Copulation in the Cricket is Performed by Chain Reaction

Authors: Sakai, Masaki, and Kumashiro, Mikihiko

Source: Zoological Science, 21(7) : 705-718

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.21.705

ZOOLOGICAL SCIENCE **21**: 705–718 (2004)

[REVIEW]

Copulation in the Cricket is Performed by Chain Reaction

Masaki Sakai* and Mikihiko Kumashiro

Department of Biology, Faculty of Science, Okayama University, Tsushima-Naka-3-1-1,
Okayama 700-8530, Japan

ABSTRACT—The male and female genitalia are finely designed to match each other for copulation in the cricket *Gryllus bimaculatus*. Copulatory acts of the male, stereotyped and time-fixed, are elicited by stimulation of mechanoreceptors on particular regions of the abdomen, cerci and genitalia. Sequential execution of each motor act proceeds as a chain reaction in which one act stimulates some receptors which in turn elicits another act and so on, while the female remains immobile on the male's back. Each key stimulus for a motor act appears as a result of the male's own act, except for copulatory papilla protrusion by the female. The final sequence of spermatophore extrusion and transfer are irreversible fixed motor actions which are triggered when the female copulatory papilla stimulates the epiphallic hairs. They proceed without continual central drive from the brain, and apparently without sensory feedback. In addition, they are well coordinated with movement and posture in the entire body. Some neural mechanisms of controlling mating behavior and switching the reproductive cycle are discussed.

Key words: cricket, copulation, genitalia, spermatophore, chain reaction

INTRODUCTION

Mating behavior is critical for maintenance of the species. In insects, many aspects of mating have been studied, including the analyses of key stimuli, orientation, motivation and adaptive strategies (Englemann, 1970; Matthews and Matthews, 1978; Thornhill and Alcock, 1983; Bailey and Ridsdill-Smith, 1991; Choe and Crespi, 1997). In contrast, the genital structure and function have received little attention. Some gross anatomical studies on the genital organs (Walker, 1922; Snodgrass, 1937; Qadri, 1940; Choperd, 1951; Randell, 1964) and their innervation (Seabrook, 1968; Guthrie and Tindall, 1968) have been published for orthopteran insects, but observations of genital movements and coordination are scanty. The main reason is possibly due to the fact that the small and complex genital organs are difficult to observe from outside because they are in close contact during copulation. In crickets, mating behavior has been reported in different species since the 1950s (Khalifa, 1950; Huber, 1955; Hörmann-Heck, 1957; Alexander, 1961; Alexander and Otte, 1967; Beck, 1974; Loher and Rence 1978, Evans, 1988). Among these, Alexander and Otte (1967) extensively studied the structure and function of the genitalia mainly using *Acheta domesticus* and various species of *Gryllus*, and discussed the results from the viewpoint of evolution of the genitalia and mating behavior in orthopteran insects. We have been studying mating behavior in the cricket *Gryllus bimaculatus* and we have shown, for the first time, causal relationships between sensory input and motor output underlying each copulatory act, which should form the basis for a complete neuroethological analysis (Sakai and Ootsubo, 1988; Sakai *et al.*, 1991; Sakai *et al.*, 1995; Matsumoto and Sakai, 2000a, b; Matsumoto and Sakai, 2001). More recently, we examined the structure and function of the male genitalia in detail, and analyzed movement and coordination of the genitalia during copulation from the viewpoint of motor control (Kumashiro and Sakai, 2001a, b).

Here, we review our studies of mating behavior and its mechanisms in the cricket *Gryllus bimaculatus*. Experiments were carried out by video recording of mating behavior, stimulation and ablation of mechanoreceptors, and examination of the genital movement during tethered copulation with artificial stimulation in abdomen-opened males. To facilitate understanding of the dynamic aspects of copulation, full illustrations are employed.

Structure of the genitalia

Male genitalia

The male genitalia are shown in Fig. 1A. The structures

---

* Corresponding author: Tel. +81-86-251-7871;
Fax. +81-86-251-7876.
E-mail: masack@cc.okayama-u.ac.jp

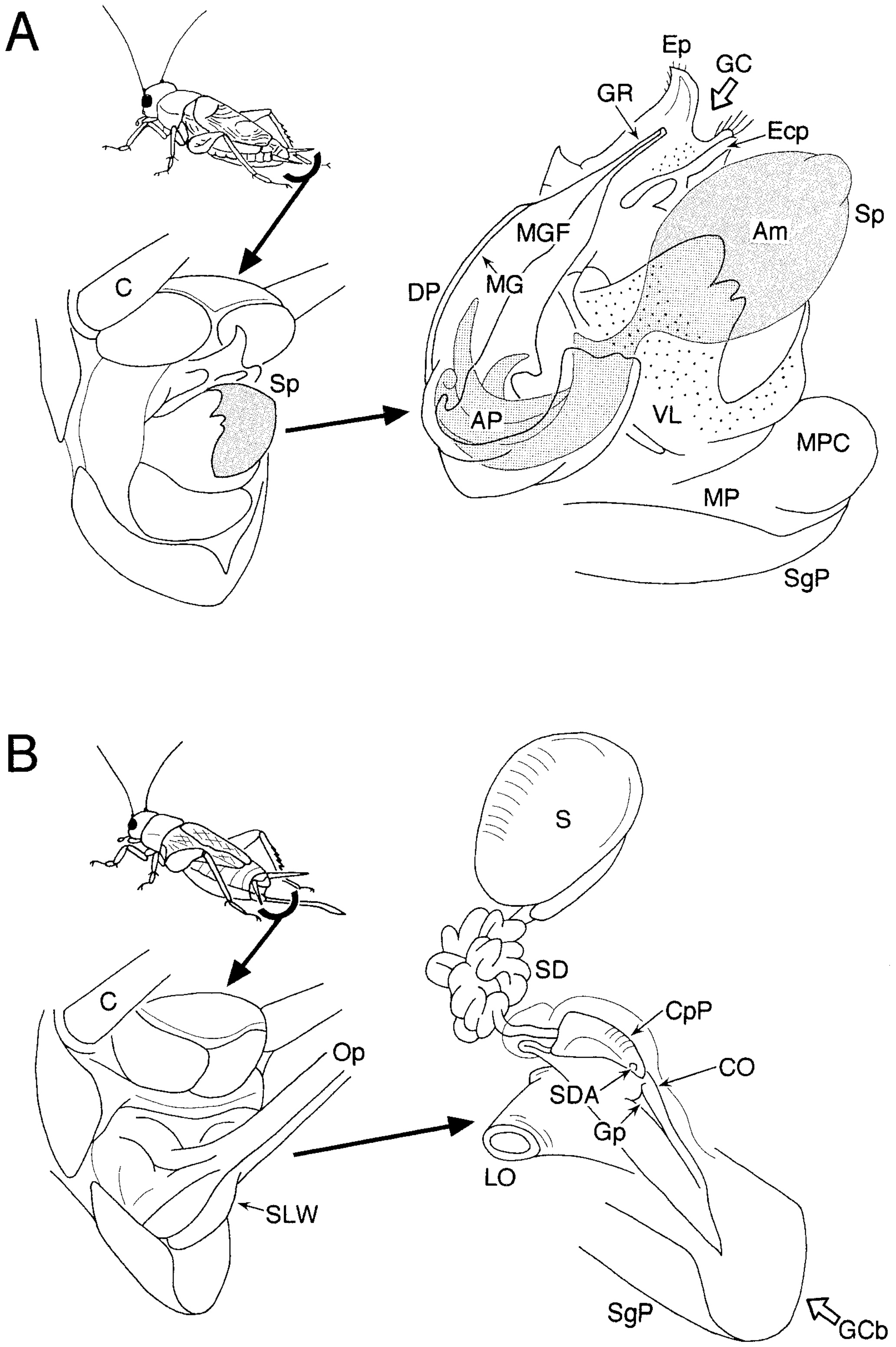

Fig. 1. The genitalia of the male and female cricket *G. bimaculatus*. **A** male genitalia used for copulation, showing external aspect of abdomen tip viewed from posterior (left) and enlarged detail of genital organs (right). In right figure, the dorsal pouch is illustrated as a perspective drawing. Dorsal pouch muscles, membranes, left ectoparamere are omitted for clarification. See Figs. 2, 6, 7, 8 for details. **B** female genitalia. Abdomen tip viewed from posterior (left) and genital organs (right). See Figs. 6, 7, 9 for details. Am, amplulla; AP, attachment plate; C, cercus; CO, common oviduct; CpP, copulatory papilla; DP, dorsal pouch; Ecp, ectoparamere; Ep, epiphallus; GC, genital cavity; GCb, genital chamber; Gp, gonophore; GR, guiding rod; LO, lateral oviduct; MG, median groove; MGF, median grooved fold; MP, median pouch; MPC, convolution of median pouch; Op, ovipositor; S, spermatheca; SD, spermathecal duct; SDA, aperture of spermathecal duct; SgP, subgenital plate; SLW, soft lateral wall; Sp, spermatophore; VL, ventral lobe.

used for copulation are called, inclusively, the phallic complex. This consists of the epiphallus (Ep), dorsal pouch (DP), guiding rod (GR), ectoparameres (Ecp), ventral lobes (VL) and median pouch (MP) (Kumashiro and Sakai, 2001a). Normally these organs are housed inside the pouch-like last abdominal sternite (SgP, subgenital plate). The epiphallus is a hook-like structure made of a sclerotized apodeme, about 1 mm wide, with a median process and two lateral processes (see Fig. 2A). These processes bear many bristle hairs of different sizes: about 70 short hairs (10–30 μm) on the median process, and about 80 long hairs (150–200 μm) on the lateral process. The ventral aspect of the epiphallus constitutes a cavity (GC, genital cavity; Fig. 7 B, D, F) for the reception of the copulatory papilla of the female. The genital cavity continues antero-ventrally to the dorsal pouch. This sack-like structure composed of a curved elastic support

