

Copulation and sperm transfer in *Archisepsis* flies (Diptera, Sepsidae) and the evolution of their intromittent genitalia

[Kopulation und Samenübertragung bei Fliegen der Gattung *Archisepsis* (Diptera, Sepsidae) und die Evolution ihrer inneren Genitalien]

by

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Abstract

Male *Archisepsis* flies introduce their genitalia deep into the female early in copulation, then later partially retract them and form a spermatophore that largely fills the vagina and extends into the entrance of the spermathecal duct. We present the most detailed hypothesis for the mechanism of spermatophore formation proposed for any fly to date. The male genitalia are braced throughout copulation in the posterior part of the vagina with a pair of erectable structures. Two more distal spiny, apparently mobile, flexible processes are oriented in a variety of directions during copulation. They may push or pull against the female to move the distal portions of the male's genitalia within her, or stimulate her. Another surface bearing a dense array of large, stiff bristles can be at least partially everted, and is often pressed against the surface of a large vaginal sclerite, presumably to stimulate the female. None of these structures appear designed to remove sperm from the female, or abraid her vagina to allow penetration by male seminal products. Some aspects of male genitalic morphology and behaviour differ intragenerically. The ways that structures with species-specific morphology interact with the female are more compatible with the sexual selection hypothesis explaining rapid divergent evolution than with the lock-and-key or the male-female conflict of interests hypotheses.

Key words

sexual selection, genital mechanics, copulation, Sepsidae, *Archisepsis*

Zusammenfassung

Archisepsis-Männchen führen zu Beginn der Kopulation ihre Genitalien tief in das Weibchen ein, ziehen sie dann teilweise zurück und formen eine Spermatophore, welche die Vagina größtenteils ausfüllt und bis zum Eingang des Spermathekenganges reicht. Wir stellen die bisher detaillierteste Hypothese zum Mechanismus der Spermatophoren-Bildung bei Fliegen vor. Während der Kopulation sind die männlichen Genitalien mit einem Paar spreizbarer Strukturen im hinteren Teil der Vagina arretiert. Zwei distale, flexible Fortsätze finden sich während der Kopulation in mehreren unterschiedlichen Positionen. Als mögliche Funktionen kommen die Bewegung des distalen Abschnitts des männlichen Genitalapparats im Weibchen und die Stimulation des Weibchens in Betracht. Eine mit stark sklerotisierten, teils langen Zähnen und Schuppen versehene Oberfläche am männlichen Kopulationsorgan kann zumindest teilweise ausgestülpt werden, und wird oft gegen einen grossen Sklerit der Vagina gepresst, möglicherweise um das Weibchen zu stimulieren. Offensichtlich dienen diese Strukturen weder der Spermienräumung im Weibchen noch der Aufschürfung ihrer Vagina im Sinne der Ermöglichung der Penetration von Substanzen der männlichen Samenflüssigkeit. Einige Aspekte der Morphologie und des Verhaltens der männlichen Genitalien zeigen intragenerische Variation. Die Art und Weise, wie art-spezifische Strukturen des Männchens mit dem Weibchen interagieren, steht eher im Einklang mit der Hypothese der geschlechtlichen Zuchtwahl, als mit der Schlüssel-Schloss-Hypothese und der Hypothese des zwischengeschlechtlichen Interessenskonfliktes.

Stichwörter

sexuelle Selektion, Genitalien, funktionelle Morphologie, Kopulation, Sepsidae, *Archisepsis*

Introduction

Animal genitalia show a remarkable tendency to evolve rapidly and divergently, and in many groups they have extraordinarily elaborate, species-specific forms (EBERHARD 1985). Several hypotheses have been proposed to account for this evolution (summarized in EBERHARD 1985, SHAPIRO & PORTER 198, ALEXANDER et al. 1997). While several general considerations indicate that sexual selection by cryptic female choice has generally been the most important factor in producing this trend (EBERHARD 1985, 1996, 1997), it is nevertheless necessary to consider all the hypotheses in any particular case. This is especially true regarding the male-female conflict hypothesis, which has only recently been carefully presented in detail, and which generates several predictions similar to those of the sexual selection hypothesis (ALEXANDER et al. 1997). Observations such as those in the present study, that are specifically designed to test these hypotheses in particular species, may help resolve this controversy.

