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 Spermienausräumung 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 artspezifische 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 abraid 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 phallotrema 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 *Archisepsis*.

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 *Archisepsis 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* (COQUILLETT, 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 expoxy 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.
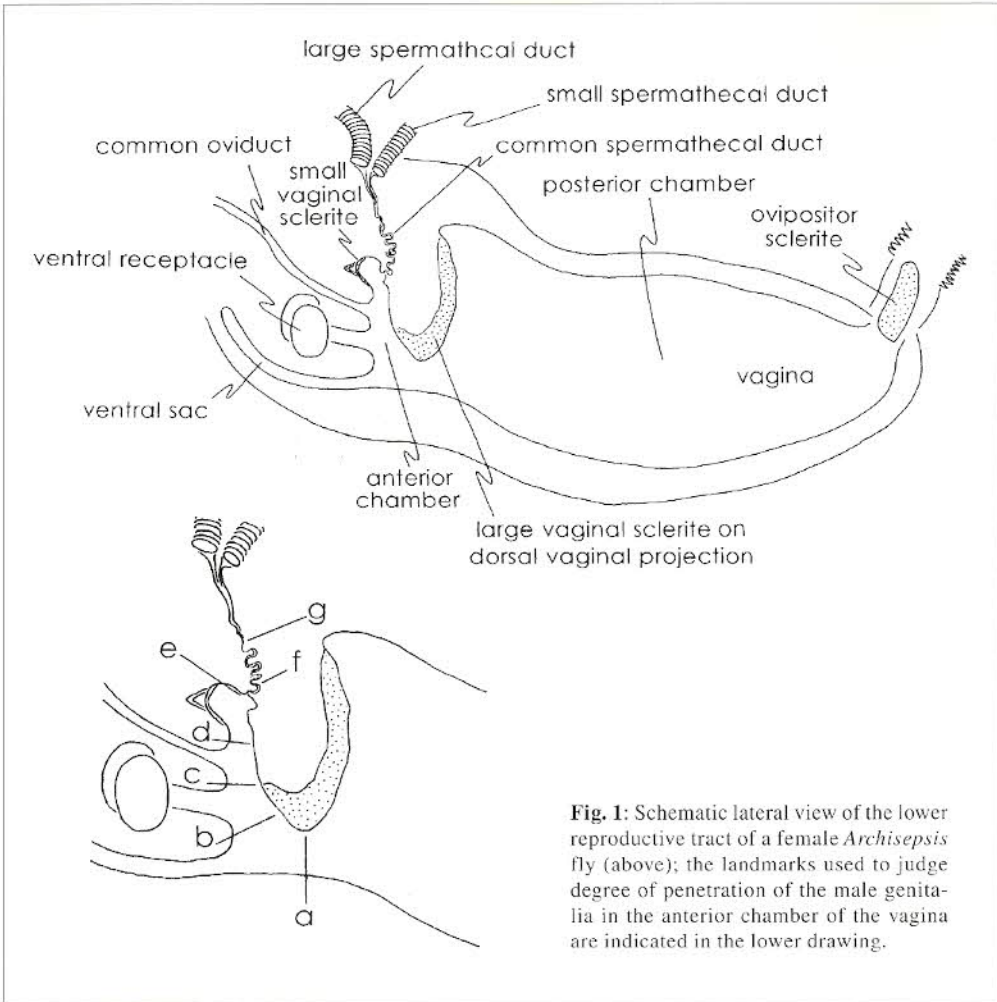
Results

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-

large spermathcal duct

small spermathecal duct

common oviduct

common spermathecal duct

small vaginal sclerite

posterior chamber

ventral receptacle

ovipositor sclerite

ventral sac

vagina

anterior chamber

large vaginal sclerite on dorsal vaginal projection

g

e

f

d

c

b

a

Fig. 1: Schematic lateral view of the lower reproductive tract of a female *Archisepsis* fly (above); the landmarks used to judge degree of penetration of the male genitalia in the anterior chamber of the vagina are indicated in the lower drawing.

ing pairs (Figs 12, 15, 54). A clear, soft tip was visible beyond the tips of the fibers in some wholemounts (e. g. Fig. 12) and, in collapsed form, in all SEM preparations (Figs 7, 10, 11). There were no obvious differences among the three species observed with the SEM in the form of the soft tip. Sections showed that the soft tip was solid rather than hollow near its tip, and that it was continuous basally with an internal, rigid structure inside the tip of the distal body (Figs 4, 5).

The basal portion of the distal body was enveloped in complexly folded membranes with deep invaginations (Figs 9, 16, 17). There was a row of prominent tooth-like projections of the membrane in *A. pleuralis* (Figs 16, 21) that was not as pronounced in the other species. The membranes of the distal body were often very difficult to resolve in whole mount preparations (e.g. Figs 3, 13, 29). Internally, the distal body had a dark, "U" shaped structure (Figs 40, 41), and at the base it was produced into a lighter, apparently flexible "stem" (Fig. 41) that articulated with the heavily sclerotized, rigid "distal rod". The distal rod in turn articulated basally (Fig. 8) with one distal corner of a thicker portion, the "central body" (Figs 3, 13, 29). This articulation was near the base of another
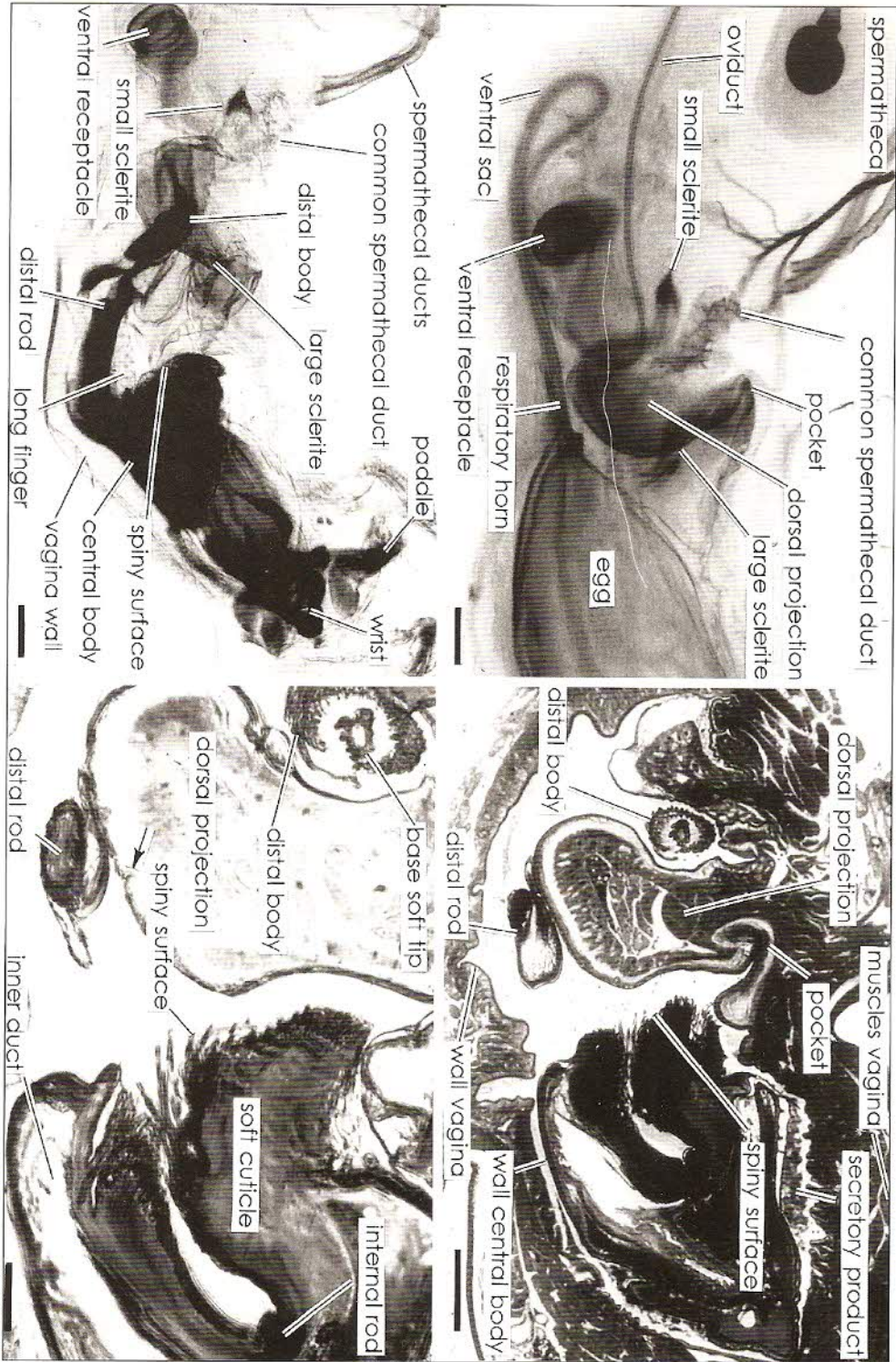
internal structure, the "distal process" (Fig. 13). Neither the "U" nor the distal rod nor the distal process were clearly visible externally in SEM images (Figs 7-9, 21). The relative positions of the distal rod and the distal process varied in whole mounts, and in combination with the fold at the base of the rod on the external surface (Fig. 8), this suggests that the articulation between the distal rod and the central body was flexible.

Except for its dorsal and distal surfaces, the central body was relatively smooth (Figs 7-9). The distal surface bore a dense, complex array short, thick, black, heavily sclerotized spines (the "spiny surface"). The shapes of the spines varied both in different portions of the distal surface and between species (Figs 18-24). The spiny surface extended deep inside the central body, and at its most basal extension was continuous with the solid, round, curved, heavily sclerotized "internal rod" (Figs 4, 5, 27). The distal end of the internal rod was hollow, and formed the base of the long finger (below). The invaginated spiny surface was underlain by a thick layer of soft cuticle (Figs 4, 5, 28). A wide, thick-walled internal duct from the neck area which had a branch extending toward the base of the distal rod lay directly posterior to this layer of soft cuticle (Figs 4, 5, 28). No muscles were found sections of the central body or in those of more distal structures. The spiny surface apparently had two portions: the "corner mass" which could be everted in the area of the distal corner of the central body opposite the articulation with the rod when genitalia macerated in KOH were placed in rain water (Figs 27, 29, 30); and the surface attached to the internal rod, which was left unmoved by the KOH-H_2O treatment (Figs 27, 29, 30). The spines on the everted "corner mass" of Costa Rican *A. diversiformis* were oriented distally.

Three apparently softer structures projected from the distal surface of the central body. A small tongue-shaped sac thickly covered with less heavily sclerotized spines directed toward its tip (the "spiny tongue") emerged laterally from the distal surface of the central body (Fig. 7). The spiny tongue in SEM specimens was not visibly wrinkled (Figs 33, 35), nor was its cuticle strongly wrinkled in sections (Fig. 28), and sections showed that it was solid (Fig. 28). Despite these indications of inflexibility, the spiny tongue was more thickly swollen in some whole mounts (e. g. Fig. 25) than in others (e. g. Fig. 43), in which its shape was similar to that in SEM preparations. An extension of a cavity

3	2
5	4

Fig. 2: Lateral view of the reproductive tract of a female Costa Rican *Archisepsis diversiformis* (OZEROV, 1993) with an egg in the posterior chamber of the vagina. The long dark respiratory horn of the egg forms a loop inside the ventral sac, passes by the entrance to the ventral receptacle, and extends up the oviduct. Scale 50 = µm.

Fig. 3: Lateral view of whole mount of a mating pair of Costa Rican *Archisepsis diversiformis* (OZEROV, 1993) prior to ejaculation. The tip of the male's distal body is just short of entering the anterior chamber of the vagina, and his spiny surface is not in contact with her large vaginal sclerite. Scale = 50 µm.

