

Behavioral and Morphological Studies of the Membranous Tergal Structure of Male *Blattella germanica* (Blattodea: Ectobiidae) During Courtship

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Abstract

The male German cockroach *Blattella germanica* (L.) exhibits a characteristic courtship display by raising its wings, turning around, and exposing its tergal glands on the seventh and eighth tergites to the female. The male secretes a courtship pheromone from the tergal glands which induces a strong feeding response in the female, facilitating copulation. Upon multiple, detailed microscopic observations of the courtship display, we found that the male markedly expanded the intersegmental area between the sixth and seventh tergites, but deflated this area as soon as it perceived a tactile stimulus on its back by the female, while continuing to raise its wings and exposing the tergal glands. The intersegmental area is composed of two parts: a membranous posterior part of the sixth tergite, and the regular intertergal membrane. The membranous posterior part was found to be crescentic in shape and clearly separated from the intertergal membrane by traverse tongue-shaped plates. Scanning electron microscopic observation revealed that there were many orifices or pores in the membranous crescentic zone, and its morphological structure was clearly different from that of the intertergal membrane. Our observations suggest that the crescentic zone is likely a secretory gland that may play an important role at the beginning of the male courtship display, different from the tergal glands.

Key words: male courtship display, crescentic zone, German cockroach, pheromone, sixth tergite

The male cockroaches of many species (Blattaria) have tergal glands on the dorsal part of the abdomen, which play an important role in the mating sequence (Roth 1969; Sreng 1993; 2006; Gemenio and Schal 2004). In the male German cockroach *Blattella germanica* (L.), there are distinct glands on the seventh and eighth tergites (Minchin 1890, Oettinger 1906, Wille 1920, Roth and Willis 1952), and the male exposes the glands by raising the wings and turning the back to the female in response to the female contact sex pheromone through antennal contact (Roth and Willis 1952, Nishida et al. 1974, Schal et al. 1990, Eliyahu et al. 2008). The male courtship pheromone is secreted from the glands and induces a strong and persistent feeding response in female. This allows the male to back up and situate itself underneath the female. The female continues to be held in the appropriate precopulatory position by feeding on the male's tergal secretions, while the male makes a genital connection (Roth and Willis 1952, Ramaswamy et al. 1980, Nojima et al. 1999a). The active substances of the male courtship pheromone have been characterized using a feeding assay as a mixture of nonvolatile phagostimulant compounds: oligosaccharides, and lipids (Nojima et al. 1999b, 2002; Kugimiya et al. 2002, 2003). Besides the phagostimulant pheromone,

some volatile chemicals have been reported from the tergal secretions (Brossut et al. 1975), but their role in the courtship sequence remains unclear. In this study, we performed detailed microscopic observations of the male courtship display, especially focusing on the beginning: when the male exposes its tergal glands to the female at a close range, but the female has not yet touched male's dorsal part including the tergal glands and its secretions. From these observations, we have specified an ultrastructure on the sixth tergite and attempted to identify it as a possible source of pheromones. Here, we report the results of our findings.

Materials and Methods

German cockroaches were purchased from Sumika Technoservice Co. (Hyogo, Japan) and maintained on rat chow and water in a dark room at 22°C. The adult males were separated from the females and maintained in a small group of 10–20 individuals.

A Teslong MS100 USB digital microscope (Teslong Technology Ltd., Shenzhen, China) was used to record microscopic-level videos of male courtship behavior for a detailed investigation. A decapitated

head of an adult female mounted on the tip of a toothpick was used to induce courtship behavior in the adult males for the observation (Roth and Willis 1952, Nishida et al. 1974).

Detailed morphological structure was investigated using a scanning electron microscope (SEM) (HITACHI S-4800; Hitachi High-Technologies Co., Tokyo, Japan). The male abdominal tergites were first dissected under a stereomicroscope and dehydrated in an alcohol series. The tergites were then sputter-coated with platinum-palladium and imaged.

Results

Our microscopic videos showed the males adopting the characteristic courtship display in response to the stimulus of the female dummy. Figure 1 shows a male squeezing the anterior part of its abdomen in order to markedly expand the intersegmental area between the sixth and seventh tergites. This expansion of the dorsal region caused the abdomen to bend downward, thus, lowering the tip of the abdomen toward the female dummy and exposing the tergal glands at an appropriate angle. The male retained this posture for several seconds; once it perceived a tactile stimulus on its back by the dummy, the male deflated and retracted the expanded parts while continuing to raise the wings and expose the glands (Supp Videos 1 and 2 [online only]).

