



Sperm transfer by spermatophore in Diptera: new results from the Diopsidae

MARION KOTRBA

*Department of Entomology, National Museum of Natural History, NHB 169, Washington,
D.C. 20560, U.S.A.*

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Sperm transfer by spermatophore is reported as a common and possibly primitive feature of the Diopsidae (Diptera, Schizophora). Spermatophores occur in four out of five investigated genera, in *Cyrtodiopsis*, *Diasemopsis*, *Teleopsis*, and in *Sphyracephala*, which is positioned close to the base of the family. Within the genus *Diopsis* the spermatophore is subject to reduction. Spermatophore size and copulation duration are positively correlated across the species. This is only partially explained by the time requirement of sperm transfer. Parsimony in time and material seems to be favoured in species with high mating frequency at special aggregation sites, whereas high investment in time and material occurs in species that do not mate preferentially at certain times or locations. The utilization of spermatophores has strong implications on the possibilities of sperm competition and cryptic female choice and may account for the occurrence of a postcopulatory passive phase or prolonged mating in some species. The increasing number of records of spermatophores in Diptera is reviewed.

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INTRODUCTION

The reproductive system of the Diptera has been the subject of recent research for two primary reasons. First, some flies are important pests or disease vectors, such as

the olive fly *Dacus oleae* Gmelin (Solinas & Nuzzaci, 1984) or the tsetse fly *Glossina austeni* Newstead (Pollock, 1972; Jaenson, 1979; Kokwaro *et al.*, 1981). Second, some dipteran species, such as *Drosophila melanogaster* Meigen (Gromko *et al.*, 1984), *Scathophaga stercoraria* Linneaus (Parker & Simmons, 1991; Ward, 1993) and *Cyrtodiopsis whitei* Curran (Burkhardt & de la Motte, 1985, 1987, 1988, 1994a; Lorch *et al.*, 1993), have become model species for studying evolutionary processes related to sexual selection. Despite these investigations, however, the actual process of sperm transfer has received comparatively little study.

It is generally agreed that sperm transfer by a spermatophore is plesiomorphic in the Insecta but secondarily reduced in the more derived Antliophora, including Mecoptera, Siphonaptera and Diptera, in favor of free sperm transfer by means of a sperm pump (Davey, 1965; Gerber, 1970; Hennig, 1973; Mann, 1984). Nevertheless, the number of reports describing spermatophores and possibly related structures in Diptera is increasing (see below) and sperm transfer by spermatophore has been reported in the mecopteran *Boreus westwoodi* Hagen (Mickoleit, 1974). Encouraged by the discovery of spermatophores in the stalk-eyed fly *Cyrtodiopsis whitei* (Diopsidae: Kotrba, 1990, 1993), mating behaviour and sperm transfer were investigated in 10 diopsid species, covering 5 of 12 recent genera (Fig. 1), to clarify the distribution of spermatophores within the family and obtain clues regarding their adaptive significance.

Spermatophores ('sperm carriers') are envelopes of male accessory gland secretions that enclose the sperm mass during transfer from the male to the female. They are formed at the opening of the male reproductive system and achieve their definite form either before or after the transfer (Weber, 1933; Gerber, 1970). As discussed below, spermatophores alter the possibilities for sperm competition and postcopulatory female choice. Understanding the distribution of spermatophores within the Diptera will enable us to predict whether such effects are to be considered in other species of interest as well.

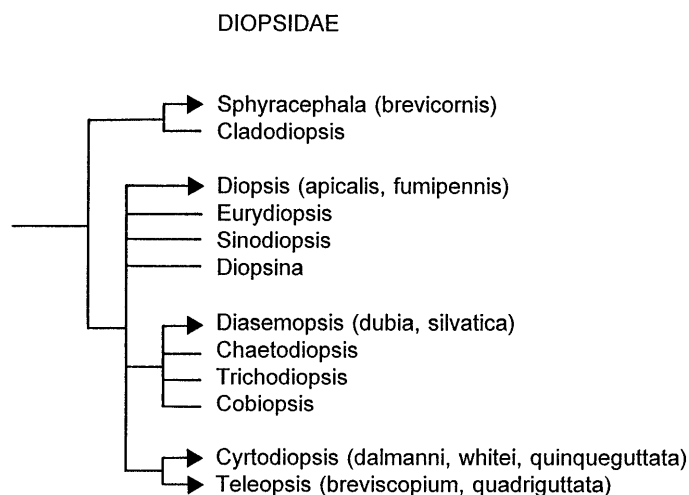


Figure 1. Recent genera of the Diopsidae according to Feijen (1989). The investigated genera (species) are marked by arrows.

The first dipteran spermatophores were found in some Chironomoidea, the Ceratopogonidae (Pomeranzew, 1932), Chironomidae (Nielsen, 1959), Simuliidae (Rubzow, 1959) and Thaumaleidae (Downes, 1968; Sinclair, 1992) and were explained as convergent, relatively recent developments. The 'mating plug' of *Anopheles gambiae* Giles (Culicidae: Giglioli & Mason, 1966) was interpreted as either the evolutionary predecessor or the remnant of a spermatophore.

Pollock (1970) reported the first spermatophore in a brachycerous fly, the tsetse fly *Glossina austeni*. Two years later he added the spermatophore of *Plecia nearctica* Hardy (Bibionidae, described by Leppla *et al.*, 1975) and discussed spermatophores as a possibly primitive feature of the Diptera (Pollock, 1972). Further findings of bibionid spermatophores were added by Kotrba (1993, *Dilophus febrilis* Linnaeus) and Blaschke-Berthold (1994, *Penthetria funebris* Meigen). Pollock (1970: 1063) speculated that the "study of sperm transfer in other genera of advanced flies would perhaps reveal similar instances". This presumption was confirmed by finding spermatophores in *Cyrtodiopsis whitei* (Diopsidae: Kotrba, 1990) and *Paratoxopoda depilis* (Sepsidae: Kotrba, unpublished). The report of a spermatophore in *Drosophila melanogaster* (DeVries, 1964; Fowler 1973) was refuted by Gromko *et al.* (1984), but Alonso-Pimentel *et al.* (1994) recently reported the occurrence of a 'sperm sac' in this and two other *Drosophila* species. The clear gelatinous material surrounding the sperm mass in the vagina of *Megaselia scalaris* Loew (Phoridae: Benner, 1991), *Dryomyza anilis* Fallén (Otronen & Siva-Jothy, 1991), *Sepsis punctum* Fabricius (Kiontke, 1989) and some *Coproica* species (Sphaeroceridae: Lachmann, 1994) might also be the relic of spermatophores.

