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Behavioral and electrophysiological studies on the sexually biased synergism between oligosaccharides and phospholipids in gustatory perception of nuptial secretion by the German cockroach

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ABSTRACT

Females of *Blattella germanica* feed compulsively on a nuptial secretion from the male's eighth tergal gland (TG-8) during courtship. Using TG-8 extract and its essential ingredients, maltotriose (MT) and 1,2-dioleoylphosphatidylcholine (PC), we investigated the perception of the secretion by gustatory sensilla in both sexes. Female-biased chemosensitivity was found in the feeding responses to the TG-8 extract. The TG-8 extract induced specific impulses in four functionally different receptor cells: the sugar, salt and other two types of receptor cells in a single gustatory sensillum on the paraglossae in both sexes. The impulse frequencies of the sugar receptor cell and a receptor cell of unknown type were significantly higher in females than those in males. There were no sexual differences in the behavioral and electrophysiological responses to MT alone; no responses were elicited by PC. However, a mixture of MT and PC elicited the behavioral responses more strongly than MT in females. The impulse frequency of sugar receptor cells toward the mixture of MT and PC also increased in females but not in males. These results suggest that the synergistic effect of PC on 'sweetness' of MT in the female cockroach contributes to her compulsive nuptial feeding. The female-biased gustatory sensitivity seems to ensure successful coupling during the nuptial feeding.

1. Introduction

Most cockroach species show sequential courtship behaviors which consist of mate finding, contact, release of tergal secretions in males, feeding on the male's secretions by the female, and copulation. Various courtship pheromones are used in each step of the behavioral sequence (Gemeno and Schal, 2004). In the German cockroach, *Blattella germanica*, a mature female produces a volatile sex pheromone 'blattellaquinone' (Nojima et al., 2005) and oxygenated derivatives of methyl-branched cuticular hydrocarbons on its body surface as a contact sex pheromone (Nishida et al., 1974; Nishida and Fukami, 1983; Jurenka et al., 1989; Schal et al., 1990; Chase et al., 1992; Elyahu et al., 2008). These female sex pheromones are received by the male antennae. Blattellaquinone attracts males from a distance. The contact sex pheromone on the female elicits a characteristic courtship behavior in males, which includes wing-raising and displaying the abdominal tergal glands (Fig. 1A) (Noirot and Quennedey, 1974; Brossut and Roth, 1977; Ramaswamy et al., 1980). After a male displays the tergal glands on both the seventh and eighth abdominal segments (Fig. 1B), the female mounts the male and inserts her paraglossae (Fig. 1C) into the cuticular openings of eighth tergal glands (TG-8) (Fig. 1A and B). The tergal secretion strongly elicits feeding behavior in the female. The act of female feeding on the male's tergal secretion has been described as 'nuptial feeding'. The mechanical movement of female's mandibles stimulates the male to extend his abdomen and to clasp the female's genitalia (Ramaswamy et al., 1980). If the female does not accept the tergal secretion, the male fails to bring her into the proper precopulatory position (Roth and Willis, 1952; Nojima et al., 1999a). Thus, the tergal gland secretion functions as a specific courtship pheromone in the courtship of *B. germanica* (Nojima et al., 1999a,b).

Several volatile chemicals have been reported from the secretion in *B. germanica* (Brossut and Roth, 1977; Brossut, 1983), but their function remains unknown (Brossut, 1983). On the other hand, non-volatile chemicals include mainly oligosaccharides and phospholipids. Cholesterol and various amino acids are also included (Nojima et al., 1999a,b, 2002; Kugimiya et al., 2002, 2003). These components are well known as general dietary phagostimulants among cockroach species. They play a role in the proper orientation of the female *B. germanica* (Tsuji, 1965; Nojima et al., 1996). Oligosaccharides consist of 0.24 μ (approx.