In order to investigate the expanded intersegmental area in detail, the abdomen of a male was fully extended and fixed on a white plastic plate with a fine thread. The morphological structure of the expanded parts was examined using the digital microscope. The expanded area between the sixth and seventh tergites was found to be composed of two parts: the posterior part of the sixth tergite and the regular intertergal membrane (Fig. 2). The posterior part was a soft, opaque, membranous structure, but distinguished and separated from the intertergal membrane by two transverse arcuate lines. The two lines were symmetric and were situated opposite to each other across the body axis, making the membranous zone appear crescentic (Fig. 2). Such membranous zones were not observed in the other dorsal segments (Fig. 3).

The morphological structure of the membranous crescentic zone was further investigated using SEM. The arcuate lines seen under the optical microscope in Fig. 2 were now observed to be the top edges

of wide, tongue-shaped plates (Fig. 4), which were pigmented only in the posterior part. These plates created a gap between the crescentic zone and the intertergal membrane. The lateral side of each plate was wide, decreasing in width closer toward the medial plane. There was no distinct structure or orifices on the surface of the plates, making their surface appear smooth. The surface of the intertergal

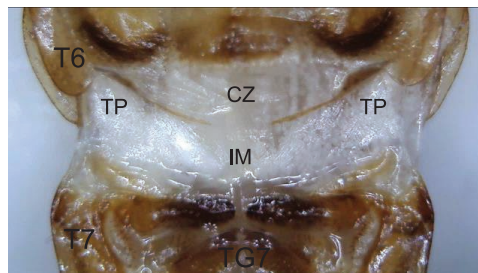


Fig. 2. Intertergal area between the sixth and seventh tergites (T6 and T7), crescentic zone (CZ), tongue-shaped plate (TP), intertergal membrane (IM), and tergal openings on the seventh tergite (TG7). Scale bar, 1.0 mm.

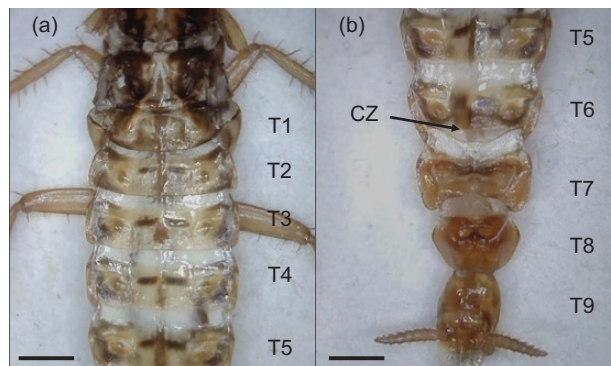


Fig. 3. A dorsal view of the stretched abdomen of a male. The wings were cut off. (a) Upper segments from the first to fifth tergites (T1 to T5); (b) Lower segments from the fifth to ninth tergites (T5 to T9), and the crescentic zone (CZ). Scale bar, 1.0 mm.

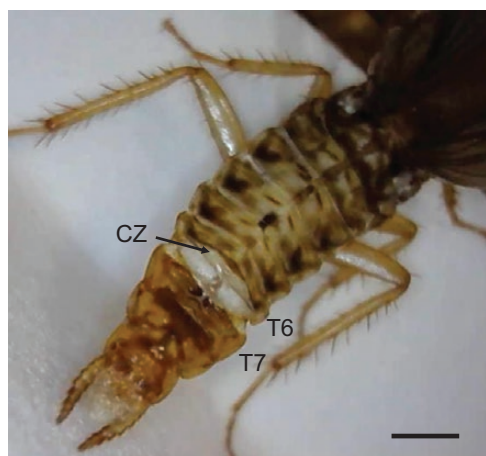


Fig. 1. Courtship display of the male German cockroach *Blattella germanica*. The male raises its wing upright, squeezes the anterior part of its abdomen, and expands the intertergal area between the sixth and seventh tergites (T6 and T7) and crescentic zone (CZ). Scale bar, 1.0 mm.

