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Sperm transfer during copulation in five *Coproica* species (Diptera: Sphaeroceridae)

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Diptera, Sphaeroceridae, genital morphology, copulation, sperm transfer

Abstract. Sperm transfer in five *Coproica* Rondani was studied by separating copulating pairs at varying time intervals from the onset of copulation. In *Coproica ferruginata* (Stenhammar), *C. hirticula* (Collin), *C. lugubris* (Haliday), and *C. vagans* (Haliday) rapid sperm transfer, which occurs shortly before the termination of copulation, is preceded by a preinsemination phase of species-specific duration. In *C. acutangula* (Zetterstedt) sperm transfer starts soon after the initiation of copulation and is accomplished slowly. At the beginning of copulation a tight fit of the secondary male gonopore or phallotreme and the openings of the spermathecal ducts, which are located at a vaginal sclerite, is established in all species. However, only in *C. acutangula*, *C. hirticula*, and *C. lugubris* sperm is transferred while those male and female structures are coupled. Males of *C. ferruginata* and *C. vagans* unhook their distiphalli before ejaculation and transfer a sperm mass into the female's vagina. In both species a mating plug blocks the female's secondary gonopore after copulation. The observed traits are discussed with respect to sperm competition and the possibility of sperm manipulation by the females. Three male behaviour patterns observed during copulation are discussed in the context of copulatory courtship: compression of the female abdomen, post-abdominal contractions which cause thrusts of the distiphallus, and drumming of the male leg on the female abdomen. The evolution of sperm transfer in *Coproica* is examined by mapping the observed sperm transfer traits onto a *Coproica* cladogram.

INTRODUCTION

Sexual selection encompasses both male competition for the fertilization of the eggs and female choice. It has been known for some time that male-male competition continues even after a male has inseminated a female successfully. Parker (1970) introduced the term sperm competition for the contest among ejaculates from different males within the female reproductive tract. Sperm competition occurs when a female remates before she has used all sperm transferred during previous copulations. Consequently, male insects have evolved different adaptations to enhance their individual reproductive success (Parker, 1984). Since males can increase their reproductive success with each successful copulation, quick and efficient sperm transfer is an important component of sperm competition (Parker, 1970). A conflict of interests between males and females arises because a female, usually, cannot increase her reproduction by copulating with additional males (Parker, 1979). For females it is more important to choose the best fathers for their offspring (Parker, 1984; Andersson, 1994). Therefore, the female body should not be seen merely as the arena for sperm competition. Probably, it is selectively advantageous for females to prejudice sperm competition. Where complex courtship signals are exchanged before copulation females may rely on them in choice of mates. In species with brief pre-

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copulatory courtship, cryptic female choice during copulation may be more important. Eberhard (1985, 1991, 1992) coined the term copulatory courtship to describe behavior patterns which occur during copulation. Copulatory courtship induces the female to accept and store the sperm of her mate and elicits many other essential reproductive processes. According to Eberhard (1985, 1990, 1991) the female makes her decision based not only on male behaviour and male external structures, but also on cues furnished by the form and movement of the male genitalia. Since it is difficult to distinguish between sperm competition and female choice during copulation, sperm manipulation by female Diptera has received little attention until Ward (1993) and Otronen & Siva-Jothy (1991) demonstrated that females can influence sperm storage.

In the present study female reproductive morphology and sperm transfer mechanisms of five closely-related dung fly species (Diptera: Sphaeroceridae) were compared in an attempt to describe possible mechanisms of sperm manipulation by the females. Five species of the monophyletic genus *Coproica* Rondani which occur in northern Europe (Richards, 1930; Pitkin, 1988) were chosen: *Coproica acutangula* (Zetterstedt), *C. ferruginata* (Stenhammar), *C. hirticula* (Collin), *C. lugubris* (Haliday), and *C. vagans* (Haliday). The adults measure between 2 and 2.5 mm in body length. The males display no discernible pre-copulatory courtship behaviour. Females copulate repeatedly and throughout their life. *C. lugubris* is typically associated with fresh cow-pats (Richards 1930; Papp, 1971, 1992). The remaining four species colonize dung-heaps near farms (Papp, 1974a,b, 1975; Pitkin 1988). Dung provides nutrition for adults and substrate and food for developing larvae (Laurence, 1955; Ferrar, 1987). Due to their huge biomass, dung-heaps can support very large dung fly populations (Papp, 1975). *C. ferruginata* and *C. vagans* adults have been reported to annoy cattle because of the often high densities of their populations (Badenhorst, 1991).

The aims of this study on five *Coproica* species were (1) description of the male and female reproductive organs, (2) determination of the timing of sperm transfer during copulation, (3) comparison of the modes of sperm transfer, (4) identification of male behaviour patterns as possible copulatory courtship, (5) discussion of the observed traits with respect to sperm manipulation and sperm competition, and (6) mapping of the observed traits onto a *Coproica* cladogram.

MATERIAL AND METHODS

Source of material and laboratory rearing

Flies were collected from their natural breeding sites as adults. In the laboratory adult flies were kept either in 12 × 16 × 20 cm plastic cages or in smaller plastic cups (diameter 9 cm) at room temperature under a 14 : 10 h LD cycle. Stocks were maintained on cow dung in combination with soya-agar (Lachmann, 1991).

Observations and dissections

For observation, virgin, sexually mature males and females were anaesthetized with CO_2 and transferred into plastic petri dishes (5.5 cm in diameter and 1.3 cm deep). Petri dishes served as mating chambers. The duration of undisturbed copulations was timed in 20 pairs per species. The females were dissected after these copulations. To determine the beginning of sperm transfer copulating pairs were separated at varying time intervals from the onset of copulation: *C. acutangula* (2, 4, 7, 10 min), *C. hirticula* (5, 7, 9, 11 min), *C. lugubris* (5, 10, 15, 20, 25, 30 min), *C. ferruginata* (3, 6, 8 min), *C. vagans* (3, 6, 9, 13 min) (10 pairs / species and interval). Females were then killed and dissected immediately. In addition, ten females of *C. ferruginata* and *C. vagans* were dissected immediately, 30 min, and

24 h after uninterrupted copulations. The reproductive tracts of females were examined under a light microscope. Spermathecae were carefully crushed under a cover-glass and examined for presence of sperm.

