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Author(s): William G. Eberhard

Source: *Evolution*, Vol. 55, No. 1 (Jan., 2001), pp. 93-102

Published by: Society for the Study of Evolution

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SPECIES-SPECIFIC GENITALIC COPULATORY COURTSHIP IN SEPSID FLIES (DIPTERA, SEPSIDAE, *MICROSEPSIS*) AND THEORIES OF GENITALIC EVOLUTION

WILLIAM G. EBERHARD

Smithsonian Tropical Research Institute and Escuela de Biología, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica
E-mail: archiseipsis@biologia.ucr.ac.cr

Abstract.—Males of *Microsepsis eberhardi* and *M. armillata* use their genitalic surstyli to rhythmically squeeze the female's abdomen with stereotyped movements during copulation. Squeezing movements did not begin until intromission had occurred and, contrary to predictions of the conflict-of-interest hypothesis for genitalic evolution, did not overcome morphological or behavioral female resistance. Contrary to predictions of the lock-and-key hypothesis, female morphology was uniform in the two species and could not mechanically exclude the genitalia of either species of male. The complex pattern of squeezing movements differed between the two species as predicted by the sexual selection hypothesis for genitalic evolution. Also, evolutionarily derived muscles and pseudoarticulations in the male's genitalic surstyli facilitated one type of movement, whose patterns were especially distinct. The data support the hypothesis that the male surstyli evolved to function as courtship devices.

Key words.—Copulatory courtship, cryptic female choice, genitalic evolution, sexual selection.

Received December 22, 1999. Accepted July 27, 2000.

Rapid and divergent evolution of male genitalia is one of the most widespread patterns of animal evolution (Eberhard 1985). The three hypotheses most often mentioned to explain this pattern are species isolation by lock and key (e.g., Shapiro and Porter 1989), male-female conflict over control of copulation (Lloyd 1979; Alexander et al. 1997), and sexual selection by cryptic female choice (Eberhard 1985, 1996, 1997; Arnqvist and Danielsson 1990). Observations of the behavior of male genitalia during copulation offer a chance to discriminate among these hypotheses.

The lock-and-key hypothesis, which has received little convincing support despite a long history of attempts to test it (summary in Shapiro and Porter 1989; also Arnqvist 1998), predicts morphological complementarity between males and females: the species-specific features of male and female genitalia should mesh or come into contact during copulation, and these features of the female should be capable of physically excluding the male genitalia of closely related species but not those of her own species. The conflict-of-interest hypothesis also predicts frequent morphological complementarity in species-specific traits (Alexander et al. 1997) and that the female will often have the morphological ability to exclude at least some males of her own species. The sexual selection hypothesis, in contrast, does not make strong predictions about morphological fit and is compatible with a lack of species-specific morphological complementarity.

Discriminating between the conflict-of-interest and the sexual selection hypotheses can be confusing, in part because the names themselves are misleading. The hypothesis of sexual selection by cryptic female choice also involves a conflict of interest between male and female: the male's best interests are favored by fertilizing all of the eggs of all of the females he encounters; the female's best interests are favored by only allowing a biased subsample of males to fertilize her eggs. The conflict-of-interest hypothesis, however, is an explicit alternative to cryptic female choice (Alexander et al. 1997). One important difference between the two hypotheses concerns the payoffs to females. Female resistance under the conflict-of-interest hypothesis is favored because she obtains

naturally selected advantages, not because the female thereby obtains superior sons that will more effectively court the females in the next generation (and, if the female ancestors of the male were especially selective, more selective daughters). For instance, if the female is able to avoid being obliged to copulate, she may gain by having more opportunity to forage or to oviposit or she may avoid increased risks of predation. Similar naturally selected payoffs to the female are also presumed to be common in other more general models of male-female conflict such as chase-away selection (Holland and Rice 1998). The advantages mentioned as likely to result from female resistance include copulation at more ideal times and places, less frequent copulation, higher survival, decreased predation, decreased energy costs, decreased exposure to parasites, and decreased harassment by other males. Conflict ideas assume that these naturally selected advantages to the female outweigh any indirect benefits she would derive from the kinds of offspring superiority assumed under sexual selection models (Alexander et al. 1997).

A second difference is that the conflict-of-interest hypothesis predicts that species-specific male genitalic structures will forcefully overcome species-specific morphological or behavioral female resistance: "The clue to this interpretation will often be evidence of forcing or manipulation by one sex, usually the male," and "the possibility must be considered that variations in success of insemination result from force or coercion rather than titillation" (Alexander et al. 1997, pp. 9, 20). In addition, such coercive behavior is predicted to occur at moments during male-female interactions when the outcome of the male's attempt to copulate and fertilize the female's eggs is being determined. Except in cases in which the female is placed under physical duress by the male, relatively direct types of male genitalic manipulation of the female are expected, such as forcing open her genital aperture to allow intromission. In contrast, the sexual selection hypothesis does not presume that overt female genitalic resistance occurs and is compatible with male behavior that cannot forcefully overcome female resistance and is apparently only stimulatory in nature. Indirect rather than direct manipula-

tions of the female are expected to be used by the male to obtain favorable responses (e.g., stroke the female to induce her to open her genital aperture and allow intromission).

Sepsid flies offer an opportunity to make these crucial observations due to a combination of traits. As is common in insects, the male genitalia of sepsids are usually species-specific in form. One portion of the male's genitalia, the surstyli, are clasping organs that are especially useful in distinguishing closely related species whose morphology is otherwise relatively similar (e.g., Duda 1925, 1926; Hennig 1949; Silva 1993; Ozerov 1992, 1993). The surstyli do not enter the female, but rather grasp the surface of her abdomen (Eberhard and Pereira 1996; Eberhard and Huber 1998; W. G. Eberhard, unpubl. ms.). Thus, in contrast to the genitalia of many other animals, which are hidden inside the female during copulation, the behavior of a male sepsid's genitalic surstyli can be observed directly. A previous study of sepsid genitalic function, however, did not include behavior and was based instead on pairs frozen during copulation. It showed that lock-and-key arguments failed to explain the species-specific surstylus forms in two species of *Archiseopsis*, because there was no female lock that could mechanically exclude males and because the site on the female abdomen that was grasped by the male's surstyli varied intraspecifically (Eberhard and Pereira 1996). Although there was also evidence arguing against male-female conflict, the overall conclusion was that surstylus function was probably a combination of physical restraint and stimulation.