Spermatophores are now known to occur in two different nematocerous lines (Chironomoidea and Bibionomorpha), one family of calyptrate and two families of acalyptrate Brachycera. Unfortunately the phylogenetic relationships between these groups remain uncertain, and it not clear whether the known instances of dipteran spermatophores are homologous. In the prevailing majority of dipteran families there are no investigations to establish the occurrence of lack of spermatophores.

The present report contributes to our understanding of spermatophore evolution in the Diptera. It shows that the spermatophore that was first detected in *Cyrtodiopsis whitei* belongs to the ground plan of the family Diopsidae. This discovery is discussed within the context of the other known instances of dipteran spermatophores. Furthermore the correlation of spermatophore morphology and reproductive behavior in the Diopsidae is discussed as well as the implications of spermatophore use on the possibilities of sperm competition and cryptic female choice.

METHODS

Ten species of Diopsidae were investigated during the summer of 1993. *Sphyracephala brevicornis* Say was captured in Maryland/USA in April 1993. All other investigated animals were obtained from laboratory cultures. *Cyrtodiopsis dalmanni* Wiedemann, *C. quinqueguttata* Walker, *C. whitei* Curran, *Teleopsis breviscopium* Rondani and *T. quadriguttata* Walker were reared from animals captured in peninsular Malaya in 1989. *Diasemopsis dubia* Bigot, *Diasemopsis silvatica* Eggers, *Diopsis apicalis* Dalman and *Diopsis fumipennis* Westwood were reared from animals captured in Natal/South Africa in April 1992.

The animals were maintained in a 25°C climatic chamber providing a 12 h

photoperiod with dawn and dusk simulated by 30 min of low intensity light. The size of the cages varied between $20 \times 20 \times 30$ cm in *Sphyracephala brevicornis* [c. 50 animals], $40 \times 40 \times 40$ cm in *Diasemopsis dubia* and *D. silvatica* [c. 50 animals each], *Diopsis apicalis* and *D. fumipennis* [c. 30 animals each], *Teleopsis quadriguttata* [c. 50 animals] and *T. breviscopium* [c. 20 animals], and $30 \times 35 \times 120$ cm in *Cyrtodiopsis quinqueguttata* [c. 50 animals], *C. whitei* and *C. dalmanni* [c. 200 animals each]. High humidity was ensured by covering the floors of the cages with wet cotton. All species were fed and bred on ground corn except for *S. brevicornis*, which was fed honey and yeast and bred on dandelion flowers (*Taraxacum officinale* Weber: Hochberg Stasny, 1985).

Mating behaviour and copulations were observed in the culture cages at 25°C. At least five observation sessions were conducted for each species at every time of the day, but more time was spent on species with small populations and low mating frequency, such as *Diopsis apicalis*, *D. fumipennis* and *Teleopsis breviscopium*. Females were dissected under a dissecting microscope within 3 min after copulation termination. The internal reproductive tract was removed, fixed in 70% ethanol (3 min) and then stained in aqueous toluidine blue (2 h). Toluidine blue stains a variety of organic matter, such as proteins and mucopolysaccharides. Staining was followed by 2–4 h in 70% ethanol until a satisfactory degree of decolorization of the vaginal tissue was achieved, leaving the internal spermatophore strongly stained in some species. The material was then dehydrated in ethanol of increasing concentration (80%, 90%, 95%, abs, 10 min each) followed by Americlear and subsequently embedded as wholemounts in Permount. The preparations were examined and drawn using a Wild M2 microscope equipped with a drawing tube. Photographs were taken with a Zeiss Fotomikroskop 2. The area of the sperm chamber was measured in drawings using a GTCO Micro digitizing tablet. The area of the sperm chamber was used as an estimation for spermatophore size (Fig. 4A) because the volume of the club-shaped and sometimes coiled sperm chambers would have been difficult to calculate. The spermatophore size shows no correlation with body size (Fig. 4B) and therefore was not corrected for the latter. Instead, the respective body length is indicated separately.

RESULTS

The mating behaviour and mode of sperm transfer was investigated in 10 diopsid species from five genera (Fig. 1). The spermatophores are illustrated in Figures 2 and 3. Figure 4 shows the correlation between spermatophore size and copulation duration as well as body size. To evaluate the possibilities for different mating strategies, sperm competition or cryptic female choice, it was recorded whether the mating activity is limited to certain times or locations and whether courtship is involved before, during or after the copulation. The major results of these investigations are presented in Table 1 together with observations of previous authors.

Mating activity and aggregation behaviour

Five of the investigated species perform aggregation behaviour, and in three of these the mating activity is especially high at the aggregation sites. In *Cyrtodiopsis*

whitei and *C. dalmanni* a high proportion of the mating activity occurs during dawn and late dusk, when the flies are aggregated at thread-like sleeping sites (Burkhardt & de la Motte, 1988; Kotrba, 1993; Lorch *et al.*, 1993, Wilkinson & Reillo, 1994). *Sphyracephala brevicornis* tended to aggregate at a sheltered part of the cage's ceiling for the night, but the mating activity showed no correlation with this behaviour. In the natural habitat *S. brevicornis* is reported to aggregate in rock crevices for hibernation. The mating activity is especially high in the first days after emerging from hibernation when the flies are still aggregated at these sites (Hochberg Stasny, 1985). In *Teleopsis breviscopium* and *T. quadriguttata* the mating activity showed no correlation with the nocturnal aggregation behaviour.

