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SPERM DISPLACEMENT BY TWO LIBELLULID DRAGONFLIES WITH DISPARATE COPULATION DURATIONS (ANISOPTERA)

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The possibility that dragonflies with frequent, brief copulations differ from those with less frequent, longer copulations in the degree of sperm precedence by the last male to mate or in the mechanism by which sperm precedence is attained is examined.The reproductive behavior, sperm displacement ability and genitalic morphology of *Celithemis elisa* and *Erythemis (Lepthemis) simplicicollis* were studied. *Celithemis* have infrequent copulations of about 5 min in duration and oviposition is in tandem, while in *Erythemis* copulation durations last about 20 sec, males mate more frequently and mates are guarded for shorter periods of time. Both spp. are primarily sperm removers, despite their differences in copulation duration and penis morphology. In *C. elisa* about 68% of the sperm in a female's bursa belongs to the last of 2 or more males to mate with her. For *E. simplicicollis* 54% of the sperm in the bursa belongs to the last male. Estimates of volume displacement for *E. simplicicollis* agree closely with data on sperm precedence for the same sp. (cf. M.E. McVEY & B.J. SMITTLE, 1984, *J. Insect Physiol.* 30: 619-628).

INTRODUCTION

Copulation in Odonata differs from other insects in a number of ways:
(1) The male copulatory organs are not homologous to those of other insects.
(2) During mating, sperm is first transfered from the testes to a temporary storage organ and then to the female's bursa copulatrix.
(3) In many species, some of the morphology of the male's penis functions to remove the sperm of previous males from the female's sperm storage organs or to reposition it in them prior to insemination.

Sperm displacement (removal or repositioning) appears widespread among odonates, particularly damselflies (Zygoptera) (WAAGE, 1979a, 1982, 1984,

1986; MILLER & MILLER, 1981; MILLER, 1982a, 1987a, 1987b; FINCKE, 1984). The dragonflies (Anisoptera) have received less attention, partly because of the complexity of their genitalia, but work to date indicates sperm displacement occurs in this sub-order as well (MILLER, 1981, 1982b, 1984; WAAGE, 1984; McVEY & SMITTLE, 1984; SIVA-JOTHY, 1984, 1986a, 1986b; E. Routman, unpubl.).

Two features of odonate reproductive behavior seem to be generally correlated (WAAGE, 1979a, 1979b, 1984): (a) the frequency and duration of copulation and (b) the nature and extent of postcopulatory interactions between mates. Copulation durations vary across a wide range in both Zygoptera (< 1 min to several hrs) and Anisoptera (several sec to several hrs). Postcopulatory behavior correlates with this variation in copulation duration. Rapid maters tend to mate frequently and exhibit non-contact guarding of mates, those species with moderate copulation durations (5-15 min) generally mate less frequently and oviposit in tandem, and some species with long (1 + hrs) or infrequent copulations, for example *Ischnura* species, exhibit no postcopulatory interaction (CORBET, 1962, 1980; WAAGE, 1984, 1986).

This raises the question of whether or not species with frequent, brief copulations differ from those with less frequent, longer copulations in the degree of sperm precedence by the last male to mate or in the mechanism by which sperm precedence is attained. MILLER (1981, 1982b), SIVA-JOTHY (1984, 1986a) and WAAGE (1984) suggested that libellulids may use either sperm repositioning or sperm removal during sperm displacement. It seemed likely that repositioning would primarily be employed by rapid maters and involve relatively simple morphology of the distal penis segment. In contrast, longer copulation times would primarily involve sperm removal using more complex penis morphology.

Sperm repositioning moves previous sperm away from the region of the female's storage organs where sperms are likely to have the highest probability of fertilizing eggs during oviposition. This region is then filled with the mating male's sperm and his initial fertilization advantage (sperm precedence) may be quite high. However, the number of sperm he can introduce and the loss of precedence due to mixing of ejaculates will be influenced by the number of sperms already present and the degree to which they can be compacted when pushed aside. In contrast, removal of rival sperm from all or part of the female's storage organ(s) would, in general, result in both an initial fertilization advantage to the last male and also maintain his advantage by avoiding or decreasing the effect of mixing. This would be most dramatic if the last male were to remove all or most of the previous sperm stored by a female.

The expectation then is that when mating opportunities are frequent it generally pays to push aside previous sperm quickly and gain an initial fertilization advantage. Each female is guarded for a short while (e.g. until another potential mate appears) and fertilizations are maximized by short matings and initial

precedence with a number of mates. At the opposite extreme, removal of most or all sperm is expected to take longer and to be more advantageous where mating opportunities are less frequent. Here fertilizations are maximized by longer time investments per female. SIVA-JOTHY (1984) has examined these alternatives in two libellulids and found evidence for repositioning in the species with shorter copulation durations and removal in the species with the longer copulation durations.