RESULTS

Female reproductive system (Fig. 1)

The female reproductive system consists of the vagina, a vaginal sclerite, three spermathecae, two spermathecal ducts, paired accessory glands with paired ducts, a common oviduct, paired lateral oviducts and paired ovaries. An ovipositor is not developed.

The vagina is sac-like and surrounded by a weak muscular sheath. Several muscles connect the vagina to the body wall. A small ventral receptacle (diameter circa 30 μm) is embedded in the vaginal muscles. Dorsally, the vaginal wall forms a species-specific vaginal sclerite which bears the openings of the spermathecal and accessory gland ducts (Fig. 1). In *C. lugubris* the dorsal vaginal wall is enlarged to form a sac (0.2 mm long) which houses the vaginal sclerite at its apex.

There are three highly sclerotized sperm storage organs or spermathecae, two of them attached distally to a common duct (Figs 2a,b). The intima of the spermathecal ducts is surrounded by several layers of muscles. Each duct bears a small sclerotized capsule at its tip (diameter 13–15 μm, Figs 2a,b). These are referred to as spermathecal valves (Figs 2a,b). The spermathecal ducts continue inside the spermathecal valves which have a membraneous middle portion and are surrounded by muscles. It is probable that, upon contraction of the surrounding muscles, the spermathecal ducts can be squeezed shut. Therefore, the spermathecal valves could control sperm transfer into and out of the spermathecae. The spermathecae are embedded in a glandular tissue. Pores in the basal portion of the spermathecae serve as openings for the glandular cells (the end apparatus of Filosi &

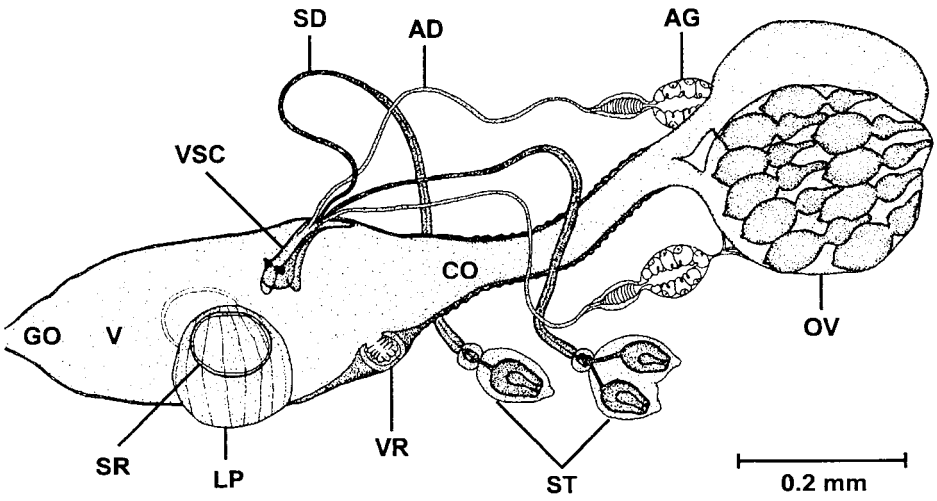


Fig. 1. Internal female genitalia of *C. ferruginata*. AD – accessory duct; AG – accessory gland; CO – common oviduct; OV – ovary; GO – secondary genital opening; LP – lateral pad; SD – spermathecal duct; SR – sclerotized ring; ST – spermathecae; V – vagina; VR – ventral receptacle; VSC – vaginal sclerite.

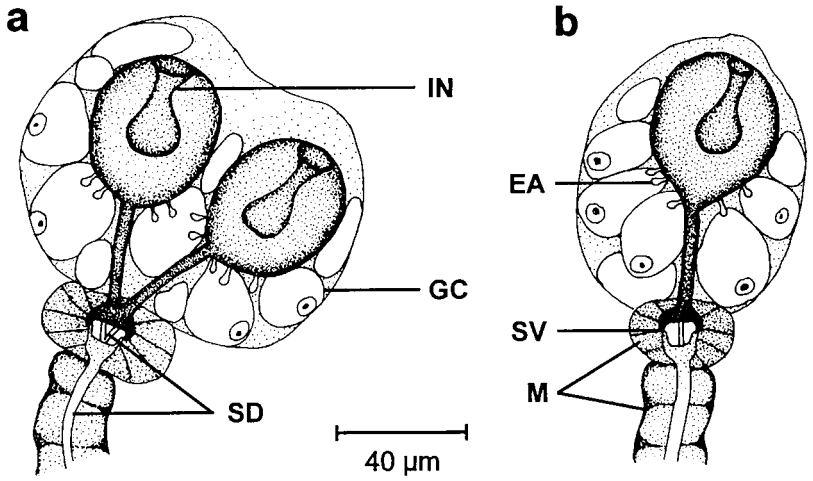


Fig. 2. *C. acutangula* female. (a) paired spermathecae; (b) single spermatheca. EA – end apparatus; GC – gland cell; IN – invagination; M – muscles; SD – spermathecal duct; SV – spermathecal valve.

Perotti, 1975). Each spermatheca has a thumb-shaped invagination (Fig. 2) which extends from the apex inward.