The present study, which describes the movements of the male surstyli just prior to and during copulation in *Microseopsis eberhardi* Ozerov and *M. armillata* (Melander and Spuler) suggests that this conclusion should be changed. Both the movements themselves and the contexts in which they occur support the idea that the male surstyli function to stimulate the female, as proposed by the sexual selection by cryptic female choice hypothesis, rather than to mesh with a species-specific female structure or to forcefully overcome her resistance by grasping her, as would be supposed by the lock-and-key and male-female conflict hypotheses.

MATERIALS AND METHODS

Behavioral observations involved virgin flies raised from fresh cow dung in which females of *M. eberhardi* collected in Panama (Barro Colorado Island, elevation 20 m) and *M. armillata* in Costa Rica (San Antonio de Escazu, 1400 m) had oviposited. Newly emerged adults (< 12 h old) were separated by sex, and kept in small cages with honey, water, and dung for 3–8 days before being placed in pairs in small petri dishes. Genitalic behavior during copulation was observed ventrally at 20× and 40× in 11 pairs of *M. eberhardi* and 20 pairs of *M. armillata* by placing the petri dish under a dissecting microscope. Continuous behavioral records of genitalic movements were obtained from taped verbal descriptions. The force of the male's squeezes on the female was judged on the basis of the displacement of the female sternite VI and the depth to which the surstyli pressed on the membranes surrounding this sternite.

Copulating pairs to be examined with the scanning electron microscope were frozen using ethyl chloride spray and im-

mediately dropped into 80% ethyl alcohol at room temperature. A previous study of *Archiseopsis* spp. showed that the positions of male surstyli are not altered by this treatment (Eberhard and Pereira 1996). This lack of effect was confirmed directly in both species of this study, because there were no noticeable differences in the positions of the surstyli or their deflections of the female's abdominal sclerites when living and preserved pairs were compared. The positions of the male's intromittent genitalia, which are tightly braced within the female (Eberhard and Huber 1998), were presumed to also be unaffected by freezing. Whole mounts to be examined with a compound microscope were dissected in 80% ethyl alcohol and transferred to euparal on microscope slides.

All orientations mentioned in descriptions of pairs (e.g., anterior, posterior) refer to the perspective of the female. Sample sizes for different aspects of behavior vary because of differences in female mobility that impeded observation, angles of viewing, and different attention to different details. Means are followed by one standard deviation, and times are given as minutes:seconds. Statistical tests employed Wilcoxon ranks tests unless specified otherwise. Names for genitalic structures follow McAlpine (1981) and Eberhard and Huber (1998). There is no comparative study of male sepsid intromittent genitalia that permits determination of homologies with those of other flies.

RESULTS

Male Morphology

As in other sepsids, the male genitalia of both *Microseopsis* species consist of intromittent structures (including the aedeagus), which are introduced into the female's body during copulation, and clasperlike epandria, whose distal portions, the surstyli, grasp the external surface of her abdomen. The male's intromittent genitalia differ only slightly from those of *Archiseopsis* (Eberhard and Huber 1998). In contrast, the surstyli differ from those of *Archiseopsis* spp. (Ozerov 1992, 1993; Silva 1993; Eberhard and Pereira 1996) in that they are strongly asymmetrical in both size and form (Fig. 1). The smaller, left surstylus is a single rigid structure (Fig. 1A, C), as is typical in other sepsids (e.g., Pont 1979; Silva 1993, Eberhard and Pereira 1996). It is forked distally and the tip of the shorter inner process is somewhat expanded and flattened (Figs. 1A, 2B). This surstylus was inserted between the tergites of the female during copulation (Fig. 2C). The tip of the longer outer process of the left surstylus is cylindrical and bears a series of long socketed setae (Fig. 1A, D). The larger right surstylus is more complex and has two features that are apparently designed to permit flexion. The lateral surface of the long, right, outer process has a slit (outer pseudoarticulation in Fig. 1D), in which an apparent membrane is visible. The tip of this process is cylindrical and bears a series of fine setae (Fig. 1A, C). The massive inner process (the finger) bears a second, inner pseudoarticulation near its base, where the cuticle is thin and sharply folded (Fig. 1A, B, D). The inner process itself has a flat inner surface, a central ridge on the outer (lateral) surface, and lacks setae (Fig. 1A, B).

The musculature is relatively simple on the left side of the epandrium of *M. armillata* with a single fan of fibers di-

