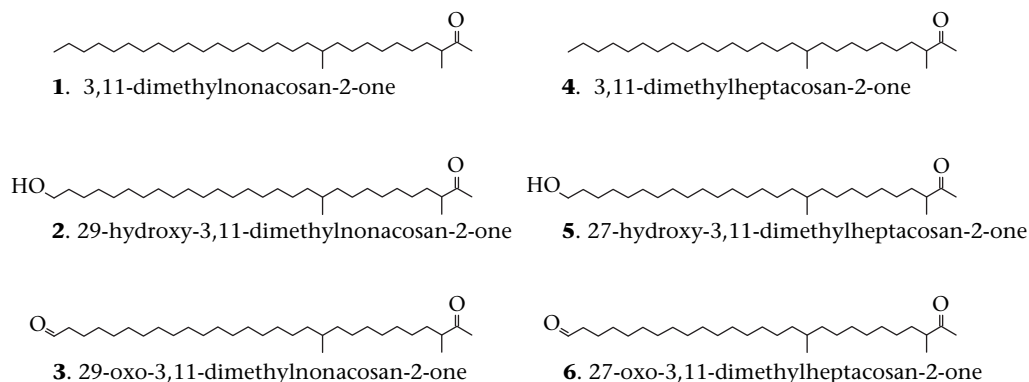


Figure 1. Components of the contact sex pheromone of female *B. germanica*.

early stadium feeding-stage nymphs, and late stadium nonfeeding nymphs that were preparing for the next moult. All assays were conducted during mid-scotophase, avoiding the first and last two hours of the scotophase. A positive response was recorded when the male executed a courtship response, rotating his body relative to the stimulus and raising his wings within 30 s. This is an unmistakable response that occurs only in a sexual context.

For measuring feeding on tergal secretions, nymphs that were starved for 0, 24, and 48 h were introduced to individually housed males as described above, except that food and water were removed prior to introduction of nymphs. Nymphs cease feeding before they moult, so only nymphs with a flattened abdomen, before they fed after the moult, were selected for this experiment. The amount of time that each nymph spent feeding, or attempting to feed, was recorded during a total of 120 s in which the male courted and thus exposed his tergal glands in close proximity (<1 cm) to the nymph's antennae or mouth parts.

Extraction and Chemical Fractionation

Nymphs of various ages were separated by sex and extracted in groups of 100 in a 20 ml vial with ~6 ml hexane for 1 min. The extract was transferred to a new vial and dried under a gentle stream of high purity N₂ to ~100 µl. The extract was then fractionated by flash chromatography: disposable borosilicate glass Pasteur pipettes with 200 mg of chromatographic silica gel (100–200 mesh, Fisher Scientific, NJ, U.S.A.) were activated at 110 °C for 30 min and washed with ~1 ml hexane (optima, Fisher Scientific) prior to application of the extract. The extract was eluted with 4 ml hexane, 2 ml of increasing amounts of diethyl ether (Fisher Scientific) in hexane (1, 2, 5, 10, 20 and 40% diethyl ether) and 2 ml of diethyl ether, 2 ml of ethyl acetate (optima, Fisher Scientific) and 2 ml of methanol (HPLC grade, Fisher Scientific). Each chromatographic fraction was tested in the behavioural assay on at least 30 males.

Behaviourally active fractions were further fractionated with high-performance liquid chromatography (HPLC). In normal-phase HPLC (Partisil silica column, 250 × 4.6 mm, 5 µm), the sample was eluted at 1 ml/min with a constant mix of 99% hexane and 1% 2-propanol (both HPLC grade, Fisher Scientific). One-minute fractions (1 ml) were collected, gently blown down to dryness, resuspended in hexane and behaviourally tested on at least 30 adult males. Because the *B. germanica* pheromone components

(Fig. 1), and related compounds, cannot be monitored with a UV detector, we used an internal standard, supellapyrone (Charlton et al. 1993), to clearly delineate the retention times of fractions.

Chemical Analysis

We used an HP6890 GC coupled to an HP5975 mass selective detector (Agilent, Palo Alto, CA, U.S.A.) to identify chemical structures in bioactive fractions. The GC was operated with splitless injection and fitted with a 30 m × 0.25 mm ID × 0.25 µm film thickness DB-5ms column (Agilent). The oven was programmed from 60 °C to 300 °C at 15 °C/min after an initial delay of 2 min, and held at 300 °C for 20 min. Injector temperature was 280 °C, MS quad 150 °C, MS source 230 °C, and transfer line 250 °C. Oxodimethyl ketones were derivatized with 1,1-*N,N*-dimethylhydrazine (DMH, 98%, Sigma-Aldrich, St Louis, MO, U.S.A.) for added thermal stability prior to GC-MS analysis. Behaviourally active HPLC fractions were dried under N₂ to ~50 µl in a conical glass reaction vial and 5 µl of DMH were added. The vial was incubated in a 60 °C glass bead bath for 30 min. Presence of DMH-derivatized oxodimethyl ketones was confirmed by selected ion monitoring (SIM) MS mode, monitoring *m/z* 72 (ketone group), 86 (DMH adduct) and 127 for both aldehyde compounds, 435, 462 and 506 (M+) for compound 3 and 407, 434 and 478 (M+) for compound 6. Sexually mature adult females were similarly extracted and their extract fractionated and treated in a similar manner for comparison. Compounds were also compared with authentic samples of the dimethyl ketones, prepared as reported by Mori (2008).