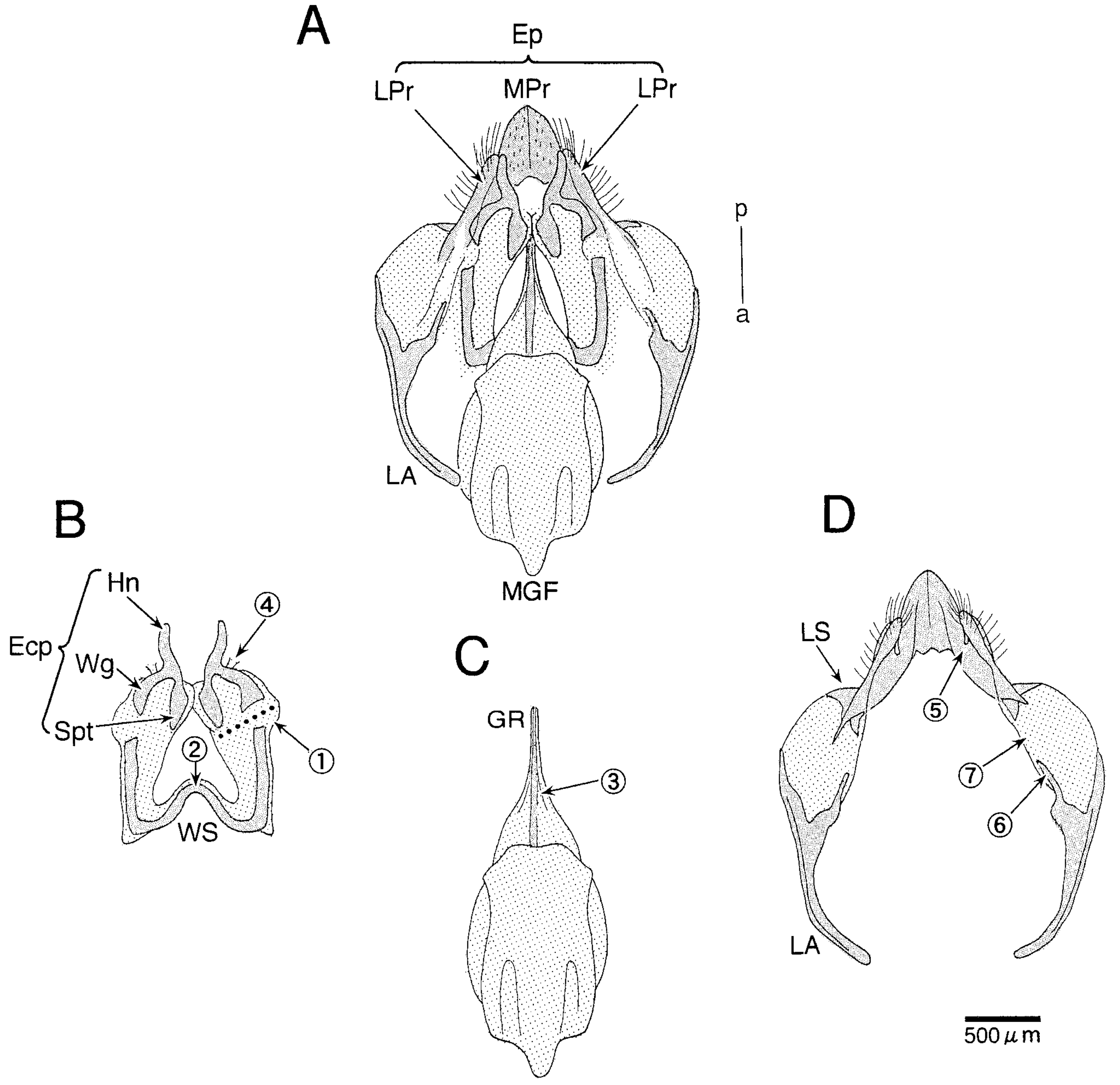

Fig. 2. Ventral view of exoskeleton of the phallic complex in the male. Up is posterior (p) and down anterior (a). The phallic complex (**A**) consists of 3 parts: ectoparamere-complex (**B**), guiding rod–dorsal pouch complex (**C**), and epiphallus-lateral arm complex (**D**). The epiphallus consists of 3 processes: median process (MPr) and two lateral processes (LPr) in (**A**). The ectoparamere is composed of 3 parts: horn (Hn), wing (Wg) and spatula (Spt) in (**B**). The ectoparameres and W-shaped hard cuticle (WS) are connected by the soft cuticle, to form the ectoparamere complex in (**B**). Hard cuticles, lateral arm (LA) and latch-like structure (LS) are shown in (**D**). Soft cuticle is shown by dots. Circled numbers indicate particular regions of soft (① and ⑦) and hard (②–⑥) cuticle. Note that ectoparameres are distorted (see Fig. 8) when soft cuticle-① is bent to the opposite side along dotted line.

plate (MGF, median grooved fold), transparent membranes covering the lateral and ventral aspects of the MGF, and four thin dorsal pouch muscles attached to the MGF (omitted in Fig. 1A, refer to Kumashiro and Sakai, 2001a). The inside of the pouch (dorsal cavity), serves as a template for the attachment plate of the spermatophore. On the midline of the inner wall of the MGF is a thin conduit-like groove (MG, median groove, 30 µm wide and 200 µm deep) that serves as a template for the spermatophore tube (ST, see Fig. 3). The groove extends from the ventral lip of the MGF to its dorsal nib in the genital cavity. The terminal region of the median groove forms a needle-like structure, the guiding rod (GR) or virga (Fig. 2C), that serves as a sheath when the spermatophore tube is inserted into the spermathecal duct of the female during copulation.

The dorsal pouch continues ventrally to membraneous structures (the ventral lobes) that serve as a template for the ampulla of the spermatophore. The ventral lobes join ventrally to another membraneous structure, median pouch, that forms the floor of the subgenital plate. The ventral lobes and median pouch expand with hemolymph during spermatophore transfer. The central part of the median pouch (MPC, median pouch convolution) maximally expands into a balloon shape during spermatophore transfer and fills the inside of the vacant dorsal pouch after copulation.

On the ventro-lateral regions of the inner surface of the genital cavity, a pair of sclerotized apodemes, the ectoparamers are attached (Fig. 1A). Each consists of 3 parts, the horn (Hn), the wing (Wg) and the spatula (Spt), that are connected by elastic soft cuticle to the W-shaped hard cuticle (Fig. 2B). These are together called the ectoparamere complex. Each part of the ectoparamere complex changes its position and shape at spermatophore extrusion as described later. On either side of the proximal regions of the horns are found about 30 small bristle hairs (5 µm long and 3 µm thick) called cavity hairs (CH, see Fig. 7F). These play a key role in triggering spermatophore extrusion and transfer.

Spermatophore

The morphology, function and formation of the cricket spermatophore have been described previously (Snodgrass, 1937; Khalifa, 1949; Alexander and Otte, 1967; Beck, 1974; Mann, 1984; Simmons, 1986; Ootsubo and Sakai, 1992; Hall *et al.*, 2000). During production by the male genitalia, the spermatophore develops an elaborate shape which plays a functional role in spermatophore fixation in the genital chamber. Its structure will be reviewed briefly to facilitate the following descriptions of copulation. The spermatophore consists of the ampulla (Am), attachment plate (AP), neck

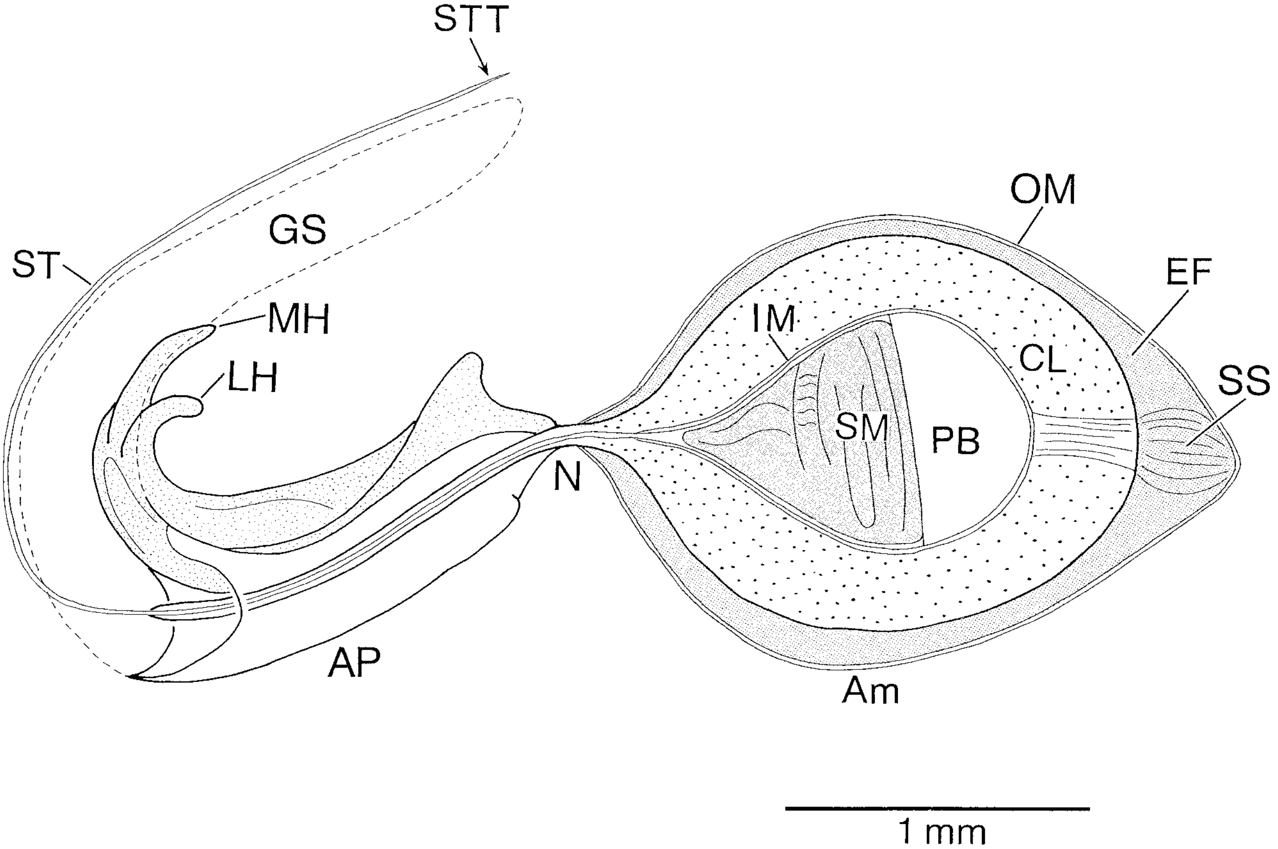

Fig. 3. Spermatophore structure. Am, ampulla; AP, attachment plate; CL, crystalline layer; EF, evacuating fluid; GS, gelatinous substance; IM, inner membrane: LH, lateral hook; MH, medial hook; N, neck; OM, outer membrane; PB, pressure body; SM, sperm mass; SS, sperm sac; ST, spermatophore tube; STT, terminal region of the spermatophore tube. These abbreviations are adopted in the following figures.