In this paper, I compare the reproductive behavior, sperm displacement ability and genitalic morphology of two additional libellulid dragonflies, *Celithemis elisa* and *Erythemis (Lepthemis) simplicicollis. Celithemis* have infrequent copulations of about 5 min in duration and oviposition is in tandem, while in *Erythemis* copulation durations last about 20 sec, males mate more frequently and mates are guarded for shorter periods of time. These species are then used to examine the prediction that sperm repositioning is more likely for species with more frequent, shorter copulations. I find that both species are primarily sperm removers despite their differences in copulation duration and penis morphology.

METHODS

Celithemis elisa were observed and collected at the Carratunk Wildlife Refuge in Seekonk, Massachusetts during June and July, 1980-82. *Erythemis simplicicollis* were observed and collected on Fisheating Creek, west of Venus, Florida (Highlands Co.) during April and May, 1981. Behavioral observations included pair formation, copulation, postcopulatory and post-oviposition behavior of males and females. For *C. elisa* observations were of both marked and unmarked individuals and were part of a larger study of their reproductive behavior (Waage, unpublished). For *E. simplicicollis* observations were of unmarked individuals and were made only during periods when specimens were being collected for sperm displacement studies.

Specimens of both species were collected before, during and after copulation and immediately preserved in 70% ethanol for later dissection. Sperm storage organs were dissected out and surrounding tissues removed. In both species, unlike zygopterans, sperm morphology changed upon exposure to acetic acid. Therefore, removal of tissues surrounding the bursa and spermathecae was accomplished without the aid of the acetic acid/ethanol solution described by WAAGE (1984). Once tissues are removed, the sperm mass is visible within the female's storage organs.

The volume of sperm stored in the bursa copulatrix and spermathecae of females was estimated using methods described in detail by WAAGE (1979a, 1982, 1984). Each sperm mass was irregularly shaped but had a large surface (side view) and a variable width (end view). These views were drawn to scale using a camera lucida and dissecting scope. The area of the side was estimated using the drawings and a digitizer and this area was multiplied by the average width, obtained by digitizing the end view drawing, to obtain a volume estimate. For *Erythemis* there was noticeable variation in the opacity of the sperm mass in 28% of the specimens. Therefore the relative translucence of each specimen was estimated (0.5 to 1.0 = opaque) without knowing the mating context of the female involved. This value was multiplied by the calculated sperm volume in order to approximately compensate for differences in sperm density assumed responsible for the differences in opacity. Each of the three mating categories had approximately the same number of specimens with less than maximum opacity. SIVA-JOTHY (1984) suggests that the relationship between sperm numbers, density and volume is more complex than I have assumed. However, my results for *Erythemis* agree

well with those of McVEY & SMITTLE (1984) who used irradiation techniques with the same species (see below).

Actual volumes were not calculated for *Celithemis*. Instead the digitized volumes were treated as a unitless index for comparison of sperm volumes. There were no noticeable differences in opacity for *C. elisa* specimens.

Three categories of specimens were compared for *C. elisa:* precopula, in (interrupted) copula, and postcopula. Precopula females were those collected away from the pond, early or late in the day, who had 100 or more mature eggs (mean = 331, n = 11, S.E. = 81, range 126-1098). They represented females who had mated previously and were soon going to mate again prior to oviposition (females were not observed to oviposit without mating). In copula and postcopula females averaged 808 eggs (n = 23, S.E. = 79, range = 291-1518). In contrast, females collected immediately after oviposition averaged 36 eggs (n = 14, S.E. = 13, range = 0-168). Interrupted pairs were taken at various times during mating, generally while the pair were still in the rocking or pumping phase of copulation. Postcopula pairs were usually taken by lowering an insect net over a copulating pair and waiting until copulation ended. Otherwise, the pair would immediately fly off to the water in tandem. The netting technique had no obvious effect on copulation duration. Occasionally, pairs arriving at the water were caught before they began oviposition and were included in the postcopula category.

Erythemis were collected in the same three categories. Precopula females were those found near the water and who contained mature eggs, plus females collected at the water during oviposition. This latter category was used since most observed matings occurred between males and ovipositing females whose mates were unsuccessful at guarding them. In copula pairs were usually taken after 5-15 sec of mating. Postcopula females were obtained by catching the female of a pair immediately after separation. In addition, post-oviposition females were obtained by catching ovipositing females and dipping their abdomens in a vial of river water until egg flow ceased. In all cases, these females contained few or no mature eggs upon dissection.