Statistical Analysis

We compared the mean times that nymphs spent feeding on tergal glands of a courting male using one-way ANOVA and Tukey-Kramer HSD in JMP 7.0 (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Nymphs Elicit Courtship throughout Their Development

Antennae taken from both male and female nymphs of various ages elicited courtship responses in 80–100% of adult males (Fig. 2). As expected, teneral adults also elicited courtship responses, especially when still unsclerotized. The capacity of female antennae

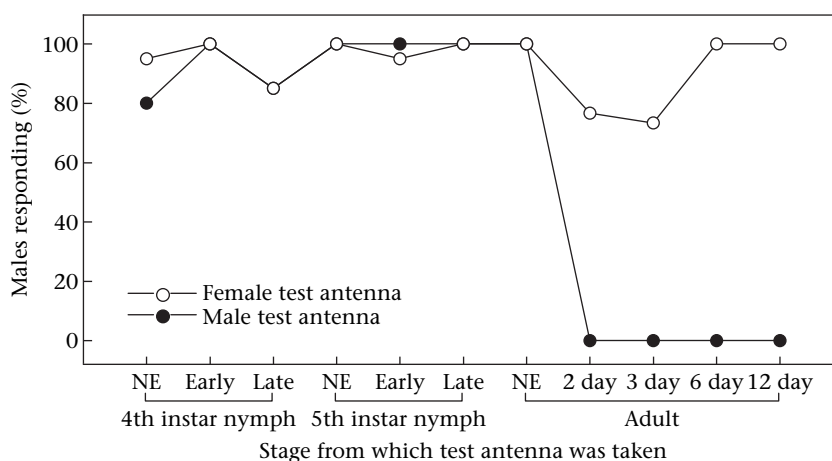


Figure 2. Percentage of adult males ($N = 30$ per data point) responding to test antennae taken from males and females of various stages in development. NE = newly emerged within 6 h of the moult; 'early' = feeding-stage nymph; 'late' = nonfeeding premoult nymph.

to stimulate courtship diminished slightly with age, only to increase again to a maximum level as they reached sexual maturity 6 days after eclosion. Adult males completely lost the capacity to elicit courtship 2 days after emergence (Fig. 2).

Fractionation of Courtship-eliciting Compounds in Nymphs

Figure 3 depicts the activity of extracts of adult females (as pheromone standards), males and nymphs following fractionation by flash chromatography. Three bioactive fractions were consistently recovered from hexane extracts of adult females: the 2% diethyl ether fraction contained the C₂₇ and C₂₉ dimethyl ketones (compounds 1 and 4), while the more polar 20% diethyl ether fraction contained the 27- and 29-oxo-dimethyl ketones (compounds 3 and 6; aldehydes) and the 100% ether fraction contained the most polar compounds, 27- and 29-hydroxy-dimethyl ketones (compounds 2 and 5) (Fig. 3a). Each of these three fractions elicited nearly 100% response in males at a dose equivalent to the extract of 0.05 of one female. As expected, extracts of adult males did not elicit courtship responses, even at 10-fold higher amounts (Fig. 3b). Two active fractions were recovered from the extracts of last-instar females, one corresponding to the dimethyl ketone fraction and the other to the oxo-dimethyl ketone fraction (20% diethyl ether) of adult females (Fig. 3a). The behaviourally active compounds that eluted with 10% ether appeared to be the early eluting leading edge of the 20% ether fraction because active compounds did not consistently elute in this fraction. Extracts of last-instar male nymphs, on the other hand, contained only a single active fraction, eluting in the same fraction as the oxo-dimethyl

ketones of the adult female (Fig. 3b). Extracts of early instar female and male nymphs contained the same behaviourally active fraction at 20% ether as last-instar nymphs.