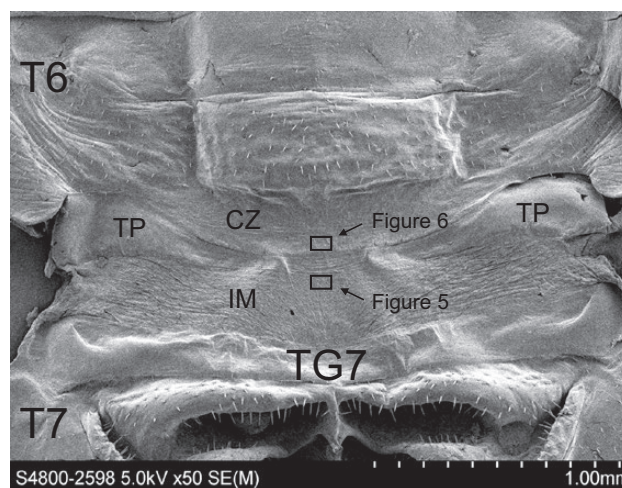


Fig. 4. Intertergal area between the sixth and seventh tergites and its ultrastructure. The sixth tergite (T6), crescentic zone (CZ), tongue-shaped plate (TP), intertergal membrane (IM), seventh tergite (T7), and tergal openings on the seventh tergite (TG7); the small rectangles are enlarged in Figs. 5 and 6.

membrane between the sixth and seventh tergites was also lacking in any specific structure or orifices, except for a general undulating texture (Fig. 5) likely due to the process of sample preparation for SEM, such as dehydration and drying.

In contrast, high magnifications of the crescentic zone surface revealed many scale-like structures containing micro-pores along the edges (Fig. 6). These pores were 0.3–0.5 μm in diameter and were spaced either individually or in groups of two or more. The pores were more concentrated toward the posterior of the crescentic zone. The other intersegmental membranes, in particular in the equivalent region of the crescentic zone were investigated, and it was also found that there were no such specific structure or orifices (Fig 7).

Discussion

Intersegmental telescopic expansion during the male courtship display is considered a simple mechanical function to expose the tergal glands toward the female. However, our microscopic observations revealed that about one-third of the expanded area is a glandular-like structure, and the area remained expanded only during the

beginning of the courtship display. The male deflated and retracted the membranous ultrastructure and the intertergal membrane when it perceived a tactile stimulus on its back, keeping it closed during the female's examination, palpations, nibbling, and feeding behavior on the tergal secretions (Supp Videos 1 and 2 [online only]). Thus, the female was given almost no opportunity or time to make physical contact with the crescentic zone. It appears that the male may be releasing a volatile signal—possibly an excitant or attractant—from the membranous crescentic ultrastructure to draw the female's attention to the tergal secretions and then immediately retracts the soft structure to protect from mechanical damage (feeding by female).

Additionally, although the specimen in Fig. 2 was fully stretched to examine the details of the structures, the crescentic zone still had some slacks. These slacks probably make it possible to expand this part markedly at the courtship display. Minchin (1890) described a structure transverse furrow on the sixth tergite and an isolated thickened membrane between the sixth and seventh tergites without detailed descriptions. The transverse furrow and the membrane are probably the crescentic zone in this study, because there was no such structure on the sixth tergite other than the slack and membranous crescentic zone. On the other hand, the morphological characteristics of the intertergal membrane between the sixth and seventh tergites were similar to those of other intertergal membranes. It should also be noted that the sixth tergite was mis-specified as the fifth tergite in the Minchin's 1890 original report but was later rectified to sixth tergite by Wille (1920).

Although both the mating behavior and the tergal glands on the seventh and eighth tergites of male German cockroaches have been studied for over a century by many researchers (Haase 1889, Minchin 1890, Oettinger 1906, Sikord 1918, Wille 1920, Roth and Willis 1952, Roth 1969, Brossut and Roth 1977, Sreng 1979, Ramaswamy et al. 1980, Nojima et al. 1999a, Belles and Ylla 2015), to the best of our knowledge, this is the first study to specify the characteristic membranous ultrastructure on the sixth tergite. It has probably gone unnoticed in the past because there are striking tergal specializations on the seventh and eighth tergites that have strongly drawn the attention of researchers. Moreover, the ultrastructure is only visible and noticeable under microscopic-level observation of the beginning of the male's courtship display and is otherwise retracted under the tergite.