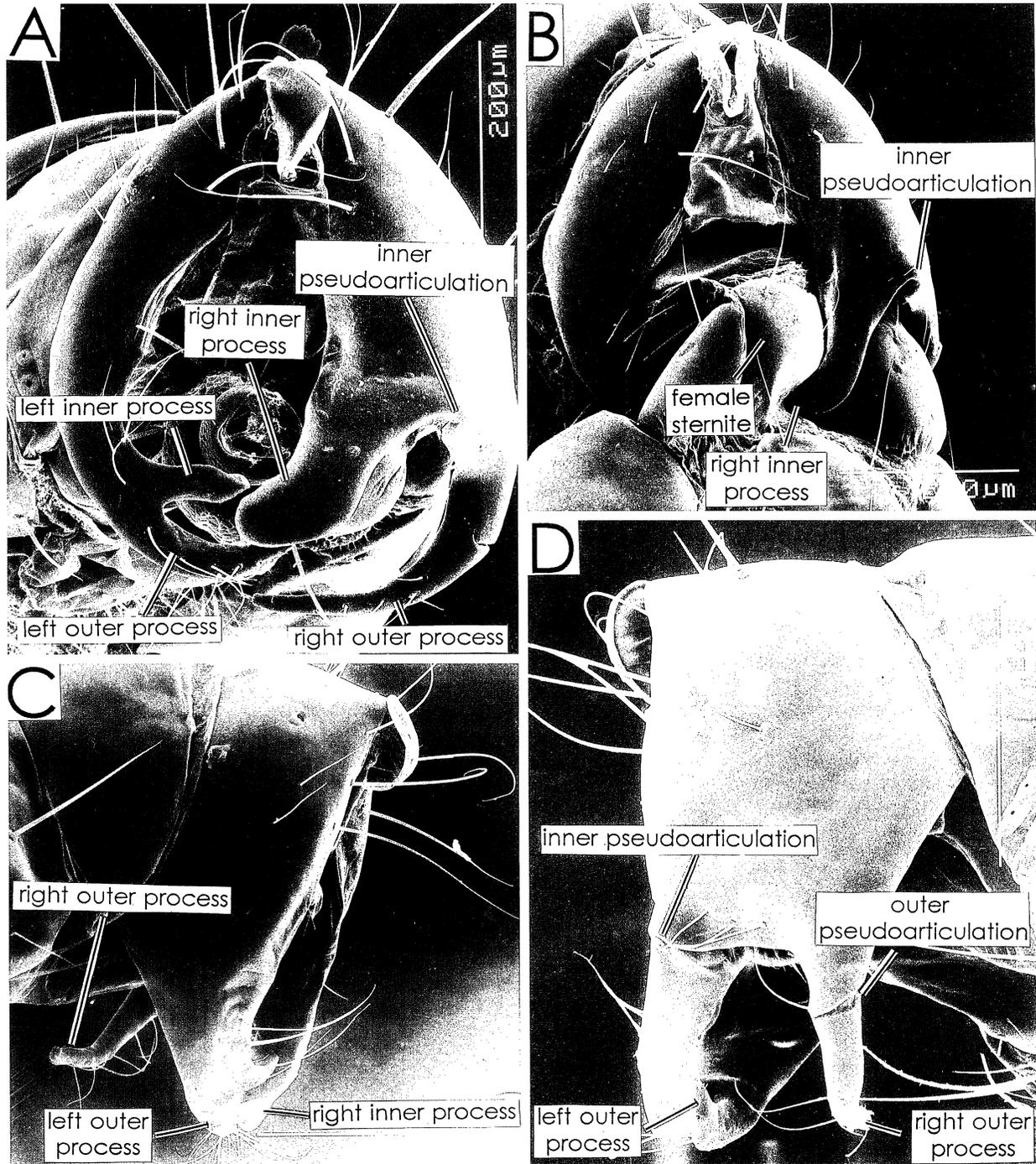


FIG. 1. (A) Epandria of a male *Microsepsis armillata*, with the surstyli flexed away from the ventral surface of the abdomen. (B) Antero-ventral view of tip of female abdomen being squeezed asymmetrically during a finger flex by the male's right surstylus; note flexion of right inner process at the inner pseudoarticulation (compare with A) and the resulting asymmetrical displacement of the female's sternite. (C) Lateral view of left epandrium and its surstylus. (D) Lateral view of right epandrium and its surstylus.

verging from the central area to attach along the lateral surface of the epandrium and the base of the surstylus (Fig. 3). Their contraction presumably caused the entire structure to close medially (and thus squeeze the female, see below). The musculature on the right side consists of two fan-shaped groups of fibers. One group diverges from a central area as in the left epandrium and probably caused medial movements.

The other group diverges from the inner pseudoarticulation and presumably caused the movements of the finger with respect to the rest of the surstylus (finger flex movements in the descriptions below; compare Fig. 1B with 1A). No muscles were seen that could cause movement at the outer pseudoarticulation. In some copulating pairs this process bent rearward at this pseudoarticulation (Fig. 2B).

Behavior of Male Surstyli Just Prior to Intromission

As in other sepsids (e.g., Parker 1972a), the male mounted the female with little preliminary courtship and clamped her wings with his modified front legs. Periodically he then courted her (Eberhard, unpubl. ms.) and pressed the tip of his abdomen against hers in apparent attempts to intromit. His surstyli were flexed away from the ventral surface of his abdomen during intromission attempts. Often, but not always, they made a few closing movements, but their tips did not engage the female as they would later (Fig. 1B, Fig. 2A–C), but instead slipped across the ventral surface of her abdomen.

During most genitalic contacts that preceded intromission, the tip of the female's abdomen (her proctiger) was directed posteriorly, in its usual resting position. In four pairs of *M. eberhardi* and three pairs of *M. armillata*, favorable angles of view allowed me to confirm that the male's surstyli did not contact the female as intromission occurred. In three pairs of *M. armillata*, I could see that just before intromission the female's proctiger was flexed dorsally about 90° from its usual posteriorly directed position, thus exposing her vulva (Eberhard, unpubl. ms.). In one pair, the female's proctiger remained immobile in this acceptance posture for several seconds before the male intromitted. The tips of the male's genitalic surstyli never touched her proctiger and they probably did not touch her abdomen at all during this period. The male apparently *induced* the female to raise her proctiger and thus expose her vulva and allow intromission, rather than forcing her to do so.

Behavior of the Male and His Surstyli during Copulation

Similarities between the species

Wing-wiping copulatory courtship movements, which resulted in transfer of liquid from the male's abdomen and hind legs to the female's wings (W. G. Eberhard, unpubl. ms.), were indistinguishable in the two species. The duration of wing wiping was also not significantly different (means for *M. eberhardi* and *M. armillata* were, respectively 3:00 ± 0:34 and 2:39 ± 0:53), but wing wiping began earlier in copulation in *M. eberhardi* (0:49 ± 0:10 after intromission, as compared with 2:01 ± 1:00 in *M. armillata*, $P = 0.007$).