(N) and tube (ST)(Fig. 3). The ovule-shaped ampulla contains the sperm mass (SM) or spermatozoa and the pressure body (PB) in a central cavity partitioned from the crystalline layer (CL) by the inner membrane (IM). The crystalline layer, located between the inner and outer membrane (OM), is a glassy hard shell. Early in spermatophore maturation the sperm mass is contained within the sperm sac (SS) in the tip of the ampulla. The opposite end of the ampulla gives off a curved, spatulate cartilage, the attachment plate, that bears medial and lateral hooks (MH and LH). The long slender spermatophore tube (ST) in the light brown color (70 μm in diameter and 2 mm long) extends from the inner membrane of the ampulla, exits through the neck, and passes along the interior of the outer side of the attachment plate (Fig. 3). The tube then leaves the attachment plate and further extends while curving along the surface of the gelatinous substance (GS) on the attachment plate. The terminal region of the tube (STT) is tapered over the last 10 μm and finally closed. Spermatozoa are ejected by internal pressure thought to be produced by osmotic interaction between the pressure body and the evacuating fluid (EF) when the tip of the spermatophore tube is broken in the spermathecal duct of the female (Khalifa, 1949).

Female genitalia

The female genitalia (Fig. 1B) have been described for *Acheta domesticus* (Hustert and Toppel, 1986) and *Teleogryllus commodus* (Sugawara, 1993). The main organs used for copulation are the last abdominal sternite (SgP, subgenital plate), the copulatory papilla (CpP) and the soft lateral walls (SLW) in the genital chamber (GCb). The genital chamber comprises an inner space covered by the subgen-

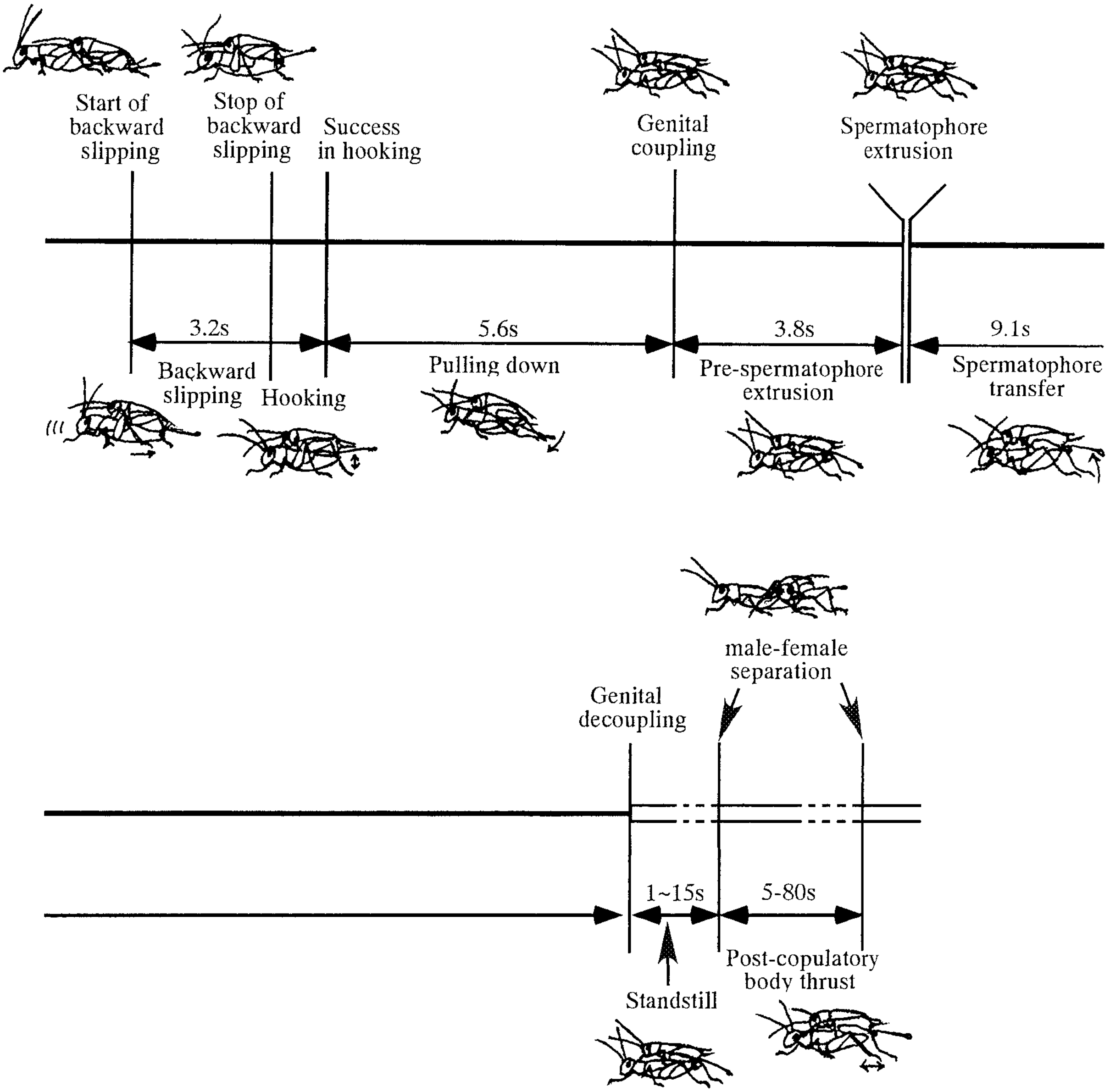

Fig. 4. Copulatory sequence and average periods for sequential behavior patterns. Onset at upper left.

ital plate just beneath the base of the ovipositor (Op). The posterior margin of the subgenital plate serves as an anchoring site for the male epiphallus to hook during copulation. The copulatory papilla (CpP) is a partially sclerotised perturbulance, about 1 mm long and 0.5 mm wide (see Fig. 7A, C, F), which is normally anterior to the soft lateral walls. There is a small opening, the aperture of the spermathecal duct (SDA) on the ventral side of the distal region of the copulatory papilla (Fig. 7C).

Copulatory sequence

Mating behavior in the cricket is highly stereotyped and time-fixed. In *G. bimaculatus*, it proceeds as follows (Sakai and Ootsubo, 1988; Sakai *et al.*, 1991). The sexually active male in the mating stage sings a calling song to attract females. When a female approaches, the male recognizes her by antennal contact chemoreceptors (Rence and Loher, 1977) and changes the song to a courtship song, turns his body away and backs toward her. When the female mounts the male, copulation is started (Fig. 4). At first, the male exhibits slightly extended posture (the “intense posture”) with the body partially raised when the female presses the elytra and the dorsal regions of the abdomen with her forelegs. Following this, the male slips backward under the female with the abdomen extended and flattened, and with the cerci vibrating (backward slipping) as they approach her genitalia beneath the base of the ovipositor. Backward slipping stops as soon as the male’s abdomen tip reaches the female’s, and then hooking starts. The male attempts to hook the female’s subgenital plate with his epiphallus. This hooking is normally repeated approximately 15 times at 0.6/s (Matsumoto and Sakai, 2001). These two phases, the backward slipping and hooking phase, last 3.2±1.2 s (n=10) on average. When the male succeeds in hooking, he begins pulling down the subgenital plate of the female (pulling down phase). After several repeats, the male and female genitalia are coupled (genital coupling). The median of the pulling down phase is 5.6 s (C.I. 4.0–8.7, n=20). At the moment of genital coupling, all the bodily movements cease except for the antennae and cerci. This immobile state (pre-spermatophore extrusion phase) continues for 3.8 s (C.I. 2.5–5.0, n=20). At the end of the immobile state, the male suddenly