Tethered *Erythemis* females also were used to obtain controlled matings. A female was tethered by gluing (with Duco Cement) 2 lb test, monofilament fishing line to the front of her thorax. Females collected near the water were then assigned randomly to one of four categories: (a) mated once (postcopula = POST 1X in Tab. II); — (b) mated once followed by a second, interrupted copulation (= INT 1X); — (c) mated twice (= POST 2X); — and (d) mated twice followed by a third, interrupted copulation (= INT 2X). This provided in copula specimens known to have recently received sperm from one or more previous males. Also, if sperm removal or repositioning does not occur, twice mated females should, on average, carry more sperm than once mated females or exhibit denser (more opaque) sperm stores. See SIVA-JOTHY (1984, 1986a) for a more detailed discussion of the relationship among sperm number, density and volume with repeated copulations.

Unless otherwise stated, all means are given with their standard errors. All statistical analyses were type II ANOVA with planned contrasts (Duncan-Waller multi-range tests give the same results). Computation was done using the GLM routine of the SAS statistical package.

RESULTS

RELEVANT REPRODUCTIVE BEHAVIOR — *CELITHEMIS ELISA*

C. elisa males are not territorial. Males localize at, but do not defend perching areas around the edges of ponds or in adjacent fields. In fact, most males are perched away from the water in open areas where they appear to scan the sky for approaching females (they generally face away from the water). Pair-formation can occur at the water, but usually takes place in adjacent fields or open areas.

Females are taken in tandem without any pair-forming displays.

If the female is receptive, mating takes place in low vegetation and lasts 4.6 ± 0.2 min (n = 14). Rocking or pumping movements of the male's proximal abdominal segments occurred during the first min of copulation (mean = 0.75 ± 0.09 min, n = 14). These movements are presumed to be related to sperm displacement in other odonates (MILLER & MILLER, 1981; WAAGE, 1984). One-half of the copulations seen during collection of specimens occurred away from the water in an adjacent field. Copulations and ovipositions occur within a several hour period each sunny day. Following copulation, the pair immediately flies to the water in tandem, often at considerable heights. Oviposition, once the pair has reached the pond, occurs in tandem into open water around the edges of the pond and occasionally over the entire pond surface.

The duration of tandem oviposition for a sample of 22 pairs was 3.0 ± 0.3 min. Females occasionally oviposited alone following tandem oviposition. A sample of 12 did so for an average of 1.8 ± 0.4 min. When finished ovipositing, females flew rapidly upward and away from the pond. Approximately 700-800 eggs are oviposited (the difference in mean egg number between females taken in copula and those taken post-oviposition). *Celithemis* females rarely mated more than once per oviposition. Males and females probably rarely mated more than once per day. The total time a female was at the water was generally under 5 min and most of it was spent in oviposition.

RELEVANT REPRODUCTIVE BEHAVIOR — *ERYTHEMIS SIMPLICICOLLIS*

The following comments are for an unmarked population observed at Fish-eating Creek during the collection of specimens. McVEY (1981) has studied this species in great detail in northern Florida. Pair formation in my population was usually with ovipositing females. In contrast to *Celithemis, E.simplicicollis* males and females paired at the water, mating was rapid, oviposition occurred near the site of mating, and males non-contact guarded their ovipositing mates.

Males localized at the water, perching on algal mats or on the shore. Because of the high density (> 20 males per 25 m^2) and the fact that individuals were not marked, it was impossible to tell if males were territorial. McVEY (1981) has shown that elsewhere males of this species are usually territorial in the sense of defending areas to which they return. Copulating occurred both in flight or while perced on algal mats. Sperm translocation (male to himself) occasionally occurred in flight immediately after tandem formation. Otherwise, it probably occurs prior to pair formation. Copulation durations, including pairs that were disturbed one or more times, averaged 19.5 ± 0.8 sec (n = 207). Copulation durations for tethered females were nearly identical (20.8 ± 0.9 sec, n = 66).

The duration of oviposition for undisturbed females was 0.63 ± 0.08 min (n = 63). However, females were often disturbed by males and the time it took them to

complete their oviposition ranged from a mean of 1.86 ± 0.24 min (n = 30) for females who mated only once to 13 min for a female who mated 7 times before completing oviposition or leaving the water. It is likely that the effects of disturbance were underestimated since females may have left the water when disturbed by males before completing their oviposition. Ovipositing females would attempt to avoid males by perching on algal mats at the water, but they were never seen to resist mating once taken in tandem.

Because females mated and oviposited several times per visit to the water, it is difficult to estimate the total number of eggs oviposited. For a sample of 19 females from whom eggs were collected (oviposition into vials while held) a range of 198 to 1280 eggs were obtained (mean = 495.4 ± 69.8). McVEY (1984) estimates egg deposition rates from 6-13 per sec in Florida for temperatures ranging from 28 to 38° C. Assuming 10 eggs per second as the appropriate rate for the population I studied, females would have oviposited 380 eggs in 0.63 min (the average undisturbed oviposition duration for my population).