Courtship-eliciting Compounds in Last-instar Female Nymphs

The behaviourally active 2% ether fraction from last-instar females was further fractionated by HPLC (data not shown). Two dimethyl ketones, identical to those of the adult female (compounds 1 and 4), were found in the active HPLC fraction when analysed by GC-MS (Fig. 4b), and the identities were confirmed using standard chemicals (Fig. 4c, d). The amounts of 3,11-dimethylheptacosan-2-one and 3,11-dimethylnonacosan-2-one were estimated at 2 and 4 ng per female nymph, respectively. This corresponded well with behavioural dose-response studies showing that ~1 ng 3,11-dimethylnonacosan-2-one elicited responses in 50% of males, and this response increased sharply to 100% at ~2 ng (Elyahu et al. 2004).

The behaviourally active 10% and 20% ether fractions were combined and the resulting fractions of both adult females and last-instar female nymphs were further fractionated by HPLC. Both adult females and last-instar female nymphs had two active HPLC fractions, one eluting in the same fraction as the two oxo-dimethyl ketones (compounds 3 and 6), 1 min before the supellapyrone internal standard, and a second, new active component eluted 2 min after the internal standard (data not shown). The behaviourally active compound(s) in the latter HPLC fraction remains to be identified.

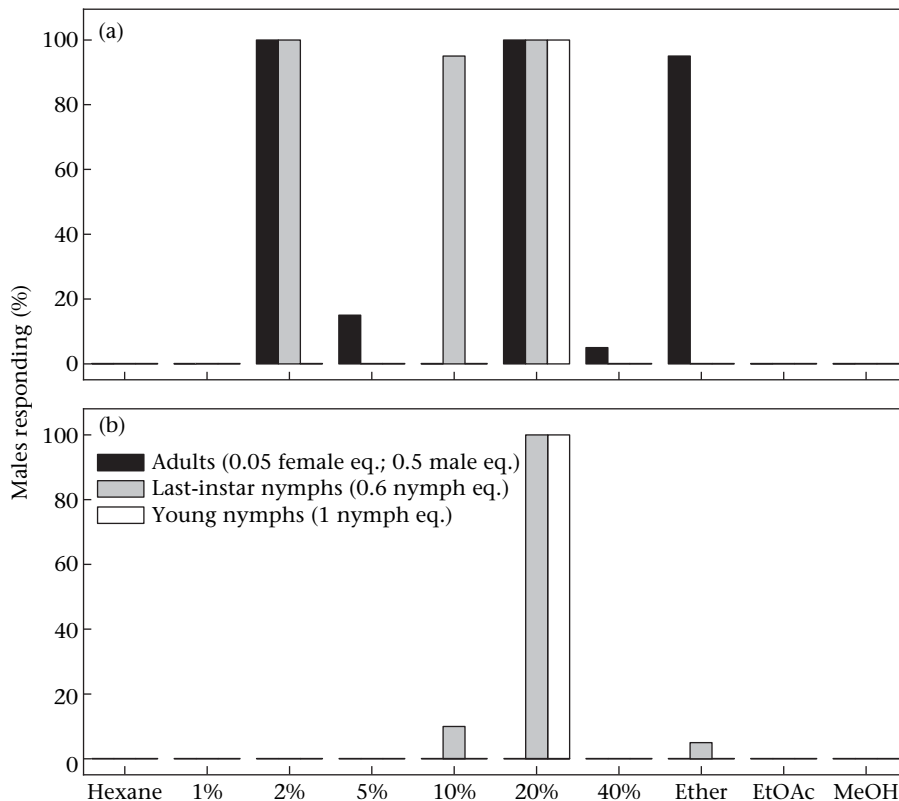


Figure 3. Percentage of males ($N > 90$ per fraction) responding to flash chromatography fractions of extracts from females and males. (a) Fractions from adult females were assayed at 0.05 female equivalents, whereas fractions from last-instar female nymphs and early instar female nymphs were tested at 0.6 and 1.0 nymph equivalents, respectively. (b) Fractions from adult males, last-instar males and early instar males were assayed at 0.5, 0.6 and 1.0 insect equivalents. Numbers (fractions) between hexane and ether represent the percentage of ether in hexane. EtOAc = ethyl acetate; MeOH = methanol. All fractions were assayed with all the indicated life stages, and zero response to each tested fraction is indicated by a line at zero response.