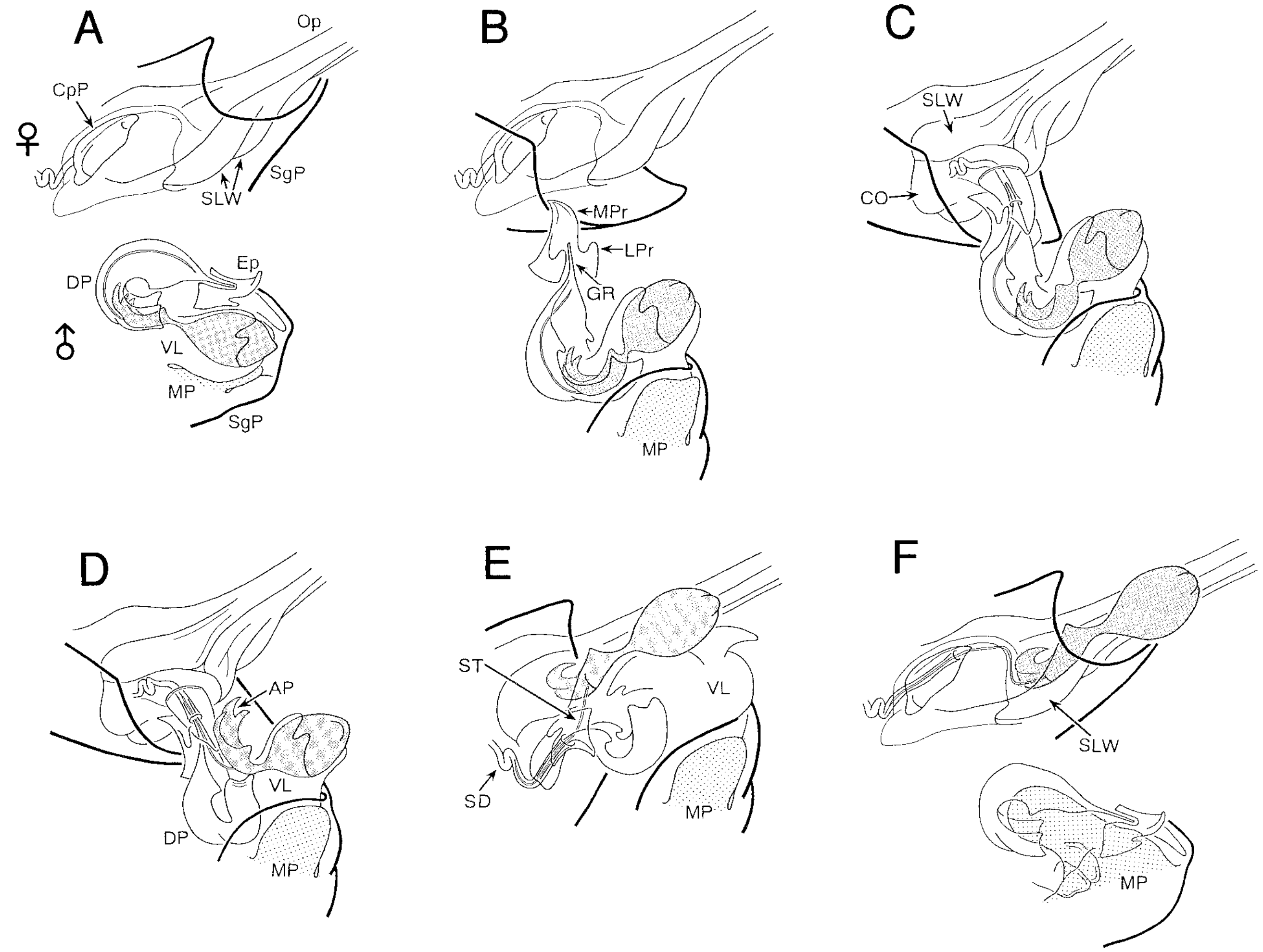

Fig. 5. Movement of genital organs and transfer of spermatophore. **A** before hooking. **B** success in hooking. **C** genital coupling. **D** dorsal pouch deformation (spermatophore extrusion). **E** spermatophore transfer. **F** after spermatophore transfer. In each pair of figures, female genitalia above and male genitalia below. See text for details.

extrudes the attachment plate of the spermatophore from the dorsal pouch (spermatophore extrusion), and transfers it to the female in 9.1 s (C.I. 6.7–11.2, n=20) (spermatophore transfer). The genitalia of the male and female are then decoupled within 1 s and all body movements stop. This immobile state (standstill phase) continues for 1–15 s. However, some males occasionally exhibit a hooking-like body movement, called the post-copulatory body thrust phase (Ootsubo and Sakai, 1992), for 5–80 s as long as the female stays on the male's back. Once the male and female are separated, the male normally shows no further copulatory actions (exception: Matsumoto and Sakai, 2000a), and he commences guarding behavior toward the female in an aggressive manner.

Movement of the genitalia during copulation

The genital organs of insects are normally housed in the abdomen. During copulation, some are exposed outside but others still remain inside. We developed new technique to induce spermatophore extrusion and transfer in the dissected condition, thus allowing us to observe all movements normally hidden in the abdomen during these phases (Kumashiro and Sakai, 2001a, b).

During hooking, the epiphallus is upwardly everted due to the expansion of the median pouch by hemolymph. After successful hooking (Fig. 5B), the male pulls down the subgenital plate of the female with the epiphallus. A few seconds later, the copulatory papilla of the female moves posteriorly through the soft lateral walls of the genital chamber, slipping out of its membraneous sheath (Figs. 5C and 6C). Protrusion of the copulatory papilla is not simply a passive reaction to the pulling down the subgenital plate but an active response, because an artificially applied stretch to the subgenital plate can not induce the observed protrusion. The protruded papilla naturally enters the genital cavity of the underlying male epiphallus (see Fig. 5C). Here, genital coupling is achieved (Fig. 7F). The male then stops movement except for rhythmic movement of the antennae and cerci. It is apparent that this quiescent state (pre-spermatophore extrusion phase) stabilizes genital coupling.

Following the pre-spermatophore extrusion phase for about 4 s, a highly stereotyped fixed action pattern of genital movements commences. First, the guiding rod is jerkily inserted into the aperture of the spermathecal duct of the copulatory papilla (Figs. 5C, 7F). This is caused by contraction of the guiding rod muscles, located just posterior to the dorsal pouch, connecting the epiphallus and the W-shaped hard cuticle (Fig. 2A). Subsequently, the strong contraction of dorsal pouch muscles bends the elastic median grooved fold (MGF), and deforms the dorsal pouch, causing the ejection of the attachment plate of the spermatophore (Fig. 5D). Coincidentally, the right and left ectoparameres are distorted by contraction of the guiding rod muscles: the horns slides posteriorly into the curved area between the medial and lat-

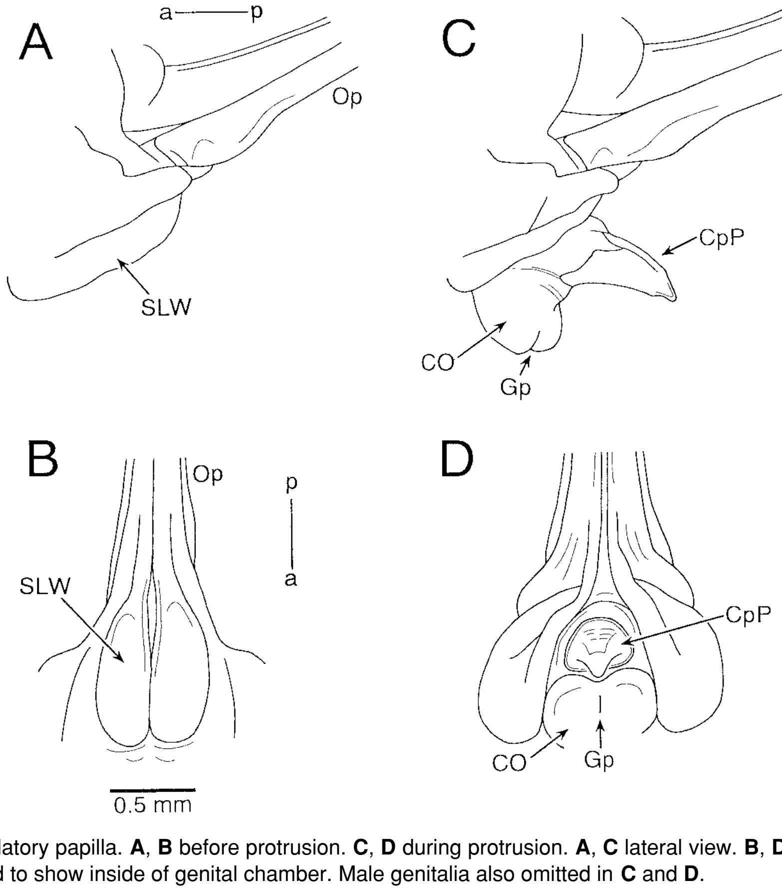

Fig. 6. Protrusion of copulatory papilla. **A**, **B** before protrusion. **C**, **D** during protrusion. **A**, **C** lateral view. **B**, **D** posterior view. The subgenital plate of female is eliminated to show inside of genital chamber. Male genitalia also omitted in **C** and **D**.