The genitalic movements of the two species were also similar in several respects. During the first minute or more following intromission, the tips of the male's surstyli did not squeeze the female's intersegmental abdominal membranes, as they would later in copulation (Figs. 1B, 2A–C). Instead they were held open and were either completely out of contact with her or resting lightly on her sternites posterior to where they would later grasp her. Soon after intromission, the male began to intermittently push his surstyli forward and then close them slightly as he drew them back posteriorly, causing their tips to slide across the surface of the female's sternite VI. Each cycle of this sort lasted 2–4 sec.

Usually these movements ended and the surstyli were then completely quiet in an open position for a short period (means of 0:33 ± 0:14 and 0:31 ± 0:16 for *M. eberhardi* and *M. armillata* before they once again began to move forward periodically and close and open. At this stage the male pushed them farther forward on the female's abdomen prior to each

closure, and soon the tip of the finger reached past the anterior margin of the female sternite VI and pressed inward on the intersegmental membrane (Figs. 1B, 2A, 2B). When the surstyli reached this position, they squeezed the female more tightly. They remained in this anterior position during the rest of the copulation. The only clear difference between the two species at this stage was that this first tight squeeze occurred earlier in *M. eberhardi* (0:54 ± 0:23 after intromission) than in *M. armillata* (2:10 ± 0:44 after intromission; $P = 0.011$). In both species rhythmic squeezing movements began very soon after (or in some cases slightly before) the moment when wing-wiping movements began (means 0:10 ± 0:20 and 0:04 ± 0:27 after wing wiping commenced, no significant difference).

As soon as the male firmly grasped the female with his surstyli, he began to pinch her rhythmically, using two types of movements. Squeezing involved moving the surstyli medially in a simultaneous closing movement. Because the surstyli were asymmetrical and grasped the female's abdomen asymmetrically, squeezes caused the female's cuticle (especially her sternite VI) to be twisted as well as squeezed (Fig. 1B, 2A). The second type of movement, finger flexing, involved only the large inner finger of the right surstylus, which moved dorso-ventrally with respect to the rest of the epandrium (Figs. 1A, B). When the finger flexed, it caused the cuticle of the female's sternite VI to bend. It was not always easy to distinguish a single squeeze from a single finger flex (in the descriptions below, "closing" refers to both types of pressure on the female and "opening" to relaxation of both types of pressure). Differences between squeezes and finger flexes were clearest when it was possible to see the inner surface of the smaller, left surstylus, and watch its movement with respect to the female tergite just below it. During a squeeze, this margin moved medially with respect to the tergite, while during a finger flex it did not.

Both squeezing and finger flexing occurred more or less continually during the long period (11:47 ± 2:50 in *M. eberhardi*, 21:18 ± 7:44 in *M. armillata*, $P = 0.005$) between intromission and the transition (below). During most of this time the surstyli were in nearly constant motion, increasing and decreasing their pressure on the female (Fig. 4). Although both squeezing and finger flexing often occurred more or less simultaneously, they also occurred separately (Fig. 4). In both species squeezing became relatively less common than finger flexing in the latter half of the pretransition phase. Some closures and flexes were less intense than others. Pauses of more than a second in the open position before beginning to close again were rare, whereas sustained closures of several seconds occurred more often. The intensity of the squeezes was usually only moderate compared with squeezes that occurred later. The intensity of finger flexes became weaker near the end of the pretransition phase.

During the entire pretransition phase the male's black sperm pump, which was usually visible through the dorsal membrane just anterior to his epandria, vibrated continuously about two to five times per second.

The transition phase lasted about 30–60 sec in both species. The male's surstyli closed very tightly early in the transition and were held immobile, while his entire abdomen made a series of about 20 to 40 strong pushing, thrusting, or exten-