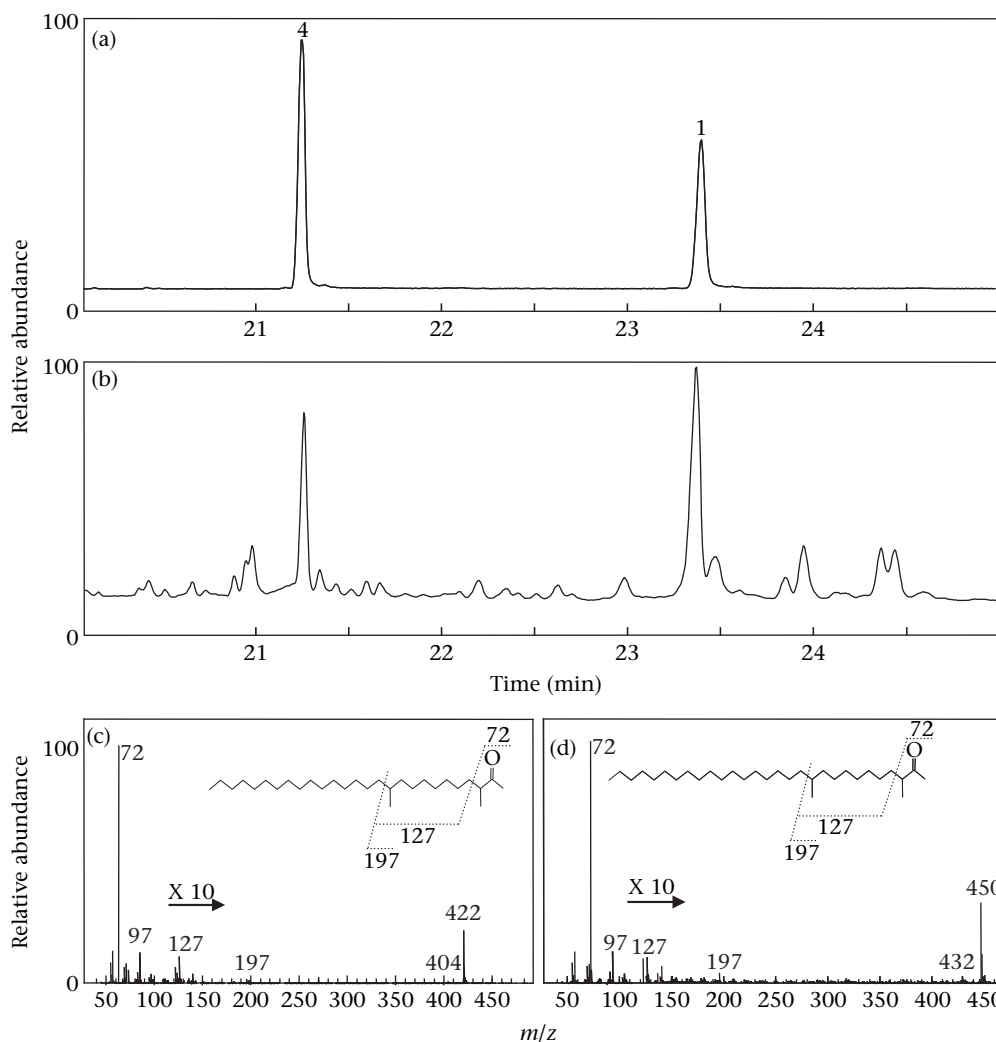


Figure 4. (a) Total ion chromatogram of 100 ng of the synthetic 3,11-dimethyl ketones 1 and 4. (b) The 2% ethyl ether fraction of extract of 100 last-instar female nymphs fractionated by flash chromatography. (c, d) Electron ionization mass spectra of peaks in the nymph extract corresponding with the authentic compounds 4 and 1, respectively.

The female nymph fraction corresponding to the oxo-dimethyl ketones was analysed in SIM mode on the GC-MS following derivatization with DMH to add thermal stability to the compounds and increase the detection limits for their reliable identification. Both 27-oxo-3,11-dimethylheptacosan-2-one (compound 6) and 29-oxo-3,11-dimethylnonacosan-2-one (compound 3) were detected in extracts of both adult females and last-instar female nymphs. The identities of both compounds were confirmed using standard chemicals (Fig. 5). These results demonstrate that last-instar female nymphs contain both C₂₇ and C₂₉ homologues of the aldehyde pheromone, as do adult females.

Courtship-eliciting Compounds in Young Nymphs and in Last-instar Male Nymphs

The behaviourally active flash chromatography fractions from extracts of last-instar males (10% and 20% diethyl ether; Fig. 3) were combined and further fractionated by normal-phase HPLC. A single behaviourally active fraction eluted 2 min after the internal standard, at the same retention time as the newly discovered courtship-eliciting compound(s) found in last-instar and adult females. This fraction was also active in extracts of younger male and female nymphs, showing that all life stages of the German cockroach have

one or more unidentified courtship-stimulating compounds in this 1 min (=1 ml) HPLC fraction. Structural elucidation of this 'universal courtship elicitor' is underway.

Relationship between Starvation and Illicit Feeding on Male Tergal Secretion

Both fed and unfed last-instar nymphs were vigorously courted by adult males. However, last-instar nymphs that were starved for 48 h spent significantly more time feeding, or attempting to feed, on male nuptial secretions than did satiated nymphs ($N = 20$; one-way ANOVA followed by Tukey–Kramer HSD: $P < 0.01$). An intermediate period of starvation, of only 24 h, resulted in an intermediate amount of feeding relative to the other two treatments that was not significantly different from either treatment (Fig. 6).