The remaining five species displayed no aggregation behaviour in the laboratory, but the few available field observations indicate that laboratory observations are insufficient in this respect. During the non-reproductive dry season (Feijen, 1989) in South Africa *Diopsis apicalis* was observed to aggregate on patches of vegetation during the day and in an extremely dense aggregation on a sheltered stem of grass during the night. However, no copulations were seen at these aggregations. Wilkinson & Dodson (1995) reported nocturnal aggregations of *Diasemopsis fasciata* on the surface of leaves.

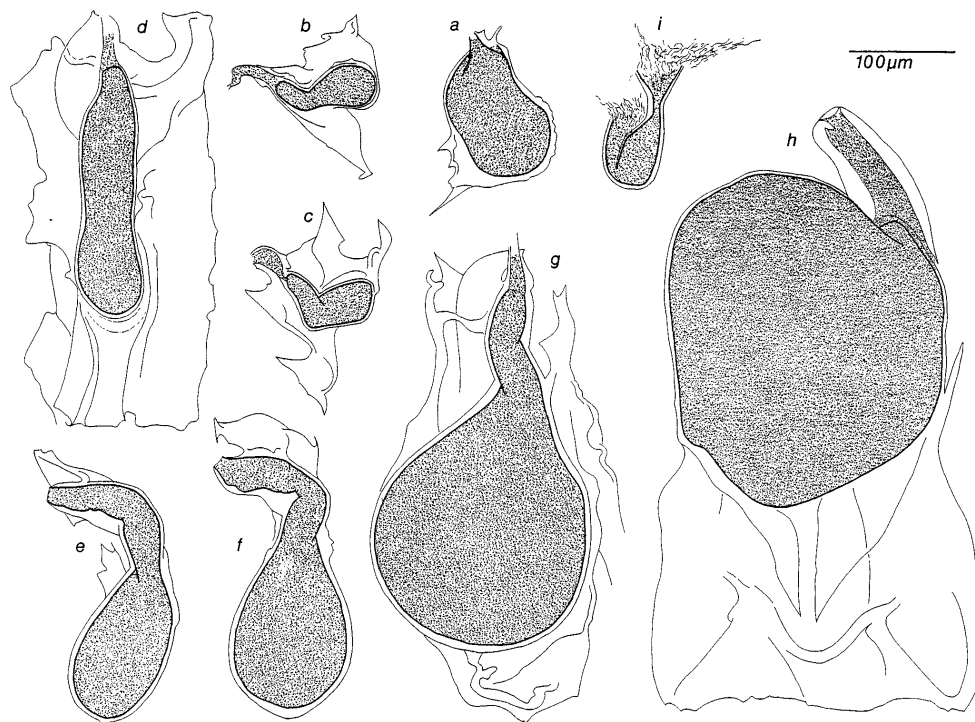


Figure 2. Spermatophores of Diopsidae. (a) *Sphyracephala brevicornis*, (b) *Cyrtodiopsis whitei*, (c) *Cyrtodiopsis dalmanni*, (d) *Cyrtodiopsis quinqueguttata*, (e) *Diasemopsis silvatica*, (f) *Diasemopsis dubia*, (g) *Teleopsis breviscopium*, (h) *Teleopsis quadriguttata*, (i) *Diopsis fumipennis*. Magnification is equal in all drawings. Scale bar = 100 μ m.

