Morphology and Function of Male Genitalia (Spermatophores) in *Euscorpius italicus* (Euscorpiidae, Scorpiones): Complex Spermatophore Structures Enable Safe Sperm Transfer

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ABSTRACT The structure and function of the spermatophore of *Euscorpius italicus* are analyzed. We show how the spermatophore gets shaped from two hemispermatophores and for the first time the sperm transfer mechanism is shown in detail, illustrating function and importance of all complex lobe structures of an euscorpiid spermatophore. A detailed description of the interaction of spermatophore and female genitalia is given. The capsular region of the spermatophore bears different lobes: The distal and basal lobes hook into two cavities on the inner side of the female's genital operculum. A so-called "crown-like structure" hooks into a membranous area in the genital atrium. During sperm transfer, these crown-like structures move backwards, in this way widening the female's genital opening. The sperm duct of the spermatophore is coated with numerous spicules on its outer side, which could serve as a sealing mechanism and/or may stimulate the female. Furthermore, we conclude that "safeguarding of sperm transfer" is one driving force for evolution of male genital complexity in scorpions, but also sexual selection by cryptic female choice could partly play a role. J. Morphol. 260:72–84, 2004.   © 2004 Wiley-Liss, Inc.

KEY WORDS: Scorpiones; *Euscorpius;* spermatophore; genital organ; safeguarding of sperm transfer

There exists an enormous variety of mating behaviors, strategies, and mechanisms in the animal kingdom. Thus it is not surprising that sexual reproduction involving mating is one of the best-investigated topics in biology. An essential part of mating is sperm transfer. Sperm transfer by spermatophores is a widespread and effective mechanism in arthropods (reviewed by Proctor, 1998) and especially in arachnids (summarized by Schaller [1979]; scorpions [Angermann, 1957], amblypygids [Weygoldt et al., 1972], pseudoscorpions [Weygoldt, 1969], spiders [Legendre, 1981], uropygids [Schaller, 1971], and mites [Witte, 1991; Alberti and Coons, 1999]).

Sperm transfer in scorpions is managed with the help of a spermatophore. Two different types of spermatophores have been described: the flagelliform type present only in buthids (Francke, 1979), and the lamelliform type characteristic for all other scorpions (Francke, 1979; Polis 1990). The flagelliform type with a peculiar flagellum connecting the spermatophore with the male genital region during mating is apparently unique. The sperm transfer of lamelliform spermatophore functions due to a lever mechanism pressing the sperm into the female genital tract (Angermann, 1957). Similar ways of sperm transfer are widespread among arthropods and can be found, for example, in pseudoscorpions and amblypygids (see above-mentioned citations). Spermatophores in the form of simple droplets or stalked spermatophores with droplets (Schaller, 1979) do not occur among scorpions.

The elaborate scorpion courtship behavior preceding sperm transfer has been described for many different species and need not to be repeated here. For a review on this topic, see Shulov and Amitai (1958), Polis (1990), and Benton (2001). Immediately before sperm transfer, the male grasps the female more tightly, leads it precisely over the spermatophore, then pushes her back and downwards, in this way eliciting the lever mechanism of the spermatophore (in non-buthid scorpions) and the sperm transfer (Angermann, 1957; Rosin and Shulov, 1963; Benton, 1993; Peretti, 1992). The importance of female cooperation during this part of courtship is not yet well understood.

The definite scorpion spermatophore is built from two hemispermatophores, which are formed in two lateral paraxial organs (Pawlowsky, 1921; Angermann, 1957) of the male genital tract (Polis, 1990).

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Both halves are pressed out of the genital pore simultaneously, glued together by glandular secretions, and fixed on the substrate by a so-called "pedicel" (Francke, 1979; Polis, 1990; Peretti, 1992; Farley, 2001).

Maccary (1810) and Fabre (1923) were the first to describe parts of mating in scorpions, but another 30 years passed before detailed descriptions were presented that included spermatophores (Bücherl, 1955/56; Alexander, 1956, 1957, 1959; Shulov, 1958; Shulov and Amitai, 1958, 1960). Sperm transfer mechanisms involving flagelliform spermatophores are described for many different buthids by Bücherl (1955/56), Alexander (1959), Probst (1972), Maury (1975), Peretti (1991), and Benton (2001). Lamelliform spermatophores before and after sperm transfer were figured by Alexander (1957), Angermann (1957), Rosin and Shulov (1963), Francke, (1979), Stockwell (1989), Benton (1993), Peretti (1996), and Peretti et al. (2000). However, the precise functions of the different parts of the complex capsular region of lamelliform spermatophores are not yet sufficiently understood. An exception is the most detailed functional description presented by Peretti (1992) for *Bothriurus bonariensis*. These data show that spermatophores are most complex in structure as well as in function.

In our study we investigate the structure and the function of the spermatophore of *Euscorpius italicus*. We show how the spermatophore gets shaped from two hemispermatophores and for the first time we show the sperm transfer mechanism in detail, illustrating function and importance of all complex lobe structures of a euscorpiid spermatophore. Furthermore, we propose different explanations for the evolution of the spermatophore structures.