Fig. 4: Saggital section of the male and female genitalia of a mating pair of Panamanian *Archisepsis diversiformis* (OZEROV, 1993) prior to ejaculation. The male's distal body, which is sectioned transversely near its tip, is deep in the anterior chamber, and the spiny surface of the male's central body is just posterior to the large sclerite of the dorsal vaginal projection. A possible secretory product of the male lies between the dorsal surface of the male's central body and neck and the wall of the vagina. Scale = 50 µm.

Fig. 5: Closeup of central portion of Fig. 4. The spiny surface is deeply invaginated within the central body and is continuous with the internal rod. The base of the soft tip of the male's distal body is hollow, and appears to have incorporated fibers that are similar to those in the fibrous tip. The arrow marks a pit in the large female sclerite. Scale = 20 µm.
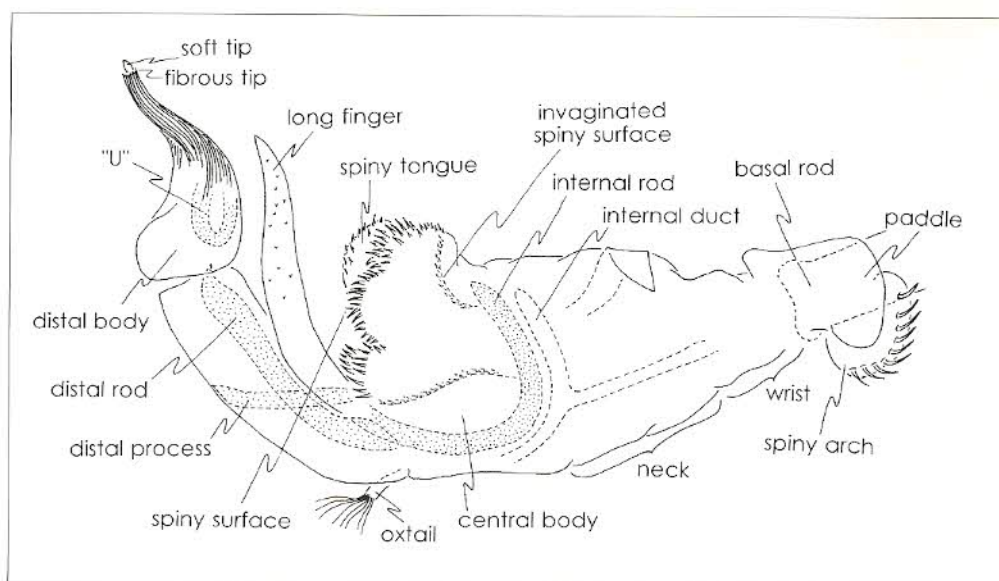
Fig. 6: Schematic lateral view of the extended intromittent genitalia of a male *Archisepsis* with the paddle and spiny arch folded basally.

in the central body ended just at the base of the spiny tongue (Fig. 28). The spines on the spiny tongue of *A. diversiformis* where shorter and more blunt than those on the tongues of *A. pleuralis* and *A. discolor* (Figs 33-35).

A long, flexible, tapering, blind-ended cylinder (the "long finger") emerged more centrally, near the base of the rod (Figs 7, 9, 21). It was covered with variable numbers of more or less prominent, small, sclerotized, basally directed teeth (Figs 36-39) whose tipes were dark (Figs 12, 14, 40-43). These teeth were especially prominent in *A. pleuralis* (Fig. 38-39), and least prominent in Costa Rican *A. diversiformis* (Fig. 36). Sections showed that most of the long finger was solid and of a uniform consistency similar to that of the thick soft cuticle in the spiny tongue (Fig. 28) and on the inner side of the spiny surface (Fig. 28), except for a small cavity near the base which was continuous with the cavity at the tip of the internal rod. As with the spiny tongue, the long fingers of specimens that had been dried for observation in the SEM were shrunken; the distal portion was twisted sharply in Costa Rican *A. diversiformis*, but not in *A. pleuralis* or *A. discolor* (Figs 36-38). Manipulation of the genitalia of a living fly showed that the long finger was stiff, but not inflexible, and this impression was confirmed in wholemounts in which the long finger deformed the walls of the vagina (Figs 42).

The third structure projecting from the distal surface of the central body was a small, more or less cylindrical structure (the "oxtail"), which bore a brush of long flexible hair-like extensions on its distal surface (Figs 44, 45). In both *A. dorsalis* and *A. pleuralis* many (perhaps most) of these extensions had short lateral branches (Fig. 44), while in Costa Rican *A. diversiformis* branches were apparently less common. The tips of the extensions were hook-like in *A. discolor* (Figs 46, 47), but simple in Costa Rican *A. diversiformis* (Fig. 45). The bases of the extensions did not have sockets (Figs 44, 45). In sections the oxtail appeared to be a solid extension of the thick, soft cuticle on the inner side of the invaginated spiny surface (Fig. 52), and it was shrunken in specimens pre-
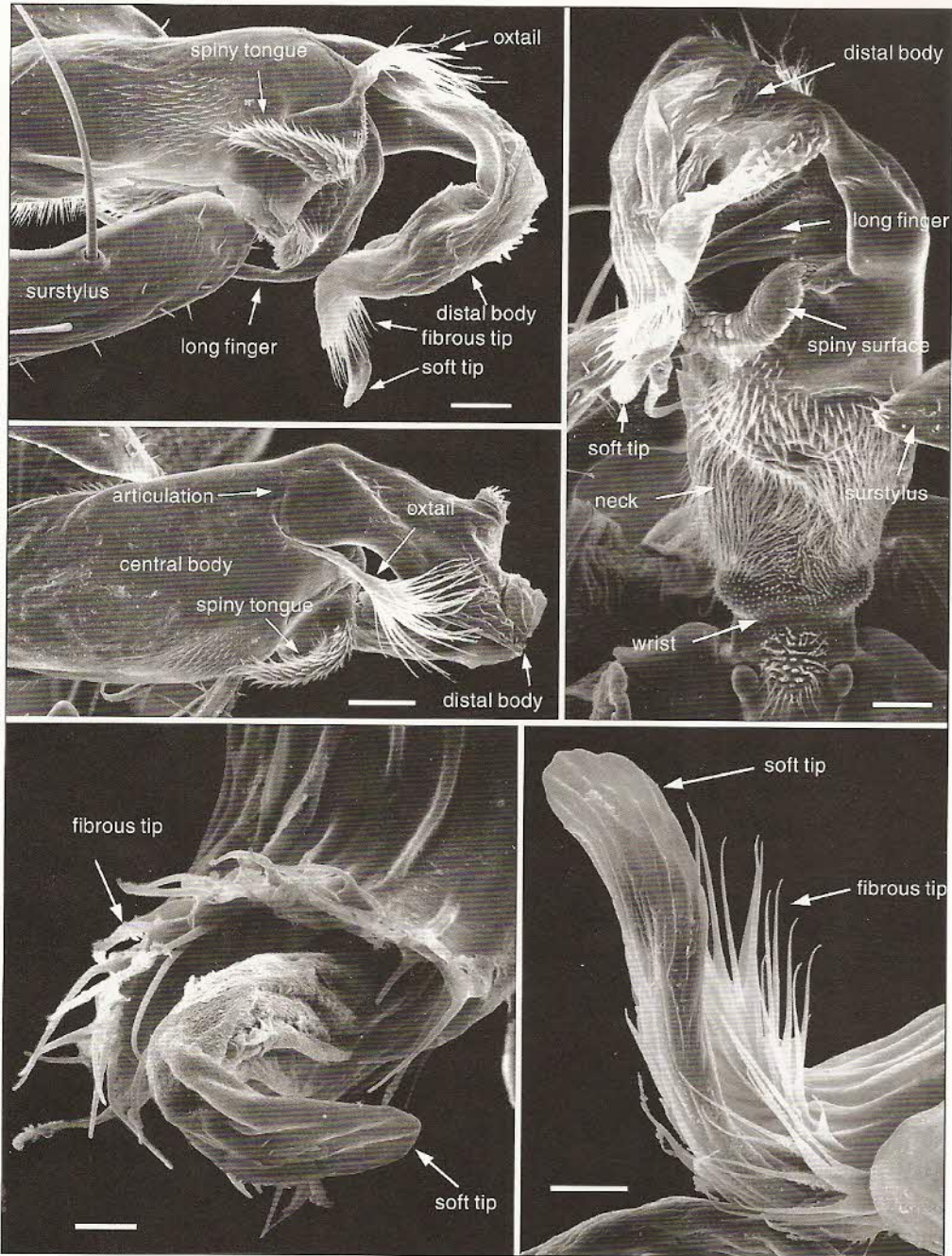
Figs 7-9: Intromittent genitalia of male *Archisepsis pleuralis* (COQUILLETT, 1904) in lateral (7), ventral (8) and dorsal (9) view (orientation with respect to their position in the female during copulation). Scales: Figs 7, 8 = 30 µm; Fig. 9 = 40 µm.

Figs 10-11: Distal portions of the distal body of *Archisepsis discolor* ((BIGOT, 1857) (10) and *Archisepsis pleuralis* (COQUILLETT, 1904) (11) seen, respectively, in anterior and lateral views. The soft tip is in the opening formed by the flexible filaments of the fibrous tip, from which ejaculate emerges (see Fig. 54). Scales: Fig. 10 = 4 µm; Fig. 11 = 8 µm.
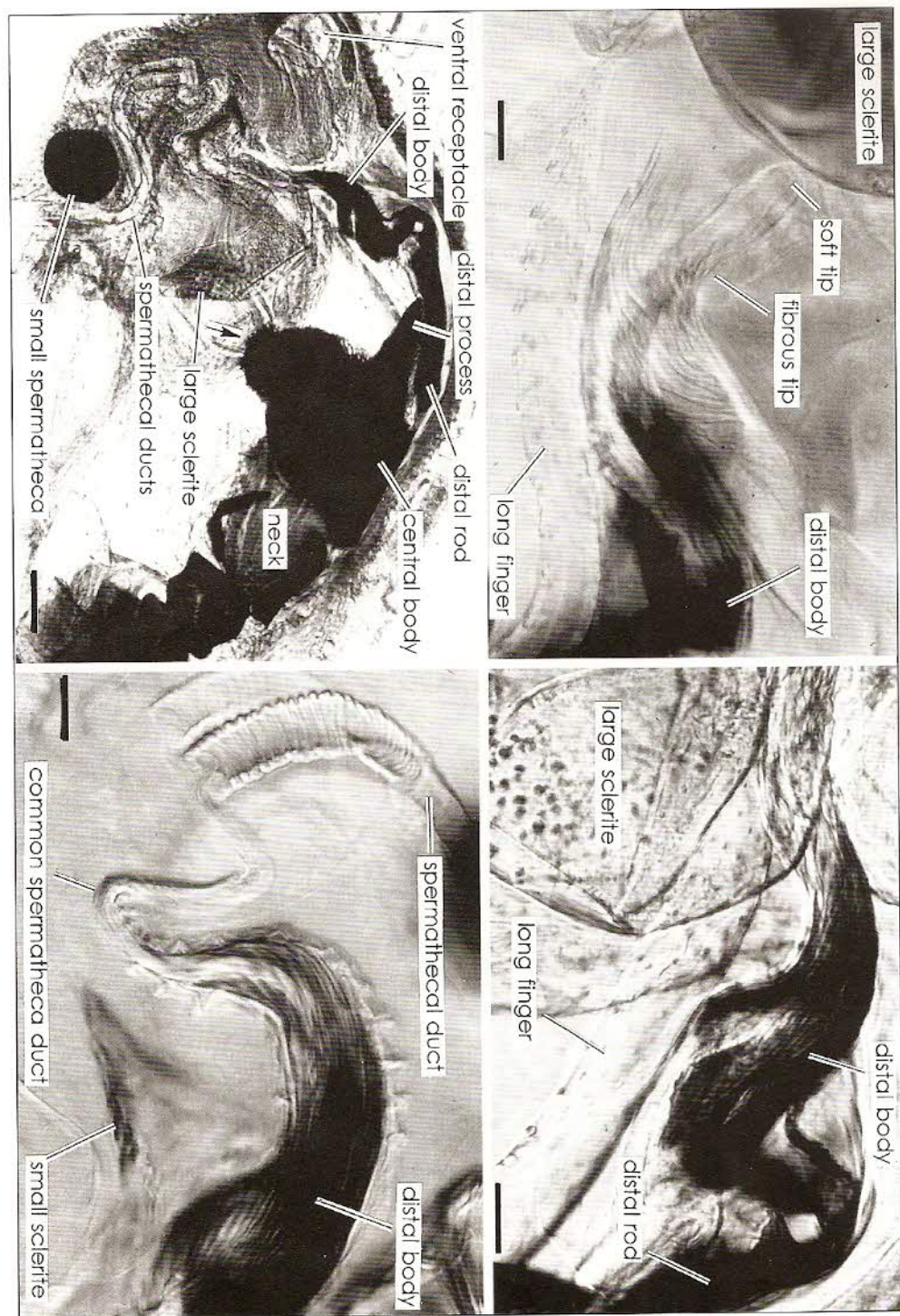
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pared for SEM study (Figs 44, 45). The oxtail was not visible in many whole mounts of copulating pairs, presumably because it was obscured by other parts of the male's genitalia and by the walls of the vagina.