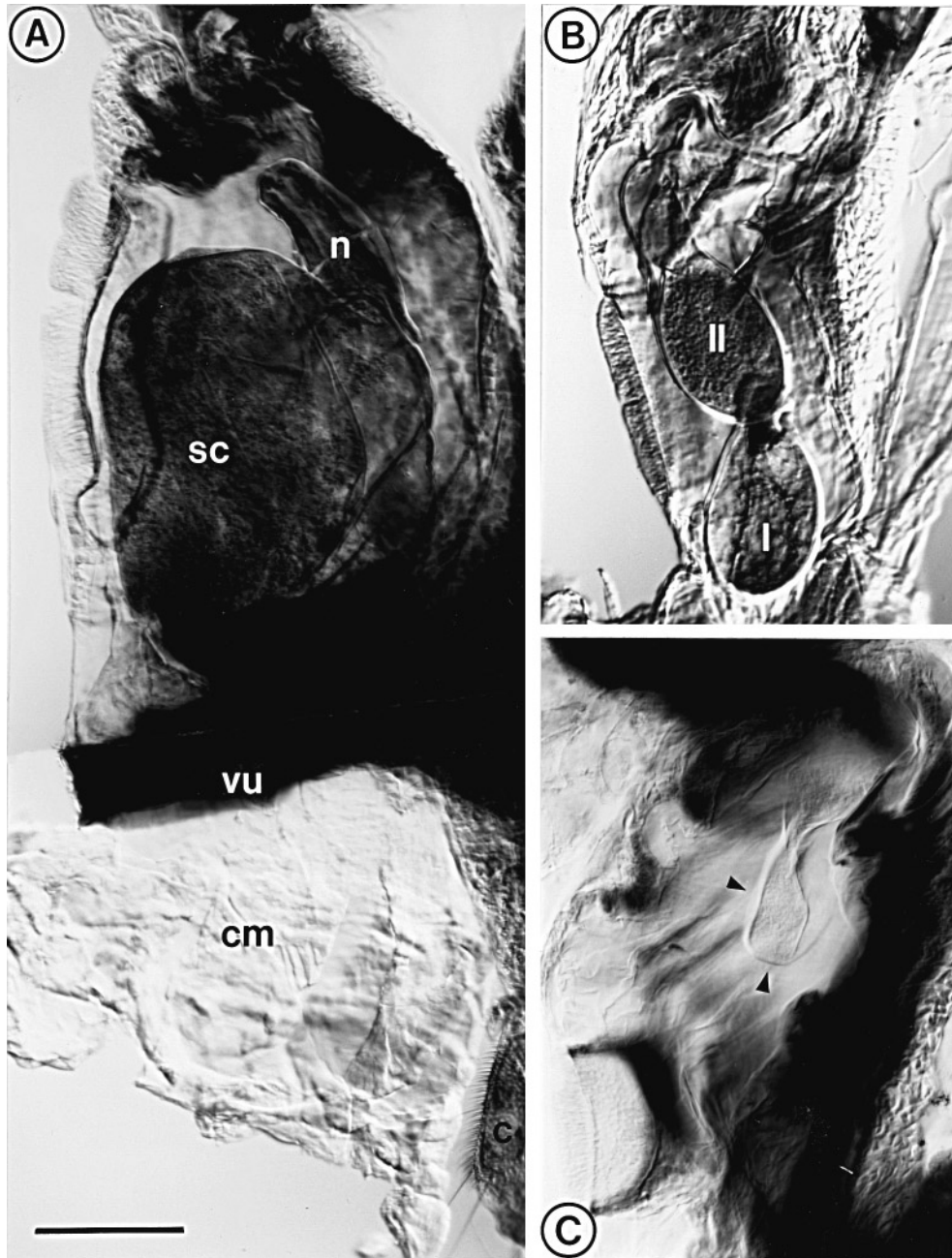


Figure 3. Spermatophores of Diopsidae. (A) *Teleopsis quadriguttata*; c: cerci; cm: clear material of outer spermatophore layer; n: neck portion; sc: sperm chamber; vu: vulva. (B) *Diasemopsis silvatica*, I: displaced first spermatophore; II: second spermatophore in proper position. (C) *Diopsis fumipennis*; arrows: spermatophore relic. Scale bar = 100 μ m.

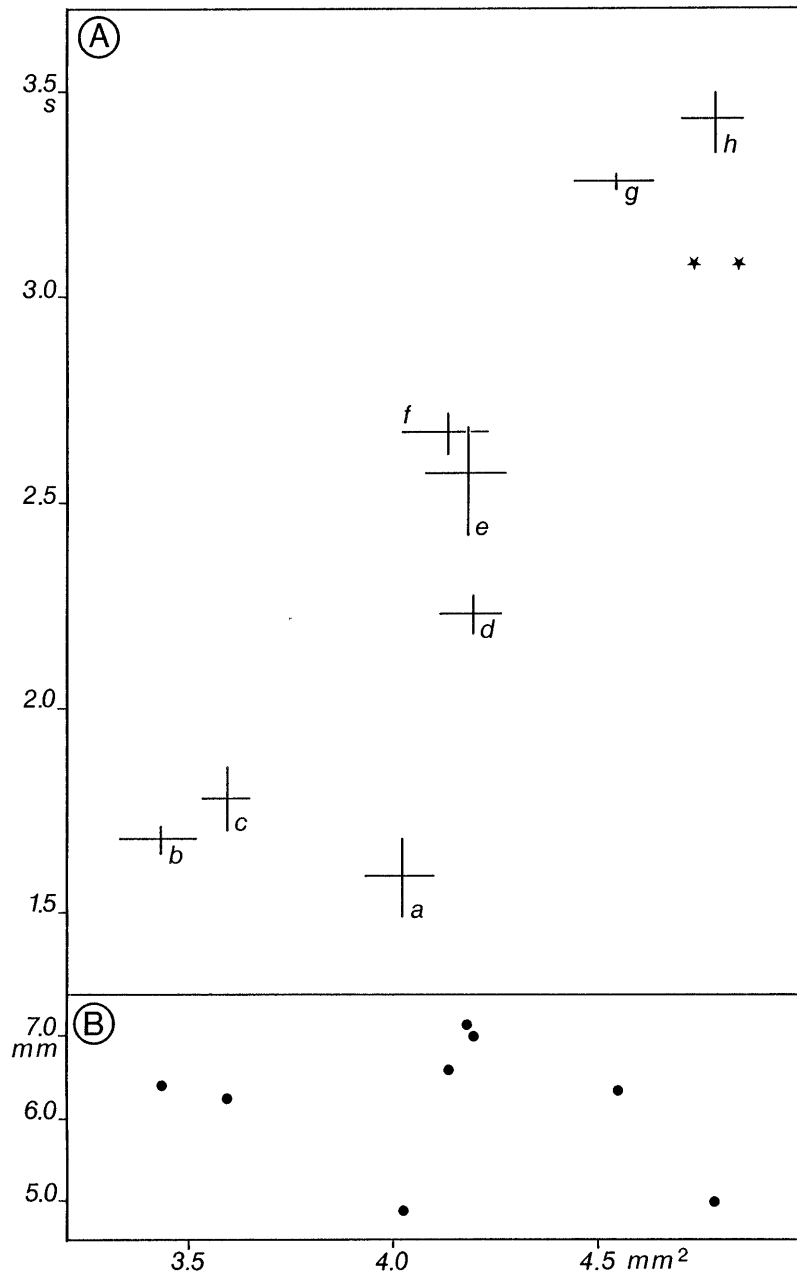


Figure 4. Correlation of spermatophore size, copulation duration and bodylength in Diopsidae. Horizontal axis: log spermatophore size; vertical axis: (A) log copulation duration, (B) bodylength (Wilkinson & Dodson 1995). The length of the bars indicates the standard deviation around the mean. (a) *Sphyracephala brevicornis* (sample size: 18 copulation durations/6 spermatophores); (b) *Cyrtodiopsis whitei* (18/5); (c) *C. dalmanni* (23/12); (d) *C. quinqueguttata* (12/7); (e) *Diasemopsis silvatica* (17/4); (f) *Diasemopsis dubia* (12/5); (g) *Teleopsis breviscopium* (5/1); (h) *T. quadriguttata* (5/7). Measurements after interrupted copulations in *T. quadriguttata* are indicated by stars.

Precopulatory passive phase

Only *Diasemopsis dubia* and *D. silvatica* copulations were sometimes preceded by a precopulatory passive phase (the terms pre- and postcopulatory passive phase are used in compliance with Parker (1970) to describe a situation where the male remains mounted upon a female without genital contact). Males mounted females without discernible courtship but were sometimes vigorously shaken off by the female. If the male managed to stay mounted, copulation often did not take place immediately. Instead, the male probed the genitalia of the female with his own genitalia from time to time and sometimes stroked her abdomen with his hind legs until copulation finally was carried out. Couples in the precopulatory passive phase sometimes formed at late dusk and continued until dark. No precopulatory interaction was detected between the sexes of any other species.