MATERIALS AND METHODS

We used *Euscorpius italicus* from Tessin (Ticino) county in the south of Switzerland as a model species. Living animals for mating experiments were either from the breeding of M.E. Braunwalder or collected by A.J. and M.E.B. by hand.

To understand how the two hemispermatophores shape the spermatophore, 10 hemispermatophores of ethanol-preserved specimens (70–80%) were investigated. To uncover the paraxial organs containing the hemispermatophores, the first six mesosomal segments were cut dorsally in their middle by using a small pair of scissors. With the help of tweezers, the paraxial organ was taken out of the mesosoma and then the hemispermatophore was carefully removed by hand from the surrounding tissue. No dissolving agents, e.g., lactic acid or KOH, and no clearing agents, e.g., clove oil, were used in order to prevent artifacts of the delicate structures of the capsular region.

Furthermore, 18 spermatophores, (12 postinsemination and 6 preinsemination spermatophores) were used for the study. Spermatophores were obtained either directly after sperm transfer (for postinsemination spermatophores) or directly before sperm transfer but after spermatophore deposition (for preinsemination spermatophores) by disrupting the mating. This was done by pushing the couple away from the spermatophore with two tweezers. For fixation and to prevent drying out of the spermatophores, they were immersed in 70% ethanol. This treatment did not cause any visible changes of morphological structures. The

drawings of spermatophores and hemispermatophores were made from these whole-mounted preserved specimens using a Leica M10 stereomicroscope.

The possible sperm transfer was reconstructed with a dead female and two spermatophores (postinsemination and preinsemination) in 70% ethanol. Photos of these reconstructions were made with a stereomicroscope (Leica M10) and a Wild MPS20 camera (Fig. 7).

All 18 matings were observed in the laboratory. As substrate we used vertically placed styrofoam plates (20 × 30 cm) in 14 cases (UV-light). In the remaining four cases we used an oblique glass plate with a rugged surface (dim light) in a plastic container (14 × 18 cm). Comparing the two treatments, no behavioral or other differences could be observed. In order to test the reliability of our conclusions, we filmed (Sony digital handycam DCR-TRV900E) the first 14 successful matings of *Euscorpius italicus* totally. The remaining four matings were filmed only during spermatophore deposition and sperm transfer, respectively. Scorpions were kept separately in plastic boxes (10.5 × 8.5 cm) on gypsum ground and fed every 2 weeks with one cricket (water ad libitum).

RESULTS
Preinsemination Spermatophore

Figure 1C–F shows a hemispermatophore from different perspectives. The arrows of Figure 1B define these perspectives. The black sides of the arrows (Fig. 1B) are equal to the bottom of the four drawings (Fig. 1C–F). At the end of the lateral lobe (ll) is situated a sclerotized structure with spikes which we termed the crown-like structure (cls). In ectal direction of the crown-like structure are situated three sclerotized lobes (Figs. 1C–E, 5A–C): The outer distal lobe (lde, lobe distal extern), the inner distal lobe (ldi, lobe distal intern), and the basal lobe (lb, lobe basal) with short spikes on it (nomenclature after Vachon, 1948, and Stockwell, 1989). The lateral lobe is connected to its counterpart by a transparent membrane. Posterior to the crown-like structure a stiffer, bent membrane replaces this connecting membrane. One side of this stiffer membrane is coated with small spicules. The whole structure represents one-half of the sperm duct (sd) of the spermatophore. This half of the sperm duct connects the crown-like structure to the region with the outer and inner distal lobes (Fig. 1C–F). Figure 1F shows that the sperm duct-building structure is U-shaped. The cavity of the U is coated with small spicules but the outside is not. This inside of the U forms the outside of the sperm duct when the definite spermatophore is built (Fig. 2A,B; definite spermatophore: Figs. 3A–F, 4A–D). Thus, when the two hemispermatophores are fused to form the spermatophore, the convex parts of the U face each other (Fig. 2A). After the fusion the U-shaped sperm duct borders (sdb) form a straight line (Fig. 2B). The definite sperm duct is well protected under the lobes (Figs. 3A–C, 4A,B). No opening is visible and the spicule-coated side forms the outside of the sperm duct (Figs. 2B, 5A,D,F, 3B,C,E,F, 4A,C,D).