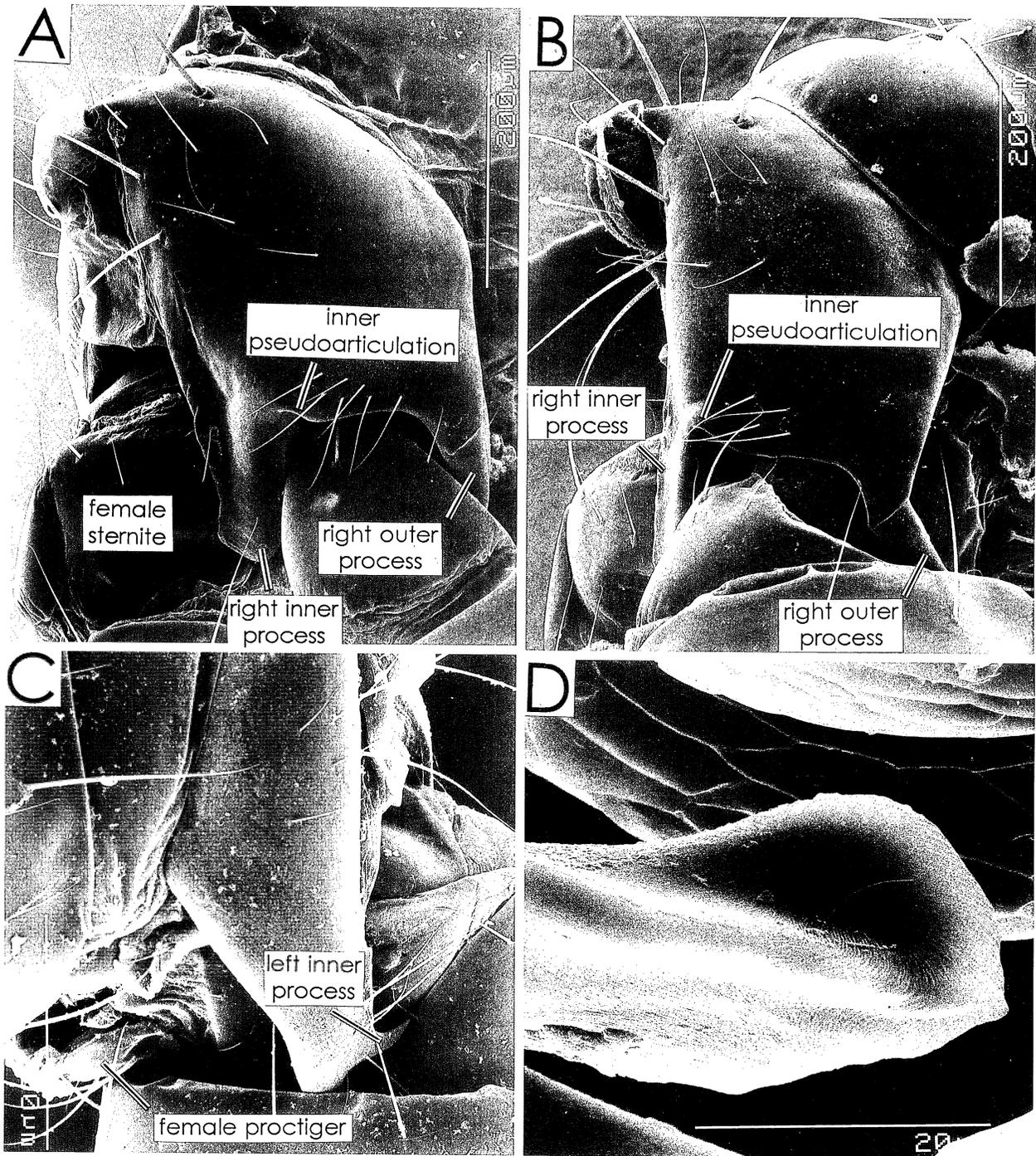


FIG. 2. Genital positions of copulating pairs of *Microsepsis armillata*. (A) Lateral and slightly posterior view of right male surstylus holding female abdomen; the male's inner process causes the female sternite VI to tilt sharply. (B) Lateral view of right male surstylus holding the female abdomen; the outer process is bent at the pseudoarticulation. (C) Lateral view of the left male surstylus holding the female abdomen; the tip of the inner process is inserted in a narrow crack between female tergites V and VI. (D) Flattened tip of left inner process.

sion movements (these movements were only consistently distinct and rhythmic enough to be counted in *M. eberhardi*, where they averaged 39.7 ± 13.0 , and occurred once every 1.1 ± 0.2 sec, speeding up somewhat in the latter portion of the transition). Often each movement of the male's abdomen was accompanied by a narrowing of its diameter; in one *M.*

eberhardi pair it seemed that the contractions were peristaltic and moved posteriorly along his abdomen. During the transition or soon afterward the male's sperm pump ceased vibrating and it remained immobile during the rest of copulation. Similar transition behavior occurs in *Archisepsis* species at about the time when sperm and spermatophore ma-

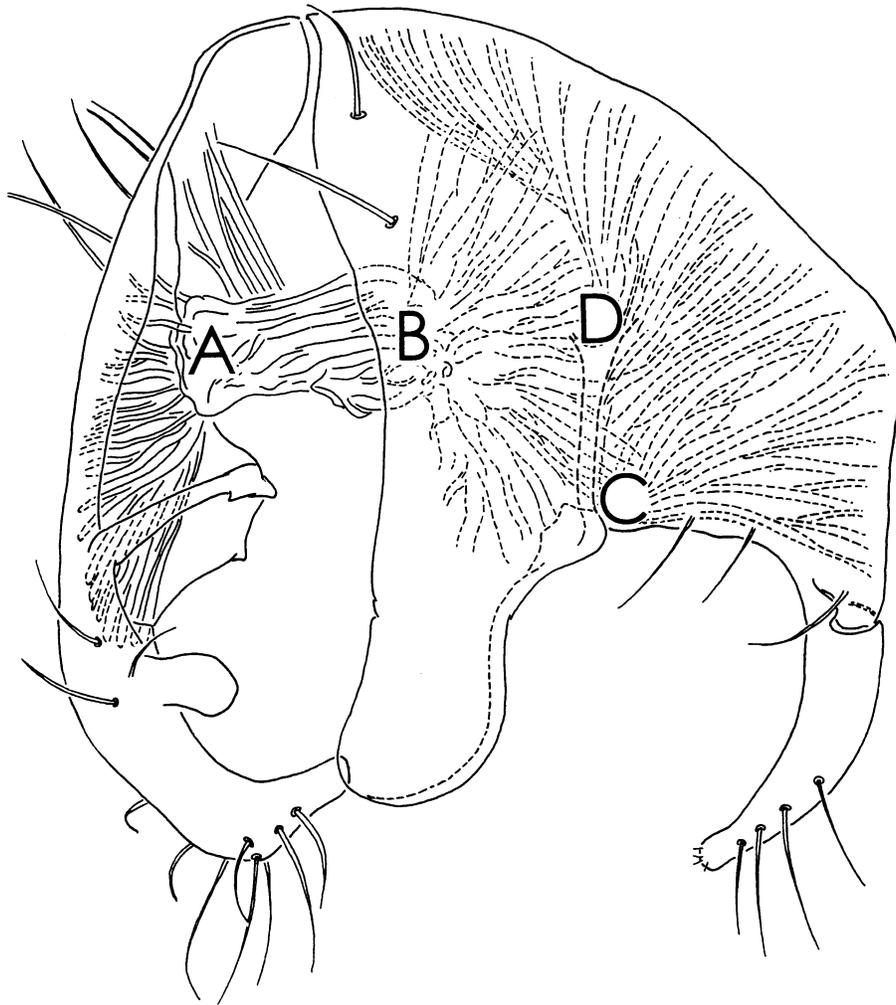


FIG. 3. Schematic posterior view of epandria and their associated muscles in a male *Microsepsis armillata*. The smaller left epandrium has a single group of muscle fibers that converge at A and presumably act to close the epandrium medially. The larger right epandrium has a similar group of fibers that converge at B. An additional group converges at C and D, near the inner pseudoarticulation, and presumably moves the large inner process with respect to the rest of the epandrium.

terial are transferred to the female (Eberhard and Huber 1998, W. G. Eberhard, unpubl. data).