Females mated an average of 2.05 ± 0.18 (n = 63, range 1-7) times per visit to the water (also an underestimate since the same female could have visited several times in a day). During oviposition the male hovered or perched near his mate, but his ability to repel other males, at least at this high density, was poor and males were rarely able to guard a female for more than 30 sec. The time devoted to guarding and the frequency of mating and remating for *E. simplicicollis* obviously varies considerably (McVEY, 1981; McVEY & SMITTLE, 1984; present study).

SPERM DISPLACEMENT — *CELITHEMIS ELISA*

WAAGE (1984) described the morphology of male and female genitalia in this species and discussed its ability to displace sperm. The data on sperm displacement are reproduced in Table I. Significant changes in total sperm volume occur among pre-, in- and postcopula females ($F = 14.39$, $df = 2,32$, $p < 0.0001$). Total pre- and postcopula sperm volumes do not differ ($F = 0.91$, $df = 1,32$, $p > 0.35$). Thus highly significant differences in total sperm volume were found between

Table I

Sperm displacement by *Celithemis elisa:* data in the table are bursa, spermatheca and total sperm volumes in the form of a relative index (cf. text for description of contexts)

Context	N	Sperm volume index — mean (s.e.) Bursa	Spermathecae	*Total*
Precopula	11	1066 (135)	239 (46)	*1281 (151)*
In copula	12	334 (95)	294 (30)	*629 (88)*
Postcopula	10	1049 (74)	391 (82)	*1440 (97)*

interrupted copula and pre- plus postcopula specimens ($F = 28.14$, $df = 1,32$, $p < 0.0001$). Similar changes occur in bursa copulatrix volumes during copulation, with pre- and postcopula volumes not different from each other ($F = 0.01$, $p > 0.91$) and in copula volumes significantly lower than pre- and postcopula ones ($F = 32.51$, $P < 0.0001$).

The volume of sperm in the spermathecae did not change significantly during copulation ($F = 1.94$, $p > 0.162$). However, there was a trend toward increasing spermatheca volume from pre-copula to in copula and to postcopula indicating that some sperm may be pushed from the bursa into the spermatheca during sperm removal. The spermatheca volume for postcopula females is 27% of the total (37% of the bursa volume).

Although there were no obvious changes in sperm density within the bursa and spermathecae among the copulation stages, it is not possible to attribute all volume changes to removal of sperm and some repositioning may occur as well. Approximately 68% of the sperm (by volume) in the bursa of a postcopula female belong to the last male to mate with her and these sperm initially are closest to the valve between the bursa and vagina where fertilization occurs during oviposition. This value for last-male sperm volume is slightly higher than reported by WAAGE (1984) due to the addition of new data since the calculations for that paper were made.

Because most of the morphology of the distal segment of the libellulid penis is erectile and collapses when the pair is separated or killed, it is difficult to determine the positioning and function of the various appendages. However, comparing sizes of the inflated distal segment and its appendages and the female's reproductive organs, it is likely that some of the male's morphology does enter the bursa copulatrix of the female and part of the medial process may enter the spermathecae.

The male and female reproductive morphology of *C. elisa* has been described by WAAGE (1984) and is similar to those of *C. eponina* as described by MILLER (1981). Figure 1A shows the elevated medial process (MP) rising above an inflatable, tongue-like apical lobe (AL) and between two chitinous lateral lobes (LL). The apical lobe is covered by spines similar to those thought to be associated with sperm removal in other odonates (Fig. 1B); but this lobe probably does not enter the bursa of the female. Males from in copula and postcopula pairs often had a thin crust of sperm on or around the apical lobe. The apical lobe may only remove sperm from the vagina after its removal from the sperm storage organs by other parts of the penis. Scanning electron micrographs (e.g. Fig. 1B) reveal that all other surfaces seem to lack spines. In this respect, *C. elisa* differs from *C. eponina* whose inner lobes and medial process (sperm tube) are spinous (MILLER, 1981).