One of the basic problems in discriminating among the possible explanations of genitalic evolution is the difficulty of observing the behaviour of male genitalia as they interact with the female during copulation. Direct observation of the behaviour of a male's genitalia within the female is usually not possible (for exceptions see MASTERS & JOHNSON 1966, WHITMAN & LOHER 1984, EBERHARD 1993). Indirect evidence of movements can be obtained, however, by studying animals frozen at different stages during copulation. Careful observation of deflections and deformations of particular structures in such pairs can also allow one to deduce at least some details of the forces exerted by males within the female. Study of multiple pairs also helps in avoiding the problem of typology, which has plagued accounts of the internal events associated with copulation (EBERHARD 1996).

The behaviour of the male genitalia of cyclorrhaphan flies while inside the female has been little studied, but appears to be diverse. The tephritid *Ceratitis capitata* (WIEDEMANN, 1824) has two inflatable toothed sacs that probably serve to move the male's distiphallus inside the female (EBERHARD & PEREIRA 1995). Calyptrate flies of the genera *Glossina*, *Musca* and *Lucilia* apparently lack such sacs (POLLOCK 1974, LEWIS & POLLOCK 1975, MERRITT 1989). In *Lucilia* the spines on the lateral barbs of the male genitalia abraided the portions of the female reproductive tract where male accessory gland products are deposited that probably have powerful effects in inducing female oviposition and inhibiting female remating (LEOPOLD et al. 1971, LEOPOLD 1976, SMITH et al. 1989, 1990). Sphaerocerid flies of the genus *Coproica* apparently lack both inflatable sacs and abrasive spines. The male uses his telomeres to spread the ventral portions of female tergite 8, and his postgonites to spread her soft vaginal tissue, and then simply inserts his distiphallus directly into the vagina where the aedeagus locks with the female's vaginal sclerite (LACHMANN 1996). In two of the five species studied by LACHMANN, the male then folds his distiphallus and postgonites rearward, pulling the female's vaginal sclerite and her spermathecal and accessory gland ducts out of her body and into his ventral genital pouch. There appear to be no published accounts of the process of intromission and sperm transfer in any sepsid, other than the mention in *Sepsis punctum* (FABRICIUS, 1794) of a vaginal sclerite bearing spermathecal ducts which is apparently hooked tightly by the male's phallosome during copulation (KIONTKE 1989), and the speculation, based on spiny male aedeagal structures, that males remove sperm from females (WARD et al. 1992).

The present study uses evidence from pairs frozen in copula to describe the behaviour of the male genitalia inside the female in two species of the sepsid genus *Archiseopsis*.

Previous studies of these and other sepsids (PARKER 1972 a, b; MANGAN 1976, PONT 1979, WARD et al. 1992, WARD 1993, W. EBERHARD in prep.) have shown that males and females meet at oviposition sites, that the male mounts and courts the female while she lays her eggs, and that copulation occurs after the female has laid all her mature eggs. Copulation only occurs if the female cooperates: she can prevent mounting by kicking the male or shaking him off, and can prevent intromission by a mounted male by lowering her abdomen; she probably must also extend her ovipositor or otherwise provide access to her internal genitalia if intromission is to occur. Once intromission has occurred, however, the female seems unable to dislodge the male even with vigorous shaking movements; all copulations ended with the male first climbing off the female and then pulling his genitalia free (W. EBERHARD, unpub.). During copulation the male genitalic surstyli grasp membranous areas near the female's 6th abdominal sternite (EBERHARD & PEREIRA 1996). There are no clear thrusting movements with the male's intromittent genitalia (W. EBERHARD, unpub.). During approximately the first third of the 15-20 min. copulation the male performs copulatory courtship movements with his middle legs, and during about the first two thirds of copulation his surstyli squeeze her abdomen rhythmically in species-specific patterns (EBERHARD in prep.). The descriptions presented below do not represent complete accounts of the morphology of male and female reproductive organs, but only of those aspects of their morphology that are closely related to behavioural events.

Materials and methods

Adults of *Archiseopsis diversiformis* (OZEROV, 1993) from Costa Rica (about 1000 m, Central Valley near San José) and from Panamá (about 20 m, Barro Colorado Island in Lake Gatun), *A. armata* (SCHINER, 1868) from Costa Rica (about 1200 m, 10 km NE of San José, San José Province), *A. pleuralis* (COQUILLET, 1904) from Costa Rica (about 1300 m, near San Antonio de Escazu, San José Province), and *A. discolor* (BIGOT, 1857) also from near San Antonio de Escazu, were raised from eggs on moist, previously frozen cow dung in small petri dishes. Since there are behavioural differences between the two populations of *A. diversiformis* studied (W. EBERHARD in prep.), the sites of origin of specimens of this species are noted throughout. Flies of *A. armata* and Panamanian *A. diversiformis* that were to be mated were separated by sex within 24 hrs of emergence, and fed moist dung and honey. Individual pairs of adults that were at least three days old were aspirated into small petri dishes and watched until copulation began. Copulating pairs of Costa Rican *A. diversiformis* were collected in the field.