Discussion

Sexual communication in the German cockroach involves reciprocal chemical signalling between the female and male. Ultimately, upon perceiving a blend of six contact sex pheromone components on the female's cuticle, the male orients his abdomen forward of the

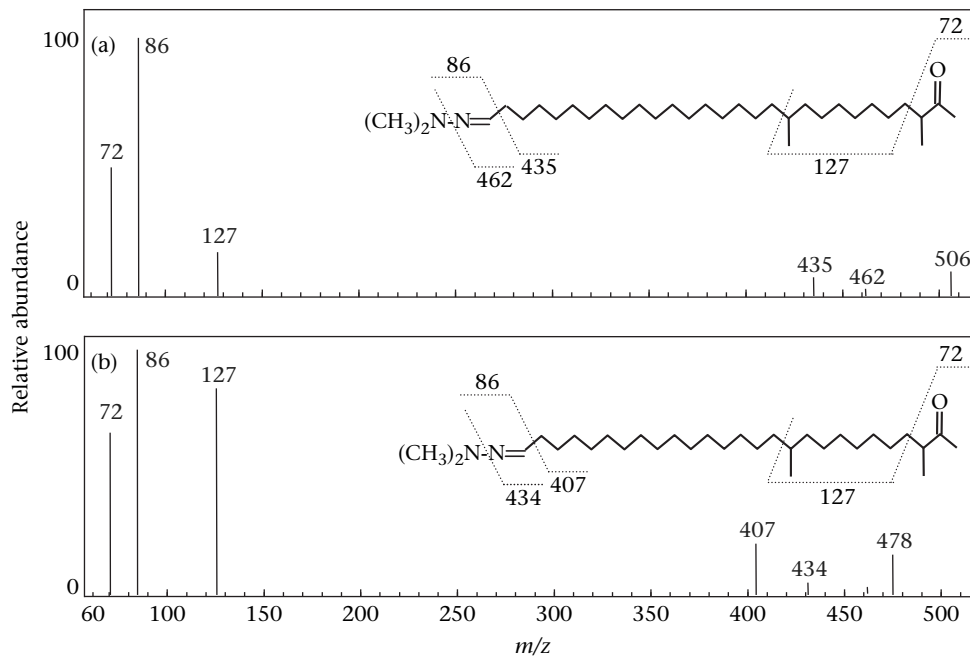


Figure 5. Selective ion mass spectra of two compounds recovered from the HPLC fraction eluting 1 min before the internal standard. A cuticular extract of 200 last-instar female nymphs was fractionated by flash chromatography, and the 10% and 20% ether fractions were combined and fractionated by normal-phase HPLC. The behaviourally active 1 min (= 1 ml) fraction was derivatized with DMH, resulting in the mass spectra corresponding to (a) authentic compound 3 and (b) authentic compound 6.

female's head and presents her with tergal secretions that act as a nuptial gift and arrests the female in a position appropriate for copulation. Our results demonstrate that immature males and females of this cockroach have infiltrated the sexual communication system, and thus may profit from their illicit access to the male's sexual offerings, which are normally covered by his wings.

Courtship of immature animals is an uncommon phenomenon in natural systems, and in most cases, neither proximate nor ultimate mechanisms have been elucidated. Three prominent examples, all in insects, are grubs of the southern masked chafer *Cyclocephala lurida* (Haynes et al. 1992), immature males of the staphylinid beetle *Aleochara curtula* (Peschke 1985), and late-emerging adult males of the parasitic wasp *Lariophagus distinguendus* (Steiner et al. 2005). In all three cases the mimic resembles adult females, possibly by producing female sex pheromone.

In contrast, we found that while males and females of all nymphal stages of the German cockroach can elicit sexual responses in adult males, only last-instar females produce some of the adult sex pheromone components; earlier instars elicit sexual displays with novel compound(s) that are not known to be components of the female contact sex pheromone. We suggest that two major forces have shaped this complex mimicry system. First, the mating system of the German cockroach favours highly sexually responsive and promiscuous males within aggregations that are numerically dominated by sexually unreceptive females because of a long period of gestation. Second, the unique human-built environment, characterized by ephemeral food resources, favours nymphs that opportunistically acquire resources, including male nuptial gifts.

Adult Female Sex Pheromone Components in Last-instar Females

Roth & Willis (1952) found that *B. germanica* males courted teneral (newly moulted) male and female nymphs, teneral adult females, as well as the isolated antennae of newly emerged adult

males. We have extended these observations and show that nymphs of all ages and stages elicited male courtship, but not all used the same stimuli.