Basal to the central body was a "neck" covered with a membrane bearing numerous hair-like processes which varied in length and thickness (e. g. Fig. 9). None of these processes had sockets at their bases (e. g. Figs 48, 50). In fact, no obvious sensory structures (socketed or hollow setae) were seen anywhere on any part of the intromittent male genitalia, with the possible exception of a pair of circular structures at the base of the central body in one *A. diversiformis* (Fig. 50) (similar structures were not seen in other specimens of this species or in other species - e. g. Figs 7-9). This lack of sensory setae contrasted sharply with their abundance on the tips of the male surstyli (Fig. 49) and other external surfaces of his body.

Basal to the neck was the "wrist" (Fig. 9) where the genitalia folded when they were not erected, and a pair of flattened structures that could be erected or extended laterally (Figs 3, 31, 32). The distal portion of the more distal "paddle" was flattened with rounded edges (Fig. 6). The more basal "spiny arch" structure bore a row of strong, curved, socketed spines (Fig. 6). The differences between species in the forms of the paddles and spiny arches were relatively small compared with intraspecific variation; when drawings were made of several specimens of each of five species and then mixed together, it was not possible to reliably distinguish species on the basis of either of these structures. Basal to the wrist was the sclerotized "basal rod" (Fig. 6).

Manipulations of intact genitalia in living flies under a dissecting scope failed to elicit any movements, other than occasional unfolding and folding in the wrist area. Erection of the paddles and spiny arches occurred when the genitalia unfolded.

Events during copulation

Although there was substantial intraspecific variation in their timing, the basic sequence of events during copulation was similar in *A. armata* and Panamanian *A. diversiformis* (Fig. 51): the distal body and the central body of the male entered the vagina nearly immediately. As copulation proceeded, the distal body was then more and more often found deeper in the anterior chamber of the vagina. Then ejaculation occurred as the distal body was withdrawn to the posterior chamber of the vagina, and it remained there for several minutes before copulation ended.

The male usually introduced his genitalia within a minute through the female's ovipositor and the posterior chamber of her vagina, so that the tip of his distal body reached or moved past the ventral edge of the large female sclerite. The basal paddles and spiny

Fig. 12: Lateral view of both the long finger and the soft tip of the distal body of a male Costa Rican *Archisepsis diversiformis* (OZEROV, 1993) which extend beyond the fibrous tip of the distal body to touch the large sclerite in the vagina. Scale = 15 µm.

Fig. 13: Lateral view of the distal body of the male genitalia in *Archisepsis armata* (SCHINER, 1868) which is part way past the large sclerite and into the anterior chamber of the vagina, and is folded back at its base onto the distal rod (arrow markes everted corner mass of spines) (ventral side is upward in the figure). Scale = 50 µm.

Fig. 14: Closeup view of the distal body and long finger that is pressed against the dorsal projection of the vagina in Fig. 13 (ventral side is to the right in the figure). Scale = 20 µm.

Fig. 15: The tip of the distal body of a male *Archisepsis armata* (SCHINER, 1868) has entered the distal portion of the common spermathecal duct, causing its walls to stretch. Scale = 15 µm.

Fig. 16: The complex membranous folds on the basal portion of the distal body of *Archisepsis pleuralis* (COQUILLETT, 1904) (arrow marks one of many tooth-like membranous processes). Scale = 20 μm).

Fig. 17: The basal portion of the distal body of *Archisepsis discolor* (BIGOT, 1857). The complex membranous folds do not completely envelope some internal structures. Scale = 20 μm).

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arches were spread laterally, with the distal portion of each paddle pressing on the spiny arch (Fig. 31). Sections showed that a large fold of the posterior wall of the vagina was pinched tightly between the paddle and the spiny arch, and that the paddle pressed rearward so forcefully that its tip was folded back on itself (Fig. 32). Similar folding of the tip of the paddle was seen in some whole mounts.

This pinch on the rear wall of the vagina was apparently maintained throughout copulation. There was no tendency in sectioned flies for males whose distal body was deeper in the female to pinch the female farther anteriorly in the vagina. There was, however, substantial variation in the angle between the paddle and the longitudinal axis of the basal rod, suggesting that the paddles and spiny arches were flexed during some stages of copulation. Prior to ejaculation, when the distal portions of the male genitalia were deeper in the female, the paddles and spiny arches were directly more posteriorly than after ejaculation had occurred. In 73 % of 26 Panamanian *A. diversiformis* pairs that were frozen prior to ejaculation the rearmost tip of the spiny arch was > 30 % along the length of the basal rod (maximum was 70 %); in only 8 % of 13 pairs in which ejaculation had occurred was the spiny arch this far rearward ($p < 0.001$ with Chi^2 Test).

Early in copulation the basal portion of the male's distal body was sometimes tightly folded posteriorly on itself and the distal rod (Fig. 40). In other cases only the junction with the distal rod was folded (Figs 13, 14, 41). In a few cases the crescent-shaped tip of the rod seemed to fit against the side of the distal body and may have supported it (Fig. 41). Presumably such folding resulted from the male forcefully pressing his genitalia anteriorly, deeper into the female. Such folding tended to occur early in copulation (in 9 of 14 pairs after < 7 min, vs. 0 of 12 pairs after 9-16 min but prior to ejaculation in *A. armata* ($p < 0.001$ with Chi^2) (a similar, but statistically insignificant trend occurred in 33 pairs of Panamianian *A. diversiformis*). Folding occurred at depths of penetration ranging from *a* throught *g* (Fig. 1), and as late as after 15 min. of copulation.

When the tip of the distal body extended past the ventral edge of the large vaginal sclerite, the spiny surface of the central body was generally close to or in contact with the posterior surface of the large female sclerite (Figs 3-5, 13, 26). In some cases the dorsal projection of the vaginal wall was apparently clamped between the spiny surface and the distal body (Fig. 26). In some whole mounts there was a partial eversion of the corner mass of the spiny surface (Figs 3, 13) that appeared to be similar to that seen in macerated specimens (Fig. 30) (problems with precision of orientation and the lower visibility in some whole mounts precluded certainty on this point). In one Panamanian *A. diversiformis* the male's spines were deflected by the female sclerite, indicating that the spiny surface was pressing forcefully on the sclerite. A slight eversion of the rest of the spiny surface was suggested in some whole mounts by the apparent distal displacement of the internal rod so there there was no longer a clear space between the rod and the black distal portion of the central body (compare Figs 3, 13, and 53 with Fig. 29). Again possible differences in the orientation of different specimens precluded certainty on this point.

Figs 18-20: Different types of spines on the spiny surface of Costa Rican *Archisepsis diversiformis* (OZEROV, 1993) that include short, long, sharp, rounded, and ramified forms. Scales: Fig. 18 = 5 μm; Fig. 19 = 8 μm; Fig. 20 = 4μm.

Figs 21-23: Ventral overview showing location of spiny surface (21), and details of further forms of spines on the spiny surface of a male *Archisepsis pleuralis* (COQUILLETT, 1904). Scales: Fig. 21 = 20 μm; 22 = 2 μm; 23 = 5 μm.

Fig. 24: Rounded, flattened spines on the spiny surface of a male *Archisepsis discolor* (BIGOT, 1857). Scale = 1 μm.
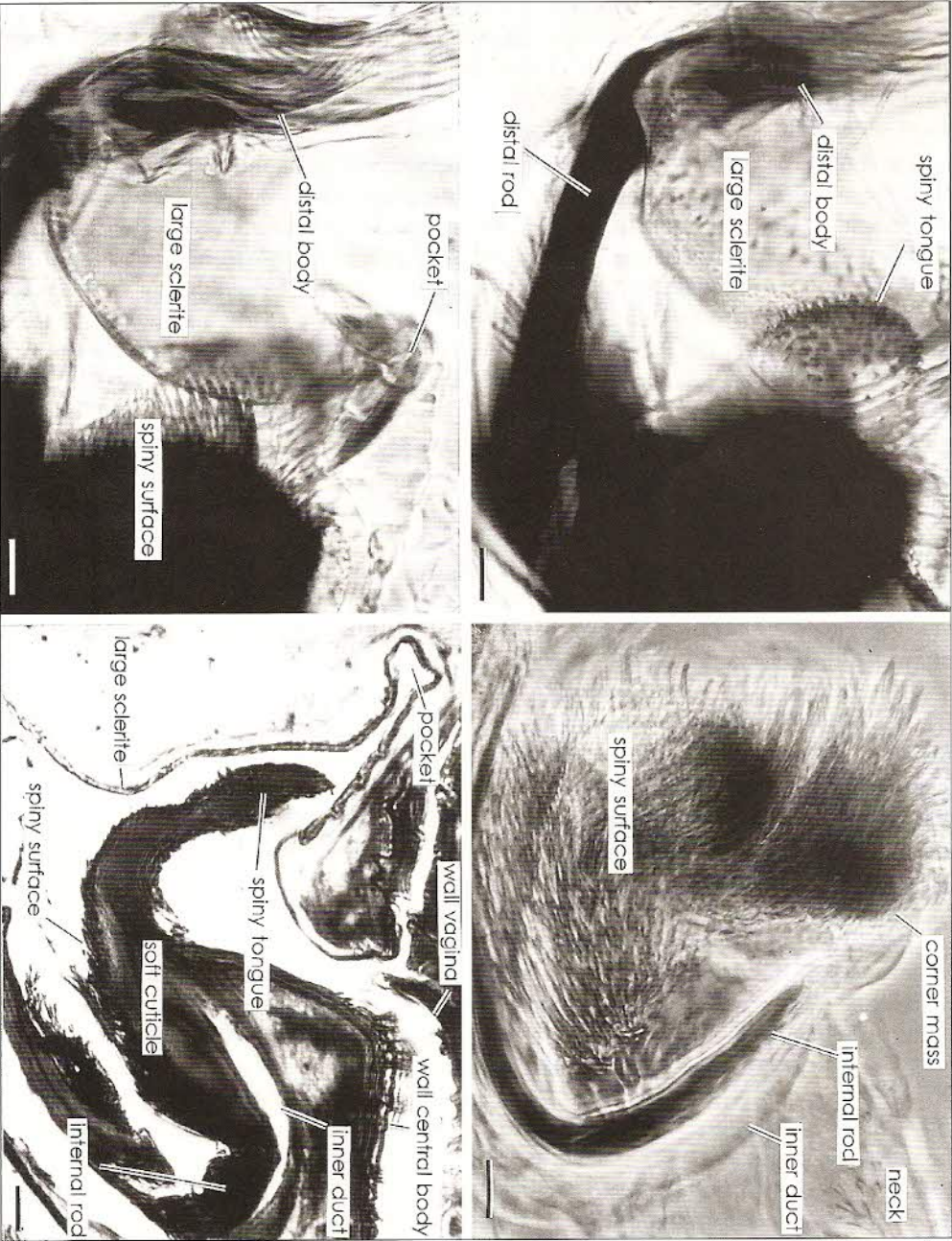
26 25
28 27

Fig. 25: Lateral view of the spiny tongue of a male Panamanian *Archisepsis diversiformis* (OZEROV, 1993) that appears to press on the large female sclerite on the dorsal projection of the vagina. The male's distal body is in the anterior chamber of the vagina and apparently presses on the anterior surface of the dorsal projection. Scale = 15 μm.