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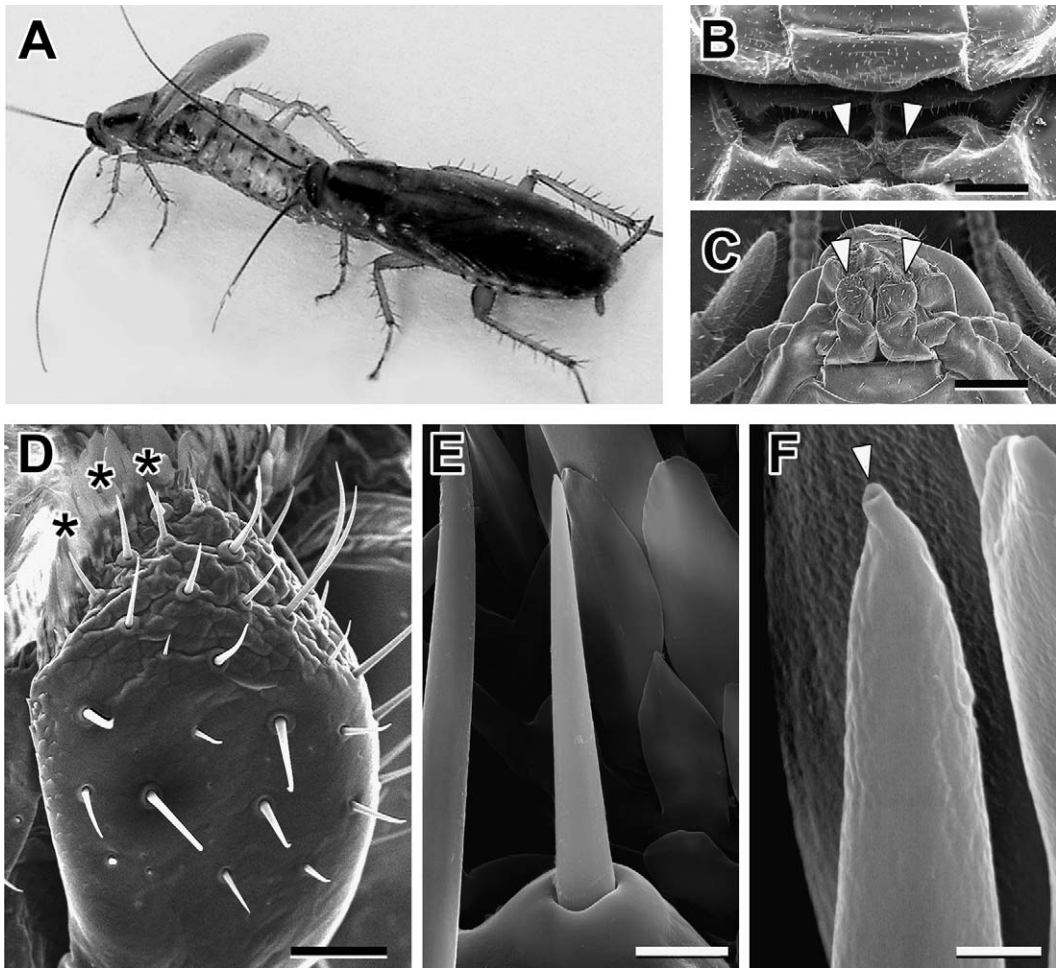

Fig. 1. Nuptial feeding and scanning electron microscopic images of the male's tergal glands and the female's chemosensory organs on the mouth parts. (A) Nuptial feeding by a female (right) on a male's (left) TG-8 secretion serves to place her in the optimum position for copulation. (B) Dorsal view of the abdomen of a male. The eighth tergal gland-openings are indicated by arrowheads. Scale bar = 200 μm. (C) Ventral view of a female's mouth parts without the labial palps. The paraglossae are indicated by arrowheads. Scale bar = 200 μm. (D) A left paraglossa of a female. The chemosensilla used in the electrophysiological tests are indicated by asterisks. Scale bar = 20 μm. (E) A TG-8 extract-sensitive sensillum on the frontal area of a female's paraglossa. Scale bar = 5 μm. (F) The tip of the sensillum shown in (E). A distal pore is indicated by an arrowhead. Scale bar = 500 nm.

18 mmol l^{-1}) of maltose, 0.15 μg (approx. 7 mmol l^{-1}) of maltotriose, 0.81 μg (approx. 40 mmol l^{-1}) of O-α-D-glucopyranosyl-(1,4)-α-D-glucopyranosyl α-D-glucopyranoside and 4 μg (approx. 200 mmol l^{-1}) O-α-D-glucopyranosyl-(1,6)-α-D-glucopyranosyl α-D-glucopyranoside as main oligosaccharides (Nojima et al., 1999b, 2002). Phospholipids consist of 7.8 μg (approx. 240 mmol l^{-1}) of phosphatidylcholines and 5.0 μg (approx. 160 mmol l^{-1}) of phosphatidylethanolamines (Kugimiya et al., 2002, 2003). Phospholipids do not induce the female feeding behavior by themselves but act synergistically with the oligosaccharides (Nojima et al., 1999b, 2002; Kugimiya et al., 2002, 2003).

In *B. germanica*, there is no information on what sensory organ on paraglossae is involved in the nuptial feeding and how females perceive information of TG-8 secretion. In this study, we focused our attention on the sensory physiology of *B. germanica* in the nuptial feeding. We assessed the female's gustatory sense involved in nuptial feeding by electrophysiological experiments coupled with behavioral tests. The behavioral responses were examined in detail by observing the movements of the mouthparts, because the mechanical movement of female's mandibles was initiated immediately after contact of her paraglossae with the TG-8 openings. We attempted to identify the chemosensilla which are responsible for perceiving the TG-8 secretion and to specify a subset of phagostimulants by morphological and behavioral observation in conjunction with electrophysiological measurements. Moreover, synergistic interactions of phagostimulants were compared in behavioral and electrophysiological responses of males and females. The gustatory specificity between the sexes and the functional role of the tergal gland secretion in the mating system of B. germanica are discussed.

2. Materials and methods

2.1. Insects

A laboratory strain of *B. germanica* (Nojima et al., 1999a) was reared on water and food pellets (MF, Oriental Yeast Co., Tokyo) at 29 ± 1 °C and 60% r.h. on 14 L:8 D photoregime. Newly emerged adults were sexed and the males and females were kept separately in small groups (7–10 insects) with water and food until use. Virgin females in the 5th day after the imaginal molt and virgin males in the 10th day after eclosion were used in behavioral and electrophysiological tests.

2.2. Stimuli

The TG-8 reservoir contains about 40–50 nl secretion per male as a transparent fluid. In order to obtain the male tergal secretions, 150 virgin males of *B. germanica* (10-day-old) were briefly chilled on ice, and the cuticular plate including TG-8 reservoirs were dissected using fine forceps and scissors. After homogenization of