The crown-like structures are both in plane and each nearly forms a closed circle (Figs. 3C, 4A,B).
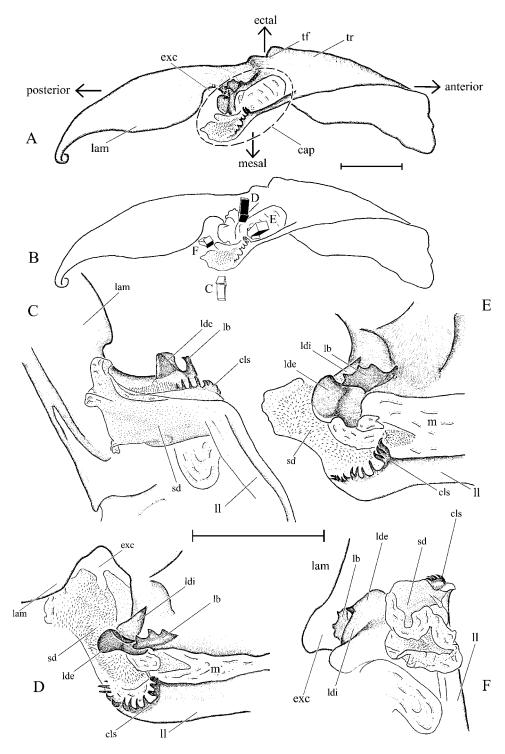
Fig. 1. Hemispermatophore of *Euscorpius italicus*. **A:** Overview. **B:** Simplified overview. Arrows indicate views of the four drawings **C–F**, (black side = bottom of drawings **C–F**). **C–F:** Four different views of the capsular region. cap, capsule; cls, crown-like structure; exc, excavation; lam, distal lamina; lb, basal lobe (lobe basal), lde, outer distal lobe (lobe distal extern); ldi, inner distal lobe (lobe distal intern); ll, lateral lobe; sd, sperm duct; tf, trunkal flexure; tr, trunk. Scale bars = 1 mm.

The distal lamina is more or less unidirectional to the upper part of the trunk (Fig. 3A,B).

Postinsemination Spermatophore

The most obvious difference noted when comparing Figure 3A,B (preinsemination) with Figure 3D,E (postinsemination) is the angle between the distal lamina (lam) and the trunk: The distal lamina of the postinsemination spermatophore lies at a right angle to the trunk. The lower part of the distal lamina is pressed into the upper part of the trunk where the sperm reservoir (sr) is situated, in this way forming a dent. The reduction in angle between distal lamina and trunk causes a backward movement of the sclerotized crown-like structures, while the outer and
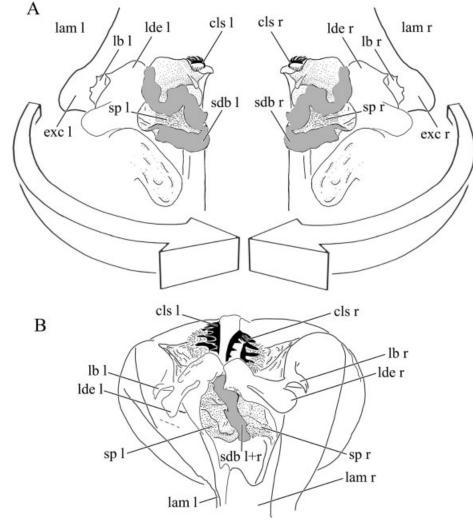
Fig. 2. Fusion of the two hemispermatophores forming the spermatophore of *Euscorpius italicus*. **A:** Same view as in Figure 1F but both opposing hemispermatophores are shown. **B:** The spermatophore formed from the two hemispermatophores. The sperm duct borders are indicated in gray. The arrows indicate the theoretical direction of the hemispermatophores in the spermatophore-building process. An "l" is added to all abbreviations of the left hemispermatophore and an "r" to all of the right hemispermatophore. cls, crown-like structure; exc, excavation; lam, distal lamina; lb, basal lobe, lde, outer distal lobe; sd, sperm duct; sdb, sperm duct bordering; sp, spicules.

inner distal lobe as well as the basal lobe stay in the same position as before (Fig. 3C,F). The distance and relative position of the paired sclerotized lobes towards each other is the same before and after sperm transfer (Fig. 4A,C). The seemingly different distance between the two outer distal lobes in Figure 4A,C is caused by the difference in perspectives. However, the distance of the lobes (outer distal, inner distal, and basal lobe) to the crown-like structure increases remarkably. The lobes of one side of the spermatophore remain connected to the crown-like structure by a spread membrane (m, Fig. 3F). The sperm duct that had been embedded under the lobes before sperm transfer is seen in a very exposed position at the highest point of the spermatophore (Figs. 3D–F, 4C,D) after sperm transfer. Figure 4C shows that the sperm duct (sd) has an opening (the sperm duct opening [sdo]) after sperm transfer. Traces of sperm (s) can be seen.

The outsides of the two side walls of the sperm duct are fully expanded and coated with numerous spicules (Fig. 5A,D) in the form of more or less slender cones (Fig. 5F). The position of the spicules depends on the folding of the membranous sperm duct; however, they are pointing at a right angle to the membrane (Fig. 5D–F). At the sperm duct opening, the spicules appear in the form of shorter, wider cones (Fig. 5E), while on the outer side of the sperm duct they are longer and more slender (Fig. 5F).

The former circles formed by the crown-like structures are open circles after sperm transfer as the expansion of a membranous slit in the circular crown-like structure leads to a semicircular appearance of the crown-like structure (Figs. 3F, 5C). As this happens, the under parts of both crown-like structures turn outwards and the spikes move in an exposed position (Figs. 4B,D, 5C).