Copulation

During copulation the partners usually remain motionless for most of the time, but in all species some kind of massage, tapping or stroking is performed at intervals, especially if the female gets restless or towards the end of copulation. In *Sphyracephala brevicornis* the male repeatedly massages the tip of the female abdomen with his middle and hind legs (Hochberg Stasny, 1985). Temporary massage of the female abdomen is also performed during copulation in *Cyrtodiopsis quinqueguttata*. In other species the male taps or strokes those parts of the female body where his forelegs come to rest during copulation (Table 1). In *Teleopsis breviscopium* the male performs a distinct pattern of motion: he taps on the female's thorax with both forelegs and rhythmically flicks one foreleg forward towards the female's eyestalk about once a second. In both *Diasemopsis* species the male repeatedly buzzes his wings in an anteroventral direction toward the eyes of the female below. Most peculiarly in the two *Diopsis* species the roles seem reversed. The female lifts her hind legs against the ventral side of the male's abdomen and massages it gently from beneath. This behaviour has also been reported in *Diopsis sulcifrons* Bezzi and *D. somaliensis* Johnson (Wickler & Seibt, 1972).

The copulation duration is comparatively constant within species but varies substantially between them (Table 1, Fig. 4). It is positively correlated with the size of the transmitted spermatophore ($r = 0.97$, $P < 0.01$). Neither copulation duration nor spermatophore size are related to body size (Fig. 4b). In *Cyrtodiopsis whitei* and *C. dalmanni* the copulation duration follows a bimodal distribution, but the category of much shorter copulations results in no sperm transfer (Lorch *et al.*, 1993; Wilkinson & Reillo, 1994) and was not included in the results.

Postcopulatory passive phase

At copulation termination the partners usually separate quickly, with the exceptions of *Sphyracephala brevicornis* and the two *Diasemopsis* species, where copulation is often followed by a postcopulatory passive phase. In *S. brevicornis* the male remains astride the female for up to 1 h or more after disengaging his genitalia. He repeatedly flicks his middle legs toward the female's eyes and massages the tip of

the female's abdomen with his middle and hind legs. This behaviour has been described in detail by Hochberg Stasny (1985, "postcopulatory pairbonding ritual"). In *Diasemopsis dubia* and *D. silvatica* the males remained in postcopulatory passive phase up to 25 min, during which they performed wing buzzing from time to time. In all three species the postcopulatory passive phase may lead to another copulation. Thus, precopulatory and postcopulatory passive phase are only to be distinguished by the sequence of events.

Sperm transfer by spermatophore

Sperm is transferred by spermatophore in all species except *Diopsis fumipennis*, where only the remnant of a spermatophore is transferred, and *D. apicalis*, where free sperm is transferred into the vaginal cavity. The spermatophore is constructed during copulation within the female's vagina from secretions of the male's accessory glands (Kotrba, 1993). The single sperm chamber is surrounded by a distinct wall of dense, translucent material and a second, outer layer of less dense material. It contains a mixture of filamentous spermatozoa and varying amounts of accessory substances that are emptied into the spermathecal ducts.

The shape and size of the spermatophore is relatively constant within species but differs between them, especially between genera (Figs 2, 3, 4). In *Sphyracephala brevicornis* the spermatophore is a simple ovoid sack (Fig. 2a), tightly packed with spermatozoa but seemingly containing only little accessory secretions. The opening is directed anterodorsally towards the spermathecal ducts but might allow spermatozoa to reach the ventral receptacle (see Kotrba, 1993) as well.

The spermatophore in *Diasemopsis*, *Cyrtodiopsis* and *Teleopsis* is more elaborate and contains considerable amounts of accessory secretions, which are strongly stained by toluidine blue. In *Diasemopsis* (Figs 2e, f, 3B) the tapered end of the club-shaped sperm chamber itself connects to the openings of the spermathecal ducts, while in *Cyrtodiopsis* (Fig. 2b–d) and *Teleopsis* (Figs. 2g, h, 3A) a separate tubular neck portion is formed. Invariably the neck establishes a close connection with the spermathecal ducts, into which the contents of the sperm chamber are emptied. The openings of the ventral receptacle, accessory glands and oviduct are concealed by the outer layer of the spermatophore that forms an irregular funnel towards the opening of the spermathecal ducts.

The small spermatophores of *Cyrtodiopsis whitei* and *C. dalmanni* are deposited in a characteristic dorsoventral orientation in the anterior part of the vagina, so that they are directly aligned with the spermathecal ducts (Kotrba, 1993). In *Diasemopsis*, *Teleopsis* and *C. quinqueguttata* the spermatophore is larger and oriented more or less longitudinally within the vagina. The voluminous spermatophore of *Teleopsis quadriguttata* (Figs. 2h, 3A) fills and extends most of the vagina, and parts of its outer layer even protrude from the vulva.

Only a small spermatophore relic is found in the vagina of *Diopsis fumipennis* after copulation (Figs. 2i, 3C). It has the form of a bent tube. Both openings are directed cranially and streams of spermatozoa protrude from there to the spermathecal ducts and the ventral receptacle. In its congener *Diopsis apicalis* no spermatophore is formed. Instead, a mass of free sperm is found in the middle portion of the vagina after copulation, and streams of sperm protrude from there towards the spermathecal ducts and the ventral receptacle.

Even though copulation usually lasts about 45 min in *Teleopsis quadriguttata*, full-sized spermatophores can already be found when copulations are interrupted after about 20 min (Fig. 4h). By interrupting copulations earlier than this, the process of spermatophore formation was reconstructed. After the copulatory apparatus is inserted into the vulva, material for the outer layer of the spermatophore streams out of the male's phallosome. This material is moulded between the inner surface of the vagina and the male copulatory apparatus pushing into it. The neck portion of the spermatophore forms around an elongate process of the aedeagus. Subsequently the contents of the sperm chamber are ejaculated into the prepared cavity. As the male withdraws his genitalia, the neck portion becomes vacant, allowing the contents of the sperm chamber to stream through the resulting tube into the spermathecal ducts. A very similar process of spermatophore formation was described in *Cyrtodiopsis whitei* (Kotrba, 1993).