Fig. 26: Lateral view of the same pair as in Fig. 25, with the male's spiny surface in focus, showing that it also apparently contacted the female's sclerite. Scale = 15 μm.

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In sectioned specimens there was a mass of material between the anterior region of the neck of the male and the dorsal wall of the posterior chamber of the vagina (Fig. 4). In some specimens tissues within the neck region appeared to contain the same substance, but this could not be confirmed.
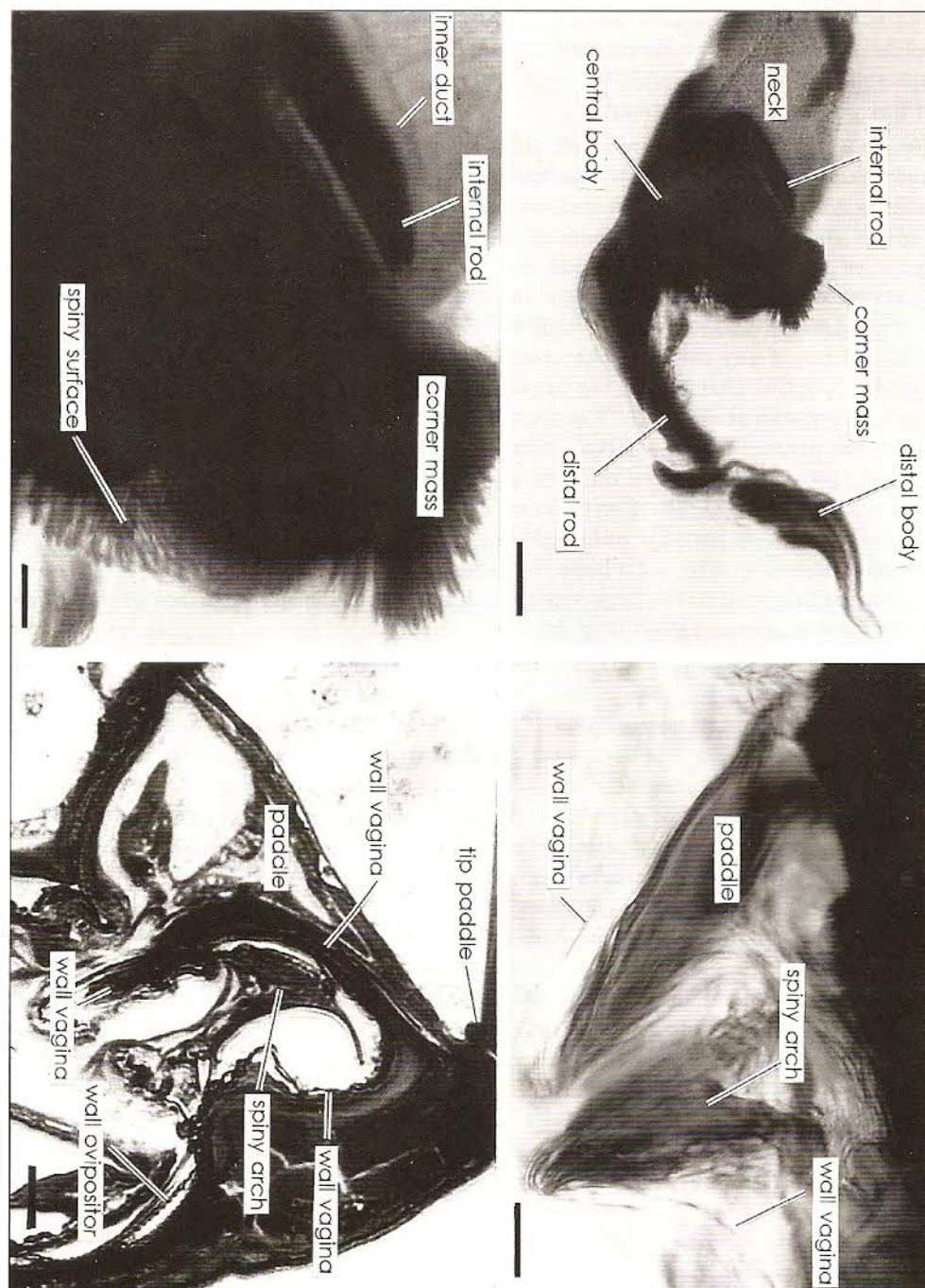
After about 15-20 min of copulation, all males ejaculated (Fig. 51, 53, 54), producing a large spermatophore (Fig. 55). The sperm reservoir of the spermatophore was in the posterior chamber of the vagina, and was more or less filled with a loosely packed mass of sperm and surrounded by a homogeneous wall (Fig. 58). In pairs fixed in ethanol a "froth" of what appeared to be wall material plus large cavities of irregular size and shape surrounded the wall (Fig. 58). In pairs fixed in Bouin's this material was also distinguishable from the reservoir wall in being more darkly and less uniformly stained, but it had few if any cavities. The anterior portion of the spermatophore more or less filled the anterior chamber of the vagina, engulfing the dorsal projection bearing the large vaginal sclerite (Fig. 56). The spermatophore duct, whose walls stained somewhat darker, ran parallel to the anterior surface of the dorsal projection of the vagina (Fig. 56), and entered the mouth of the common spermathecal duct and extended about up it about 1-5 μm (Fig. 57). The distal portion of the spermathecal duct was swollen, and its wrinkled walls were partially unfolded (Fig. 55). The vagina was usually swollen by the spermatophore (Fig. 55), and a large "wrinkle" in the wall was usually (perhaps always) present just posterior to the large vaginal sclerite where the spiny surface of the male's central body had been earlier (Fig. 59).

In only two cases was a pair frozen when it was clear that the male was in the process of ejaculation (Figs 53, 54), though there were a few other cases in which ejaculation may have been in progress. The positions of the male genitalia during different stages of the production of the spermatophore were thus not determined. Presumably the tip of the male's distal body was in or near the mouth of the common spermatheca duct (e.g. Fig. 15) during the first part of ejaculation, when probably spermatophore material rather than sperm was transferred (below). This would explain how spermatophore material entered and unfolded the walls of the common spermathecal duct. During at least the latter stages of apparent sperm emission, the distal body was withdrawn from the anterior chamber of the vagina to near the middle of the posterior chamber, where it remained during the rest of copulation. In all pairs in which ejaculation had occurred, the distal body was extended anteriorly in the direction of the distal rod (e.g. Fig 53), rather than being folded back over the rod (e. g. Figs 40, 41). The withdrawal of the distal body from the anterior to the posterior chamber was accompanied by a distal flexion of the paddle and spiny arch which may have provided the motive force for the withdrawal (below).

The position of the long finger varied widely. Prior to ejaculation, when the distal body had entered or was entering the anterior chamber of the vagina, the long finger usually projected anteriorly either parallel to or at an acute angle with the distal body (Figs 12,

Fig. 27: Lateral view of the transparent central body of a male *Archisepsis diversiformis* (OZEROV, 1993) whose genitalia had been macerated in KOH. Both the invaginated spiny surface which extends all the way to the internal rod, and the partially evaginated corner mass of spines are illustrated. Scale = 15 μm.
Fig. 28: Saggital section of the spiny tongue of a male Panamanian *Archisepsis diversiformis* (OZEROV, 1993) that is in the outer portion of the pocket of the posterior chamber of the vagina at the base of the large female sclerite. The tongue appears to be an extension of the thick layer of soft cuticle that lines the invaginated spiny surface. Scale = 16 μm.

Labels in figure (top left): inner duct, internal rod, spiny surface, corner mass

Labels (top right): central body, neck, internal rod, corner mass, distal body, distal rod

Labels (bottom left): paddle, wall vagina, tip paddle, wall vagina, wall ovipositor, spiny arch, wall vagina

Labels (bottom right): wall vagina, paddle, spiny arch, wall vagina

30	29
32	31

Fig. 29: Male genitalia of Costa Rican *Archisepsis diversiformis* (OZEROV, 1993) which were treated with KOH and then immersed in rain water to increase internal pressure, resulting in the eversion of the corner mass of spines. Scale = 50 µm.

Fig. 30: Closeup of the everted corner mass in Fig. 29. Scale = 15 µm.

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14). Most often it appeared to contact the anterior surface of the dorsal vaginal projection (Figs 14, 40, 41) (59 % of 17 pairs of *A. armata*, 54 % of 28 pairs of Panamanian *A. diversiformis* whole mounts in which the position of the long finger was clear). In several other cases (9 % of the total of 45) it bent back on itself up to 180°, and projected basally, contacting the large female sclerite, the vaginal wall, the distal rod, or the spiny tongue (Fig. 43). Other positions included a variety of angles with the distal body and contact with the posterior surface of the large vaginal sclerite, touching the wall of the vagina (Fig. 42), or out of contact with the female. In some cases the long finger exerted appreciable force on the female (Fig. 42). After the distal body had been withdrawn to the posterior chamber for ejaculation, the long finger was often directed at angles of up to 90° with the long axis of the distal body (Fig. 43). In 44 % of 16 pairs of Panamanian *A. diversiformis* it touched and perceptibly deformed the wall of the vagina.

The spiny tongue extended anteriorly beyond the spiny surface prior to ejaculation (3 % of 29 pairs of *A. armata*; 70 % of 33 pairs of Panamanian *A. diversiformis*; 67 % of 15 paris of Costa Rican *A. diversiformis*; *A. armata* differs significantly from each of the others, both $p < 0.001$ with Chi^2). The spiny tongue sometimes contacted the female, most often on or near the large vaginal sclerite (Fig. 25), and occasionally on the lateral walls of the posterior chamber of the vagina. In other pairs the spiny tongue was not touching the female. In only two pairs of *A. diversiformis* did the form of the spiny tongue or the surface it contacted suggest that it exerted appreciable force on the female. The tongue's relatively short length suggests that it could not cause substantial movements of the distal or central body within the female.

The oxtail was most often distinguished in whole mounts late in copulation. In many pairs only the tips of the long, hair-like extensions were visible. The oxtail generally did not touch either the female or other portions of the male's genitalia; the extensions most often contacted the spermatophore after it had been produced.

Discussion

How insertion and withdrawal of the distal body occur

The male genitalia are relatively inflexible and rigid, while most portions of the walls of the female vagina are highly flexible and extensible. The insertion and withdrawal of the male genitalia can be likened to inserting and withdrawing one's foot from a stocking. In general it was not clear whether the male genitalia moved with respect to a static female vagina, or vice versa (or both). The descriptions below are expressed in terms of male rather than female movement for the sake of convenience, and are not meant to exclude the possible movement of female structures past immobile male structures.