Various types of chemical signals are involved in the sexual behavior of the German cockroach. These chemical signals have been characterized as the volatile female sex pheromone, which attracts the male from a distance (Liang and Schal 1993, Nojima et al. 2005); the nonvolatile female contact sex pheromone, which elicits male courtship behavior via antennal contact (Roth and Willis 1952, Nishida et al. 1974, Schal et al. 1990, Eliyahu et al. 2008); and the nonvolatile male courtship pheromone, which elicits the female

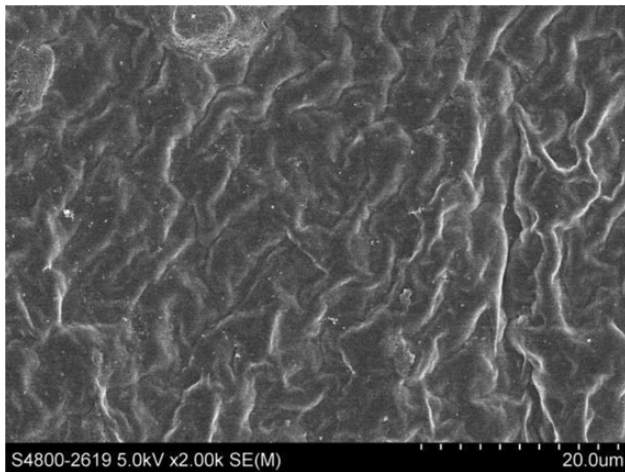


Fig. 5. The intertergal membrane (IM). White small particles on the surface are debris.

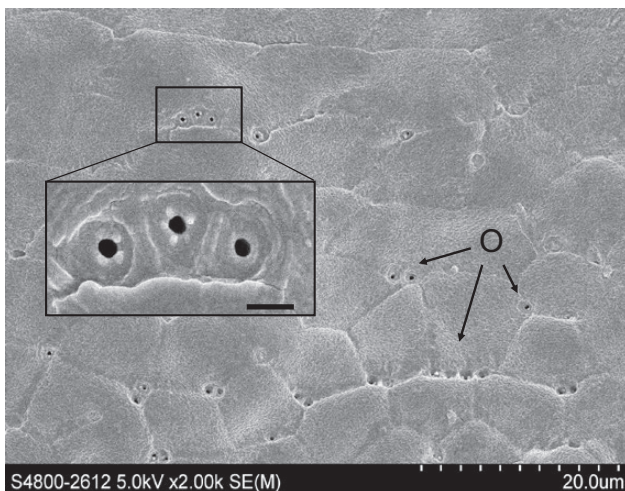


Fig. 6. The crescentic zone (CZ), plaques or scales with micro-textural wrinkles, and orifices (O); group of orifices in the inserted rectangles. Scale bar, 1.0 μm .

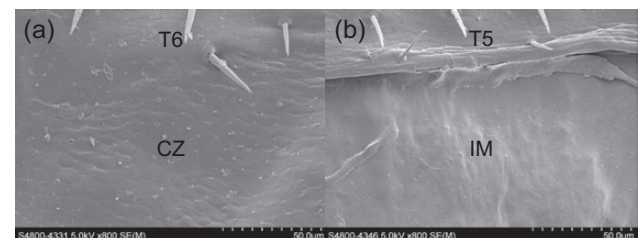


Fig. 7. A comparison of the morphological characteristics between the crescentic zone and the equivalent region of an intertergal membrane. (a) The sixth tergite (T6), and the crescentic zone (CZ); (b) The fifth tergite (T5), and its intertergal membrane (IM).

feeding response (Nojima et al. 1999b, 2002; Kugimiya et al. 2002, 2003). However, there is no clear evidence indicating the involvement or existence of a volatile sex pheromone for male German cockroaches (Roth and Willis 1952, Brossut et al. 1975, Gemen and Schal 2004).

The SEM observations indicate that there are significant morphological differences between the membranous crescentic zone and intertergal membrane. Similar membranous ultrastructures with pores associated with the release of pheromonal signals are known in many insect species, including cockroaches (Roth 1969; Sreng 1993, 2006, Ma and Ramaswamy 2003, Gemen and Schal 2004). Sreng (2006) stated that 'the close relationship between release of pheromone and glands demonstrates that calling posture may indicate perfectly gland location in the body and vice versa.' This statement appears to support the association between the morphological characteristics of the crescentic zone and its role in the male courtship display. Our observations suggest that the crescentic zone of the male German cockroach is possibly a gland that releases volatile pheromonal signals during the male's courtship display. Further study will be carried out to clarify the function and secretions of this membranous ultrastructure in the mating sequence of the German cockroach.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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