After different intervals of time in copula, each pair was gently induced to walk into a small vial, where the flies were killed nearly instantaneously with a freezing spray (ethyl chloride). A previous study (EBERHARD & PEREIRA 1996) showed that genitalic structures are immobilized in natural positions with this spray.

Three techniques, whole mounts, sections, and SEM were used to observe morphology. For whole mounts, frozen pairs of *A. diversiformis* (both sites) and *A. armata* were immediately transferred to 80 % ethanol at ambient temperature. After several weeks (to allow tissues to harden), the female's lower reproductive tract was dissected free and placed in HOYER'S medium on a microscope slide and covered with a coverslip. To avoid possible displacement of male genitalia, most dissections left the inner layer of the muscles associated with the vaginal walls intact, and small pieces of glass were placed on the slide around the specimen so that it would not be pressed when the coverslip was

added. Male genitalia were also pulled from the female, mounted in HOYER'S, and drawn using a camera lucida. Some isolated male genitalia were macerated in KOH to observe internal structures. Some macerated genitalia were transferred to rain water to raise internal pressures and cause expansion of internal structures. Wholemount preparations were examined under 100x and 400x magnification using NOMARSKI interference contrast optics after many of the tissues had cleared.

For sections, copulating pairs of Costa Rican and Panamanian *A. diversiformis* were frozen as above, fixed in Dubosq-Brasil, then embedded in ERL-4206 epoxy resin, and serially sectioned with a diamond knife in a REICHERT ultramicrotome. Sections were either 1.3 or 1.5 μm thick, and every fourth section was mounted on a slide and stained with methylene blue in aqueous borax solution (1%) (see HUBER 1993).

For examination with the SEM, the genitalia of Costa Rican *A. diversiformis*, *A. pleuralis*, and *A. discolor* were fixed in alcohol, unfolded and then sublimation dried and coated with gold and studied with a Hitachi S-570.

The homologies of different portions of the intromittent male genitalia of sepsids have apparently never been determined (A. NORRBOM pers. comm.), as taxonomists have used male front legs and surstyli to distinguish species. Genitalic structures are thus given only descriptive names in the accounts that follow. Multiple figures of some structures are presented to emphasize and document variation. All orientations of genitalia refer to positions during copulation. Voucher specimens have been deposited in the U. S. National Museum and the Museo de Insectos of the Universidad de Costa Rica.

Results

Morphology

The general layout of the portions of the female reproductive tract that were involved in copulation is shown schematically in Fig. 1, along with the different landmarks (a-h) in the anterior chamber of the vagina that were used to judge the degree of penetration of the male's genitalia. When an egg was in the vagina (Fig. 2), its long respiratory horn extended far up the oviduct, and served to trace the connections between the vagina, the ventral sac, and the common oviduct, as well as their relationships with the apparently two-chambered ventral receptacle and its duct (sections of the ventral receptacle showed complex additional subdivisions within the two chambers), the entrance to the common spermathecal duct, and the large and small female vaginal sclerites. The large vaginal sclerite on the posterior side of the dorsal projection of the vaginal wall bore a complex array of small indentations (Figs 5, 25, 40, 41) as well as some irregular larger indentations (Figs 2, 14, 41). The dorsal vaginal projection separated the anterior chamber of the vagina, into which the ducts of the ventral receptacle and the spermathecae, as well as the oviduct empty, from the posterior chamber which communicated posteriorly with the ovipositor. The anterior and posterior chambers were joined via a wide passage on the left side of the dorsal projection, and a narrow space on the right.

The general layout of the distal portions of the intromittent genitalia of the male is shown in whole mounts and sections (Figs 3-5), schematically (Fig. 6), and in SEM preparations (Figs 7-9). Starting from the distal tip and moving basally, the following structures could be distinguished. The tip of the distal body was composed of many flexible cuticular fibers embedded in a membrane (Figs 4, 10, 11). These fibers were flexible, and were splayed apart and bent to varying degrees in wholemounts of copulat-