The female storage organs of *C. elisa* (Fig. 1C) consist of a club-shaped bursa copulatrix (B) into which two elongated spermathecae (ST) enter. The sperma-

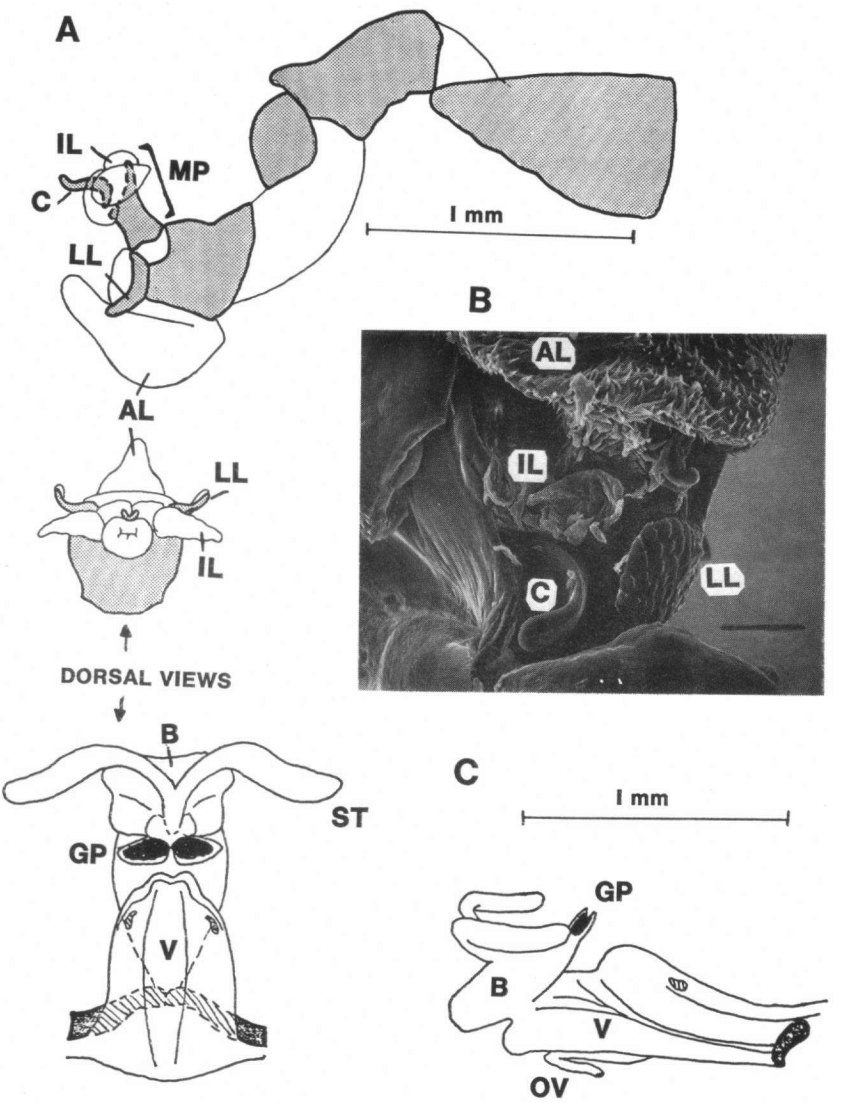

Fig. 1. Male and female reproductive morphology of *Celithemis elisa*. (A and C modified from WAAGE, 1984): (A) Lateral and dorsal views of expanded penis showing erectile appendages of the 4th segment, (black and shaded areas are chitinous, C cornu, MP medial process, IL inner lobes, LL lateral lobes, and AL apical lobe); — (B) Electron micrograph of the collapsed distal segment (scale bar = 100 μm); spines are obvious on the apical lobe (AL), but there are none on the cornu (C), lateral lobes (LL) or inner lobes (IL); — (C) Lateral and dorsal views of the female genitalia (B bursa copulatrix, ST spermatheca, V vagina, OV oviduct, and GP genital plate).

thecae are long and narrow which may prevent any part of the medial process from entering them. However, MILLER (1981) suggests that the inner lobes and medial lobes of *C. eponina* enter, respectively, the bursa and spermathecae.

SPERM DISPLACEMENT — *ERYTHEMIS*

Table II presents spermatheca, bursa and total sperm volumes for both natural copulations and those resulting from tethered female experiments. Natural precopula females include (a) females arriving at the water and carrying sperm from previous mating/oviposition bouts (= female alone and post-oviposition in Table II) and (b) postcopula females (since females mated 2 or more times per oviposition). In *Erythemis,* spermatheca volumes were small (8% of the bursa and 7% of the total volume in postcopula females) but there were significant differences in volume among pre-, in and postcopula females ($F = 3.24$, $df = 2,110$, $p < 0.04$). In copula and postcopula females did not differ ($F = 0.03$, $P > 0.86$) but both differed from the smaller precopula volumes ($F = 4.92$, $P < 0.029$).