One certain movement occurred during the first several minutes of copulation when the distal body moved more or less gradually from the posterior chamber of the vagina into the anterior chamber after the paddles and spiny arches had seized the vaginal wall. The distal body was found deep in the anterior chamber more and more often as copulation

Fig. 31: Lateral view of the the paddle and spiny arch of a copulating male Panamanian *Archisepsis diversiformis* (OZEROV, 1993), showing how they pinch the wall of the female's vagina. Scale = 15 µm.
Fig. 32: Approximately saggital section of paddle and spiny arch of male Panamanian *Archisepsis diversiformis* (OZEROV, 1993) which are pinching a fold in the wall of the female's vagina. Note the curl at the tip of the paddle, which suggests that strong pressure was being applied by the male. Scale = 15µm.
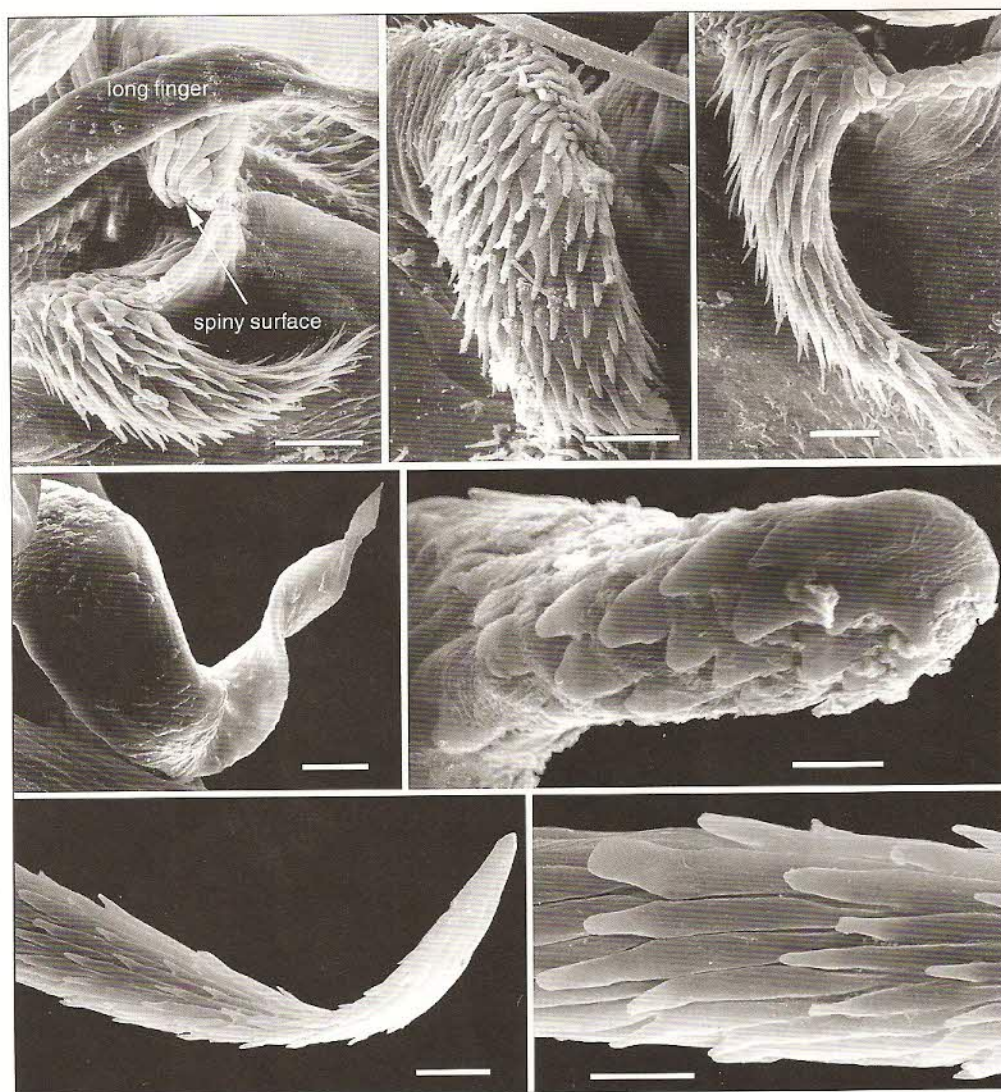
33	34	35
36	37	
38	39	

Figs 33-35: The spiny tongues of *Archisepsis* species. - **33**: *Archisepsis pleuralis* (COQUILLETT, 1904); - **34**: Costa Rican *Archisepsis diversiformis* (OZEROV, 1993); - **35**: *Archisepsis discolor* (BIGOT, 1857). The spiny tongue of *A. diversiformis* was relatively thicker, and its spines were relatively shorter than those of the other species. Scales: Figs 33, 34 = 8 μm; Fig. 35 = 5 μm.

Figs 36-39: The tips of the long fingers of *Archisepsis* species. - **36**: Costa Rican *Archisepsis diversiformis* (OZEROV, 1993); - **37**: *Archisepsis discolor* (BIGOT, 1857); - **38**: *Archisepsis pleuralis* (COQUILLETT, 1904). The long finger of *A. pleuralis* was more thickly covered with longer, basally projecting teeth (detail in Fig. 39). Scales: Fig. 36 = 5 μm; Fig. 37 = 3 μm; Fig. 38 = 4 μm; Fig 39 = 2 μm.

progressed (Fig. 51). The sharp folding back of the distal body on the distal rod in some pairs (Figs 13, 14, 40, 41) indicates that substantial inward forces were sometimes exerted. Using this folding as an indicator of exertion of inward force, one can say that inward thrusts were more common earlier in copulation, but also sometimes occurred as late as after 15 min. of copulation, and that they occurred at a variety of degrees of penetration (from *a* to *g* in the anterior chamber of the vagina).

We do not understand exactly how the inward movement of the distal body was produced, but some details are clear. The consistent positions of the paddles and spiny arches at the rear of the vagina in whole mounts, and the lack of any correlation between their exact position with regard to the rear opening of the vagina and the degree of penetration of the distal body in sectioned pairs argue that the pinch at the rear of the vagina was maintained for the duration of copulation. The posterior deflection of the paddles and spiny arches early in copulation prior to ejaculation suggest that the paddles and spiny arches were folded basally when the central body and neck were first thrust as a unit deeper into the female early in copulation. The motive force for this thrust could come from the muscles attached to the bases of the paddles, or could originate deeper within the male and be transmitted to the central body.

An alternative possibility, that the distal body is driven deeper into the female by elongation of the genitalia themselves once they are inside the female, seems remote. The distal body, the distal rod, and the central body are all stiff and sclerotized. No significant elongation or changes in the overall shapes (other than the possible movements at the hinge of the rod with the central body) was seen in any whole mounts. In fact, the one type of length change observed was a **shortening** that resulted from the distal body being folded rearward on the rod when it had penetrated only part way past the dorsal projection of the vagina (Figs 13, 40).

Another, non-exclusive, possibility is that the movement of the distal body deeper into the female vagina occurs due to movements of portions of the genitalia brought about by internal pressure changes in the central body. The fact that the eversions of the corner mass of the spiny surface seen when macerated genitalia were transferred to water also appeared to occur during copulation seems to confirm that increases in the internal pressure of the central body do occur during copulation. The wide, strong-walled internal duct just basal to the spiny surface (Figs 4, 5) may be the structure within which the pressure rises. The hypothesized eversion of the spiny surface would be in the opposite direction needed to pull the distal body deeper into the vagina, but the thick layer of cuticle on the inner surface of the spiny surface suggests that when the pressure returned to previous levels, the ensuing invagination of the spiny surface could be powerful, and result in a strong pulling action of the long finger.

It is obvious that these proposals are only tentative, since we never observed the predicted eversion of the spiny surface. On the other hand, if our interpretation is correct, it may be impossible to observe such eversion with the techniques we used. It seems reasonable to suppose that somehow the elaborate invaginated array of spines on the central body is at least sometimes evaginated, and does not remain hidden and functionless inside the male's genitalia throughout copulation.

In sum, it is clear that the distal body gradually moves deeper into the female during the first several minutes of copulation, and it is probably sometimes pressed quite forcefully into the female. The mechanisms responsible for this movement are, however, still a mystery.

A second certain movement that occurs during copulation is the withdrawal of the distal body from the anterior into the posterior chamber of the vagina that occurs around the time of ejaculation (Fig. 51). But, paradoxically, any movement inward or outward by the distal body would seem to be ruled out by the very firm and apparently immobile anchoring of the basal portion of the male genitalia at the posterior end of the vagina by the pinching action of the paddle and spiny arch. At least part of the probable explanation for how withdrawal occurs is that the male's genitalia are essentially immobile, and
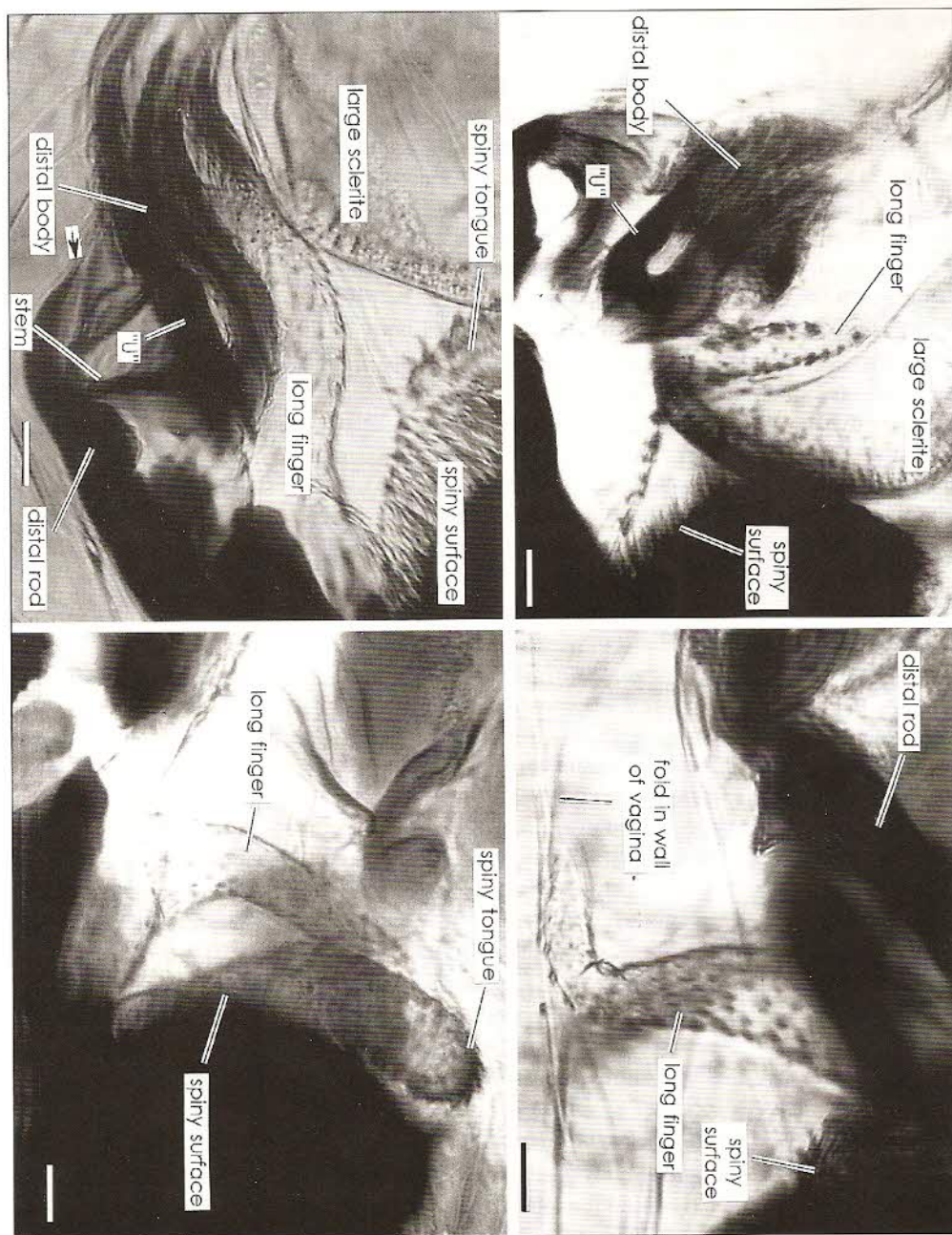
Fig. 40: Ventro-lateral view of the long finger of a male Panamanian *Archisepsis diversiformis* (OZE-ROV, 1993) that bends sharply as it apparently presses forcefully on the ventral and anterior surface of the dorsal projection of the vagina. The distal body is folded sharply basally on itself, and is also folded basally on the distal rod as it also apparently pushes forcefully against the dorsal projection. Scale = 13 µm.