Last-instar female nymphs, and no other nymphal stage, produced four of the six contact sex pheromone components of sexually mature females, albeit at much smaller quantities. Using analytical approaches, we could not detect the two alcohols, 27- and 29-hydroxy-dimethyl ketones (compounds 2 and 5), which presumably serve as metabolic precursors of the oxo-dimethyl ketones (Schal et al. 1991). Nearly 100% of males responded to the ethyl-ether fraction of adult females, which contains hydroxy-dimethyl ketones, but none responded to the equivalent fraction from last-instar female nymphs. Nevertheless, we refrain from the conclusion that these long-chain alcohols are absent in last-instars because fatty acids in this fraction might have masked the behavioural activity of the pheromone, as has been shown for 3,11-dimethylnonacosan-2-one (Nishida & Fukami 1983).

Behavioural activity of the HPLC fraction that contained the two oxo-dimethyl ketones was as high as that of the less polar fraction containing the two dimethyl ketones. Yet the aldehydes were represented in much smaller amounts in both last instars and adult females than were the dimethyl ketones (Elyahu et al. 2008b). These results could be explained by several observations. First, the oxo-dimethyl ketones are rather unstable and readily decompose even at ambient temperature during HPLC fractionation and solvent removal, and therefore are less represented in analytical procedures. Second, the 1 min HPLC fraction that contains these aldehydes might also contain related, yet unidentified, compounds that contribute to its overall courtship-stimulating bioactivity. We know, for example, that the cuticular surface of the cockroach *Blattella orientalis* contains 27-oxo-11-methylheptacosan-2-one, which can elicit courtship in *B. germanica* males (Elyahu et al. 2008a). The cuticular surface of nymphs and adults of the German cockroach contains both 11-methylheptacosane and 11-methylnonacosane (Jurenka et al. 1989), which could be metabolized to the respective alcohol and aldehyde analogues.

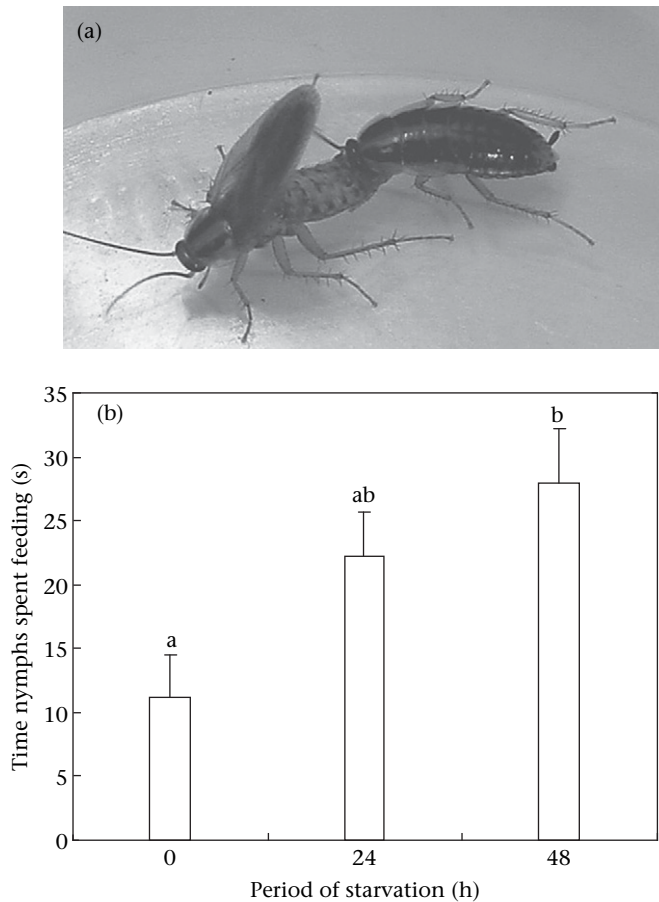


Figure 6. (a) A nymph (right) feeding on tergal secretions exposed by a courting male (left). (b) Mean \pm SE time spent by satiated and starved nymphs feeding or attempting to feed on tergal secretions of a courting male. Different letters indicate statistically significant difference ($N = 20$, one-way ANOVA followed by Tukey–Kramer HSD: $P < 0.01$).

It is important to mention that in conducting these experiments we adopted special precautions to avoid chemical cross-contamination. We used nymphs that had no contact with adults since hatching, and nymphs were sexed and separated before they moulted to the fourth instar. Also, every GC-MS analysis of nymph extract was preceded by a blank run. Hence, the sex pheromone analogues found in last-instar female nymphs could not have originated from contamination from adult females.