Table II

Sperm displacement by *Erythemis simplicicollis:* data are estimated sperm volumes for natural and tethered females collected in various contexts (cf. text) and are presented as mean (standard error) for the bursa copulatrix, spermatheca and total volumes

Context	N	Sperm volume (mm^3 x 100) Bursa	Spermatheca	*Total*
Precopula:				
Female alone	20	34.8 (5.7)	1.8 (0.3)	*36.7 (5.9)*
Post-oviposition	15	19.0 (4.3)	1.3 (0.2)	*20.3 (4.3)*
Pooled precopula	35	28.0 (3.9)	1.6 (0.2)	*29.7 (4.1)*
In copula:				
Natural pairs	23	12.6 (2.7)	2.2 (0.4)	*14.8 (2.6)*
Tethered: int. 1x	11	15.6 (6.6)	2.5 (0.3)	*17.9 (6.7)*
Tethered: int 2x	10	18.3 (6.6)	2.6 (0.4)	*20.9 (6.7)*
Pooled in copula	44	14.6 (2.6)	2.3 (0.2)	*16.9 (2.6)*
Postcopula:				
Natural pairs	12	25.5 (3.8)	2.0 (0.5)	*27.6 (3.9)*
Tethered: post 1x	8	37.0 (7.6)	2.1 (0.6)	*39.1 (3.9)*
Tethered: post 2x	12	34.9 (6.6)	3.0 (0.5)	*37.9 (6.7)*
Pooled postcopula	32	31.9 (3.4)	2.4 (0.3)	*34.3 (3.5)*

For natural pairs, there was no significant difference in total sperm volume (bursa + spermathecae) among these three categories of "precopula" females ($F = 2.72$, $df = 2,46$, $p > 0.77$). There were, however, significant differences among the total sperm volumes of pre-, post- and in copula females ($F = 4.33$, $df = 2,69$,

$p < 0.017$). Postcopula and precopula sperm volumes did not differ ($F = 0.11$, $p > 0.74$), but both differed significantly from in copula ones ($F = 7.20$, $p < 0.009$). The magnitude of total sperm volume decrease from precopula to in copula is 41% for natural pairs. Sperm density varied among specimens with 27 of the total 111 specimens being recorded as having sperm of lower density. There was, however, no pattern to density variations and no obvious changes in density that would reflect sperm repositioning (e.g. sperm in tethered females mated twice in a row did not appear denser than those mated once).

There were no significant differences in total sperm volumes for natural and tethered in copula pairs ($F = 0.45$, $df = 2,43$, $p > 0.64$) or for natural and tethered postcopula females ($F = 1.14$, $df = 2,31$, $p > 0.33$). Therefore the data for tethered and natural pairs were pooled. The difference in total sperm volume between pooled postcopula and pooled in copula specimens was highly significant ($F = 14.68$, $df = 1,110$, $p < 0.0002$). This difference reflects a 48% decrease in sperm volume during copulation (presumably an underestimate since pairs were interrupted during the displacement phase).

Comparison of postcopula and in copula volumes provides the best estimate of sperm displacement potential since postcopula females carry the most sperm and they are likely to mate prior to completion of oviposition. Approximately 54% of the sperm in the bursa of a postcopula female belong to the last male to mate with her (range 48% to 58% for tethered females mated twice and natural pairs, Tab. II). Sperm in the bursa initially are closest to the valve between the bursa and vagina where fertilization occurs during oviposition.

Crusts of sperm were found on the distal penis segment of 12 of 14 in copula and postcopula males examined. This and the lack of an obvious density change suggests that displacement primarily involves removal of sperm rather than packing it into a more dense mass prior to insemination.

Comparison of the volumes for tethered females mated once and those mated twice reveals no significant increase in volume for the twice mated females ($F = 0.02$, $df = 1,31$, $p > 0.89$). Sperm therefore, does not appear to be simply added to what was previously there. Similarly, for in copula specimens following one and two matings, there was no difference in sperm volume ($F = 0.15$, $df = 1,43$, $P > 0.70$). Thus 40-48% of the sperm present at the outset of mating must be removed rather than just pushed aside or compacted in the bursa.

Table III shows that there was no tendency for the second of two successive copulations of a female to be longer than the first. This indicates that copulation duration is fairly independent of the amount of sperm a female carries or the recentness of her mating. Despite their brevity, copulation durations did vary at the water. Copulations of undisturbed pairs ranged from 7 to 61 sec for natural pairs and 7 to 46 sec for tethered pairs. Disturbed pairs ranged from 22 to 149 sec. When I examined the relationship between copulation duration and the volume of sperm in natural and tethered postcopula females, no significant increase in