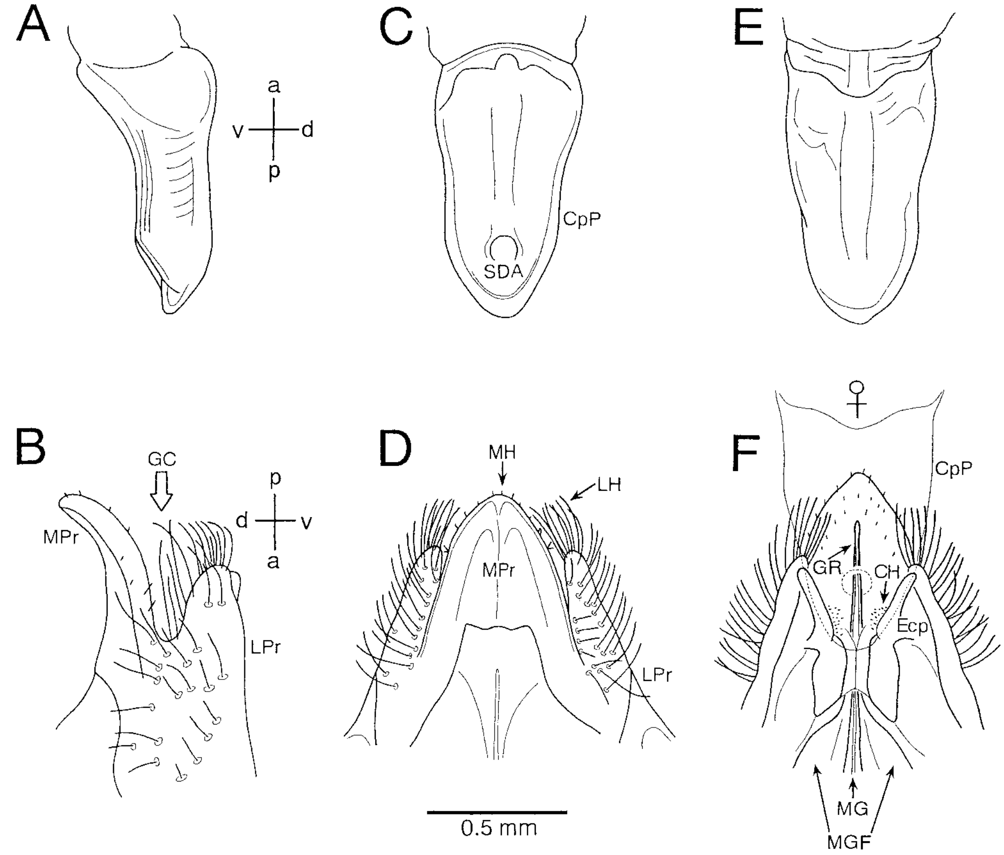

Fig. 7. Morphology, and interaction of copulatory papilla and epiphallus in genital cavity. **A**, **C**, **E** copulatory papilla. **B**, **D**, **F** epiphallus. **A**, **B** lateral aspect. **C**, **F** ventral aspect. **D**, **E** dorsal aspect. Position of copulatory pupilla in genital cavity during genital coupling is shown in F. MH, median hair, LH, lateral hair, and CH cavity hair. See Fig. 1 legend for abbreviations.

eral processes of the epiphallus, the wings become twisted, as the lateral edges of the spatulas are raised (Fig. 8B). These are due to stress changes in the soft cuticle rather than in the ectoparameres themselves, as described below. At the beginning of this distortion, the W-shaped cuticle is pulled backward by contraction of the guiding rod muscles. The ectoparamere complex, however, does not move because the soft cuticle (Fig. 2B-①) between the wings and W-shaped cuticle is blocked by the soft cuticle (Fig. 2D-⑦) connecting the latch-like structure (LS) and the lateral arm (LA). When the epiphallus muscle connecting the LA and LS contracts, the LA slides posteriorly, which causes displacement of the cuticle-⑦ and bending of the cuticle-①, resulting in distortion of the ectoparameres. This causes the spatulas to stand, and they press the MFG on both sides (Fig. 8B), which stabilizes the projected guiding rod in the spermathecal duct during spermatophore transfer (even during rhythmic vibration of the abdomen tip). Previously, the function of the ectoparameres was thought only to hold the copulatory papilla (Alexander and Otte, 1967; Snell and Killian, 2000) .

Next, as spermatophore extrusion occurs, the flaps of the ventral lobes holding the ampulla of the spermatophore suddenly open to the left and right as they fill with hemolymph (Fig. 5E). The median pouch, together with the ventral lobes, is expanded by hydrostatic pressure in the abdomen. The passages for the hemolymph to the ventral lobes and median pouch, which are normally closed, are opened at dorsal pouch deformation via displacement of the lateral arms (Kumashiro and Sakai, 2001a). The spermatophore is slowly pushed up toward the genital chamber of the female, and the attachment plate enters the genital chamber. The slow rising of the abdominal tip is supported not only by the six stretching legs against the increasing load of the female but also by other parts of the body including the head, thorax and abdomen. All of these contribute to maintain the state of genital coupling. After separation of the male and female, the convolution of the expanded median pouch enters the dorsal cavity and stays tamped (dotted region, Fig. 5F). Interestingly, the median pouch always moves to the right and left at a rate of 0.16 Hz (C.I. 0.15–0.19, n=9). It may be involved in cleaning the inside of the dorsal pouch and subgenital plate for the next spermatophore preparation.

The spermatophore is held in the genital chamber by the pillow-like lateral walls that firmly catch the attachment plate (Fig. 9). The copulatory papilla retracts to its original anterior position in the genital chamber, and the subgenital plate closes to further secure the spermatophore. The ampulla remains outside (Fig. 9A). Meanwhile, the spermatophore tube passes through the 2 mm straight part of

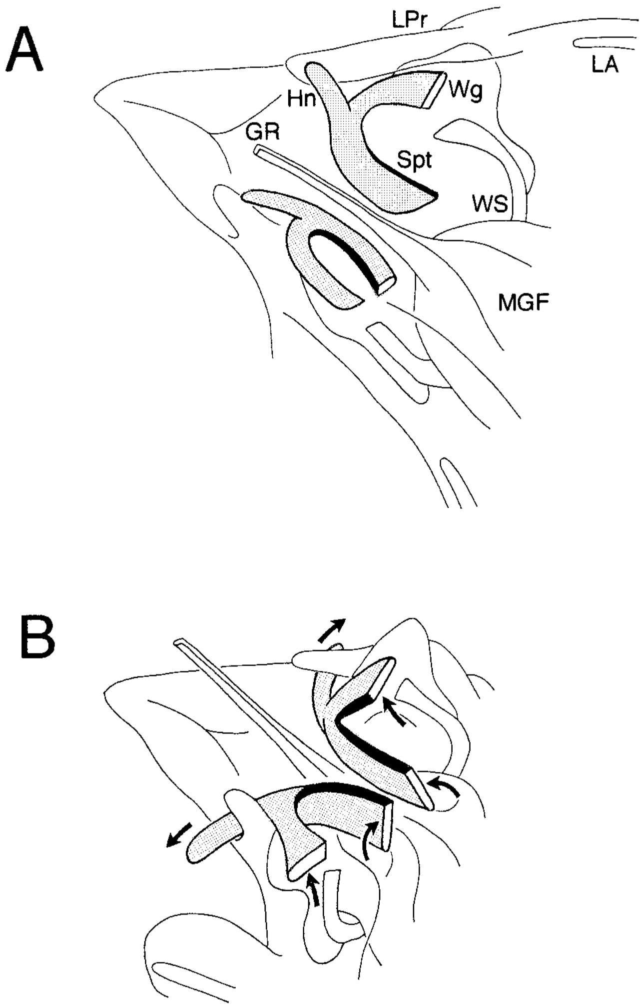

Fig. 8. Changes in position and shape of ectoparamere complex during spermatophore extrusion and transfer. **A** before spermatophore extrusion. **B** during spermatophore extrusion and transfer. Each arrow indicates movement of horn (Hn), wing (Wg) and spatula (Spt) of ectoparamere. The female copulatory papilla is not illustrated. Refer to Fig. 5 for position and direction of phallic complex during genital coupling.

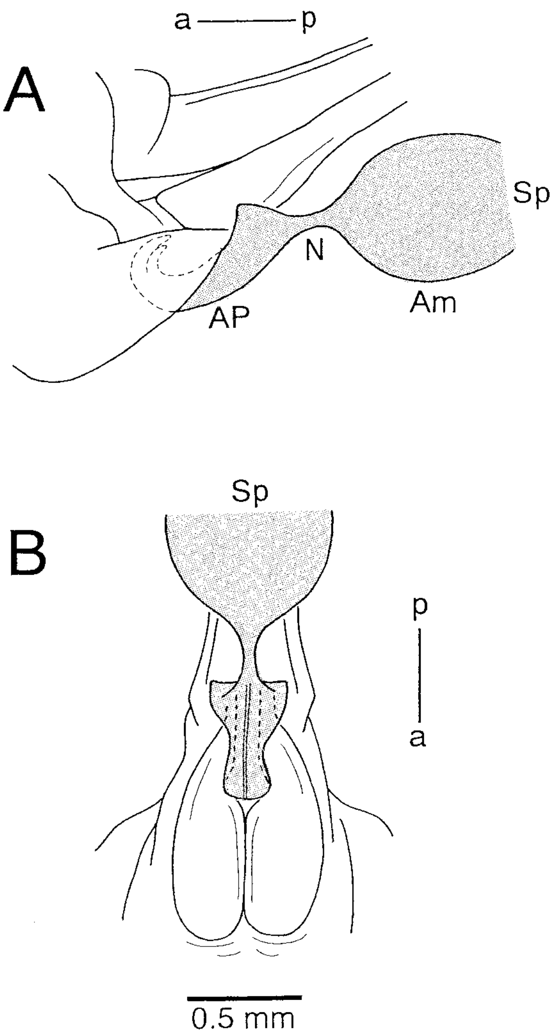

Fig. 9. Fixation of spermatophore in genital chamber of female. **A** lateral view. **B** posterior view. Subgenital plate of female is eliminated to show inside of genital chamber. Note, that attachment plate of spermatophore matches part of soft lateral walls of genital chamber. a, anterior. p, posterior.

spermathecal duct during spermatophore transfer which lasts for 9.1 s (Fig. 5E, F). This prolonged period may be needed to thread the slender spermatophore tube deep into the thin spermathecal duct. Cercal oscillation produces rhythmic movement of the phallic complex and abdominal tip during spermatophore transfer, which seems to facilitate the smooth advancement of the spermatophore tube.

Sensory input and motor output

The causal relationships between stimulation and motor response in copulation were investigated by stimulation and ablation of sensilla (Sakai and Ootsubo, 1988; Sakai *et al.*, 1991; Kumashiro and Sakai, 2001a). First of all, slight contact of the back with a cotton bud induced an intense posture in males restrained on the rotatable Styrofoam sphere. During this intense posture, backward slipping was elicited when the middle and distal regions of the cerci were contacted with the same stimulus, while hooking was elicited when the proximal cerci and epiproct were touched. Elimination of the middle and distal regions of the cerci caused the lack of backward slipping and removal of sensilla on the dorsomedial regions of the cerci resulted in the deficiency of hooking. The surgical rotation (180°) of the cerci abolished normal backward slipping and hooking. On the other hand, ablation of the female subgenital plate caused the failure of copulatory papilla protrusion. For the epiphallus of the male, removal of the lateral hairs (Fig. 7D) gave rise to a difficulty in pulling down of the female subgenital plate and that of the cavity hairs (Fig. 7F) produced inability to perform spermatophore extrusion. Finally, stimulation of the cavity hairs with a model of the copulatory papilla led to the extrusion of the spermatophore. Based on these results, the stimulus-response chain was constructed as follows.