During most of the posttransition period the male's surstyli were immobile. In *M. eberhardi* and in some pairs of *M. armillata* (below) this immobility was interrupted by a period of rhythmic finger flexing (Fig. 4), during which the outer processes of the surstyli remained tightly squeezed shut. In *M. eberhardi* the temporal pattern of this posttransition finger flexing differed from pretransition finger flexing. The finger was unflexed for about as long as it was flexed, and the mean ratio of unflexed to flexed during posttransition was 1.02 ± 0.14 to 1.0, as compared with 0.52 ± 0.11 to 1.0 during the pretransition phase ($P = 0.012$). The rate of posttransition finger flexing in this species (one each 5.3 ± 0.9 sec) was similar to that of pretransition flexing (one each 4.9 ± 0.9 sec; not significantly different). When posttransition finger flexing ceased, the male slowly tightened his grip over a period of several seconds, returning to a very tight closure, which he then held motionless until copulation ended.

In both species copulation ended when the male opened

his surstyli and released his grip on the female's abdomen, climbed off the female, turned 180° , struggled for several seconds to pull his genitalia free (a process that caused the female's ovipositor to be everted), and then walked away.

Differences between the species

The genitalic behavior of the two species differed in several respects in addition to the differences just noted. In *M. armillata* there were multiple, rapid finger flexes imposed on the rhythmic pretransition open-close movements (mean one every 0.39 ± 0.09 sec; inset in Fig. 4). These rapid finger flexes were essentially continuous during the first two-thirds or more of the pretransition phase and were especially pronounced during slow closing movements. Rapid finger flexing occurred in only two of 10 pairs of *M. eberhardi* and in both cases was very brief and infrequent. In one pair, it only occurred in a few bursts lasting 10–15 sec during the last 60 sec of the pretransition phase.

A second difference was that the open-close cycles during

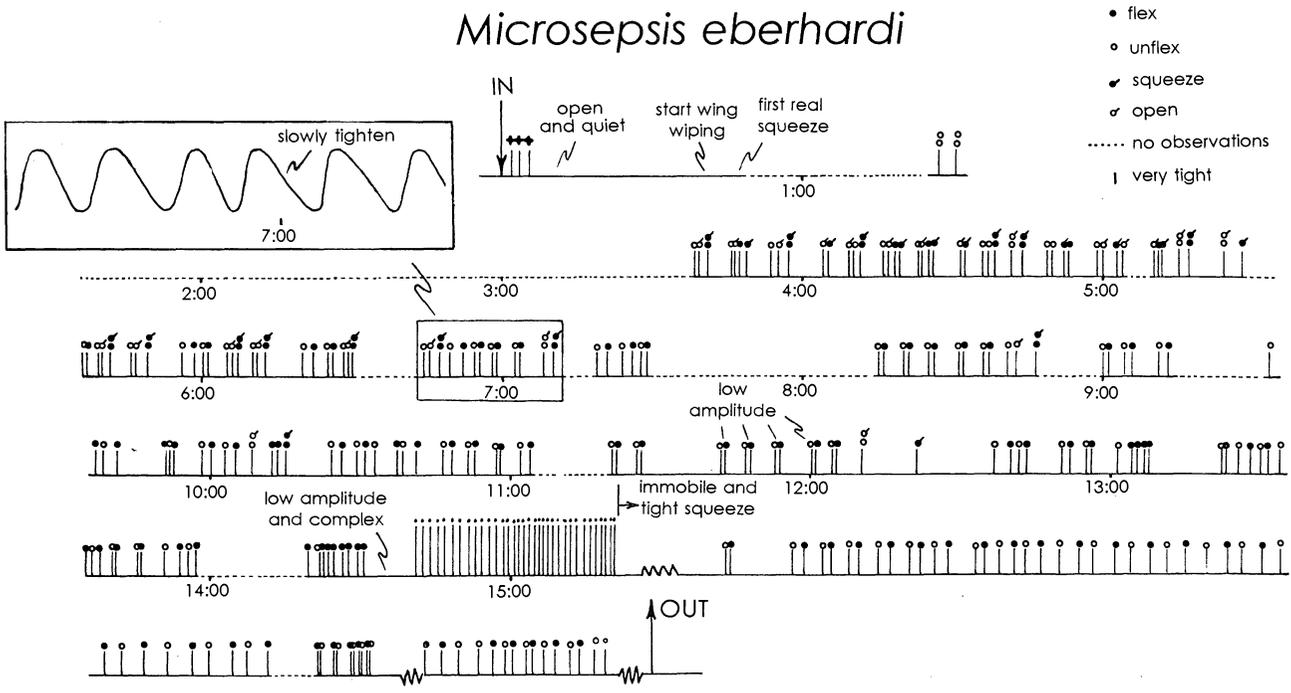
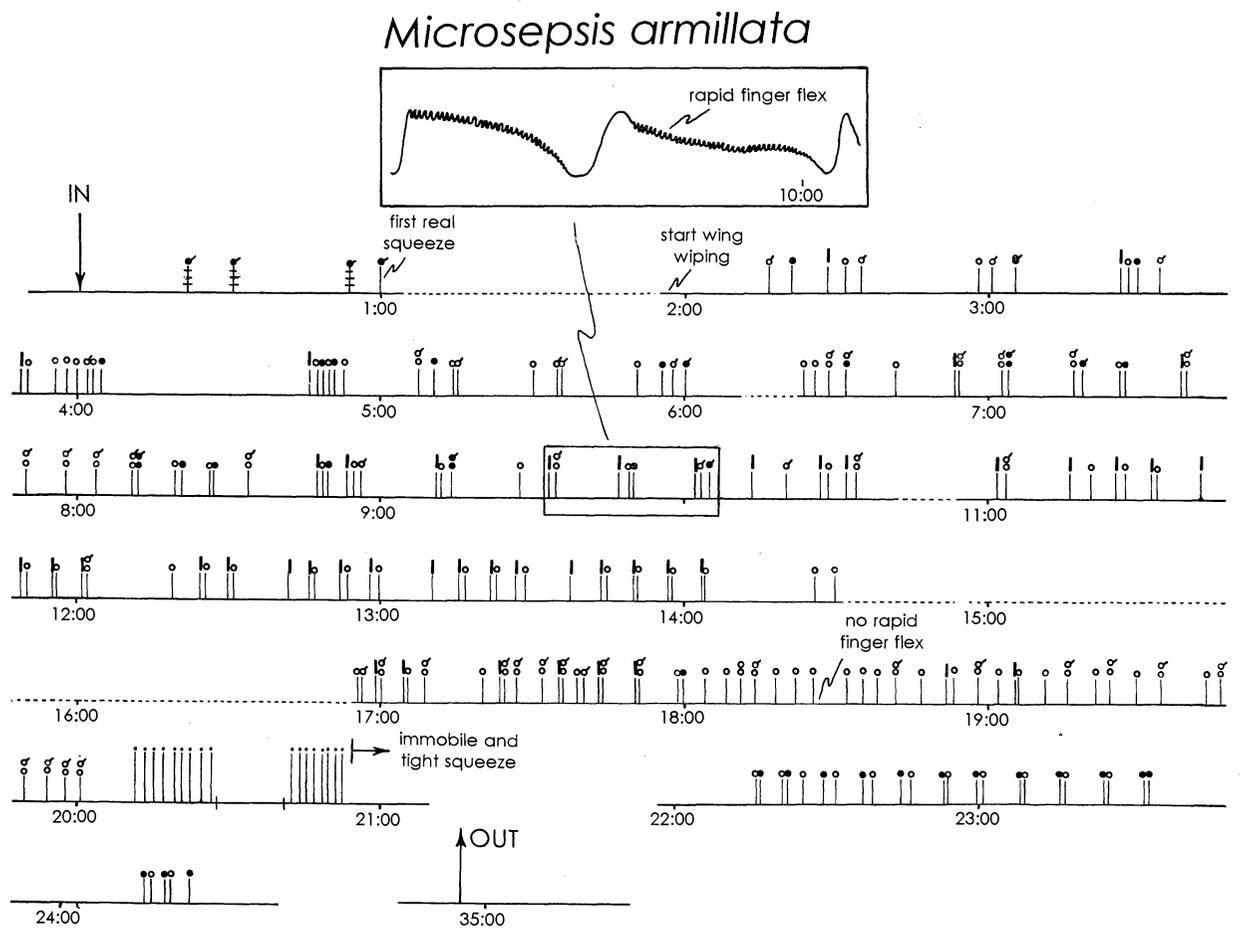