Probably in all cases the remains of the spermatophore are expelled by the female sooner or later. In *Cyrtodiopsis whitei* the partially emptied spermatophore is expelled within a few minutes to one hour after copulation (Kotrba, 1993). Expelled spermatophores can be found adhering to the sleeping threads of *C. whitei* and *C. dalmanni*. In *Diasemopsis dubia* no spermatophores were found in the vagina of mated females 24, 29 and 35 min after the copulation, but masses of spermatozoa in the spermathecal ducts proved that spermatophores had been transferred and meanwhile expelled.

In *Diasemopsis silvatica* it was observed once that immediately after one copulation another male approached the female and performed a second copulation that lasted unusually long (15 min). After this second copulation two spermatophores were found in the female's vagina: one almost empty and dislocated posteriorly (Fig. 3B,I), the other full and in proper alignment with spermathecal ducts (Fig. 3B,II). The second male presumably had dislocated the first male's spermatophore.

DISCUSSION

Sperm transfer by spermatophore is a common feature of the Diopsidae. Evaluating the occurrence of spermatophores within the Diopsidae in the light of what is presently known about the family's phylogeny, their phylogenetic significance on family level, and evolutionary trends within the family, can be inferred. Furthermore the occurrence of spermatophores in diopsids and other Diptera can be discussed comparatively under the aspects of Gerber's (1970) hypothesis about spermatophore evolution. In the second part of the discussion some hypotheses about the implications of spermatophores for different mating strategies, sperm competition and postcopulatory female choice are set forth, by relating spermatophore morphology with differences in reproductive behaviour.

Phylogenetic aspects

The phylogeny of the Diopsidae has not been fully clarified, but it is agreed that the genus *Sphyracephala* should be positioned near the base of the family (Fig. 1, Shillito, 1971; Steyskal, 1972; Feijen, 1989). As almost all investigated Diopsidae,

including *Sphyracephala brevicornis*, utilize spermatophores, this feature can be inferred as belonging to the ground plan of the family Diopsidae.

A simple, sac-like spermatophore, tightly packed with spermatozoa, but accompanied by little accessory secretions, as found in *Sphyracephala brevicornis* (Fig. 2a), might resemble the spermatophore of the common ancestor. Within the family two evolutionary trends can be recognized. One results in much more elaborate spermatophores that contain relatively large amounts of accessory substances, as found in *Diasemopsis*, *Cyrtodiopsis* and *Teleopsis* (Fig. 2b–h). The other trend leads toward spermatophore reduction within the genus *Diopsis*.

The transition from a spermatophore preformed by the male and superficially attached to the female genital opening to a spermatophore formed only within the female genital chamber during copulation, and finally to the transfer of free sperm to the female reproductive tract, is believed to be a common evolutionary trend (Gerber, 1970; Parker, 1970; Mann, 1984). Hypothetically, the known cases of dipteran spermatophores can be ordered according to such a trend (Pollock, 1972; Kotrba, 1993). In the nematocerous instances the spermatophore is more or less preformed in the paired primary section of the male ejaculatory duct and transferred through a relatively wide duct without the involvement of a sperm pump (Nielsen, 1959; Linley, 1981). In some cases it can be recognized that these spermatophores are originally composed of two chambers (Nielsen, 1959; Davies, 1965; Linley, 1981). At least part of the spermatophore remains outside of the vagina after copulation. As opposed to this, in the brachycerous instances a one-chambered spermatophore is inserted deeply into the vagina (Pollock, 1970; Kotrba, 1990). This shift could have coevolved with the internalization of the openings of the spermathecal ducts in the female, as dipteran spermatophores invariably connect with these openings. According to Pollock (1972) the spermatophore of *Glossina austeni* is constructed during copulation within the female's vagina, while Tibayrenc & Itard (1970) reported that the spermatophore is preformed within the male. In *Glossina pallidipes* Austen the intraspecific shift from spermatophore formation to transfer of free sperm has been reported (Jaenson, 1979) and explained as an alternative strategy when male accessory secretions are lacking. This could be interpreted as approaching the last step in spermatophore reduction. In the Diopsidae the one-chambered spermatophore is constructed during copulation in the anterior portion of the vagina. The components of the spermatophore are transferred successively by a sperm pump through a narrow ejaculatory duct (Kotrba, 1990, 1993). Spermatophore reduction in favour of free sperm transfer occurs within the genus *Diopsis*.

Pollock (1972) proposed that sperm transfer by spermatophore might be a primitive feature of the Diptera, a hypothesis that has been reiterated by recent authors (Blaschke-Berthold, 1994; Lachmann, 1994). It is corroborated by the following findings: (1) spermatophores are commonly considered plesiomorphic in the Insecta and occur in almost all insect orders (Mann, 1984; Kotrba, 1993); (2) their occurrence is being discovered in an increasing number of dipteran families; (3) in the Diopsidae spermatophores belong to the ground plan of the family; (4) their appearance in the various dipteran instances can be ordered according to a widely accepted trend of spermatophore evolution; (5) while the presence of a sperm pump and/or narrow ejaculatory ducts was formerly considered contradictory to the occurrence of spermatophores (Downes, 1968; Hennig, 1973; Mickoleit, 1974;

Blaschke-Berthold, 1994), the findings in the Diopsidae show that the two features can coexist.

However, it seems premature to classify spermatophores as a primitive feature of the Diptera. The phylogenetic relationships between the taxa known to utilize spermatophores remain uncertain, and it is also uncertain whether these spermatophores are homologous. In the prevailing majority of dipteran families there are no comparable investigations to establish the occurrence or lack of spermatophores. Thus the scattered distribution of spermatophore occurrences within the Diptera might be due to several cases of homoplasy (convergence), as well as to homology in combination with several cases of reduction.