Male reproductive system

The external male reproductive organs consist of two pairs of claspers, the postgonites and the telomeres, the unpaired aedeagus and a spine comb on sternum 5 (Swann, 1993, terminology after Roháček, 1982). The male internal genitalia consist of paired orange testes and paired vasa deferentia, a pair of elongate accessory glands, an unpaired terminal (accessory) bulb, a bulbus communis and the ejaculatory duct (Figs 3a,c). The bulbus communis is muscular and receives the vasa deferentia, the paired accessory glands and the terminal (accessory) bulb. In *C. hirticula* males only, the distal portion of the vasa deferentia is enlarged to a seminal vesicle. In males of the other species the vasa deferentia form seminal vesicles over their full length (Figs 3a,b). Immediately before and following copulation, no sperm were found in the seminal vesicles or the ejaculatory duct. Thus, the seminal vesicles do not serve as storage organs for mature sperm. Distally the bulbus communis opens into the ejaculatory duct which has a muscular sheath. At a position, approximately two thirds of its length, the ejaculatory duct is enlarged slightly and forms a small muscular ejaculatory bulb, which houses the saddle-shaped ejaculatory apodeme (30–35 µm in length, Fig. 3b). It is probable that the ejaculate is pumped into the female reproductive tract by the muscular coat of the ejaculatory duct and the ejaculatory bulb. The posterior portion of the ejaculatory duct enters the phallophore, marking the primary gonopore. The phallophore represents the basal portion of the aedeagus (Figs 3b, 4a,b). The terminal portion of the aedeagus is called the distiphallus and represents the actual intromittent organ (Figs 4a–d). The distiphallus carries the secondary gonopore, the phallotreme (Fig. 4c). Anterior to the phallotreme, two ridges project to the apex of the distiphallus. The outside of the distiphallus is ornamented with spines and barbs. The distiphallus, telomeres

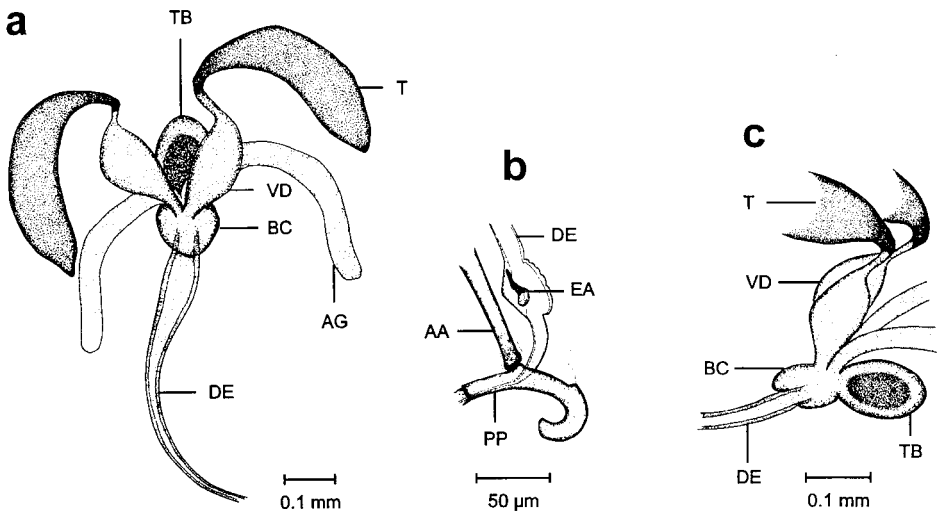


Fig. 3. Internal male genitalia of *C. lugubris*. (a) dorsal view; (b) ejaculatory bulb, lateral view; (c) lateral view. AA – aedeagal apodeme; AG – accessory gland; BC – bulbus communis; DE – ductus ejaculatorius; EA – ejaculatory apodeme; PP – phallophore; TB – terminal (accessory) bulb; T – testis; VD – vas deferens.

and postgonites are species-specific in shape and provide important characters for species identification (Pitkin, 1988; Swann, 1993).

Copulation

The engagement of male and female genitalia during copulation is described in more detail elsewhere (Lachmann, 1994, 1996). The features important for the understanding of sperm transfer are summarized here: during copulation the male mounts the female and holds her with his foretarsi at the wing bases; the male's middle- and hindtarsi rest upon the female's abdomen (Fig. 5b); the telomeres spread the secondary female gonopore, which is situated between sterna 8 and 9; the postgonites and distiphallus are inserted into the vagina; the postgonites spread the soft vaginal tissue and the phallotreme is hooked to the spoon-shaped vaginal sclerite, thus achieving a tight fit of the male phallotreme and the openings of the spermathecal ducts (Fig. 5a). In *C. hirticula* the copulatory position achieved initially is maintained until the end of copulation (Fig. 5a). Males of *C. ferruginata* and *C. vagans* unhooked their distiphalli from the vaginal sclerite before termination of copulation. Even though the two structures disconnect, the distiphallus remains inside the vagina.

In *C. lugubris* the distiphallus is not long enough to reach the vaginal sclerite which is located at the apex of the vaginal sac. The initial step in copulation must involve a retraction of the sac into the main vaginal chamber so that the distiphallus can contact and hook the vaginal sclerite. It is probable that this is achieved by contraction of muscles attached to the dorsal apex of the sac and the vagina. Once the phallotreme is hooked to the vaginal sclerite, males of *C. lugubris* and *C. acutangula* pull their distiphallus and postgonites