FIG. 4. The temporal patterning of genitalic behavior during copulation in one pair each of *Microsepsis armillata* and *Microsepsis eberhardi*. Distinctions between squeezes and finger flexes were sometimes difficult (see Materials and Methods). The two insets illustrate diagrammatically the approximate changes in pressure over time in typical cycles of squeezing and finger flexing, as deduced from changes in the degree of deformation of the surface of the female's abdomen.

the pretransition phase in *M. eberhardi* were both more frequent and more constant in frequency than those in *M. armillata* (Fig. 4). In *M. eberhardi* cycles averaged one every 4.9 ± 0.9 sec and successive opening movements were never regularly separated by more than 10 sec. In contrast, in *M. armillata* there were always periods of at least 5 sec and often much longer between successive openings (Fig. 4). In *M. armillata* the male's surstyli often opened several times in relatively rapid succession and then closed slowly and remained closed for 10 sec or more (Fig. 4).

An additional difference concerned the pattern in which closures were tightened. In all pairs of *M. armillata* the male often closed especially tightly during the second or so at the end of a long closure, just before he opened again (Fig. 4). These especially tight closures were so regular that it was often possible to predict when the next opening movement would occur. In some (but not all) pairs the male also leaned laterally when he squeezed the female especially tightly, apparently twisting her abdomen further with his surstyli as a result. This pattern of closing down especially tightly just before opening was not seen in *M. eberhardi*.

Behavior during the transition phase also differed between the two species. In *M. eberhardi* the male's abdomen moved in an easily distinguished rhythmic pattern, whereas in 60% of the *M. armillata* pairs I was not able to even distinguish individual pushing movements. The transition began sooner after intromission in *M. eberhardi* ($11:47 \pm 2:50$) than in *M. armillata* ($21:18 \pm 7:44$; $P = 0.005$). The duration of transition was slightly longer in *M. eberhardi* (mean $0:44 \pm 0:12$) than in *M. armillata* (mean $0:30 \pm 0:07$; $P = 0.04$).

All male *M. eberhardi* performed a burst of posttransition finger flexing behavior (mean duration $2:11 \pm 0.49$ sec). In contrast, posttransition finger flexing was usually entirely absent in *M. armillata* (70% of the pairs; $P = 0.003$ with χ^2 test), and when it did occur it involved only a very few flexions (as few as two or three) that lasted for a shorter time (mean duration $1:04 \pm 0.57$).

Positions of Male Intromittent Genitalia within the Female

Examination of nine pairs of *M. armillata* frozen during copulation indicated that the basic morphology of the male intromittent genitalia and the overall course of events inside the female during copulation is probably very similar to that in *Archiseopsis* (Eberhard and Huber 1998). In six pairs, the fibrous tip of the distal body of the male was inserted to or beyond the dorsal projection of the bursal wall. At the maximum insertion the tip was near the mouth of the spermathecal duct. There was a large spermatophore in the bursa of the other three females, and the male intromittent genitalia were partially withdrawn. The male's genitalia also had pairs of similarly shaped spiny arches and paddles near the base as in *Archiseopsis*, where they grasped the female's bursal lining and braced the genitalia tightly inside the female (Eberhard and Huber 1998).