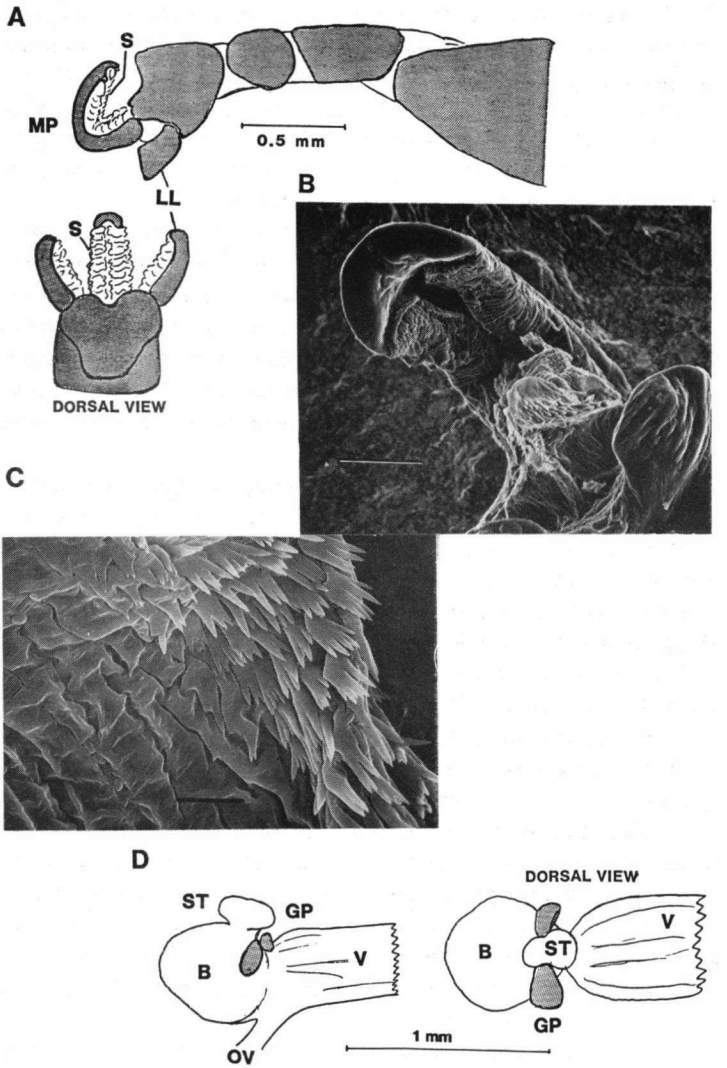

Fig. 2. Reproductive morphology of male and female *Erythemis simplicicollis:* (A) Expanded penis showing erectile structures as in Figure 1. There is no apical lobe and no cornu; — (B) Electron micrograph of the partially expanded 4th segment of the penis, with lateral lobes removed, showing the medial process with its collapsed sacs (scale bar = 100 μm); — (C) Enlarged view of the sacs at the base of the medial process (scale bar = 20 μm) showing spination and folds of the collapsed sac; — (D) Lateral and dorsal views of the female reproductive organs (cf. Fig. 1).

Table III

Number of times that the duration of the second of two successive matings by *Erythemis* females was shorter, longer or equal to the duration of the first

Number of second copulations that were:	Natural pairs	Tethered pairs	*Total pairs*
> 2 sec shorter	29	5	*34*
= +/- 2 sec	21	9	*30*
> 2 sec longer	23	9	*32*
Total	*73*	*23*	*96*

bursa or total volume was found for increasing duration ($F = 0.017$, $df = 1,31$, $p > 0.80$). Females occasionally had no sperm in the bursa when examined. Eleven of 43 in copula females had none, and 2 of 37 postcopula females. No mature precopula females had an empty bursa. The absence of sperm in postcopula females may represent (a) accidental separation of pairs prior to insemination, (b) matings with males who had temporarily exhausted their sperm supply, or (c) matings with males who had failed to transfer sperm properly from their testis to the penis vesicle.

The penis of *Erythemis simplicicollis* (Fig. 2A-C) differs from that of *Celithemis* in lacking an apical lobe. Instead there are two chitinous lateral lobes and a curved medial process on which are two inflatable sacs (S). Near the base of the medial process these sacs are very spinous (Fig. 2C), but along most of the length of the lobe there are no spines. When collapsed, the sacs on the medial process are folded in such a way that they might trap or remove sperm (Fig. 2B & C). There are also spines on the base of the medial process and lateral lobes that might function in providing traction for movement of the medial process into the bursa, or for removing sperm from the bursa into the vagina.

The female also differs from *Celithemis* (Fig. 2D) in having a large sac-like bursa (B) to which is attached a small, single spermatheca (ST). The bursa of one specimen of *Erythemis simplicicollis* had a shape that appeared to have been molded to the expanded sacs on the medial process of the penis. I assume that only the medial lobe of the penis enters the bursa and that no access to the spermatheca is possible for the male.