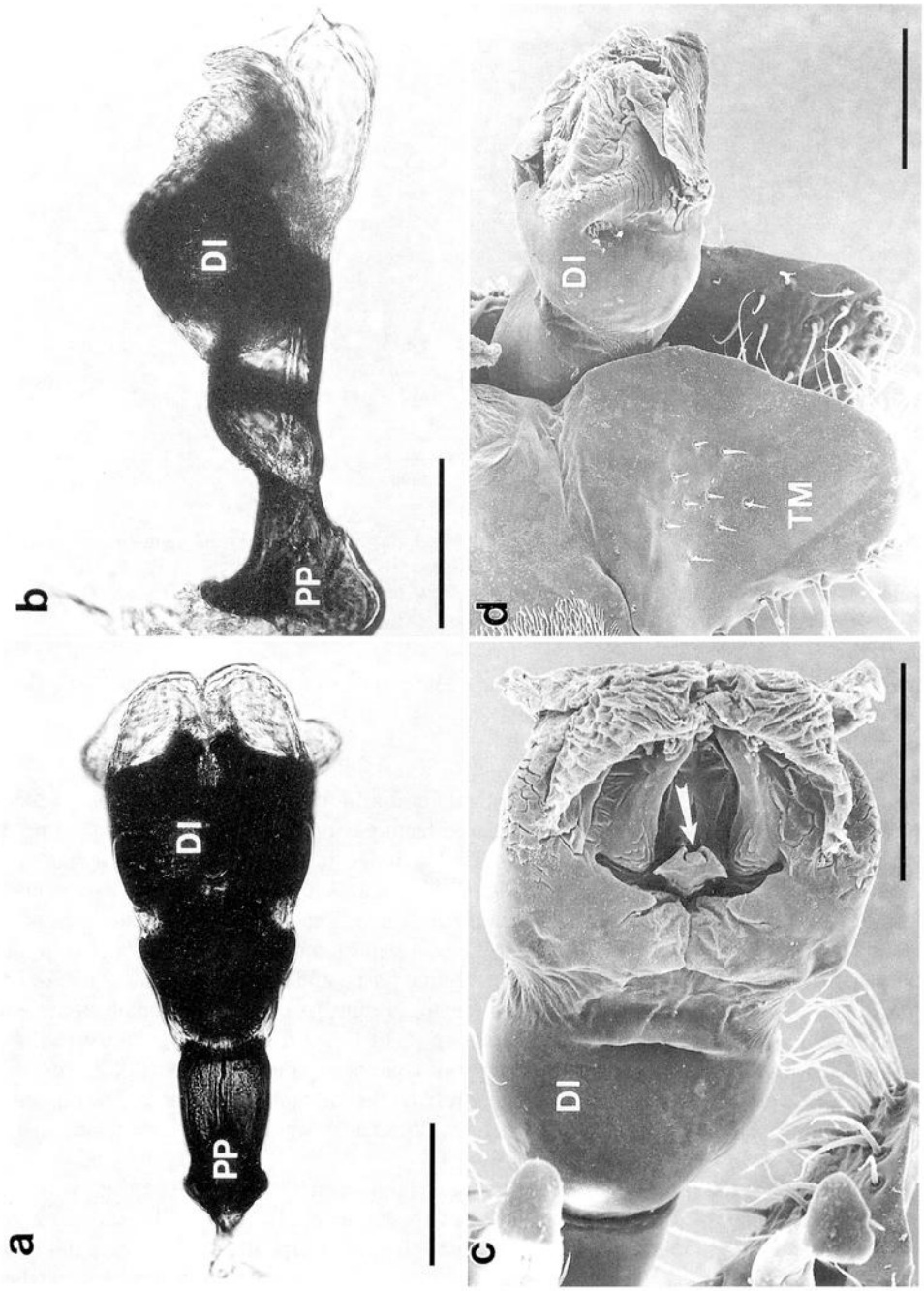


Fig. 4. Aedeagus of male *C. ferruginata*. (a) dorsal view in light microscope; (b) lateral view in light microscope; (c) dorsal view in SEM, the white arrow points to the phallotreme; (d) lateral view in SEM. DI – distiphallus; PP – phallophore; TM – telomere. Scale = 50 μm.

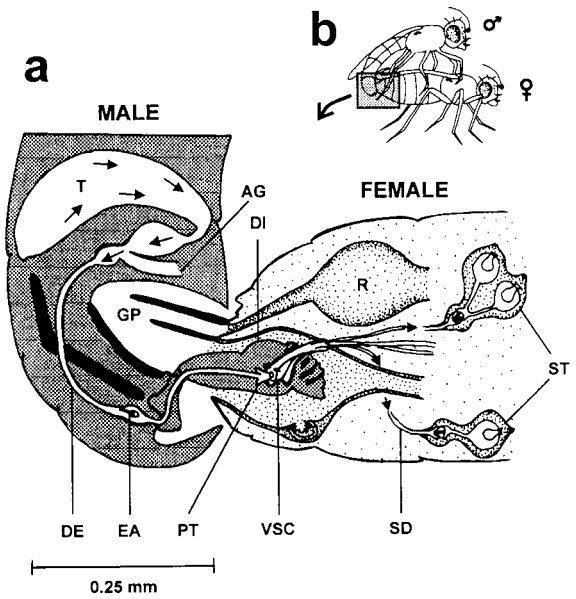


Fig. 5. (a) Sperm transfer via the "closed system" in *Coproica*. Arrows show the passage of sperm from the testes to the spermathecae. (b) Copulating *Coproica* pair. AG – accessory gland; DE – ductus ejaculatorius; DI – distiphallus; GP – genital pouch; PT – phallotreme; R – rectal bladder; SD – spermathecal ducts; ST – spermathecae; T – testis; VSC – vaginal sclerite.

back into a genital pouch located ventrally in the male 5th abdominal segment. In *C. lugubris* the dorsal vaginal sac is everted and the vaginal sclerite is pulled out of the female body (Lachmann, 1994, 1996). After copulation females of *C. lugubris* retract the vaginal sclerite by means of muscles attached to the apex of the vaginal sac. Females of *C. acutangula* do not have their dorsal vaginal wall transformed into a sac. Consequently, the vaginal sclerite is pulled out only a short way, putting a great strain on the dorsal vagnial wall. In *C. acutangula* and *C. lugubris* the distiphallus is hooked to the vaginal sclerite until the end of copulation.