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that the anterior portion of the female's vagina is in essence pushed anteriorly past the male's distal body by the spermatophore material emerging from the tip of the distal body. However, the fact that the distal body was always extended anteriorly after ejaculation, rather than being at least partly bent back on the rod argues against this as the sole explanation of the movement. A second possible motive force would be a rearward pull on the entire central body and neck. The differences in the positions of the spiny arches at different stages of copulation are in accord with this idea, and contraction of the muscles associated with the bases of the paddles could produce this movement.

In either case the elastic lining of the vagina would be stretched as a result of the male movements. This stretching may explain the otherwise mysterious but consistent dorsal "wrinkle" in the vaginal lining just posterior to the base of the dorsal projection (Fig. 59). A wrinkle of this sort was never seen in pairs frozen prior to ejaculation, but was visible in all sectioned material and in most wholemounts after ejaculation occurred. During the first portion of ejaculation, while the anterior chamber of the vagina was filling with spermatophore material, the male's distal body would move ventrally as well as posteriorly with respect to the female's vagina. This ventral movement of the male's distal body and rod would stretch the ventral surface of the lining of the posterior chamber of the vagina tight, and at the same time reduce the tension on the dorsal surface. Any anterior movement of the paddle would also reduce tension, especially on the dorsal wall. The resulting slack in the dorsal wall would accumulate just anterior to the central body, at the base of the less flexible large sclerite on the dorsal projection.

How the spermatophore is formed

In none of the copulating pairs was the male in the process of forming the anterior portion of the spermatophore when the pair was frozen. This suggests that the first stage of spermatophore production is probably relatively rapid, in contrast with the apparently drawn-out ejaculations of diopsids (KOTRBA 1996). It also means that we have only indirect data on how the process occurs. The following hypotheses are based on several details of what we observed.

Ejaculation probably begins after the fibrous tip of the distal body has been inserted into the mouth of the spermatheca duct (Fig. 15). Since the tip sometimes reached the spermathecal duct after as little as 8 min of copulation but ejaculation never occurred before 14 min. in *A. armata* (corresponding times in Panamanian *A. diversiformis* were 10 and 15 min.), ejaculation is not necessarily triggered by arrival of the tip of the distal body at the spermathecal duct.

Ejaculation probably begins with the emission of liquid spermatophore material from the fibrous tip of the distal body, forming a tube around the soft tip of the distal body.

Fig. 41: Lateral view of the long finger of a male Panamanian *Archisepsis diversiformis* (OZEROV, 1993) that extends anteriorly and is apparently pressed by the distal body against the large sclerite of the dorsal projection. The stem at the base of the distal body is folded back on the distal rod, and the crescent-shaped tip of the rod (arrow) appears to fit against the rounded surface of the base of the distal body. Scale = 15 μm.
Fig. 42: Lateral view of the long finger of a male Panamanian *Archisepsis diversiformis* (OZEROV, 1993) that extends laterally and presses forcefully on the wall of the posterior chamber of the vagina (note the bend and wrinkle in the finger, and the folds in the wall). Scale = 15 μm.
Fig. 43: Lateral view of the long finger of a male Panamian *Archisepsis diversiformis* (OZEROV, 1993) that is directed basally, and apparently presses on the spiny tongue which in turn apparently presses the wall of the vagina. Scale = 10 μm.

Figs 44-47: Oxtails of *Archisepsis* species. - **44**: *Archisepsis pleuralis* (COQUILLETT, 1904); - **45**: Costa Rican *Archisepsis diversiformis* (OZEROV, 1993). They are showing the branching structures on the hair-like extensions (arrows). - **46, 47**: The tips of the extensions were sharply hooked in *Archisepsis discolor* (BIGOT, 1857). Scales: Figs 44, 45 = 10 µm; Fig. 46 = 3 µm; Fig. 47 = 0.8 µm.
Fig. 48: Spine-like extensions on the neck of *Archisepsis pleuralis* (COQUILLETT, 1904) (see also Fig. 9), showing the lack of sockets at their bases. Scale = 2 µm.
Fig. 49: Two setae on the surstylus of *Archisepsis pleuralis* (COQUILLETT, 1904), showing the sockets at their bases. Scale = 4 µm.
Fig. 50: Hair-like extensions on the neck of Costa Rican *Archisepsis diversiformis* (OZEROV, 1993), showing the lack of sockets at their bases. The arrows mark circular structures of unknown significance (see text). Scale = 10 µm.

Perhaps an additional material is added to the interior surface of the tube that causes it to harden and to stain a darker blue. The emergence of the spermatophore material probably displaces the tip of the distal body away from the mouth of the spermatheca duct and into the anterior chamber of the vagina. Judging by the position of the spermatophore duct, the tip of the distal body travels along or very near the anterior surface of the dorsal projection of the vagina wall. As the spermatophore material emerges, it gradually fills the inner end of the anterior vagina chamber nearest the entrance to the spermathecal duct. Either as a result of this filling, or of movements of the paddles and spiny arches (above), the distal body is gradually displaced rearward into the posterior chamber of the vagina, with the soft tip leaving behind a hollow tube (the spermatophore duct) in the spermtophore material as it goes. An apparently similar process is used by the diopsid *Teleopsis quadriguttata* (WALKER) to form the neck of the spermatophore (KOTRBA 1996).

When the tip has moved past the dorsal projection and into the posterior chamber of the vagina and a certain amount of spermatophore material has accumulated in the posterior chamber, the male transfers his sperm. During sperm transfer the tip of the distal body is embedded in the spermatophore material, and the sperm and liquid in which they occur emerge to form a round or oval mass within the still liquid spermatophore material. The tip of the distal body is then withdrawn completely from the spermatophore and the spermatophore material closes around the exit site, forming the sperm reservoir. This leaves the sperm mass in the reservoir connected with the spermatophore duct that leads to the spermatheca duct; as seen in some sectioned material, the spermatophore duct was still empty or nearly empty of sperm (Figs 56, 57). The spermatophore material immediately surrounding the sperm reservoir becomes modified to form the homogeneous inner layer of the spermatophore wall which stains lighter blue (Fig. 58). The transfer of first spermatophore material and then the sperm is the same order as probably occurs in several flies, including a nematoceran (LINLEY 1981), the phorid *Megaselia scalaris* (BENNER 1991), the diopsids *Cyrtodiopsis* (KOTRBA 1993) and *Teleopsis* (KOTRBA 1996), and tse-tse flies in the genus *Glossina* (SAUNDERS & DODD 1972, POLLOCK 1974), as well as in many other insects (GERBER 1970). In *Teleopsis*, however, a cavity is apparently prepared in the spermatophore material before the sperm are transferred into it (KOTRBA 1996).

Species differences

The overall similarity between the events observed in field and laboratory matings of *A. diversiformis* indicates that copulation with virgin and non-virgin females are similar. Most if not all of the Costa Rican *A. diversiformis* females were already mated previously (nearly all females collected prior to copulation at dung in nature were inseminated - W. EBERHARD unpub.). There were, however, several differences in the details of copulation between the species. Males of *A. armata* apparently penetrated deeper into the female more quickly than those of Panamanian *A. diversiformis*: after 4-7 min. of copulation, the tip of the distal body was past the entrance of the oviduct in 5 of 6 *A. armata* but 0 of 8 *A. diversiformis* were (p = 0.003 with FISHER's exact test). In copulations which had lasted 9 min. or longer and in which the male had not yet ejaculated, the distal body was more often folded back on the rod in Panamian *A. diversiformis* than in *A. armata* (6 of 14 vs. 0 of 12, p = 0.013 with FISHER's exact test). As mentioned above, such folding probably results from thrusting pressure by the male. The spiny tongue
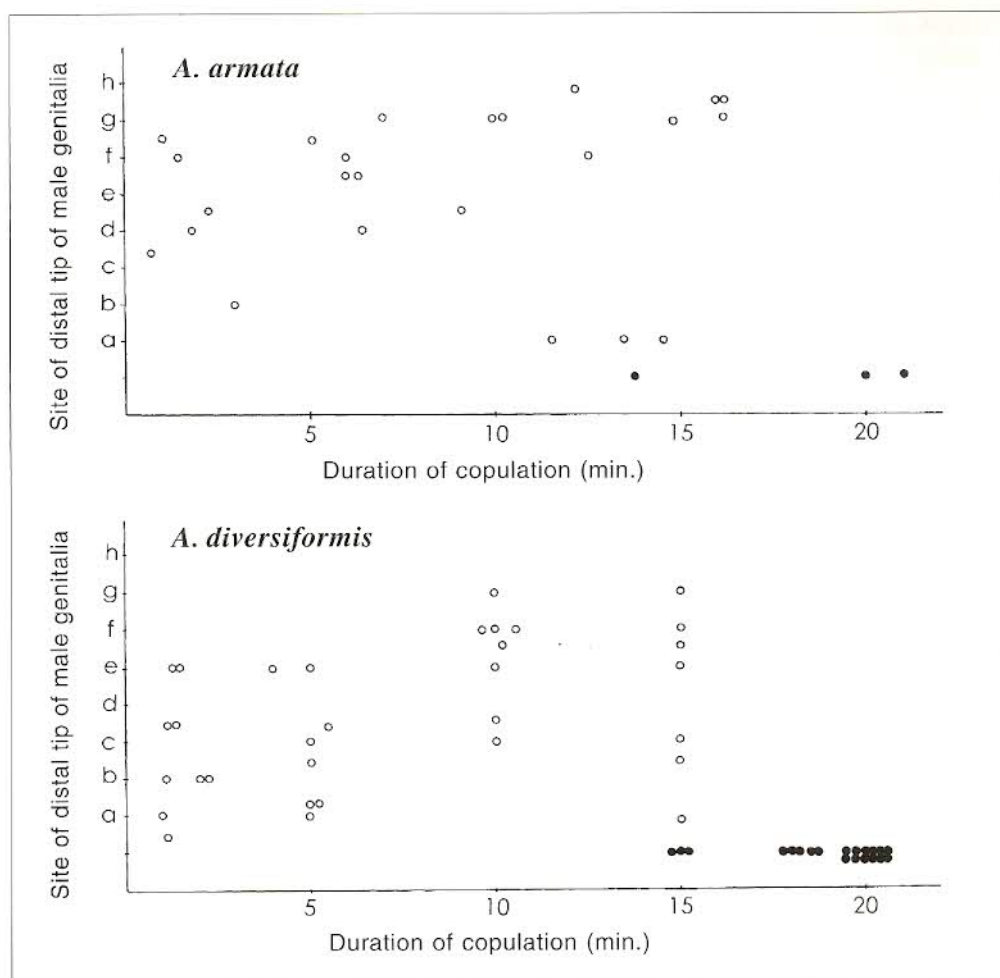
Fig. 51: Graphical representations of the positions of male genitalia within the female in pairs of *Archisepsis armata* (SCHINER, 1868) (N = 27) and Panamanian *Archisepsis diversiformis* (OZEROV, 1993) (N = 52) frozen after different lengths of time in copula. Filled circles represent pairs in which ejaculation had occurred. The sites represented by the letters (shown in Fig. 1) are the following: a - posterior chamber of vagina posterior to the ventral corner of the dorsal projection; b - past this ventral corner; c - past the mouth of the duct of the ventral receptacle; d - past the mouth of the oviduct; e - past the small vaginal sclerite; f - at the mouth of the common spermathecal duct; g - barely within the common spermathecal duct; h - deep in the common spermathecal duct.

projected anteriorly beyond the spiny surface more often in both Panamanian (N = 33) and Costa Rican (N = 15) *A. diversiformis* than in *A. armata* (N = 29) (p < 0.001 in both cases with Chi^2). In some pairs of Costa Rican *A. diversiformis* the tips of at least some of the fibers of the distal body and the soft tip were inserted into the entrance of the ventral receptacle duct, a position never seen in the other species. Although the data from Costa Rican *A. diversiformis* are not strictly comparable because of less precise measurements of copulation durations, the males appear to penetrate even less deeply throughout the pre-ejaculatory phases of copulation (only 7 % of 15 past *d*, compared with 42 % of 33 Panamanian *A. diversiformis* and 62 % of 29 *A. armata* - p < 0.01 and < 0.001 respectively with Chi^2).

