Animal Behaviour 78 (2009) 1471–1475

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Homosexual interactions in bed bugs: alarm pheromones as male recognition signals

Camilla Ryne*

Department of Ecology, Lund University

ARTICLE INFO

Article history:
Received 6 February 2009
Initial acceptance 27 March 2009
Final acceptance 17 September 2009
Available online 24 October 2009
MS. number: 09-00079R

Keywords:
alarm pheromone
bed bug
Cimex lectularius
Cimicidae
sex recognition

Homosexual mounting is a common behaviour in bed bugs as male sexual interest is directed towards any newly fed individual. The only mode of copulation in the common bed bug, *Cimex lectularius*, is by traumatic insemination, where the male pierces the female abdomen with his needle-like penis. Homosexual mating would result in abdominal injuries in mounted males, as males lack the female counteradaptive spermalege structure. I here show that bed bug alarm pheromones, previously hypothesized to be a predator chemical defence, can be used by newly fed males to signal their sex and reduce the risk of homosexual mating. Mechanical blocking of the male pheromone glands significantly increased homosexual mounting duration compared to control males, while applying male extracts containing mainly alarm pheromone onto male–female mating pairs completely interrupted or shortened mating duration and reduced sperm transfer. Males confined with other males received piercing scars, demonstrating that homosexual mating occurs. The focal males in the all-male confinement experiment had reduced longevity compared to singly held males, but why this reduction in longevity occurred is not clear. Mounted males thus benefit from being able to discharge alarm pheromones, while mounting males consider the alarm signal a major sex identification cue, suggesting that male bed bugs use alarm pheromone communication to avoid homosexual harassment and mounting.

Homosexual behaviour implies direct and indirect costs for both participants without any obvious fitness benefits, yet the behaviour persists in populations and across animal classes (Edwards & Todd 1991; Harari et al. 2000). In social mammals, male–male mounting has traditionally been associated with the assertion of dominance, in which homosexual behaviour maintains social rank resulting in increased access to females for dominant males (Holeckova et al. 2000). However, several studies show that such mounting behaviour may not be an act of dominance (Reinhardt et al. 1986; Edwards & Todd 1991; Bernstein & Cooper 1999; Holeckova et al. 2000), and therefore does not lead to an increase in fitness. In bed bugs, *Cimex lectularius* (Hemiptera; Cimicidae (L.)), a potential additional cost of homosexual mounting would be wounding from traumatic insemination behaviour (Siva-Jothy 2006). In those animals in which sex-specific external characters are not apparent, such as bed bugs and other nocturnal animals, a signalling system to communicate sexual characteristics would reduce the cost of unnecessary fights or energy losses from mistaken identity (Harari et al. 2000; Switzer et al. 2004).

Common bed bugs are nocturnal, blood-feeding ectoparasites of humans that mate and lay eggs in close proximity to their hosts (Carayon 1966). The only mode of mating is through traumatic insemination (Carayon 1966) in which the male pierces the female through the abdomen at a specific site and ejaculates into the haemocoel. The female has evolved a secondary genital opening (spermalege; reviewed in Siva-Jothy 2006; Reinhardt & Siva-Jothy 2007) that decreases the immunological cost of being wounded (pierced) during insemination (Morrow & Arnqvist 2003; Reinhardt et al. 2003, 2005).

Mating is closely associated with feeding, since feeding causes an increase in body size and males are attracted to any large individual regardless of sex: it is the bloated body that increases the attractiveness (Reinhardt & Siva-Jothy 2007). Mating behaviour does not include long-distance attraction; instead males rapidly mount any large, newly fed nearby individual. The male then folds his abdomen underneath the mounted individual and probes with the paramere (penis). After this sequence of mounting, the male decides either to continue to mate or to dismount (Siva-Jothy 2006); thus sex identification is likely to occur after mounting.

Many insects use pheromones for communication (e.g. Wyatt 2003). Thus far, it has been established that bed bugs produce alarm pheromones as a defence against predation (Levinson et al. 1974a, b). In high doses, alarm pheromones cause increased activity

* Correspondence: C. Ryne, Department of Ecology, Lund University, Sölvegatan 37, SE-223 62 Lund, Sweden.
E-mail address: camilla.ryne@ekol.lu.se

0003-3472/$38.00
doi:10.1016/j.anbehav.2009.09.033

and dispersal in nymphs and adults. The alarm pheromone consists of two components, (*E*)-2-octenal and (*E*)-2-hexenal, that make up 78% of the 15 different substances found in whole body eluates (Siljander et al. 2008). The alarm pheromone is obvious and easily recognized by the human nose during emission (C. Ryne, personal observations).

Male–male interactions and mounting often result in the clearly detectable emission of alarm pheromones. The characteristic smell is, however, rarely detected in male–female interactions. Thus, the release of alarm pheromone and the behavioural responses to this signal appear to differ between males and females. I hypothesized that alarm pheromone perception by males influenced mounting behaviour and predicted that (1) addition of pheromones (i.e. mimicking male–male interactions) would decrease mating duration between males and females, and (2) failure to emit alarm pheromones (i.e. mimicking male–female interactions) would increase mounting duration in male–male interactions. The hypothesis concerning the usage of chemical signals as behavioural deterrents is predicated on an existing homosexual behaviour. To evaluate further whether homosexual encounters, such as piercing, occur at all between males, I hypothesized that male interactions would result in visible piercing scars (melanization; Siva-Jothy 2006) in the mounted (focal) male.