Males of the five *Coproica* species perform bouts of rhythmic contractions with their terminalia throughout copulation. During the contractions the male terminalia are curled under and cause strong thrusts of the distiphallus either into the female's vagina (*C. hirticula*, *C. ferruginata*, *C. vagans*) or into the male genital pouch (*C. acutangula*, *C. lugubris*). As a result the female abdomen is compressed dorsoventrally. The contractions are strongest in *C. lugubris* and *C. acutangula*. The frequency of the contractions increases toward the end of copulation in males of *C. hirticula* and *C. lugubris*, but not in *C. acutangula*. Males of *C. ferruginata* and *C. vagans* contract their terminalia constantly during the last phase of copulation. In addition to the contractions, males of *C. hirticula*, *C. lugubris*, and less often *C. vagans* drum the female's abdomen rhythmically with their hindtarsi. Since the tarsi are held at a 45° angle from the female, the thickened first hind-tarsomere is the most important male body part in drumming the abdomen of the female. The duration of copulation is species-specific (Table 1) and does not depend on the mating status of the female. Post-insemination associations (Alcock, 1994) were not observed in the *Coproica* species studied here.

Timing of sperm transfer

A comparison of the species-specific duration of copulation and the onset of sperm transfer shows that males of *C. hirticula*, *C. ferruginata*, *C. lugubris* and *C. vagans* transfer sperm shortly before the copulation is terminated. In these species sperm transfer is

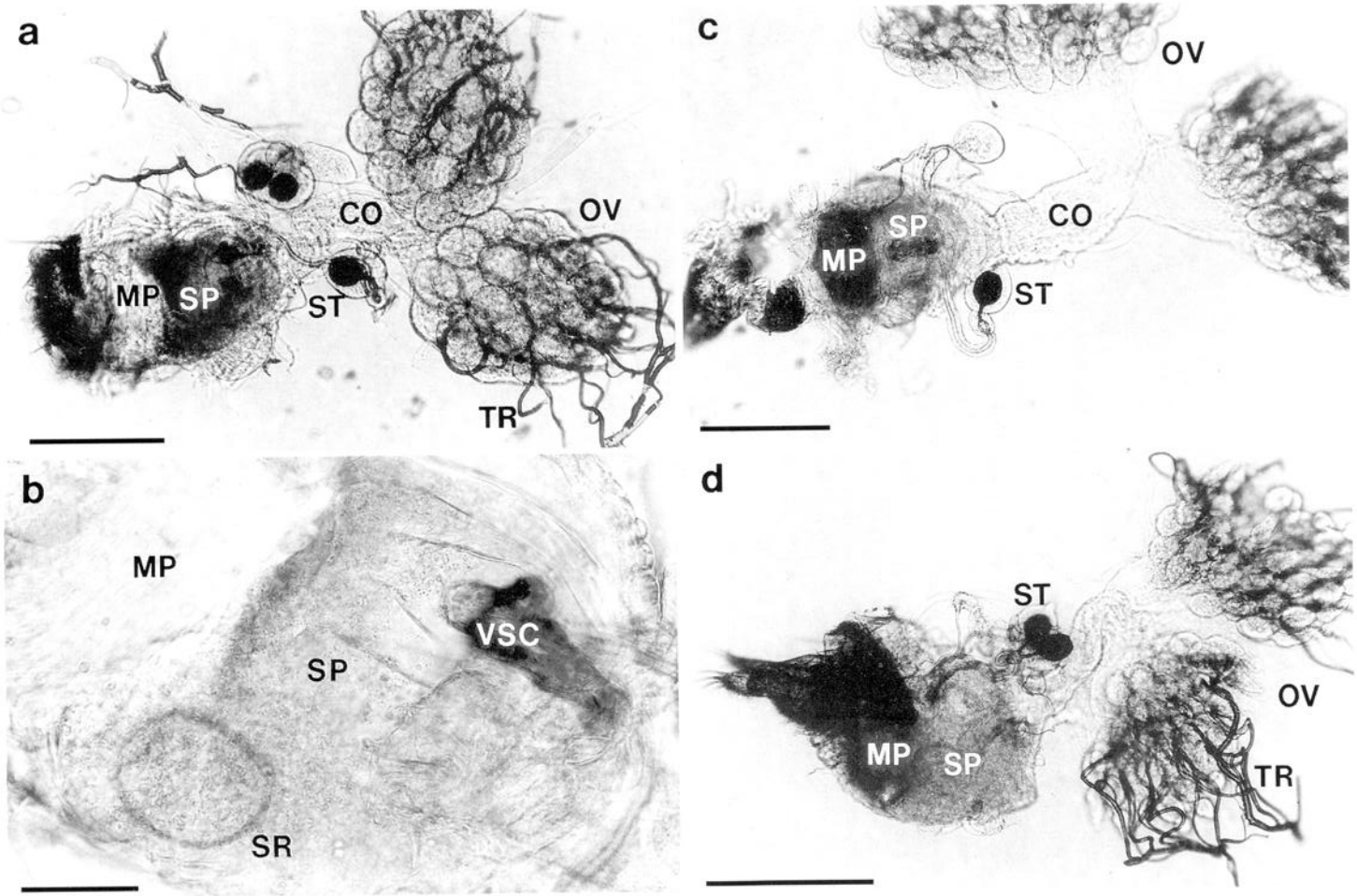
completed quickly. By contrast, in *C. acutangula* sperm transfer starts much earlier and continues gradually until the end of copulation.

TABLE 1. Timing and mode of sperm transfer in five *Coprɔica* species. Per time interval 10 pairs were studied and the females of those pairs dissected immediately. Duration of copulation was determined in 20 pairs / species (a.c. – after copulation termination, i.a.c. – immediately after copulation termination).