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Functions of male genitalic structures and movements

The flattened form of the paddle, the strong setae on the edge of the spiny arch, the strong pinching action of the two structures on the female's vaginal wall (Fig. 32), their positions at the posterior end of the vagina throughout copulation, and the apparent force they exerted on the female, as indicated by the curled tip of the paddle, suggest strongly that they function as holdfast devices to brace the male's genitalia firmly in the vagina. Withdrawal of the distal body from the anterior to the posterior chamber of the vagina may be produced at least in part by distal flexion of the paddles and spiny arches (above).

The multitude of different positions of the male's distal body in the female suggests two possible, non-exclusive functional interpretations. Males may have difficulty locating the entrance of the common spermathecal duct and inserting the tip of the distal body into it, and may thus sometimes insert the tip of the distal body into both the mouth of the common spermathecal duct and other openings while searching. Alternatively, touching other sites within the female with the tip of the distal body may serve to stimulate the female. A stimulatory function in the spermathecal duct would seem to be supported by the fact that *A. armata* males frequently succeeded in inserting the tip into the common spermathecal duct relatively early in copulation (as early as 7 min. - Fig. 51), but did not ejaculate until much later (15-20 min.). Similar variation in positions of intromittent male genitalia also occurs early in copulation in the tse-tse fly *Glossina austeni* (POL-LOCK 1974), and stimulation of the female by insertion of genitalic structures in the spermathecal duct has been proposed in cicindellid beetles (FREITAG et al. 1979).

The distal orientations of the spines of the evaginated corner mass of the spiny surface in some copulating pairs suggests that the spines contact the "pocket" at the base of the dorsal projection of the vagina (Figs 1, 2), with which the corner mass was often aligned. The rest of the spiny surface of the central body was never seen everted, but we presume that eversion does occur, probably due to higher pressure within the central body (above). Judging by the positions and forms of the spines, possible functions of both the corner mass and the rest of the spiny surface, as well as the spines and hair-like extensions on the neck, include both stimulation of the female, or abrasion of her vaginal lining to allow penetration of male accessory gland products as in muscoid flies (LEWIS & POL-LOCK 1975, MERRETT 1989). We found no sign, however, of abrasion of the female walls in sectioned material; an abraiding function also seems to be ruled out for the flattened, cobblestone-like spines of *A. pleuralis* (Fig. 24). The possible male product found alongside the neck region (Fig. 3) was not appropriately positioned to be introduced into abrasions made by the stronger spines of the spiny surface or the corner mass. The strong spines on the neck region of *A. pleuralis* (Fig. 9) might serve to introduce this material into the female; but this leaves unexplained the complete lack of such spines combined with production of the material in Costa Rican and Panamanian *A. diversiformis*.

The lack of abrasion also fails to support the hypothesis of WARD et al. (1992) that female avoidance of copulation serves to reduce the risk of internal injury by the male's genitalia. Also seemingly ruled out is the possibility that these, or any other male structures served to remove sperm stored from previous males (WARD et al. 1992), since no male structure ever penetrated to a site where sperm are stored (spermathecae) or might be stored (ventral receptacles). It is still possible that the spines remove sperm that is displaced by the female into the vagina from storage sites, but the variety of spine shapes on different portions of the male genitalia and in different species (Figs 18-24) remains unexplained by this hypothesis.
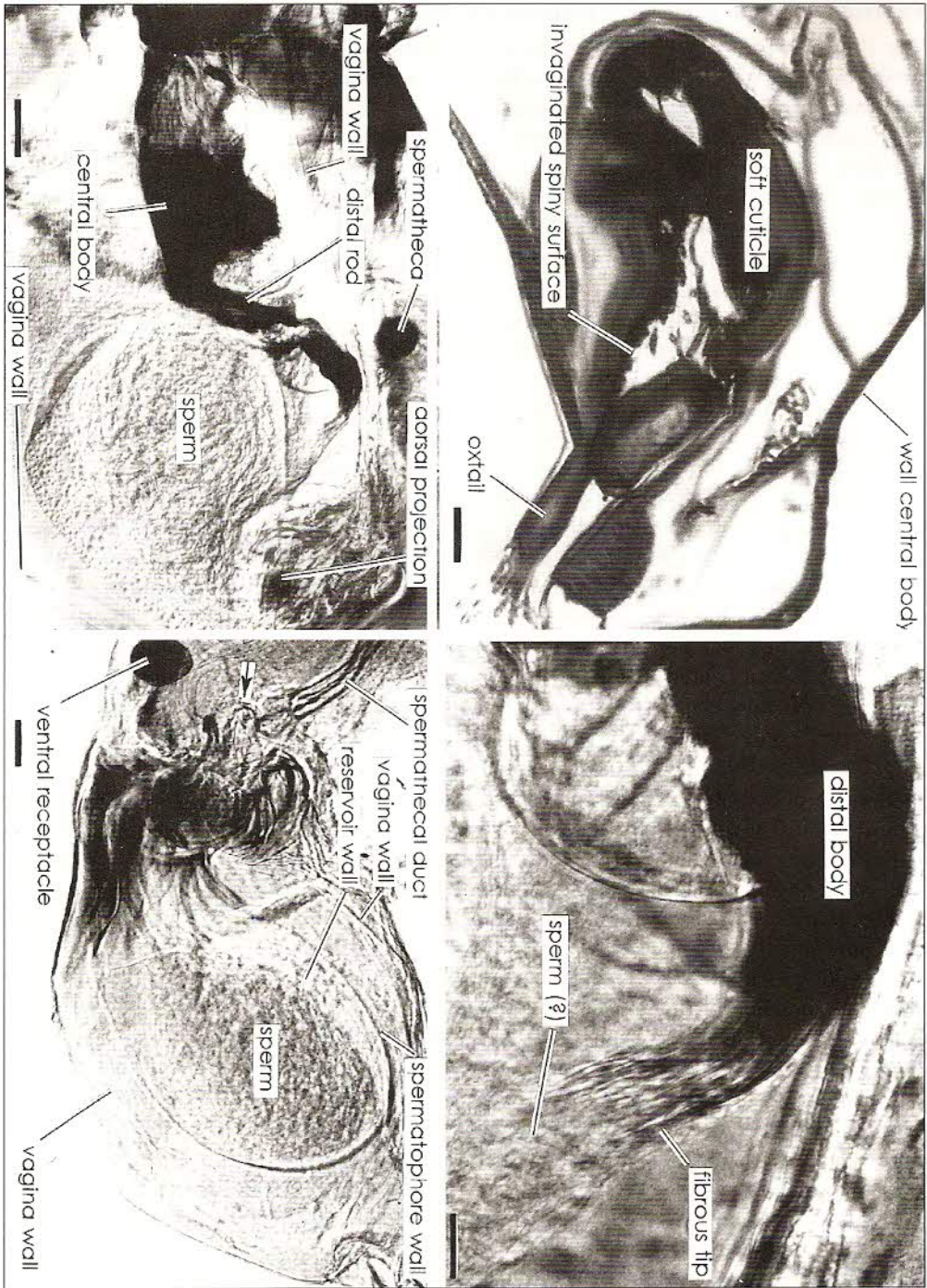
The postulated eversions and powerful spring-like retractions of the spiny surface and internal rod could explain both the structure and the variety of positions of the long finger. Each eversion would produce a distal movement of the long finger, whose base is continuous with the internal rod. The basally directed, cuticle-tipped spines on the finger could function as anti-slip devices that snag against the vaginal lining during the spring-like retractions, and thus help pull the male's genitalia deeper into the female. Some of the orientations and details of the long finger's position with respect to the female suggested that it exerted force on the female. The most common pre-ejaculatory position of the finger involved contact with the anterior surface of the dorsal projection of the female, a position that would be particularly appropriate for pulling the distal body deeper into the female. It appears, however, that the male is not able to move the finger directly. There were no muscles anywhere in the male genitalia distal to the artic-ulations of the paddles. The finger was composed of solid soft cuticle for nearly its entire length. This cuticle may be capable of being stiffened or inflated slightly by fluid pressure, however, as it contracted when dry.

There are still many mysteries. The lack of socketed setae or any other obvious sensory receptors anywhere on the intromittent genitalia distal to the wrist leaves the question of how the male orients his genitalia inside the female unanswered. There was no obvious function for the oxtail, or the branched and hooked tips of its hair-like extensions, or the complexly folded membranes enclosing the basal portion of the distal body (Figs 16, 17) or the small vaginal sclerite or the ventral sac of the female. No ejaculatory duct was identified in the male genitalia.

Implications for hypotheses explaining genitalic evolution

Some of the hypotheses that have been proposed to explain the rapid divergent evolution of animal genitalia make predictions that are clearly not in accord with observations reported here. A tight mechanical fit between rigid male and female structures, as pre-dicted by the species isolation and cryptic female choice via mechanical lock-and-key (EBERHARD 1985, SHAPIRO & PORTER 1989) clearly does not occur. Most female structures contacted by the male's intromittent genitalia during copulation are soft and flexible, and could mesh with a wide variety of male forms. The major rigid internal female structure, the large vaginal sclerite, does not fit even approximately with the shape of any male structure, nor is it physically capable of impeding the male's access to the mouth of the common spermathecal duct. The relatively inaccessible location of the mouth of the spermathecal duct, at the innermost end of the anterior chamber of the vagina, could, however, result in more general selection favoring males with structures, such as the long finger and the paddles and spiny arches, that may have enabled them to position, brace, and move the distal portions of their genitalia appropriately. But the