The variation found in the mating behaviour and spermatophore morphology of the investigated Diopsidae (Figs 2, 4) is only partially consistent with what is known about their phylogenetic relationships (Fig. 1). While intrageneric comparison between *Diasemopsis dubia* and *D. silvatica* and between *Cyrtodiopsis dalmanni* and *C. whitei* reveals no significant differences, *C. quinqueguttata* differs considerably from its congeners. Indeed, the placement of *Cyrtodiopsis quinqueguttata* in the genus *Cyrtodiopsis* has previously been questioned by Burkhardt & de la Motte (1983a). The possibly closely related genera *Cyrtodiopsis* and *Teleopsis* (Feijen, 1989) are almost opposites in mating behaviour and spermatophore size, while these same features place the comparatively distantly related *Sphyracephala* next to *Cyrtodiopsis*.

The positive correlation between spermatophore size and copulation duration in the investigated diopsid species (Fig. 4) is only partially explained by the actual time requirement of sperm transfer. *Cyrtodiopsis whitei* and *C. dalmanni* copulate slightly longer than *Sphyracephala brevicornis*, but transfer smaller spermatophores. Furthermore, the spermatophore of *C. whitei* is completed only a few seconds before copulation termination, while that of *Teleopsis quadriguttata* is already completed after half the average copulation duration (Fig. 4). In the following sections these inconsistencies are discussed in combination with observed differences in mating activity and behaviour as possible adaptations to different mating strategies.

Implications of spermatophore use for different mating strategies, sperm competition and cryptic female choice

Spermatophores and short copulation durations

Sperm transfer via a closed system directly into the narrow spermathecal ducts is presumably a time-consuming process. If, on the other hand, the sperm mass is simply deposited in the vagina, it is prone to disperse throughout the reproductive tract, leak out of the vulva, or be diluted by sperm of other males, even before reaching the spermathecae. However, fast and effective sperm transfer can be achieved if a spermatophore is utilized. After the spermatophore is deposited in the vagina, its neck portion and outer layer funnel the sperm into the spermathecal ducts, while the sperm chamber surrounds the sperm mass posteriorly. A closed system is established between sperm chamber and spermathecae, which persists after copulation termination and replaces functions otherwise continuously performed by the male's copulatory apparatus, while the male is free to pursue other mating opportunities.

Spermatophore utilization might actually constitute a precondition for short copulation durations (Kotrba, 1993). Very short copulations in the range of 30–60 s

occur in *Sphyracephala brevicornis*, *Cyrtodiopsis whitei* and *C. dalmanni*. Moreover, other Diptera with very short copulation durations, such as *Glyptotendipes paripes* Edwards (3–5 s, Chironomidae: Nielsen, 1959) and *Simulium decorum* Walker (75 s, Simuliidae: Linley & Simmons, 1983), utilize spermatophores as well.

Short copulations at aggregation sites

Those diopsid species with very short copulation durations are also those with high mating activities at special aggregation sites (Table 1). This allows numerous matings with different partners during limited times of high population density. As promiscuity is high, males face an enhanced risk of sperm competition. But they gain opportunities to inseminate a large number of females, achieving either a high number of progeny through first male precedence (Lorch *et al.*, 1993; Burkhardt & de la Motte, 1994b), or at least some through other forms of sperm competition.

In *Cyrtodiopsis whitei* a large proportion of the copulations [25% (Burkhardt & de la Motte, 1988), 94% (Lorch *et al.* 1993)] occur during the short periods of dawn and dusk, when the animals are aggregated at nocturnal aggregation sites. Males and females can copulate up to 40 times a day and promiscuity is high (Lorch *et al.*, 1993; Kotrba, 1993; Burkhardt & de la Motte, 1994a). That *C. whitei* and *C. dalmanni* also transfer the smallest spermatophores (Fig. 4) and thus invest only a small amount of sperm per mating, might be a further adaptation to multiple matings within a short time.

Sphyracephala brevicornis is reported to perform multiple hasty copulations without 'pairbonding' during the first 3–4 days after emerging from hibernation, when the animals are still aggregated at the hibernation site (Hochberg Stasny, 1985). Unfortunately these sites could not be included in the present investigation, and the size of spermatophores actually transferred in this context remains unknown. Under laboratory conditions the spermatophores of *S. brevicornis* were larger than those of *C. whitei* and *dalmanni*, but still smaller than those of the other investigated species.

Spermatophores and sperm competition

Spermatophores distinctly alter the possibilities of sperm competition. When multiple promiscuous matings occur within a short time, transfer of free sperm into the vagina is associated with a high risk of sperm mixing, even before the spermatozoa reach the spermathecae. This risk is eliminated if spermatophores are utilized, as only one spermatophore at a time can be properly positioned at the entrance of the spermathecal ducts. Spermatophores might even function as temporary mating plugs (Lorch *et al.*, 1993). As a result spermatophores may be responsible for first male precedence in *Cyrtodiopsis whitei* (Lorch *et al.*, 1993; Burkhardt & de la Motte, 1994b) and other spermatophore-using Diptera such as *Culicoides melleus* Coquillett (Linley, 1975) and *Glossina austeni* (Curtis, 1968). As opposed to this, last male precedence is reported in species not using spermatophores, such as *Rhagoletis pomonella* Walsh (Myers *et al.*, 1976), *Ceratitis capitata* Wiedemann (Katiyar & Ramirez, 1970) and *Scathophaga stercoraria* (Parker, 1970; Ward, 1993).

While spermatophores reduce the risk of sperm competition, they bear the risk of being displaced or removed as a whole by succeeding males. Until now the only evidence for such displacement in Diopsidae is a single observation in *Diasemopsis silvatica* (see above). In the carrion beetle *Thanatophilus sinuatus* Fabricius (Silphidae)

males remove the spermatophores of predecessors from the female vagina by means of their spiny aedeagus (Neuner & Peschke, 1994).

Spermatophores and female choice

Spermatophores also alter the possibilities for postcopulatory 'cryptic' female choice (Thornhill & Alcock, 1983; Eberhard, 1985). In *Cyrtodiopsis whitei* the interval between copulation termination and spermatophore expulsion ranges from a few minutes to more than half an hour (Kotrba, 1993). By controlling the time lapse until spermatophore expulsion, females might influence the amount of spermatozoa reaching their reproductive system. In the field cricket *Gryllus bimaculatus* DeGeer females are reported to selectively remove the spermatophores of small males before sperm transfer is completed (Simmons, 1986).