Species	Copulation duration (min)	Separation of copulating pairs (min)	Percent of females				Preinsemination phase (min)
			Sperm mass in vagina	Spermathecae densely filled with sperm	Sperm in spermathecae	Mating plug	
C. acutangula	13.3 ± 2.2	2	0	0	80	0	0
		4	0	0	100	0	
		7	0	0	100	0	
		10	0	100	100	0	
C. hirticula	12.8 ± 1.1	5, 7, 9	0	0	0	0	≥ 11
		11	0	80	80	0	
		i.a.c.	0	100	100	0	
C. lugubris	33.8 ± 4.8	5, 10, 15, 20, 25	0	0	0	0	≥ 30
		30	0	80	80	0	
		i.a.c.	0	100	100	0	
C. ferruginata	9.2 ± 1.2	3, 6	0	0	0	0	≥ 8
		8	40	0	0	20	
		i.a.c.	100	0	100	100	
		30 a.c.	100	100	100	100	
C. vagans	16.9 ± 3.0	3,6	0	0	0	0	≥ 9
		9	50	0	0	0	
		13	80	0	0	70	
		i.a.c.	100	0	100	100	
		30 a.c.	100	100	100	100	

In *C. lugubris* no sperm was transferred during the first 25 min of copulation, but 80% of the females that were allowed to copulate for 30 minutes, had sperm in their spermathecae (Table 1). In 80% of female *C. acutangula*, sperm could be detected 2 min, and in 80% of female *C. hirticula* 11 min after the onset of copulation. In *C. ferruginata* 40% and in *C. vagans* 50% of the females were inseminated after 8 min and 9 min of copulation respectively (Table 1). 80% of female *C. vagans* that were allowed to copulate for 13 min were inseminated. Since the average duration of copulation in this species is 16.9 ± 3.0 min, sperm transfer is completed earlier with respect to the termination of copulation compared with the other species. The longest pre-insemination phase occurs in *C. lugubris* and lasts approximately 30 minutes. In *C. acutangula* a pre-insemination phase does not exist. Even though sperm were not counted, a positive correlation between numbers of sperm

Fig. 6. Photomicrographs of the internal genitalia of recently mated females carrying a sperm mass and a mating plug. (a) *C. vagans* female, dorsal view, a translucent mating plug is blocking the genital opening. (b) Vagina of a *C. vagans* female filled with sperm and blocked by a mating plug. (c) Dorsal view of female *C. ferruginata* genitalia, the mating plug is dark. (d) Lateral view of *C. ferruginata* female genitalia. CO – common oviduct; MP – mating plug; OV – ovary; SP – sperm; ST – spermatheca; SR – sclerotized ring; TR – trachea; VSC – vaginal sclerite. Scale: a, c, d = 0.2 mm, b = 50 μm.


a
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transferred and duration of copulation over the range of 13.3 ± 2.2 min may be assumed for this species.

It was not possible to detect fluid male accessory secretions in the female reproductive tract during or after copulation by light microscopy.

Modes of sperm transfer

Two modes of sperm transfer were found in the *Coproica* species studied: 1) males of *C. acutangula*, *C. hirticula* and *C. lugubris* deliver their ejaculate directly from their phallotreme to the spermathecal ducts, and 2) males of *C. vagans* and *C. ferruginata* transfer a sperm mass into the female's vagina and a mating plug blocks the female's secondary genital opening after copulation.

In the reproductive tracts of dissected *C. acutangula*, *C. hirticula* and *C. lugubris* females, sperm was always exclusively found in the spermathecae or the spermathecal ducts which originate at the vaginal sclerite. It may be concluded that males delivered their ejaculate directly from their phallotreme to the spermathecal ducts.

Females of *C. ferruginata* and *C. vagans* dissected shortly before the natural termination of copulation never contained sperm in their spermathecae (Table 1). Instead a large sperm mass was present in the vagina (Figs 6a–d). As sections of copulating pairs have shown, males of *C. vagans* and *C. ferruginata* unhook their distiphalli before ejaculation from the vaginal sclerite and deliver a sperm mass into the vagina (Lachmann, 1994, 1996). Immediately after copulation, few spermatozoa had traveled into the spermathecae. 30 min later the spermathecae were densely filled with sperm, but a considerable amount of spermatozoa was still present in the vagina. In 20% of *C. ferruginata* females dissected after 8 min in copula and in 80% of *C. vagans* females dissected after 9 min, a compact plug blocked the female's secondary gonopore (Table 1, Figs 6a–d). A certain percentage of the *C. ferruginata* and *C. vagans* females killed during copulation was inseminated, but did not carry a mating plug (Table 1). Thus, the mating plug was formed after sperm transfer had been completed. The plug observed in *C. vagans* females was translucent and kept its shape even when it was dissected out (Fig. 6a). It appeared granular at high magnification (Fig. 6b). 24 h after copulation the plug and spermatozoa were still present in the female's vagina. The mating plug found in *C. ferruginata* females was darker and softer but also granular when examined at high magnification (Figs 6c,d). 30 min after copulation the plug and spermatozoa were still present in the vagina; 24 h after copulation only dark remnants of the plug and few spermatozoa could be found in the vagina. This suggests that the plug is dissolved in the female tract.

Dissections proved that individual variation in spermathecal filling occurred in females of the five *Coproica* species. But the three spermathecae of any given female were evenly filled with sperm in most cases. Spermatozoa were never found in the common oviduct. It is not certain whether the small ventral receptacle functions as sperm storage organ. It is assumed to be the fertilization site in *Dacus oleae* (Diptera: Tephritidae: Solinas & Nuzzaci, 1984) and *Cyrtodiopsis whitei* (Diptera: Diopsidae: Kotrba, 1993) and could have the same function in the *Coproica* species.