In the early stage of copulation, there are six steps (Fig.

10), each consisting of mechanoreceptor stimulation and a resultant response. First, the female steps on the male's back (Fig. 10, S_1). The sensory stimulus consists of mechanical contact of the dorsal region of the male's abdomen and the elytra by the female's venter. As a result, the male intense posture, with the abdomen slightly raised, is triggered (Fig. 10, R_1). Such a strained posture helps to bring the male's abdomen and cerci into close contact with the venter of the female. Mechanoreceptors are abundant on the surface of the abdomen (Gnatzy and Hustert, 1989)

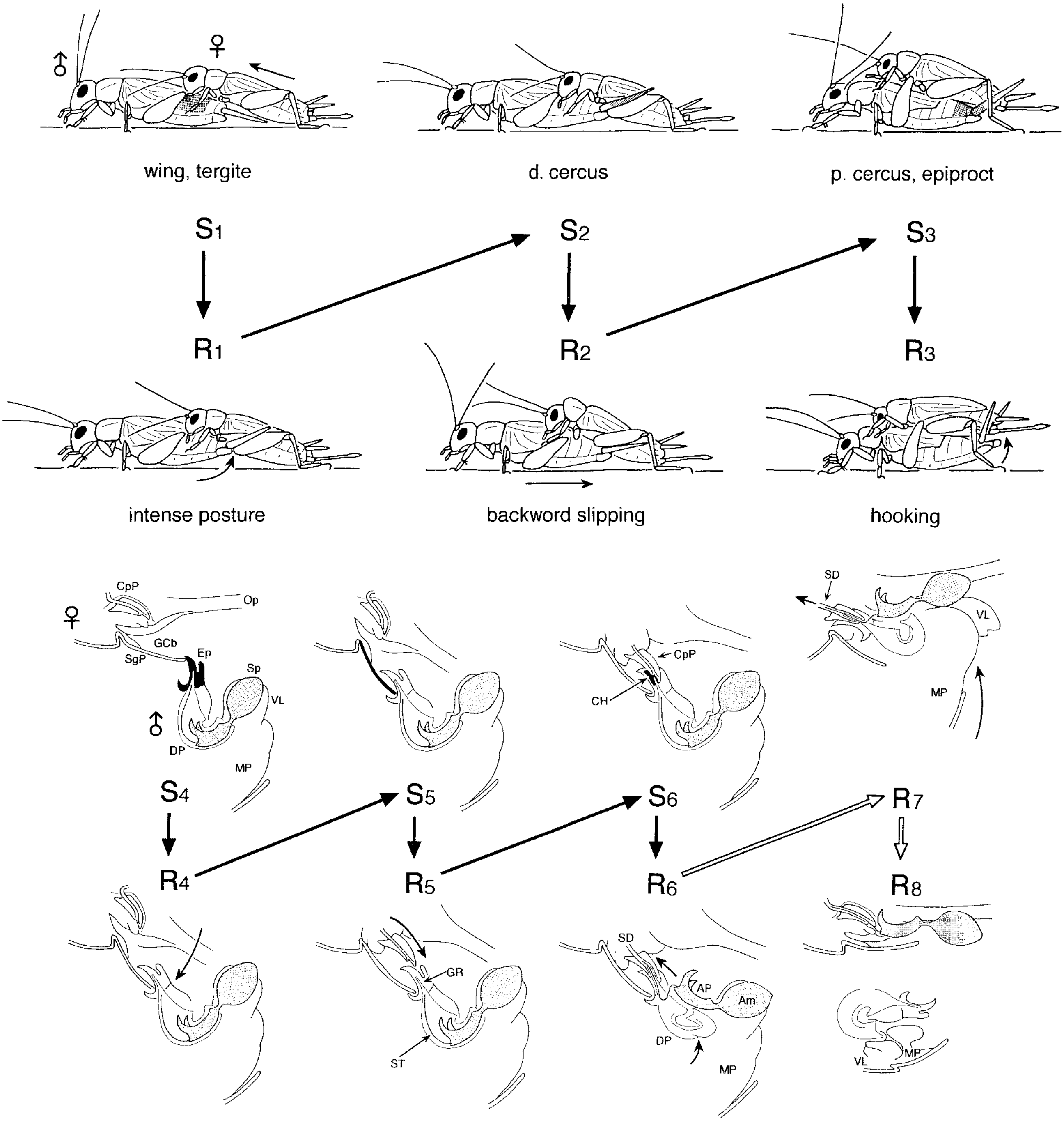

Fig. 10. Chain reaction in cricket copulation. Three steps leading to success in hooking (S_1–R_3). Upper three figures (S_1, S_2 and S_3) show locations (gray) of key stimulus detection by male, and lower three figures (R_1, R_2 and R_3) show male responses to respective stimulation. In S_1, tergites and wings are stimulated. In S_2, distal region of cercus (d. cercus) is stimulated. In S_3, proximal region of cercus (p. cercus) and epiproct (last abdominal tergite) are stimulated. Large black arrows show stimulus-response chain.

Three steps after success in hooking (S_4–R_8). Upper figures (S_4, S_5 and S_6) except R_7 show locations (black) of key stimulus detection. Lower figures (R_4, R_5 and R_6) except R8 show responses to respective stimulation. Large black arrows show stimulus-response chain, large white arrows show sequence of irreversible fixed actions (R_6, R_7 and R_8).

and elytra (Schäffner and Koch, 1987; Hiraguchi *et al.*, 2003). Specifically, abdominal sternites 4–7 are sensitive sites for eliciting the intense posture, though there is no particularly dense distribution of bristle hairs in that region. Second, as a result of the first step, bristle (trichoid) hairs (Schmidt and Gnatzy, 1972; Sakai and Ootsubo, 1988) in the dorsomedial subdivision of the distal regions of the cerci come into contact with the female abdomen (S2). In response to this contact, backward slipping is initiated (R2). Third, when the male reaches the end of the female's abdomen, backward slipping stops. This is because contact between the distal cercal hairs and the female venter is lost and, instead, bristle hairs of the proximal cerci and the last abdominal tergite (epiproct) come into contact with the female's abdomen (S3). Contact with bristle hairs on this region causes both opening of the subgenital plate and hooking with the epiphallus (R3). Fourth, when hooking succeeds, the epiphallus contacts the subgenital plate of the female, thus stimulating the bristle hairs on the processes of the epiphallus (S4). This, in turn, elicits the male pulling down movement of the subgenital plate (R4). Fifth, repeated pulling down continuously stimulates the female subgenital plate (S5) where some mechanoreceptors responsible for the next act by the female possibly lie. As a result, the copulatory papilla of the female protrudes posteriorly into the genital cavity of the epiphallus (R5). Sixth, the tip of the copulatory papilla presses small bristles (cavity hairs) on the ectoparameres (S6). This causes the stopping of all the movements except for the antennae and cerci. About 4 seconds later, the same stimulation triggers guiding rod ejection, spermatophore extrusion (R6) and transfer (R7). The final act is completed in about 9 seconds (R8).

DISCUSSION

Structure and function of the genitalia

The genitalia of the male and female crickets possess intricate inter-matching structures. The evolution of such functional complexity in these tiny organs is amazing. First, the male epiphallus hooks onto the female subgenital plate (Fig. 5). Second, the copulatory papilla precisely fits the male genital cavity (Fig. 7). Third, the aperture of the female copulatory papilla is just opposite to the guiding rod at genital coupling (Fig. 7). Fourth, the spermatophore tube is held in the straight portion of spermathecal duct along the copulatory papilla (Fig. 5). If the tube were longer, it would clog the anterior winding regions of the spermathecal duct. Fifth, the attachment plate of the spermatophore matches the soft lateral walls of the genital chamber (Fig. 9). It is obvious that this structural complementarity between the male and female genitalia guarantees the coupling of the genitalia and the transfer of the spermatophore. In addition, the ectoparamere complex of the epiphallus plays a role in stabilizing the guiding rod through a remarkable mechanical distortion in morphology during spermatophore extrusion and transfer (Fig. 8). The timely expansion of the ventral lobes and median pouch is also indispensable step for spermatophore transfer.

Morphological studies of the genitalia in insects often refer to the lock and key hypothesis as an isolating mechanism between newly separated species, although it is questioned by some researchers (Eberhard, 1993; Alexander and Otte, 1967). We can provide some insight into this matter from comparison with another species. According to our preliminary observations, *Teleogryllus emma* had a slender copulatory papilla compared with that of *G. bimaculatus*. The papilla was experimentally inserted into the genital cavity of *G. bimaculatus* but did not closely fit, suggesting that genital coupling would be unstable, and that spermatophore extrusion would be difficult because stimulation of mechanoreceptors in the genital cavity would be incomplete. It was reported that *Gryllus pennsylvanicus* and *Acheta domesticus* failed to connect the genitalic parts because the copulatory papilla is considerably smaller in *Gryllus* than in *Acheta* (Alexander and Otte, 1967). The differences in morphology of the genitalia in different species can even lead to different copulatory postures and movements in copulation (Alexander and Otte, 1967). In fact, copulatory acts were different between *T. emma* and *G. bimaculatus*, and the time from success in hooking to spermatophore extrusion was different: about 110 s in *T. emma* compared with 20 s in *G. bimaculatus*. Full knowledge of the genital structure and function in *G. bimaculatus* shown here is a basis for future comparative studies.