Because these compounds were not detected in earlier instars, we propose that their appearance in last-instar female nymphs is part of the gradual progress towards the sex-specific adult phenotype. Sexual dimorphism of several morphological and physiological features becomes evident in last instars, including body size and shape, corpora allata (which produce juvenile hormone) morphology (Chiang et al. 1991), production of juvenile hormone and its release into the haemolymph (Treiblmaier et al. 2006), and differentiation of the fat body so that upon stimulation by juvenile hormone, the fat body of last-instar females can synthesize small yet significant amounts of vitellogenin, an adult female-specific yolk protein precursor (Kunkel 1981).

Courtship-eliciting Compounds from Young Nymphs and Last-instar Male Nymphs

Last-instar male nymphs and younger nymphs of both sexes do not produce any of the contact sex pheromone components

identified in last-instar and adult females. Rather, they possess novel, unidentified courtship-eliciting compound(s) with similar chromatographic characteristics to those found in adult females (Elyahu et al. 2008b). These results indicate that adult females have at least one more pheromonal component that remains to be identified, and that this compound appears to serve as a 'universal courtship elicitor' in this species.

Why Engage in Intraspecific Sexual Mimicry?

The adaptive value of sexual mimicry by immatures has not been studied extensively, and it appears to be different in each case where such mimicry occurs. The following four scenarios may be invoked to explain sexual mimicry by immatures: (1) sexual cues produced by immatures are by-products of normal metabolism and they serve no adaptive value; (2) immatures procure nutrients from courting males or access to territorial resources controlled by adult males; (3) by eliciting courtship, immatures deflect aggression by adult males; and (4) in cases where adult males are a limited resource, immature females might exploit adult males as future mates. A special case may be 'play' or 'practise' courtship, as shown in some mammals, but this is unlikely in insect larvae and nymphs. Under any of these scenarios, vigorous courtship would be directed towards immatures in cases where the male's sexual threshold is particularly low because of a unique coupling of copulation and metabolism, as in the German cockroach (see below).

In the case of attractive immature grubs of *Cyclocephala lurida*, males normally do not come in contact with grubs (adults live above ground and grubs live underground), and therefore the larval courtship-inducing chemicals appear to have no communicative function. Haynes et al. (1992) suggested the sex pheromone evolved from larval odour, but since neither immature nor adult compounds have been chemically identified, this hypothesis remains untested, as is any speculation on the adaptive value of sexual mimicry by *C. lurida* grubs.

Similarly, in the parasitic wasp *Lariophagus distinguendus*, young adult males produce the female sex pheromone (Steiner et al. 2005; Ruther & Steiner 2008). This was shown to be costly for male mimics, leaving them unavailable to court females while they were homosexually courted by other males. It was therefore concluded that the courtship-eliciting chemicals have a non-sex-specific role, and young males are under selection pressure to inactivate or mask the chemical signals, as they get older, while females retain them and use them as a sex pheromone.

In the staphylinid beetle, *Aleochara curtula*, sexually immature adult males produce the female pheromone, and there is some evidence that this might protect them from high levels of intrasexual aggression (Peschke 1985). Many arthropod species inflict damage on vulnerable teneral individuals, especially under conditions of high density and scarceness of food. A few examples are the assassin bug, *Triatoma rubrofasciata* (Cortéz & Gonçalves 1998), the spiny lobster, *Panulirus japonicus* (Matsuda et al. 2003), the spider, *Amaurobius ferox* (Kim 2001), the corn earworm, *Helicoverpa zea* (Dial & Adler 1990), and locusts, *Schistocerca gregaria*, and cockroaches, *Blaberus atropos* (personal observations). In the lobster cockroach, *Nauphoeta cinerea*, adult males engage in high levels of intrasexual aggression that can even escalate to killing the opponents. But highly aggressive territorial males also show courtship responses upon contact with teneral individuals, both nymphs and adults (Schal & Bell 1983), suggesting that intraspecific sexual mimicry evolved in these vulnerable individuals to avert potentially fatal agonistic interactions. Moreover, at low density, *N. cinerea* males are highly territorial, and by deflecting aggression and stimulating courtship, nymphs may gain access to territorial resources. It is therefore possible that newly emerged *B. germanica*,

both nymphs and adults, also use sexual mimicry to deflect aggression and cannibalism at this vulnerable stage.