Reduction in Angle Between Distal Lamina and Trunk

Figure 3A,B and D,E indicates a reduction in angle between distal lamina and trunk during sperm transfer, shown by the white arrow in Figure 3B. Actually, the distal lamina stays horizontal during
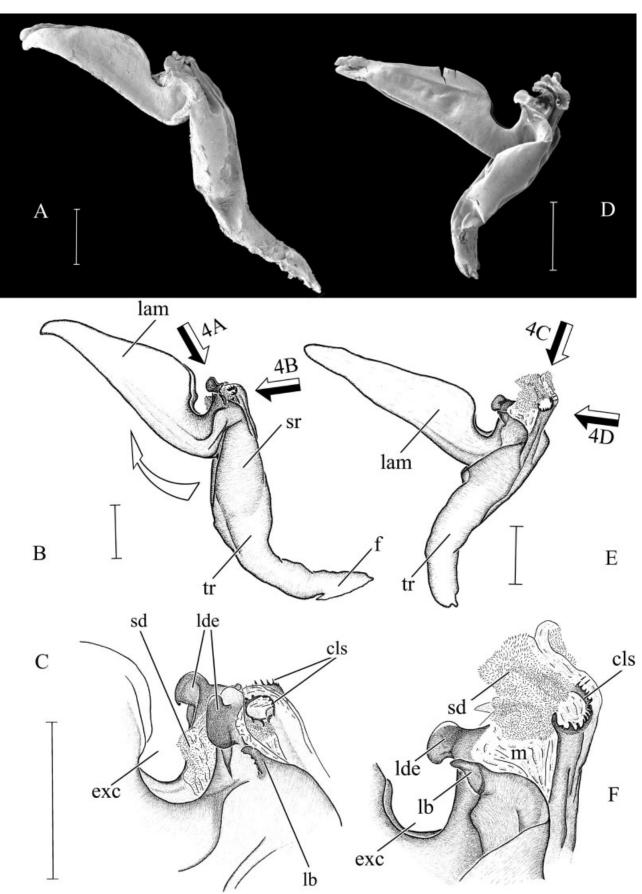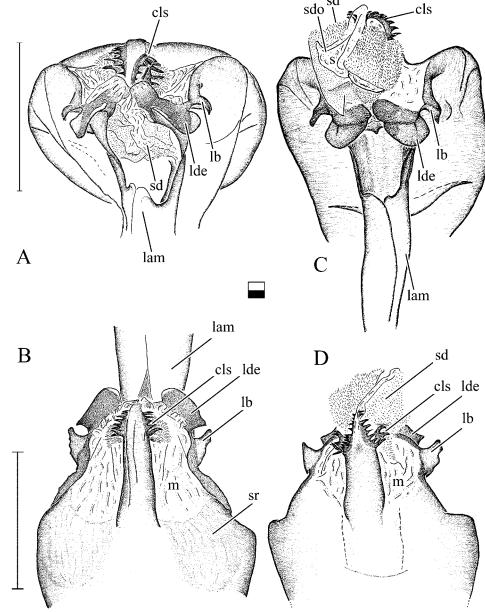
Figure 3

Fig. 4. Comparison of *Euscorpius italicus* spermatophore before and after sperm transfer. Before sperm transfer: **A:** Posterior view of capsular region. **B:** Mesal view of capsular region. After sperm transfer: **C:** Posterior view of capsular region. **D:** Mesal view of capsular region. The arrows in Figure 3 give the exact direction of the four views. The quadrate represents the arrows of Figure 3 viewed from behind. cls, crown-like structure; lam, distal lamina; lb, basal lobe (lobe basal); lde, outer distal lobe (lobe distal extern); m, membrane; sd, sperm duct; sr, sperm reservoir. Upper scale bar = 1 mm for **A**. Lower scale bar = 1 mm for **B–D**.

sperm transfer (Fig. 7A,B). Due to the down and backward movement of the female during sperm transfer (see next section), the upper part of the trunk moves towards the distal lamina (Fig. 7B). This is possible due to the flexibility and bending of the trunk. While this happens, the bending of the trunk leads to an S-shaped appearance (Fig. 7B). Furthermore, the expandable transparent membrane (m, Figs. 3F, 4B) is necessary to allow this movement.

When the angle between distal lamina (lam) and trunk is reduced, the distance between the lobes and