METHODS

Experimental Insects

The bed bug stock culture was provided by the Department of Animal and Plant Sciences, Sheffield University, and maintained at the Department of Ecology, Lund University, Sweden. The bed bugs were reared on weekly meals of fresh, defibrinated chicken blood purchased from TCS Biosciences Ltd, Botolph Claydon, Buckingham, U.K. The jars containing the bugs were fitted with a parafilm (Pechiney Plastic Packaging, Chicago, IL, U.S.A.) cover. The blood was heated to 35 °C and the jars were placed on the blood with the parafilm barrier between the blood and the bugs, enabling the bed bugs to pierce the parafilm to reach the blood. The culture was kept in a climate chamber at 70% relative humidity, 25 °C, and 12:12 h light:dark. To obtain virgins of similar age, I induced moulting of final instar nymphs with a blood meal (Carayon 1966), and the nymphs were placed into individual jars. The sex of newly moulted adults was determined under a stereomicroscope (Stutt & Siva-Jothy 2001). All adults were fed 7–10 days prior to experiments, as this is the normal feeding interval of bed bugs (Carayon 1966; Siva-Jothy 2006). I examined all individuals in all tests for presence of sperm from mating under a stereomicroscope after the experiments, since this can be observed through the cuticle (Carayon 1966).

Mating and Mounting Behaviour

Both males and females require a blood meal to produce gametes and to initiate mating behaviour (Reinhardt & Siva-Jothy 2007). *Cimex lectularius* live for approximately 200–300 days (Reinhardt et al. 2003; this study) and mate throughout adult life, with mating activity associated with feeding events. The optimal feeding frequency for *C. lectularius* is every 7–10 days; thus all males that performed the mounting in these experiments were fed at least 7 days prior to experiments. Recently fed males are unable to perform mating behaviour because of the fully bloated body, but females are mounted frequently directly after a blood meal (Siva-Jothy 2006). Experimental (focal) males and females were fed directly before the experiments to ensure that they were in the most attractive state. Mounting behaviour in this study consisted of the combined behaviours of mounting and probing with the male paramere. The two behaviours occur regardless of the focal individuals' sex and in short time intervals. Whether probing led to actual piercing was not possible to distinguish in all interactions, since the male abdomen is curved underneath the female (or male), but the duration of a mounting leading to piercing and sperm transfer is approximately 110 s in the first mating after a female blood meal (Siva-Jothy 2006). Mountings not leading to sperm transfer are usually shorter, but piercing may still occur (C. Ryne, personal observations).

Male–Male Interactions Mimicking Male–Female Interactions

The 2–3-week-old virgin males (focal) were placed dorsally in a petri dish with a silicone elastomer bottom covered with plastic foil for immobilization. The males were randomly divided into two groups: (1) operated (treatment) and (2) sham operated (control). I applied nail polish to the two large metathoracic glands between the first and second pair of legs through a small hole in the plastic foil to cause a mechanical blockage of the glands, which hinders the emission of alarm pheromones (operated group, blocked glands; Carayon 1966). The control group (sham operated) had nail polish applied between the second and third pair of legs. When the nail polish was dry, I allowed the males to feed from my arm immediately prior to the experiment. The experiment was conducted at room temperature in dimmed light. One fully satiated male (focal) was placed in a petri dish with a filter paper covering the bottom to facilitate movement, and an unfed male was introduced. Each mounting on the focal male was timed and the number of mountings was recorded in all treatments since all focal males were mounted several times as the introduced male's attention remains unchanged between mountings. The experiment lasted 3 min. Petri dishes were thoroughly cleaned and the filter paper was replaced between replicates.

Male–Female Interactions Mimicking Male–Male Interactions

Extracts and delivery system

Extracts were obtained by submerging 10 virgin males, 1 month old, in 1 ml of hexane (99% purity) for 30 min. The solvent was recovered and transferred into a new glass vial and was kept in a freezer between trials. Pure hexane (10 µl; treatment 2) or extract (treatment 3) was placed on a 0.5 cm^2 filter paper inside glass pipettes with a 5 ml rubber bulb as a delivery system for the volatile chemicals during mounting behaviour (see below).

Behavioural experiments

I used 2–3-week-old males and females, fed at least 7 days prior to experiments. Mating trials were conducted in dimmed light and at normal room temperature. The focal female bed bugs were randomly divided into three treatments groups: (1) male + female (control group), (2) male + female with hexane stimulus (solvent control), and (3) male + female with male extract stimulus (treatment). All the females were blood fed directly before the experiments. The fed female was introduced to a virgin male, which performed the mounting behaviour. The chemicals were delivered by a continuous manual puffing when the male mounted the female, creating an intermittent air flow containing volatile chemicals. Only the first mounting duration was timed in all three treatments, as male attention decreased after first mounting/mating.

Male Mating Scars

One-week-old males, previously held individually, were marked with correction fluid (Tip-ex) and randomly divided into two