Chain reaction

One of the best known models of chain reactions in animal behavior is mating in the stickleback fish studied by Tinbergen (1951). The male and female perform courtship, exchanging signals to each other: one act performed by the male functions as a key stimulus for the female, vice versa. In insects, it has been known that each species has its own mating sequence, as in the butterfly (Brower *et al.*, 1965) and fly (Brown, 1966). At the final stage of courtship, the male holds fast to the female, searches for the genital opening, inserts the appendage, and finally transfers the spermatoza or spermatophore to the female while the female remains passive and occasionally even immobile. Stich (1963) showed that mating in the male crane fly proceeds by reaction chains via contact of different parts of the male's body with those of the female's. It remains unknown, however, which mechanoreceptors are involved in eliciting each motor act.

Our study has shown that copulation in the cricket is carried out by a chain reaction: one motor act stimulates some mechanoreceptors, which in turn elicit another act and so on. The key stimulus appears as a result of his own previous act ("self-produced key stimulus"). For the male to perform each act, mechanoreceptive sensilla play a crucial role, and their strategic arrangement on the abdomen, cerci and genitalia assures the smooth execution of different motor acts step by step (Fig. 11). On the other hand, the female

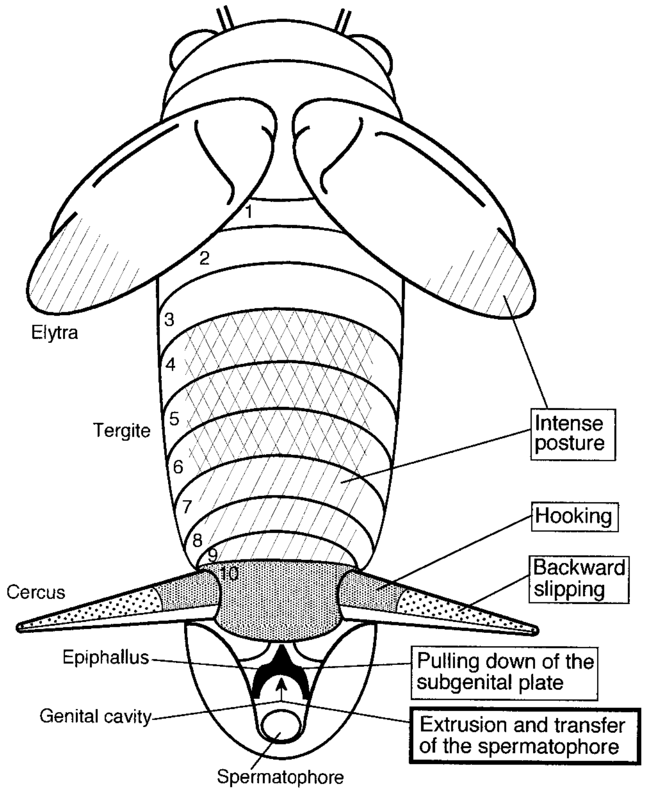

Fig. 11. Input and output relationships for copulatory acts in male cricket. Each region as shown by the different patterns has mechanoreceptors to which contact stimulation elicits a specific motor response. Numbers indicate abdominal segments (1–10). Reversible acts in early stage shown in thin-lined box, and irreversible fixed actions in later stage, in thick-lined box. See text for details.

becomes immobile after the start of copulation and shows only copulatory papilla protrusion. The hypnotic state of the female appears to be due to vibratory stimulation of her venter produced by the male's body thrusting under the female.

There may be a question whether a fixed act is still elicited if an intermediate key stimulus is artificially given, skipping the prior steps in the copulatory sequence. For example, if the proximal cerci and epiproct are stimulated directly with a female model, do males display hooking? In fact, hooking is elicited. However, the occurrence rate is only 50% (n=20) in even highly excited males (Matsumoto and Sakai, 2000a). This is because quiet or singing males tend to respond to stimulation by an evasion response rather than by a mating response. This is reasonable because there is nothing on the male's back. In contrast, when the intense posture was induced with a female model prior to test stimulation, males exhibited hooking to the same stimulation at 100%. Another example is that when cavity hairs in the epiphallus are stimulated directly with the model of the copulatory papilla, spermatophore extrusion is indeed elicited, but again the success rate is much lower (15%, n=61) than in males exhibiting the intense posture by dorsal stimulation (85%, n=23)(Sakai *et al.*, 1991). The male in the latter situation actively opens the subgenital plate upon stimulation and easily ejects the spermatophore during cavity hair stimulation, with the genital organs vigorously vibrating. From these, the intense posture elicited by the female at the start of copulation is essential, not only for making a close contact between the male's dorsum and female's venter, but also for the smooth execution of each copulatory act without fail.

The term "chain reaction" may give one the impression that males behave like an unsophisticated robot. This is incorrect, for a male shows flexible, adaptive behavior as he energetically palpates the venter of the female with his abdomen tip to search for the female's genital opening during backward slipping, and he accurately adjusts the body axis to become parallel to that of the female when she leans on the male's back. Furthermore, it is often observed that males accomplish copulation even with a sexually reluctant female which is incessantly trying to dismount from the male's back. It was observed that the male stays for a short time keeping his abdomen tip raised when artificial stimulation to the abdomen tip is suddenly removed. It is as if the male is anticipating the same stimulus returning to the previous position. These implicate a mechanism similar to "active touch" hypothesized in mammals (Gibson, 1962). The chain reaction mechanism involved in copulation is understood in insects but the control mechanisms for the motor acts still remain to be elucidated.

Mechanisms of spermatophore extrusion and transfer

It is apparent that the goal of copulation in animals is to transfer spermatozoa from the male to the female. The mechanisms of this critical behavior, however, have not been fully investigated either in invertebrates or vertebrates. In monkeys, copulation culminates at the moment of male ejaculation with body tenseness and rigidity, possibly accompanied by orgasm (Goldfoot *et al.*, 1980). In rats, the male shows jerky stretching at ejaculation following repetitive intromission, firmly holding the female body (Meisel and Sachs, 1994). Another example is observed in salmon in which the paired male and female show convulsive stretching with their mouths open wide at sperm emission and egg spawning (Satou *et al.*, 1991). Even in crickets, the male assumes a stretching posture with the abdomen tip pressing against the female subgenital plate until the spermatophore is transferred, and then suddenly he falls into a complete motionless state. These suggest that there may be a similar mechanism in the final acts of copulation across species.

Spermatophore extrusion and transfer in the cricket are irreversible fixed actions. The main acts in the genitalia are ejection of the guiding rod, deformation of the dorsal pouch, and expansion of the ventral lobes and median pouch. These are triggered by stimulation of the cavity hairs, but the movements themselves are independent of sensory input or peripheral feedback because they occur in pseudo-copulation in which the female is not present on the male's back (Sakai *et al.*, 1991), and in tethered copulation with some nerve afferents denervated in abdomen-opened males

(Kumashiro and Sakai, 2001b). According to our recent extracellular recordings, most of the genital motoneurons, including ones innervating the dorsal pouch, ventral lobe and median pouch, automatically discharge in first tonic and then rhythmic manner after being triggered (Kumashiro and Sakai, 2001b); their activity is purely centrally programmed. On the other hand, one should not overlook that the male is continuously pushing his abdomen tip onto the genital opening of the female with the six legs stretching against the substrate during spermatophore transfer. If the male failed to maintain this posture, the genitalia would decouple and the spermatophore would fall to the ground. This fact suggests that there must be an extensive coordinating system connecting the pattern generators for spermatophore extrusion and transfer with those for controlling the posture of the entire body.

The neural pathway for spermatophore extrusion and transfer has not yet been established. It has been shown that sensory neurons innervating epiphallic sensilla project ipsilaterally to the lateralmost part of the terminal abdominal ganglion (McCallum and Beck, 1988; Sakai *et al*., 1991) which corresponds to region X named by Murphey (1985). Although the dorsal pouch motoneuron dendrites arborize in a similar region (Kumashiro and Sakai, 2001b), so far an overlapping between the axon terminals and dendritic arbors was not detected (Kanazawa and Sakai, 2000). Thus, we assume that some interneurons mediate transmission from cavity hair sensory neurons to a pair of the dorsal pouch motoneurons. The long latency (4 s) to cavity hair stimulation for triggering spermatophore extrusion may be due to the high threshold of the interneurons in question (Kumashiro and Sakai, 2001b).

Control of mating behavior and the reproductive cycle

It is known that central pattern generators for copulation are totally localized in the thoracic and abdominal ganglia since all the copulatory acts are artificially elicited with a female model in decapitated males as well as decerebrated males (Sakai *et al*., 1995; Matsumoto and Sakai, 2000a,b). On the other hand, the brain plays a principal role in inhibition of the pattern generators for copulation: not only in temporary inhibition in the mating stage, but also in long-term inhibition (about 1 h) in the post-copulatory sexually refractory stage. The former is simply controlled by the brain but the latter is caused by the brain under the control of the terminal abdominal ganglion. This is evidenced by the fact that males in the sexually refractory stage just after copulation quickly resume the mating response to the female model, when they are decerebrated, decapitated or connective-cut (Sakai *et al*., 1995; Matsumoto and Sakai, 2000b). The switching of the reproductive stage at spermatophore extrusion may be underlain by a flip-flop neuronal switch (Kanzaki *et al*., 1994) which alternates between two states: the mating mode and refractory mode. Recently, we have suggested that the 1 h-time-fixed sexually refractory stage starting at spermatophore preparation is controlled by some "timer neurons" located in the terminal abdominal ganglion (Ureshi and Sakai, 2001; Ureshi *et al*., 2002; Kumashiro and Sakai, 2003). Finally, it should be mentioned that the sexual excitability of the male influencing the performance of male's copulatory acts is also regulated by the brain via octopamine (Matsumoto and Sakai, 2001). Our results indicate that mating behavior in the cricket is a very useful model for the future neuroethological study.

ACKNOWLEDGEMENTS

The authors thank Dr. Field for critical reading and comments. This project was supported by a Grant-in-Aid for Scientific Research (13640680) from the Ministry of Education, Science, Culture and Sports to MS.