Additionally, some aspects of sperm transfer were studied in three other sphaerocerid species: *Spelobia clunipes* (Meigen), *S. bifrons* (Stenhammar) and *Halidayina spinipennis* (Haliday). In these species a large sperm mass was found in the vagina of the females dissected after copulation and a mating plug blocked the female's secondary gonopore.

DISCUSSION

In *Coproica* mating systems, which may be characterized by the term scramble competition polygyny (Thornhill & Alcock, 1983), it is probable that males are selected to maximize the number of their copulations. Therefore, *Coproica* males should benefit from short copulations and sperm transfer should take place as early and rapidly as possible during copulation. Sperm should be transferred directly into the female storage organs and maneuvered into a location that guarantees a high fertilization rate.

However, copulation duration and timing of sperm transfer varied considerably among species. This leads to the conclusion that sperm transfer is not the sole function of copulation. The diversity in sperm transfer patterns and mechanisms reflects the different and sometimes conflicting interests of males and females in the outcome of a copulation (Parker, 1984; Knowlton & Greenwell, 1984).

Females concentrate on choosing the best mates possible because they can increase the quality of their offspring by using sperm of preferred males (Thornhill & Alcock, 1983; Andersson, 1994). The possibility of female sperm manipulation in the Diptera has been suggested (DeVries, 1964) but its importance had not been acknowledged until recently (Ward, 1993). Since insemination and fertilization take place within the female reproductive tract, it is possible that female control over sperm is a widespread phenomenon (Eberhard, 1985, 1990, 1991; Birkhead & Hunter, 1990; Birkhead & Møller, 1993; Ward, 1993). Due to differences in mode and timing of sperm transfer, different mechanisms of sperm manipulation by the female are possible in the five *Coproica* species and shall be discussed here.

Sperm storage and possible sperm manipulation by the female

Female morphology suggests two ways in which the passage of sperm into the storage organs may be controlled. First, the spermathecal ducts are surrounded by muscles that may play a role in sperm transport. Second, spermathecal valves at the distal end of the spermathecal ducts could control access of sperm into and out of the spermathecae.

In *C. acutangula*, *C. hirticula* and *C. lugubris* males inject their ejaculate into the spermathecal ducts. However, females could prevent sperm transfer by closing their spermathecal valves. In *C. ferruginata* and *C. vagans* the ejaculate is transferred as a large mass into the vagina. Females of the latter species may have complete control over the process of sperm storage once the male has ejaculated into the vagina. Thirty minutes after copulation a sperm mass was still present in the vagina of *C. ferruginata* and *C. vagans* females even though the spermathecae were densely filled with sperm. The sperm mass, which is deposited in the vagina, appears to be larger than the combined volume of the three spermathecae. Therefore, the spermatozoa remaining in the vagina could be digested or expelled after the mating plug, which initially blocks the female's secondary gonopore, has disappeared. Further studies are needed to document the fate of the mating plug. *C. ferruginata* and *C. vagans* females may digest or expel the plug after copulation.

The results show that there is potential for female sperm manipulation during sperm transfer and storage in *Coproica* species. Sperm manipulation by females has been explained as a mechanism of female choice. What criteria could the females employ to discriminate among males during copulation? According to Eberhard (1985, 1990, 1991) male stimuli serve as criteria for female choice. In the five *Coproica* species three male

behaviour patterns may qualify as copulatory courtship stimuli: compression of the female abdomen, postabdominal contractions which cause thrusts of the distiphallus, and drumming of the leg on the female abdomen. Those behaviour patterns are typical for copulation and occur before and during sperm transfer. While *C. acutangula* females would have to evaluate males during sperm transfer, *C. ferruginata*, *C. hirticula*, *C. lugubris*, and *C. vagans* females could use the pre-insemination phase (Table 1) to evaluate the quality of the male's copulatory stimuli.

Male insemination strategies

In contrast to the presumably female-mediated sperm storage in *C. ferruginata* and *C. vagans*, sperm is transferred directly from the phallotreme to the female spermathecal ducts in *C. acutangula*, *C. hirticula*, and *C. lugubris*. This mode is assumed to have evolved as an adaptation to efficient sperm transfer in males. According to Downes (1968) and Pollock (1972) this mode is called "sperm transfer via a closed system". The ejaculate is supposedly injected under pressure from the muscular ejaculatory duct. Due to the tight fit of the male and female organs, it is probable that sperm transfer via a closed system involves very little loss of spermatozoa and thus, is more efficient than transfer of a sperm mass. The closed system may even allow males to make an assessment of the amount of sperm to be transferred to the female. This type of insemination is reported for a number of other Diptera (Tipulidae, Trichoceridae: Neumann, 1958; Culicidae: Spielman et al., 1974; Sarcophagidae: Pollock, 1972; Calliphoridae: Merritt, 1989; Asilidae: Reichardt, 1929; Tephritidae: Solinas & Nuzzaci, 1984).

In *C. hirticula* and *C. lugubris* sperm transfer via the closed system takes place at the end of copulation and is completed quickly. Males of *Coproica acutangula* transfer sperm gradually throughout copulation. Gradual sperm transfer has been discussed with reference to sperm competition during insemination (Saul et al., 1988; Yamagishi & Tsubaki, 1990). Sperm competition in *C. ferruginata*, *C. hirticula*, *C. lugubris* and *C. vagans* might involve rearrangement of a rival's sperm during the prolonged pre-insemination phase. For example, this might be achieved by pumping secretions into the spermathecae that push a rival's sperm to the apical portion of the spermathecae. The distiphallus is too short to reach sperm stored in the spermathecae. Because the spermathecal ducts are long and very narrow it appears unlikely that stored sperm can be flushed out by copulating males. Sperm mixing appears unlikely because the spermatozoa are coiled densely around the spermathecal invaginations.