Fig. 3. Comparison of *Euscorpius italicus* spermatophore before and after sperm transfer. Before sperm transfer: **A:** Lateral view of spermatophore. SEM. **B:** Lateral view of spermatophore (drawing). **C:** Lateral view of capsular region (drawing). After sperm transfer: **D:** Lateral view of spermatophore. SEM. **E:** Lateral view of spermatophore (drawing). **F:** Lateral view of capsular region (drawing). The four black-white arrows show views of the four drawings in Figure 4A–D. Lower half of arrows (colored black) indicate bottom of the drawings in Figure 4A–D. The white arrow shows the path of the upper part of the trunk (sr). cls, crown-like structure; exc, excavation; f, foot; lam, distal lamina; lb, basal lobe (lobe basal); lde, outer distal lobe (lobe distal extern); ll, lateral lobe; m, membrane; sd, sperm duct; sr, sperm reservoir; tr, trunk. Large scale bar = 1 mm for **C,F**. Other scale bars = 1 mm.
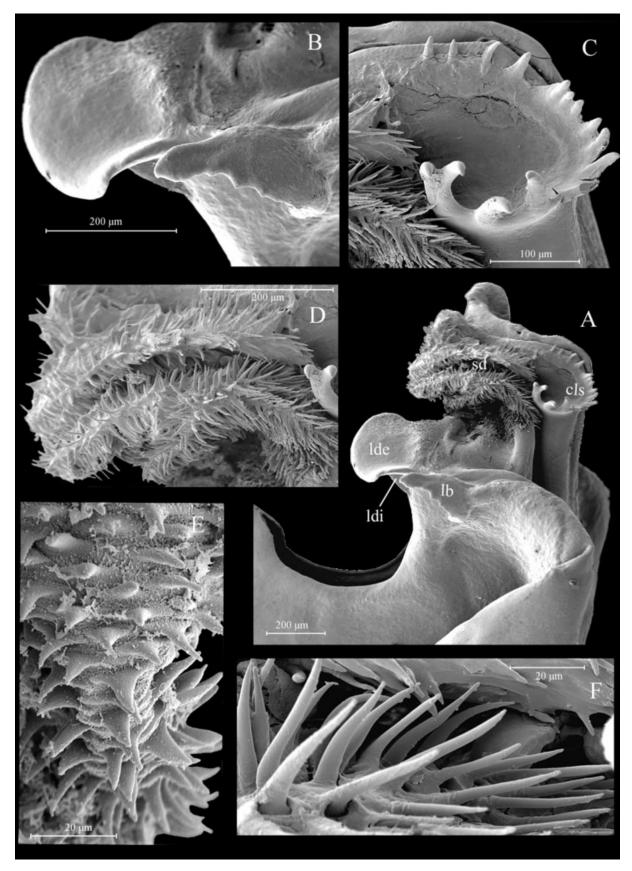
Fig. 5. *Euscorpius italicus* spermatophores after sperm transfer. SEM. **A:** Lateral overview of the capsular region. **B:** Lobe region. **C:** Crown-like structure. **D:** Sperm duct with spicules. **E:** Short spicules of the sperm duct near the sperm duct opening. **F:** Long spicules on the outer wall of the sperm duct. cls, crown-like structure; lb, basal lobe (lobe basal); lde, outer distal lobe (lobe distal extern); ldi, inner distal lobe (lobe distal intern); sd, sperm duct. Scales indicated separately.
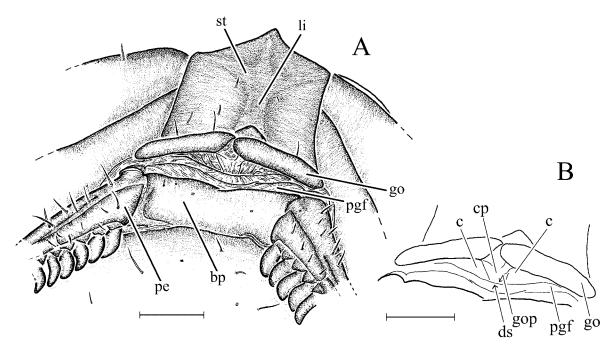
Fig. 6. Female genitalia of *Euscorpius italicus*. **A:** Detailed drawing of female genitalia showing genital operculum (go) in open position. **B:** simplified drawing. bp, basal plate; c, cavity; cp, central piece; ds, docking spot; go, genital operculum; gop, genital opening; li, longitudinal impression; pe, pecten; pgf, post genitalic fold; st, sternum. Scale bars = 1 mm.

the crown-like structures increases (Fig. 3). The sclerotized distal lamina impresses the wall of the trunk (tr) where the sperm reservoir is located (Figs. 3B,E, 7).

Spermatophore With Female Genitalia During Sperm Transfer

Figures 7 and 8 demonstrate the precise fit of the curved genital operculum (go) into the "excavation" (exc, Fig. 3C,F) between the distal lamina (lam) and the lobe region containing one outer distal and one basal lobe on each side of the spermatophore (the tiny inner distal lobes are not indicated in the figures because they mostly lie "hidden" behind the big outer distal lobes). The longitudinal impression (li) of the sternum (st) (Fig. 6A) guides the distal lamina. This "guideway" for the distal lamina continues anterior towards the mouth opening of the female. The lobes (outer distal and basal lobe) fit into the two cavities (c) (Fig. 6B) at each side of the central piece (cp) on the inner side of the genital operculum (go) (Fig. 6) and fix the spermatophore in these cavities (Fig. 8C). The genital opening (gop) is on one side bordered by the postgenital fold (pgf) and on the other side by the genital operculum; both embrace the central piece with the genital opening and the cavities (c) (Fig. 6). In the position before sperm transfer (Fig. 7A), the region with the lobes and the crown-like structure are pressed toward the genital opening (Fig. 8C). The postinsemination spermato-

phore is only fixed when the lobes are inside the cavities and the crown-like structures are in contact with the docking spot (ds) (Figs. 6B, 7B, 8). The bending of the trunk (tr) and the position of the foot (f) in relation to the female genital opening has to be like in Figure 7B at the moment of sperm transfer. The slightest deviation of that position causes an "automatic" removal of the posterior part of the distal lamina, leading to an incomplete fit and disabling the coordinated sperm transfer mechanism. When the crown-like structures are not in touch with the docking spots (ds) (Figs. 6, 8), they do not get hooked and the whole spermatophore slides posteriorly.