REFERENCES

Alexander RD (1961) Aggressiveness, territoriality, and sexual behavior in field crickets (Orthopera: Gryllidae). Behaviour 17: 130–223

Alexander RD, Otte D (1967) The evolution of genitalia and mating behavior in crickets (Gryllidae) and other Orthoptera. Misc Publs Zool Univ Mich 133: 12–18

Bailey WJ, Ridsdill-Smith (1991) Reproductive behaviour of insects. Chapman and Hall, London

Beck R (1974) The neural and endocrine control of mating behavior in the male house cricket, *Acheta domesticus*. L. Ph.D. Thesis University of Nottingham

Brower LP, Brower JVZ, Cranston EP (1965) Courtship behavior of the queen butterfly *Danaus gilippus*. Zoologica 50: 1–39

Brown RGB (1966) Courship in the *Drosophila obscura* group. II Comparative studies. Behaviour 25: 281–323

Choe JC, Crespi BJ (1997) The Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge

Choperd L (1951) Les divisions du genre *Gryllus* basees sur l'etude de l'appareil copulateur (Orth. Gryllidae). Eos 37: 267–287

Eberhard WG (1993) Copulatory courtship and morphology of genitalic coupling in seven *Phyllophaga* species (Coleoptera: Melolonthidae). J Natur Hist 27: 683–717

Englemann F (1970) The Physiology of Insect Reproduction. Pergamon Press, Oxford

Evans AR (1988) Mating systems and reproductive strategies in three Australian Gryllid crickets: *Bobilla victoriae* Otte, *Balamara gidya* Otte and *Teleogryllus commodus* (Walker)(Orthoptera: Gryllidae: Nemobiinae; Trigonidiinae; Gryllinae). Ethology 78: 21–52

Gibson JJ (1962) Observation on active touch. Psychol Rev 69: 477–491

Gnazy W, Hustert R (1989) Mechanoreceptors in behavior. In "Cricket Behavior and Neurobiology", Ed by F Huber, TE Moor, W Loher, Cornell University Press, Ithaca, London, pp 198–226

Goldfoot DA, Loon HW-V, Groeneveld W, Slob AK (1980) Behavioral and physiological evidence of sexual climax in the female stump-tailed macaque (*Macaca arctoides*). Science 208: 1477–1479

Guthrie DM, Tindall AR (1968) The biology of the cockroach. Edward Arnold Ltd, London

Hall MD, Beck R, Greenwood M (2000) Detailed developmental morphology of the spermatophore of the Mediterranean field cricket, *Gryllus bimaculatus* (De Geer)(Orthoptera: Gryllidae). Arthr Str Dev 29: 23–32

Hiraguchi T, Yamaguchi T, Takahata M (2003) Mechanoreceptors involved in the hindwing-evoked escape behavior in cricket, *Gryllus bimaculatus*. J Exp Biol 206: 523–534

Hörmann-Heck S (1957) Untersuchungen uber den Erbgang einiger Verhaltensweisen bei Grillenbastarden (*Gryllus campestris* L., *Gryllus bimaculatus* DeGeer). Z Tierpsychol 14: 137–183

Huber F (1955) Sitz und bedeutung nervöser Zentren für Instinkthandlungen beim Männchen von *Gryllus bimaculatus* DeGeer. Z Tierpsychol 12: 12–48

Hustert R, Toppel U (1986) Location and major postembryonic changes of identified 5-HT-immunoreactive neurones in the terminal abdominal ganglion of a cricket (*Acheta domesticus*). Cell Tissue Res 245: 615–621

Kanazawa R, Sakai M (2000) Neural pathways for spermatophore extrusion in the male cricket *Gryllus bimaculatus* DeGeer. Comp Biochem Physiol A 127: 384

Kanzaki R, Ikeda A, Shibuya T (1994) Morphological and physiological properties of pheromone-triggered flipflopping descending interneurons of the male silkworm moth, *Bombyx mori*. J Comp Physiol A 175: 1–14

Khalifa A (1949) The mechanism of insemination and the mode of action of the spermatophore in *Gryllus domesticus*. Q J Microsc Sci 90: 281–292

Khalifa A (1950) Sexual behavior in *Gryllus domesticus* L. Behaviour 2: 264–274

Kumashiro M, Sakai M (2001a) Reproductive behavior in the male cricket *Gryllus bimaculatus* DeGeer: I Structure and function of the genitalia. J Exp Biol 204: 1123–1137

Kumashiro M, Sakai M (2001b) Reproductive behavior in the male cricket *Gryllus bimaculatus* DeGeer: II Neural control of the genitalia. J Exp Biol 204: 1139–1152

Kumashiro M, Sakai M (2003) Auto-spermatophore extrusion in male crickets. J Exp Biol 206: 4507–4519

Loher W, Rence B (1978) The mating behavior of *Teleogryllus commodus* (Walker) and its central and peripheral control. Z Tierpsychol 46: 225–259

Mann T (1984) Spermatophores. Springer-Verlag, Berlin, Heidelberg, New York, pp 107–111

Matsumoto Y, Sakai M (2000a) Brain control of mating behavior in the male cricket *Gryllus bimaculatus* DeGeer: the center for inhinition of copulation actions. J Insect Physiol 46: 527–538

Matsumoto Y, Sakai M (2000b) Brain control of mating behavior in the male cricket *Gryllus bimaculatus* DeGeer: brain neurons responsible for inhibition of copulation actions. J Insect Physiol 46: 539–552

Matsumoto Y, Sakai M (2001) Brain control of mating behavior in the male cricket *Gryllus bimaculatus* DeGeer: Excitatory control of copulatory actions. Zool Sci 18: 659–669

Matthews RW, Matthews JR (1978) Insect behavior. John Wiley and Sons, New York

McCallum AS, Beck R (1988) Epiphallic afferent projections into the terminal abdominal ganglion of the cricket *Acheta domesticus* and *Gryllus bimaculatus*. Invert Repr Dev 14: 289–293

Meisel RL, Sachs BD (1994) The physiology of male sexual behavior. In “The physiology of reproduction vol 2”, Ed by E Knobil, JD Neil, Raven press, New York, pp 3–106

Murphey RK (1985) A second cricket cercal system: bristle hairs and the interneurons they activate. J Comp Physiol 156: 357–367

Ootsubo T, Sakai M (1992) Initiation of spermatophore protrusion behavior in the male cricket *Gryllus bimaculatus* DeGeer. Zool Sci 9: 955–969

Qadri MAH (1940) On the development of the genitalia and their ducts of orthopteroid insects. Trans R Ent Soc Lond 90: 121–175

Randell RL (1964) The male genitalia in Gryllinae (Orthoptera: Gryllidae) and a tribal revision. Can Entomol 96: 1565–1607

Rence R, Loher W (1977) Contact chemoreceptive sex recognition in the male cricket, *Teleogryllus commodus*. Physiol Entomol 2: 225–236

Sakai M, Matsumoto Y, Takemori N, Taoda Y (1995) Post-copulatory sexual refractoriness is maintained under the control of the terminal abdominal ganglion in the male cricket *Gryllus bimaculatus* DeGeer. J Insect Physiol 41: 1055–1070

Sakai M, Ootsubo T (1988) Mechanism of execution of sequential motor acts during copulation behavior in the male cricket *Gryllus bimaculatus* DeGeer. J Comp Physiol A 162: 589–600

Sakai M, Taoda Y, Mori K, Fujino M, Ohta C (1991) Copulation sequence and mating termination in the male cricket *Gryllus bimaculatus* DeGeer. J Insect Physiol 37: 599–615

Satou M, Shiraishi A, Matsushima T, Okumoto N (1991) Vibrational communication during spawning behavior in the hime salmon (landlocked red salmon, *Oncorhynchus nerka*). J Comp Physiol A 168: 417–428

Schäffner K-H, Koch U (1987) A new field of wing campaniform sensilla essential for the production of the attractive calling song in crickets. J Exp Biol 129: 1–23

Schmidt K, Gnatzy (1972) Die Feinstruktur der Sinneshaare auf den Cerci von *Gryllus bimaculatus* Deg. (Saltatoria, Gryllidae). III Die kurzen Borstenhaare. Z Zellforsch Mikrosk Anat126: 206–222

Seabrook WD (1968) The innervation of the terminal abdominal segments (VIII–XI) of the desert locust, *Schistocerca Gregaria*. Can Entomol 100: 693–715

Simmons LW (1986) Female choice in the field cricket *Gryllus bimaculatus* (De Geer). Anim Behav 34: 1463–1470

Snell LC, Killian KA (2000) The role of cercal sensory feedback during spermatophore transfer in the cricket, *Acheta domesticus*. J Insect Physiol 46: 1017–1032

Snodgrass RE (1937) The male genitalia of orthoperoid insects. Smithon Misc Collns 96: 76–80

Stich HF (1963) An experimental analysis of the courtship pattern of *Tipula oleracea* (Diptera). Can J Zool 41: 99–109

Sugawara T (1993) Oviposition behaviour of the cricket *Teleogryllus commodus*: Mechanosensory cells in the genital chamber and their role in the switch-over of steps. J Insect Physiol 39: 335–346

Thornhill R, Alcock J (1983) The evolution of insect mating system. Harvard University Press, Cambridge

Tinbergen N (1951) The study of instinct. The Clarendon Press, Oxford

Ureshi M, Sakai M (2001) Location of the reproductive timer in the male cricket *Gryllus bimaculatus* DeGeer as revealed by local cooling of the central nervous system. J Comp Physiol A 186: 1159–1170

Ureshi M, Sakai M (2002) Serotonin precursor (5-hydroxytryptophan) has a profound effect on the post-copulatory time-fixed sexually refractory stage in the male cricket, *Gryllus bimaculatus* DeGeer. J Comp Physiol A 188: 767–779

Walker FM (1922) The terminal structures of orthopteroid insects: a phylogenetic study. Ann Ent Soc Amer 15: 1–76

(Received May 7, 2004 / Invited Review)