53	52
55	54

Fig. 52: Saggital section of the central body and the oxtail of a Panamanian *Archisepsis diversi-formis* (OZEROV, 1993); internally the oxtail apparently constituted an extension of the soft cuticle associated with the invaginated spiny surface. Scale = 13 μm.
Fig. 53: A male Costa Rican *Archisepsis diversiformis* (OZEROV, 1993) ejaculates in the posterior chamber of the vagina. Scale = 70 μm.
Fig. 54: Closeup of Fig. 53, showing the material (sperm?) emerging from the expanded fibrous tip of the distal body. Scale = 15 μm.
Fig. 55: Newly formed spermatophore in the vagina of a female Costa Rican *Archisepsis diversi-formis* (OZEROV, 1993) after copulation ended. The vagina is swollen, and the common spermath-ecal duct is partially unfolded (arrow). Scale = 50 μm.
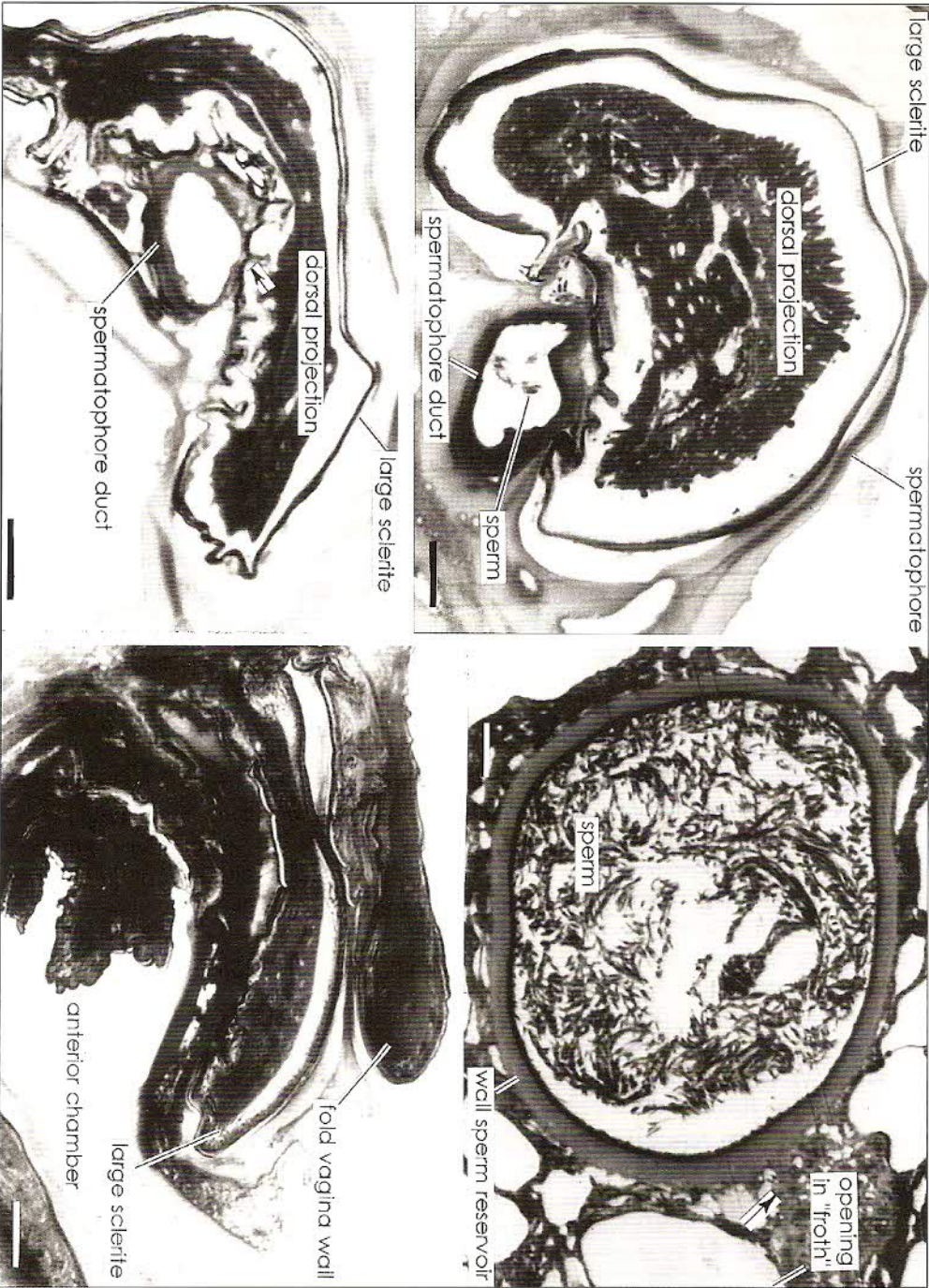
57	56
59	58

Fig. 56: Section of the anterior portion of a finished spermatophore of a Costa Rican *Archisepsis diversiformis* (OZEROV, 1993), showing the spermatophore material completely surrounding the dorsal projection of the vagina, and the spermatophore duct with a small number of sperm. Scale = 15 μm.

morphological details of these male structures could apparently differ without affecting their abilities to perform these mechanical functions.

There was also no sign that any male genitalic structures are designed to overcome or counteract internal resistance from female structures or behaviour that could prevent male coupling, as predicted by the male-female conflict of interest hypothesis (ALEXANDER et al. 1997). In fact, perhaps the most divergent portions of the male genitalia, the spines on the anterior surface of the central body, and the spines and hair-like projections on the neck (Fig. 60), are all unlikely to be involved in mechanical manipulations of the female. In contrast, the most forceful, physically manipulative genitalic structures of the male, the paddles and spiny arches, were relatively uniform in the different species. In addition, they meshed with a soft, apparently featureless portion of the female, the vaginal wall, which gives no evidence in its design of resistance to male manipulation (Figs 31, 32).

Female genitalic mechanisms to gain naturally selected advantages by rejecting males would seem superfluous in any case in these flies. Female *Archisepsis* are able to (and frequently do) prevent mounting by kicking and shaking the male off, and prevent intromission by such a simple measure as bending the abdomen ventrally (and probably also sometimes by failing to extend the ovipositor or otherwise permit intromission). Females of Costa Rican and Panamanian *A. diversiformis* in nature also often terminate mounting prior to intromission by simply walking off the oviposition substrate and into the grass or leaf litter nearby, which reliably induces the male to dismount and leave within 15-30 sec. (W. EBERHARD in prep.) (females of *A. armata* have not been observed in nature). In sum, the data do not fit well with the conflict of interest hypothesis.

The pleiotropy hypothesis makes no clear predictions, other than that the species-specific variations in male genitalic structures should be arbitratry and have no effect on a male's ability to accomplish sperm transfer. The fact that we are able to assign tentative functions to some structures does not support this hypothesis, but the functions for some of the more distinct structures (e. g. the hair-like structures on the neck) were the least certain. The prediction that the interspecific variations in these structures do not affect a male's ability to transfer sperm cannot be tested with the present data.

The hypotheses that the male genitalic structures that differ among related species function to stimulate the female (either for species recognition or to influence cryptic female choice) fit the data more easily. Some structures and movements, such as the contact and possible eversion of the spiny surface of the central body, the hair-like processes on the central body (Figs 9, 60), and the contact of the spiny tongue with the large female sclerite do not seem to have any obvious functions other than stimulation. The orientations of these spines and the ways they contact the vagina are not appropriate for holding the male's genitalia within the female (WARD et al. 1992), or moving them deeper within her.

Fig. 57: Section near the tip of the empty spermatophore duct of a Costa Rican *Archisepsis diversiformis* (OZEROV, 1993), where it extends into the membranous mouth of the common spermathecal duct (arrow marks fold in the membrane at the mouth). Scale = 15 μm.

Fig. 58: Transverse section of a spermatophore of a Costa Rican *Archisepsis diversiformis* (OZEROV, 1993), showing the loosely packed sperm in the sperm reservoir, and the inner portion of the surrounding frothy mass (arrow marks apparent extension of wall of sperm reservoir into "froth"). Scale = 15 μm.

Fig. 59: Saggital section through the anterior portion of the vagina of a Panamanian *Archisepsis diversiformis* (OZEROV, 1993) showing the prominent fold in the wall of the of vagina just posterior to the large vaginal sclerite that usually (or perhaps always?) formed after ejaculation (ventral side is to the right). Scale = 15 μm.

The likelihood that these possible types of stimulation function in species recognition seems very low. Several other species-specific male structures and behaviour patterns are routinely brought into play prior to intromission in sepsids: the male clasps the female with his modified, species-specific front legs, courts her in a species-specific manner with his middle and hind legs prior to genitalic coupling, and grasps her abdomen with his species-specific genitalic surstyli from the beginning of intromission. In addition, cross-specific mounting attempts are infrequent in nature (W. EBERHARD unpub.), and female rejection of males occurs at several stages prior to intromission when the advantage to females of rejecting males of other species is likely to be greatest (ALEXANDER 1964, EBERHARD 1985, SHAPIRO & PORTER 1989). Other male structures (middle legs, surstyli) also clearly perform courtship behaviour during copulation (W. EBERHARD in prep.). Rejection of species recognition leaves the hypothesis of stimulation to influence cryptic female choice as the one most in accord with the data.

One important limitation of this study is that our techniques allow only minimum estimates of the movements of male genitalia within the female. For instance, we would
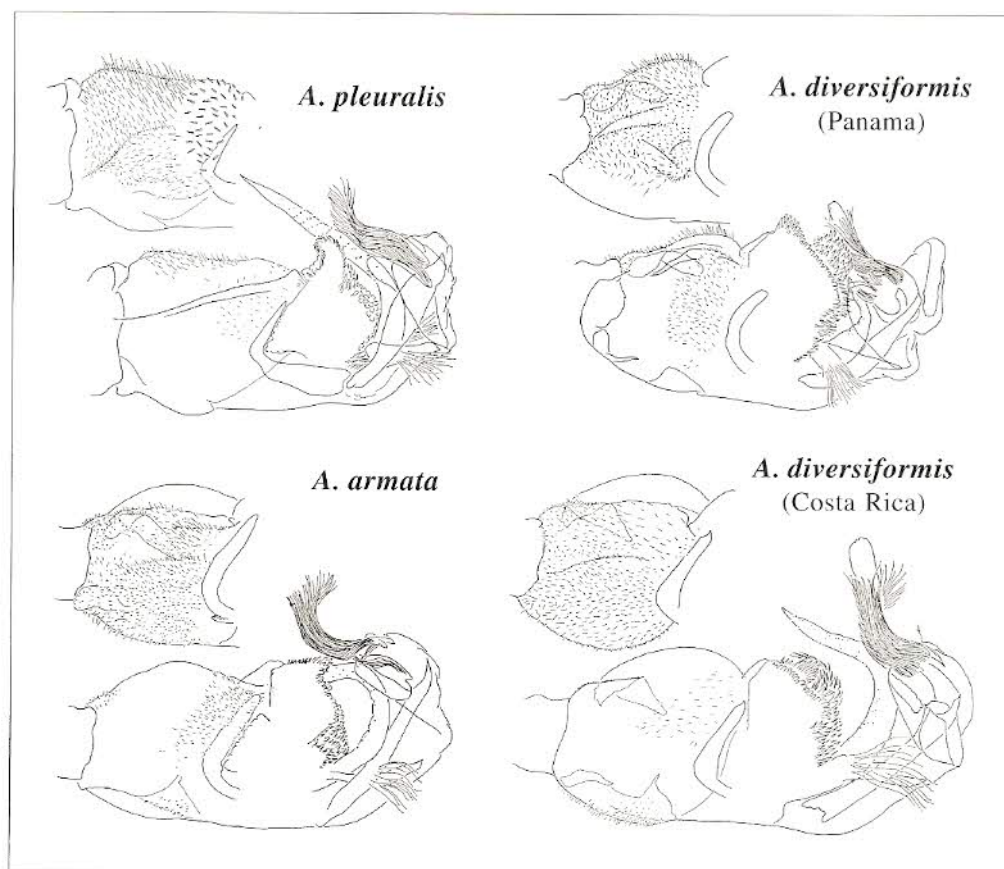

Fig. 60: The distal portions of the intromittent male genitalia in different species of *Archisepsis* (all drawn to the same scale) (no attempt was made to distinguish which structures lay over others in the portions of the genitalia distal to the long finger). In each species both sides of the neck region are drawn to show the patterns of hair-like processes.

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have missed small, rhythmic movements of internal genitalic structures if they occurred. There is evidence that this kind of movement may occur in *Archisepsis*. The ejaculatory apodeme, a sclerite in the sperm pump, was generally visible through the membrane just anterior to the male's hypandrium, and it was always in constant rapid motion during the first 10-15 min. of copulation. The apodeme consistently stopped moving just before ejaculation occurred in these and other *Archisepsis* species (W. EBERHARD in prep.). Similarly, movements of elastic structures such as the spiny surface of the central body, if they were extended or otherwise deformed during copulation but sprang back during the moment that the pair was frozen or during subsequent fixation, would also be missed. The general conclusion is that possible stimulatory movements of the male intromittent genitalia were, if anything, underestimated.

Acknowledgements

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