DISCUSSION
Functional Interpretation

The fusion of the halves of the sperm duct deserve detailed consideration. As noted before, the sperm duct is formed by fusion of two U-like structures facing each other (Fig. 2A). However, the cavities of the two U's (Fig. 2A), coated with numerous spicules, form the outer side of the sperm duct (Fig. 2B). Thus, during the building of the definite spermatophore the convex parts of the U's (which later represent the borders of the sperm duct, sdb, Fig. 2) are pressed against each other with considerable force, so that they lie in straight parallel lines in the definite spermatophore (compare Fig. 2). The spicules are situated on the outer side of the sperm
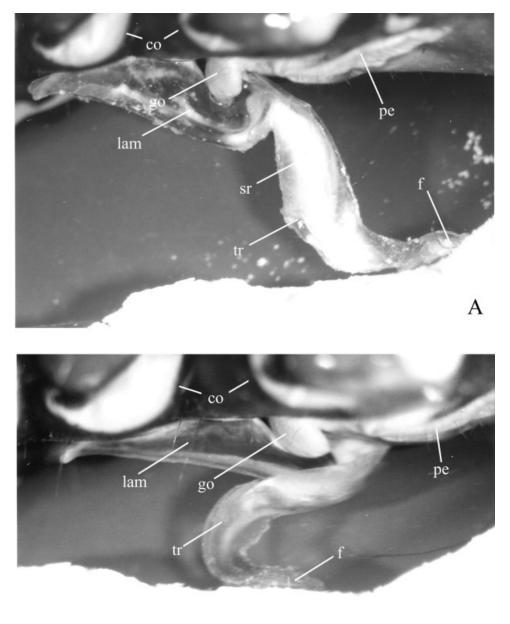
Fig. 7. Photograph of female genitalia of *Euscorpius italicus* with spermatophore during theoretical mating. **A:** With preinsemination spermatophore position directly before sperm transfer. **B:** With postinsemination spermatophore exactly at sperm transfer. co, coxae; f, foot; go, genital operculum; lam, distal lamina; pe, pecten; sr, sperm reservoir; tr, trunk.

duct. During sperm expulsion the membranes with the spicules are vaulted toward the outside (Fig. 4C,D). The fused halves will not open until high sperm pressure forces them to do so. The fact that the sperm duct is embedded under the lobe region in the preinsemination spermatophore (Figs. 3B,C, 4A,B) makes an increase of the pressure in the sperm reservoir necessary to press out the sperm. During the reduction of the angle between the distal lamina and trunk, the sperm duct becomes uncovered due to the increase of distance between the lobe region surrounding it and the crown-like structure. Additionally, the distal lamina presses against the sperm reservoir and thus increases the pressure

inside the reservoir. Consequently, the pressure on the sperm duct situated at the upper part of the sperm reservoir also increases, the sperm duct opens its exposed end (Fig. 4C), and sperm is pressed out.

For the first time, we present evidence that the sclerotized lobes of the spermatophore (dorsal and basal lobes) and the crown-like structures function as a device for opening the female genital atrium. As a first step, all these structures most probably are hooked in the soft genital region of the female. Figure 8 shows that the distal and basal lobes (Figs. 3, 4, 5B) hook into the two cavities on the inner side of the opened genital operculum of the female (Fig. 6). On the other side of the genital opening the crown-
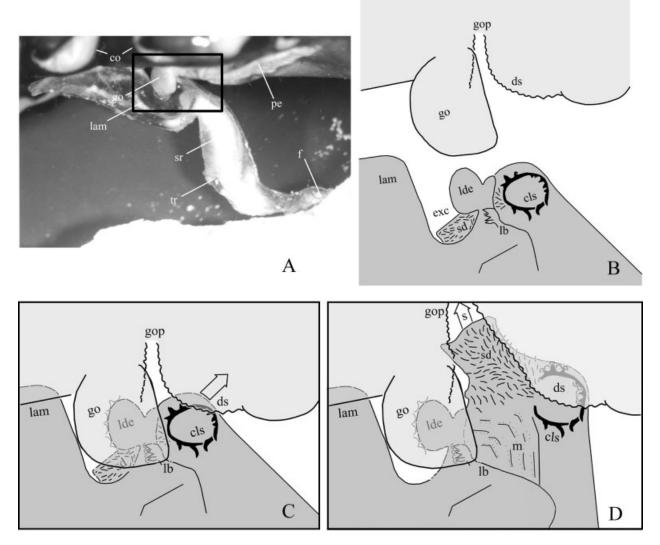
Fig. 8. Diagram of the functional relationship of spermatophore and female genitalia in *Euscorpius italicus*. **A:** Same picture as in Figure 7A. Black frame indicates the part shown in detail in **B–D**. **B–D:** Lateral views of simplified female genitalia and simplified capsular region of spermatophore. **B:** Female genitalia and spermatophore not in touch. **C:** Female genitalia and spermatophore in touch just before sperm transfer. Arrow indicates the direction of crown-like structure's movement at the moment of sperm transfer. **D:** Sperm transfer. Crown-like structures moved away from lobe region. Lobe region is hooked to inner side of genital operculum and crown-like structures are hooked at docking spots. Sperm duct is in widened genital opening. Arrow indicates sperm that gets pressed into female genital tract. cls, crown-like structure; ds, docking spot; go, genital operculum; gop, genital opening; lam, distal lamina; lb, basal lobe (lobe basal); lde, outer distal lobe (lobe distal extern); m, membrane; s, sperm; sd, sperm duct.