DISCUSSION

These observations of *Microsepsis* agree with previous data on the closely related *Archiseopsis* (Eberhard and Pereira 1996) in that they are not compatible with the lock-and-key hy-

pothesis. There was no sign of any female structure that would impede genitalic coupling with cross-specific males. The males' species-specific surstyli grasp soft, featureless membranes on the female's abdomen.

At first glance, *Microsepsis* seems to conform to the male-female conflict hypothesis of genitalic evolution (Lloyd 1979; Alexander et al. 1997). Male behavior prior to mounting and attempting intromission clearly fits the coercive (as opposed to luring) category that they propose is associated with male-female conflict. The male's surstyli are both designed and used as powerful claspers to grasp the tip of the female's abdomen. They are well-designed to function as forceful weapons in male-female conflicts by seizing the female's abdomen and restraining her so that the male's intromittent genitalia can be positioned for intromission. But the behavioral observations reported here show that this interpretation is incorrect.

The events surrounding the moment of intromission in both *M. eberhardi* and *M. armillata* indicate that male surstyli have no role in forcefully promoting copulation. The surstyli did not grip and squeeze the female while intromission was being achieved and were not used to forcefully improve the male's chances of copulating. Instead they only gripped the female many seconds later, after intromission had occurred, and this grip was only slowly tightened, long after the male's genitalia were safely braced deep inside the female. The site that they grasped was not near where sperm would be transferred (see Eberhard and Pereira 1996 on *Archiseopsis*), so male squeezes could have no direct positive effect on the course of intromission and sperm transfer. Several details of surstylus behavior, such as complex sustained rhythmic squeezes, rhythmic finger flexes, the progressively tighter grip, and the multiple species-specific differences in timing and duration are not easily explained by the idea that the surstyli serve as instruments to forcefully overcome female resistance. In contrast these details are all easily reconciled with the hypothesis that the surstyli function as stimulators.

Some aspects of the surstylus design that enable the male to produce finger flexing movements are synapomorphies of *Microsepsis*. The massively asymmetrical development of the right surstylus and its inner process (finger) distinguishes *Microsepsis* from its sister genera *Archiseopsis* and *Palaeosepsis* (Silva 1993). The additional fan of muscle fibers in the large right surstylus may constitute a second synapomorphy, because it differs from that of the less modified left surstylus, and it probably produces movements of the finger with respect to the rest of the surstylus at the inner pseudoarticulation. The pseudoarticulation at the base of the inner process is also a derived trait of *Microsepsis* compared with *Archiseopsis* and many other sepsids (Pont 1979; Ozorov 1992, 1993; Silva 1993; Eberhard and Pereira 1996). Similar apparent points of flexibility are present at the bases of the right inner processes of several other *Microsepsis* species (Ozorov 1993; Silva 1993), although perhaps not in all (e.g., *M. stenoptera*; Silva 1993). Still another possibly derived surstylus trait is the strong keel on the dorsal surface of the right inner process (Figs. 1A, 1D, 2A), a feature lacking on the left inner process (Fig. 1A) as well as on the inner processes of five *Archiseopsis* species (Eberhard and Pereira 1996; W. G. Eberhard, unpubl. data). This keel probably reinforces the inner

process and makes it less likely to bend during finger flexing behavior.

These morphological modifications appear to be correlated with behavioral differences, because finger flexing movements similar to those described here also occur during copulation in *M. furcata* and *M. mitis*, but not in several species of *Archiseopsis* (W. G. Eberhard, unpubl. ms.). Thus, these derived properties of the right surstylus of *Microsepsis* are all probably designed to allow the male to press rhythmically on the female's sternite VI and the membrane anterior to it during copulation. The functional significance of the second, outer pseudoarticulation is less clear. There were no muscles designed to move it, and it sometimes flexed passively away from the rest of the surstylus during copulation (Fig. 2B).

Genitalic movements that may stimulate females have also been seen in other flies during or following copulation (Grimaldi 1987; Otronen 1990; Eberhard 1994, 1999). In two of these groups (the drosophilid *Zygothrica* and the neriids), the genitalic movements differ between related species (Grimaldi 1987; Eberhard 1999). Genitalic movements during copulation also occur in a few other insects (beetle: Alexander 1959; butterflies: Lorkovic 1952; Platt 1978; Scott 1978). A very similar situation to that documented in this study may occur in the silvaniformes group of *Heliconius* butterflies (K. Brown, pers. comm.). The tip of the genital valve, which is species specific in form, moves independently of the rest of the valve due to special muscles and articulations. It tickles the female repeatedly during the sometimes extended period after the male seizes (or partially seizes) the female with the bases of his valves, but before intromission of his aedeagus. Still another possible case of stimulatory genitalic movements occurs in swallowtail butterflies. Processes on the inner face of the male genital valve (the harpes) are moveable and are relatively good species characters (Tyler et al. 1995). The harpes are the first male structures that seize the female, and sometimes they give her multiple rhythmic squeezes (K. Brown, pers. comm.). The female probably needs to respond appropriately for the male to be able to achieve intromission (K. Brown, pers. comm.). Previous observations on a pierid (Lorkovic 1952) also suggest male genitalic courtship behavior.

ACKNOWLEDGMENTS

I thank A. L. Ozerov for kindly identifying my flies, M. Vargas for outstanding help producing SEM images, M. L. Baena for raising flies, M. J. West-Eberhard for useful comments on a previous version of the manuscript, K. Brown for sharing his extensive knowledge of butterfly behavior, and the Smithsonian Tropical Research Institute and the Vicerrectoría de Investigación of the Universidad de Costa Rica for financial support.

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Corresponding Editor: D. Wheeler