In contrast to the almost complete lack of precopulatory courtship, a variety of courtship behaviours, such as massage, tapping, stroking, leg flicking, wing buzzing, etc. is observed in most diopsid species during or after copulation. Such 'copulatory courtship' (Eberhard, 1994) might function to influence cryptic female choice, inducing the female to allow sperm transfer to continue, maybe even stimulating sperm uptake, as suggested by Eberhard (1985).

The risk of spermatophore displacement by a succeeding male, or a choosy female itself, probably constitutes a strong selective pressure. The following two male strategies might have evolved as consequences: (1) prevention of spermatophore displacement by remaining with the female for prolonged time after sperm transfer (see following two sections), and (2) reduction of spermatophores in favour of free sperm transfer (see Reduced spermatophore, below).

Postcopulatory passive phase

After the first days of hasty copulations in the spring, *Sphyracephala brevicornis* males switch to a different mating strategy, now diminishing the risk of sperm competition by engaging in a postcopulatory passive phase ('pair bonding': Hochberg Stasny, 1985). After disengaging his genitalia the male remains astride the female for up to one hour. This corresponds to the mating behaviour observed in the present investigation. A shorter postcopulatory phase also occurs in *Diasemopsis dubia* and *D. silvatica*.

In *Scathophaga stercoraria* the postcopulatory passive phase restricts the accessibility of the female to other males between copulation and oviposition and thus reduces the risk of sperm competition (Parker, 1970). In the Diopsidae however, the postcopulatory passive phase is not extended until oviposition and thus not apt to entirely prevent further matings of the female before oviposition. Instead, the postcopulatory passive phase in Diopsidae seems primarily involved with ensuring effective sperm transfer. The postcopulatory passive phase prevents spermatophore dislocation by other males while sperm is transferred from the spermatophore to the spermathecae. Accompanied by courtship behaviour, such as massaging of the female's abdomen and leg flicking in *Sphyracephala brevicornis* or wing buzzing in both *Diasemopsis* species, it might also function to influence cryptic female choice. Otronen & Siva-Jothy (1991) have shown that the last male to mate with a previously mated female in *Dryomyza anilis* is able to increase his paternity from 18% to 70% by performing tapping sequences on the female genitalia after sperm transfer.

The postcopulatory passive phase combines some advantages of a short copulation with those of a long one. After transferring a spermatophore within relatively short

time the male has the option to leave the female and pursue a new copulation opportunity as soon as it arises. But unless this occurs, the male can reduce his risk of sperm competition and ensure sperm transfer to be completed by guarding his last mate. As the postcopulatory passive phase is sometimes followed by another copulation in *Sphyracephala brevicornis* (Hochberg Stasny, 1985), it also preserves the opportunity to occupy more of the female sperm storage organs and thus diminish the degree of sperm mixing in the spermathecae due to later matings. In the mating systems of *Sphyracephala* and *Diasemopsis* these advantages seem to outweigh the gains of immediately leaving the mated female in search for other females.

Long copulations

In contrast to the strategy involving small spermatophores, large spermatophores are transferred in copulations that last comparatively very long. Although the regarded species *Teleopsis breviscopium* and *T. quadriguttata* gather at nocturnal aggregations sites (Burkhardt & de la Motte, 1985), copulations do not occur preferentially at these locations, and copulation duration does not appear to be a limiting factor. This strategy involves high investment in time and material per copulation but ensures paternity by diminishing the risk of sperm competition. The amount of sperm transferred is probably large enough to fill the female's spermathecae to capacity with one copulation. It is accompanied by a large amount of accessory substances. Concurrently, the time investment per copulation is very high. In *T. quadriguttata* the termination of the copulation is even retarded after sperm transfer is completed. Similar to the postcopulatory passive phase, a prolonged mating prevents other copulations and allows sperm transfer into the spermathecae to be completed. It may also involve internal postcopulatory courtship. Prolonged copulations have been reported in other spermatophore-using flies as well: *Plecia nearctica* copulates for up to 56 h although sperm transfer is already accomplished after about 12 h (Thornhill, 1976). In *Glossina austeni* copulations last between 2 and 24 h (Pollock, 1974).

Reduced spermatophore

Spermatophore reduction is found within the genus *Diopsis* and is reported to occur within many other insect taxa as well (Gerber, 1970). As pointed out above, spermatophore reduction might be an evolutionary consequence of negative selective pressure due to the risk of spermatophore displacement. Further investigations have to be conducted in the Diopsidae to learn more of the behavioural context and corroborate the suggested possibility of spermatophore displacement by succeeding males or by choosy females themselves.

CONCLUSIONS

Sperm transfer by spermatophore, as found in the Diopsidae and some other flies, is possibly related to certain mating strategies. The spermatophore establishes a closed system with the spermathecae and ensures effective sperm transfer to continue even beyond copulation termination, while the male is free to pursue other mating opportunities. The utilization of spermatophores may even constitute a precondition for mating systems with multiple short copulations. Furthermore, spermatophores are likely to have strong implications on sperm competition. Spermatophores reduce

the risk of sperm competition within the vagina, but at the same time bear the risk of being displaced as a whole by succeeding males or through cryptic female choice. This risk supposedly triggers the evolution of mechanisms to prevent spermatophore displacement, such as a postcopulatory passive phase, prolonged copulation, and spermatophore reduction. The reproductive system of the Diopsidae with its various stages of spermatophore use, development, and reduction provides a model system for further investigation of these topics.

No decision can yet be made whether spermatophores represent a primitive feature of the Diptera. However, it seems likely that additional instances of spermatophores will be discovered, as more effort is put into understanding the process of sperm transfer in other flies. Hopefully this report will encourage such investigations to address the question of spermatophore evolution and, last not least, contribute to a better understanding of the phylogeny of Diptera and their relationship to other panorpoid insects.

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