like structures (Figs. 3, 4, 5C) get hooked at the docking spots (Figs. 6, 8C). This explains the presence of spikes on the basal lobes and the crown-like structures and the movement of the latter to an exposed position during sperm transfer.

When the angle between trunk and distal lamina decreases, the distance between the lobes and the crown-like structures increases (Fig. 3). Therefore, as the crown-like structures and the lobes are hooked at opposite ends of the genital opening, the increase of their distance opens the female genital opening as wide as possible (Fig. 8). This increase of distance is caused by a backward movement of the crown-like structures while the lobe region is pressed in an opposite direction against the inner side of the genital operculum and fixed there (Fig. 8). Regarding this and the location of the sperm duct at the top of the spermatophore (Fig. 3D–F), it seems obvious that most of the sperm duct is inside the female genital tract during sperm transfer (Fig. 8). During artificial sperm transfer (Fig. 7) it was quite easy to insert the sperm duct into the female genital atrium. Furthermore, the spicule-coated membranes are pressed against the walls of the female genital atrium, in this way avoiding the backflow of inserted sperm (Fig. 8). Interestingly, the spicules are considerably shorter at the sperm duct opening (Fig. 5E) than on the outer walls of the sperm duct

(Fig. 5F), where they presumably serve for sealing. This complex interaction of spermatophore and female genital opening guarantees a safe sperm transfer.

Another possibility could be that the lobes, the crown-like structures, and the spicules on the sperm duct serve for stimulating the female. However, we found no behavioral evidence for this (see below).

Angermann (1957) was the first to describe a downward movement of the distal lamina and explained it as a lever mechanism leading to expulsion of sperm. Numerous subsequent authors (see Introduction) supported this idea of a lever mechanism from Angermann's pioneer work. But as our data show, the definition of the downward movement of the distal lamina is misleading. In fact, it is not a downward movement of the distal lamina but a movement of the flexible trunk towards it (Fig. 7).

Peretti (1992) provided a detailed description of spermatophore function in Bothriuridae. As a first step, the spermatophore of *Bothriurus bonariensis* contacts the genital operculum and is "aligned" by a pair of sclerotized horns originating in the capsular region of the spermatophore. In *Euscorpius italicus* the genital operculum fits the excavation between the lamina and the capsular (lobe) region. The same pattern occurs in *B. flavidus* and *B. cordubensis*, where an excavation or depression between the lamina and the capsular region is also shown (Peretti, 1996; pers. commun.). Fixation of the spermatophore is managed by "rotation" due to capsular eversion of the two horns in *B. bonariensis* (Peretti, pers. commun.), and by the dorsal and basal lobes and the crown-like structure in *E. italicus*. Exposing and opening of the sperm duct is made possible by a downward movement of the horns in *B. bonariensis* and by a backward movement of the crown-like structures in *E. italicus*. Finally, the horns in *B. bonariensis* serve for sealing and prevention of sperm backflow, while in *E. italicus* the spicule-coated outer walls of the sperm duct serve this function. Therefore, the same functional requirements of indirect sperm transfer by spermatophores are fulfilled by different structures in the two species mentioned. The possibility should be kept in mind that the horns of *B. bonariensis* could be homologous to the crown-like structures in *E. italicus*, as both are situated just beneath the opening of the sperm duct. Possible homologies to the dorsal and basal lobes of *E. italicus* cannot be found in *B. bonariensis* (see Peretti, 1992).

As we have shown, a longitudinal impression on the female's sternum serves as a "guiding line" for the distal lamina of the spermatophore. However, a sexual dimorphism in sternum morphology, especially in the form or depth of this longitudinal impression, is not apparent in *Euscorpius italicus*. Regarding the great variation between different fossil and recent scorpion taxa in sternum morphology (e.g., Kjellesvig-Waering, 1986; Polis, 1990; Soleglad

and Fet, 2003), it would be interesting to investigate correlative differences in spermatophore structures.

Evolutionary Origin of Spermatophore Complexity

Several theories have been proposed to explain the complexity of genital organs in general. Some of them focus on species-specificity, e.g., lock-and-key theory (Dufour, 1844) or genitalic recognition theory (summarized in Eberhard, 1985). We will not treat these theories further, as *Euscorpius* hemispermatophores were shown recently to be species-specific only in certain cases (A.J., pers. obs.). The pleiotropism theory (Mayr, 1963) fails to explain the fact that female genitalia all look the same in species where spermatophores are species-specific. We do not know of any other female character (including behavioral, morphological, physiological characters) in scorpions with species-specific spermatophores that has been interpreted as pleiotropically related to male spermatophore specificity. Thus, we do not treat this hypothesis further.