Fully sclerotized nymphs of the German cockroach, on the other hand, are less vulnerable to damage from adult males. Under normal conditions in the laboratory, German cockroach males are relatively docile compared to gravid females (Breed et al. 1975). Nevertheless, adult males experience strong selection pressure to forage and procure proteins as part of a paternal investment strategy, and when food is scarce, small nymphs may become vulnerable. Towards the conclusion of copulation, adult males transfer to females a urate plug that serves as a paternal nutritional investment in the offspring (Mullins et al. 1992). Females can metabolize this postnuptial gift and incorporate its constituent nitrogen into amino acids. Moreover, females deficient in nitrogen incorporate more male-derived products into their offspring, indicating that the male's contribution may play a significant role in offspring survival and the mother's fitness (Mullins et al. 1992). A similar situation occurs in another blattellid, *Xestoblatta hamata* (Schal & Bell 1982), suggesting that in cockroach taxa, including *Blattella*, whose adult males possess well-developed uricose glands, there might be a premium on protein foraging or urate sequestration by adult males. Males with urate-laden accessory reproductive glands also are under strong pressure to mate because copulation is the only means to void potentially toxic urates. Therefore, it might not be difficult to elicit courtship behaviour from protein-rich males with a relatively low threshold for both mating and urate excretion, while eliciting courtship in aggressive protein-seeking males might protect nymphs when food resources are scarce.

Although defensive benefits cannot be ruled out, our results suggest that sexual mimicry may also provide nutritional benefits. Often, courted nymphs feed on the tergal secretion of the courting male (Fig. 6a), and males appear not to discriminate between a mounting adult female and a nymph. Moreover, starved nymphs spent more time than satiated nymphs feeding and attempting to feed on tergal secretions of courting males (Fig. 6b). The tergal gland secretion is composed of sugars (maltose and other oligosaccharides) in relatively large amounts (Nojima et al. 1999, 2002) in addition to proteins, phospholipids, fatty acids and hydrocarbons (Kugimiya et al. 2002). Note that the male's wings normally conceal the tergal secretion, which becomes accessible only during courtship. Receptive females appear to make mate choices based upon sampling this secretion (C. Schal, unpublished data), suggesting a strong selection pressure on males to forage and stockpile sufficient secretion in their large tergal reservoirs. Interestingly, the male may protect his tergal secretion against overexploitation by the adult female; the cuticular openings of the eighth tergal reservoir are small relative to the size of the female's mandibles (Nojima et al. 1999). Because nymphs have smaller mouth parts, they may gain greater access to the secretions and, in turn, acquire proportionally more nutrients. It is also useful to consider the hypothesis that the size of the tergal openings in males is subject to opposing selection forces: sexual selection may select for larger openings and more nuptial secretion, but sexual mimicry and exploitation of male resources by nymphs may provide strong directional selection towards smaller, more restricted openings.

A recent report concluded that although female *Leucophaea* (= *Rhyparobia*) *maderae* normally feed on male tergal secretions during courtship, females preferred to mate with males whose secretions had been washed away over intact males (Mondet et al. 2008). In addition, the male secretions appeared to have detrimental effects on female longevity, and no apparent benefit to her offspring (Mondet et al. 2008). Nevertheless, the *Leucophaea* tergal glands lead to single pores (secretory units) being widely distributed throughout the male's tergum, whereas in *Blattella* the gland has become highly specialized with large secretory reservoirs.

Moreover, in the German cockroach, attractiveness of the secretions is considered an important step in mating (Roth & Willis 1952; Nojima et al. 1999). The critical importance of this secretion in facilitating mating, coupled with the observation that adult males are more mobile than small nymphs, would suggest that *Blattella* nymphs may accrue significant benefit from deceiving males to offer them these prenuptial gifts, especially when food is scarce.

This sexual deception, and the male's ready acceptance of immatures as potential mates, could be further reinforced by the male-biased operational sex ratio in this species; adult females incubate the egg case for about 20 days and are sexually receptive for only 2–3 days during each 25-day gonotrophic cycle. Moreover, females store sperm and may remain completely unreceptive for several consecutive reproductive cycles, further exacerbating the male-biased operational sex ratio. Hence, the chance of obtaining a mate is low for most males, and males might have evolved a bet-hedging strategy based on relatively nondiscriminating courtship of members of its aggregation.

Although the operational sex ratio in established populations of the German cockroach is greatly skewed towards males, adult males may represent a limited resource in founding populations. Under the latter conditions, a last-instar female may benefit from engaging adult males in courtship until she ecloses and sexually matures. Although possible, we consider this a rather unlikely scenario: on average, females mate 5.7 days after adult eclosion (Schal & Chiang 1995), and there is no evidence that sexually immature females or nymphs can engage adult males in courtship for such a protracted duration.

Further studies are needed to illuminate the chemical cues that stimulate courtship of immatures in the German cockroach, the functions of this behaviour, and other possible adaptive values to the courted nymphs and detrimental effects on the courting male. How common is this phenomenon in nature? To what extent can males afford to give away their tergal secretion? How effective is deceitful stimulation of courtship at preventing aggression? More generally, how common is this phenomenon of courtship of immatures among cockroaches and related insects? Courtship is the first step in the reproductive process, and a better understanding of the former is needed to fully comprehend the latter.

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