Deposition of a sperm mass and a mating plug is described here for *C. ferruginata* and *C. vagans*. Mating plugs are known from a number of other Diptera (Culicidae: Spielman et al., 1974; Giglioli & Mason, 1966; Sepsidae: Kiontke, 1989; Drosophilidae: Alonso-Pimentel et al., 1994). It is probable that their primary function is paternity assurance (Parker, 1970); the plug prevents sperm-leaking from the vagina. Transfer of a sperm mass is presumed to be less efficient than sperm transfer via the closed system because (1) sperm are not injected into the spermathecal ducts, which leaves the female in control over the transport of sperm into the storage organs, and (2) the amount of sperm transferred to the female vagina appears to be larger than the combined volume of the three spermathecae. The latter phenomenon has also been reported for *Dryomyza anilis* (Diptera: Dryomyzidae) by Otronen & Siva-Jothy (1991), for *Glossina austeni* (Diptera: Glossinidae) by Pollock (1974) and for *Drosophila melanogaster* (Diptera: Drosophilidae) by Kaplan et al.

(1962), Gugler et al. (1965) and Gilbert (1981). Excess sperm could be either digested or expelled by *C. ferruginata* and *C. vagans* females after the mating plug has vanished. The mating plug is thought to consist of male accessory secretions. Male accessory secretions are always transferred during copulation (Leopold, 1976; Chen, 1984) and have been reported to form copulatory plugs in other Diptera (Spielman, 1964; Giglioli & Mason, 1966). Since the ejaculatory duct is narrow, solidification of the accessory secretions must occur inside the female. However, experiments with labeled males are necessary to confirm that the mating plugs found in *C. ferruginata* and *C. vagans* are male-derived.

Phylogenetic aspects

So far the present interpretation of sperm transfer in the five *Coproica* species was concentrated on the adaptive significance of the observed traits. However, those traits are products of the interplay between constraints from the past and adaptations of the present. In order to understand their evolution it is necessary to discriminate between apomorphic and plesiomorphic sperm transfer traits. In the first step the observed characters are mapped onto the phylogeny of the taxon *Coproica* as presented by Swann, (1993; Fig. 7).

Coproica is a monophyletic genus within the subfamily Limosininae of Sphaeroceridae (Swann, 1993). The *acutangula*-group which includes *C. acutangula* and *C. lugubris* is considered the basal group (Swann, 1993). Both species perform sperm transfer via the closed system, as does *C. hirticula* (Fig. 7). *C. hirticula* is placed in the *hirticula*-group which is closely related to the *ferruginata*-group (Swann, 1993). The two species in the *ferruginata*-group, *C. ferruginata* and *C. vagans*, both transfer a sperm mass and a mating plug (Fig. 7).

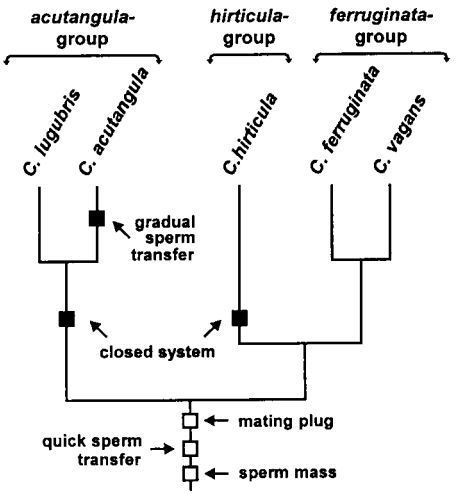

Fig. 7. *Coproica* cladogram (after Swann, 1993) showing the modes of sperm transfer found in five *Coproica* species. Quick transfer of a sperm mass and a mating plug into the female's vagina are considered ground-plan characters in *Coproica* (hypothesis 2). Sperm transfer via the closed system evolved independently in the *acutangula*- and *hirticula*-group (■ apomorphic characters, □ plesiomorphic characters).

When the data are mapped onto the phylogeny presented by Swann (1993; Fig. 7), two interpretations are possible: (1) sperm transfer via the closed system is the plesiomorphic mode in *Coproica* and consequently mating plug and sperm mass are apomorphic characters for the *ferruginata*-group, or (2) transfer of sperm mass and mating plug are plesiomorphic traits in *Coproica*. Sperm transfer via the closed system evolved independently in the *acutangula*- and *hirticula* -groups.

An outgroup comparison is necessary to identify the plesiomorphic character state in *Coproica*. So far sperm transfer has been studied in three outgroup species belonging to different taxa within the Limosininae: *Spelobia clunipes*, *S. bifrons* and *Halidayina spinipennis*. Dissections of

females killed immediately after copulation revealed that females of those three outgroup species carry a sperm mass and a mating plug in their vagina. Sperm transfer via the closed system has not been reported for sphaerocerids other than the *Coproica* species studied here. Therefore, hypothesis 2 is favoured at the moment.

This preliminary outgroup comparison suggests that transfer of a sperm mass and occurrence of a mating plug might represent the plesiomorphic character states in *Coproica* (hypothesis 2). If hypothesis 2 is correct, sperm transfer via the closed system must have evolved independently in the *acutangula*-group (*C. acutangula*, *C. lugubris*) and the *hirticula*-group (*C. hirticula*). However, further studies on sperm transfer within *Coproica* and within the Limosininae are needed.

In contrast, the following three hypotheses are well supported by the data presented here: (1) slow sperm transfer is an apomorphic character for *C. acutangula*; (2) in the five *Coproica* species, a tight engagement of phallotreme and spermathecal duct openings is established by applying the phallotreme to the vaginal sclerite of the females during copulation; this is the plesiomorphic condition in *Coproica**; (3) it is possible that the evolution of this structural fit was not linked to a certain mode of sperm transfer but its preliminary function was probably to secure the copulatory position or to stimulate the female by copulatory courtship; and the structural correspondence of phallotreme and vaginal sclerite may be interpreted as a pre-adaptation for the evolution of sperm transfer via the closed system within *Coproica*.

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