Our results show that in fact all structures of the complex capsular region are involved in ensuring the transfer of sperm (hooking in female genital region, opening of female genital atrium, sealing and preventing of sperm loss). These data are partly in accordance with the hypothesis of Kraus (1984), who claimed that complex genitalia evolved in order to maintain a complex coupling mechanism that safeguards sperm transfer. This basic idea of the Kraus hypothesis is corroborated now by data on scorpions as well. However, the hypothesis of Kraus was restricted to the evolution of spider genitalia. Due to completely different mating behaviors and mechanisms in spiders and scorpions, general conclusions should be drawn with caution.

All *Euscorpius* species investigated so far show very similar but highly complex spermatophores or hemispermatophores, respectively, with only the exception of *E. flavicaudis*, where the capsular region of the spermatophore is astonishingly simple (e.g., Vachon, 1948; Angermann, 1957; Molteni et al., 1983; Scherabon, 1987; Benton, 1993; Fet and Soleglad, 2002). We have no explanation for this simplicity: The mating behavior of *E. flavicaudis* was analyzed in detail by Angermann (1957) and Benton (1993); however, no striking differences in the decisive phase of sperm transfer as compared to the other *Euscorpius* species could be found by them. It is possible, of course, that in *E. flavicaudis* the female plays a larger targeting and release role than in other species with a more complex spermatophore. However, these problems clearly need further investigation.

Complexity of genital organs may also be explained by sexual selection on genital morphology by cryptic female choice (e.g., Eberhard, 1996). Before sperm transfer, the complicated structures of the

capsular region could serve to stimulate the female; this is especially true for the lobes, the crown-like structures, and the spicule-coated sperm duct. Theoretically, these structures, serving as courtship devices (Eberhard, 1996), could enable the female to evaluate the male's quality before sperm transfer occurs. However, in this case we would expect at least occasional disrupting of mating by the female. To our knowledge, this could never be observed in *Euscorpius italicus*.

However, cryptic female choice may also occur after sperm transfer. There are no morphological arguments such as complicated female genitalia indicating any kind of mechanism to eject previously deposited sperm or to store it under different conditions (Pawlowsky, 1925; Polis, 1990; A.J., pers. obs.).

In addition, the presence of a mating plug (spermatocleutrum) in *Euscorpius italicus* (Angermann, 1957; pers. obs.) could be evidence for only a single insemination; thus, mate choice after sperm transfer would be impossible, as no mechanisms for removal or digestion of sperm plugs are known among scorpions. However, Peretti and Acosta (1999) and Peretti and Battán-Horenstein (2003) observed multiple mating in species of *Bothriurus* where females bear a mating plug that increases in size from mating to mating. We could not find such incomplete mating plugs in *E. italicus*; even in single-mated females, the mating plugs were complete (A.J., pers. obs.). This fact probably indicates that in *E. italicus* sperm transfer occurs only once per female and mating season.

If this assumption should prove to be true, male quality assessment by the female must occur before insemination by disruption of mating. As mentioned above, we could never observe such "flubs." Also, Benton (1992) claimed that females of *Euscorpius flavicaudis* normally mate only once per season, and when mating is initiated it will be finished successfully. In contrast, females of bothriurids and iurids seem to disrupt mating regularly after spermatophore deposition but before insemination occurs (Peretti, 1996; Tallarovic et al., 2000). This could allow females to assess the male's quality on the basis of spermatophore properties. This could also explain the differences in duration from spermatophore deposition to sperm transfer in *Euscorpius* and bothriurids. While in *Euscorpius* this phase lasts just some seconds up to 1 min (according to our video sequences; see also Benton, 1993) it lasts in *Bothriurus flavidus* 4–6 min (Peretti, 1996), and in bothriurids in general 1–8 min (Peretti and Battán-Horenstein, 2003). The fact that some spermatophores of bothriurids are species-specific (Acosta and Peretti, 1998) could be another indication of this kind of cryptic female choice. However, in other bothriurid species spermatophores look very similar and do not allow species discrimination (Peretti, pers. commun.).

In conclusion, these data indicate that the complex spermatophores of at least certain scorpions (bothriurids and iurids) could have been shaped under sexual selection by cryptic female choice. However, the hypothesis that safeguarding of sperm transfer is one driving force for genital complexity (Kraus, 1984) does not contradict the cryptic female choice hypothesis. Therefore, both may well be true.

Finally, the possibility should be kept in mind that *Euscorpius* scorpions (which possibly mate only once per mating season and which show no species-specific spermatophores in several cases) stem from polyandrous species. This could mean that their complex spermatophore morphology originally had been shaped as species-specific by female mate choice. After the emergence of monoandry in *Euscorpius* (probably caused by application of a mating plug that prevents females from a second insemination) the species-specific spermatophore characters disappeared, but general complexity of the spermatophores remained because of the necessity of a safe sperm transfer.

ACKNOWLEDGMENTS

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