DISCUSSION

SPERM DISPLACEMENT

Discussion below assumes that changes in volume reflect changes in numbers of sperm. SIVA-JOTHY (1984, 1986a) has shown that this may not always be the case. I therefore assume that most of the volume is sperm and not seminal

fluids or that sperm are uniformly mixed with these fluids. Changes in density (opacity of sperm mass) reported above for *E. simplicicollis*, are also assumed to reflect changes in sperm density and number and not just amount of seminal fluids.

Given this caveat, it is evident that both *C. elisa* and *E. simplicicollis* males are capable of removing sperm stored in the bursa copulatrix of their mates. The tendency for an increase in spermatheca volumes during copulation (Tabs I, II) suggests there may also be some repositioning of stored sperm; perhaps affected by the female (P.L. Miller, pers. comm.).

The magnitude of displacement from the bursa is greater for *C. elisa*, which has the longer copulation duration (5 min vs 20 sec). Comparison of pre- and in copula bursa volumes suggests that about 68% of bursal sperm in precopula females is removed by *C. elisa* males. Given this magnitude of sperm removal, it is surprising that *E. simplicicollis* takes less than 20 sec to remove 40-48% of the sperm stored in the bursa. Since *Erythemis* females store about 1.5 times more sperm in their bursa than do *Celithemis* females, *E. simplicicollis* males probably remove more sperm in 20 sec than *C. elisa* males remove in 5 min. Recent work on *Perithemis tenera* (E. Routman unpubl.) suggests that as much as 80% or more sperm removal occurs in a 15 sec copulation. Thus it is clear that short copulations can involve substantial degrees of sperm removal.

The picture in libellulids is further complicated by the fact that copulation durations and sperm displacement can vary within a species. SIVA-JOTHY (1986a, 1986b) shows that short (21 sec) copulations by *Orthetrum cancellatum* result in only a 10-15% decrease in female sperm volume while longer ones (15 min) result in nearly 100% removal of stored sperm (cf. also MILLER, 1983 and SIVA-JOTHY, 1984). Thus, it is not yet possible to make simple generalizations across species about copulation durations and the degree and type of sperm displacement.

SPERM PRECEDENCE

A further complication is the relationship between the portion of sperm stored by a female that belongs to the last male to mate with her and the actual percentage of eggs that will be fertilized by that male, his sperm precedence. Estimates of volume or number of sperm belonging to the last male may initially underestimate his sperm precedence. For example, McVEY & SMITTLE (1984) have used x-ray irradiation techniques to show that the last male to mate in *E. simplicicollis* fertilizes 99.5% of the eggs immediately oviposited by his mate. Sperm removal and replacement by a male thus affords him greater sperm precedence than is expected based on my volume estimates, which suggest that 54-58% of the sperm in the female's bursa belongs to the last male to mate. Therefore, *Erythemis* males seem to gain nearly complete access to eggs fertilized during oviposition by removing modest amounts of sperm in a short period from

a critical location in which they leave their own sperm (cf. also FINCKE, 1984; SIVA-JOTHY, 1986a, 1986b; WAAGE, 1986).

McVEY & SMITTLE (1984) also point out that mixing of sperm of two or more males occurs such that the fertilization rate of the last male drops to 75% within 24 hrs and 64% after two days. My sperm volume data indicate that if complete mixing were to occur after two days the last male would be expected to fertilize 54-58% of the eggs; which is reasonably close to their estimate of 64%. McVEY & SMITTLE (1984) suggest that actual precedence by the last male two days later may be closer to 55%. Note that they quote an inaccurate value of 48% of the bursal sperm as belonging to the last male, which I gave in an earlier draft of WAAGE (1984). Thus, it appears that sperm removal, even when as low as 50-60%, gains fertilizations for males by both positioning their sperm in the right place for immediate access to eggs and by diluting the numbers of competitor sperm when and if mixing of sperm occurs in the bursa. In addition, repositioning of sperm would also give a large immediate fertilization advantage to odonate males such that the unique adaptive significance of removal may be in longer term precedence, the prevention of sperm mixing, or providing more room for sperm of the last male.

FEMALE AND MALE MORPHOLOGY AND SPERM DISPLACEMENT

It is not clear exactly how sperm removal occurs in either species. Two possibilities are: (a) inflation of sacs on the medial process forces sperm out of the bursa and into the vagina from which it is then pulled out of the female by spines at the base of the medial lobe and those on the apical lobe; or (b) the sperm is somehow caught in folds of the sacs when they deflate during withdrawal of the penis from the bursa. MILLER (1982) describes a similar "snapping zone" near the base of the medial lobe in *Nesciothemis farinosa* that might relate to the second possibility for sperm removal by *Erythemis*. More detailed measurements of expanded penis morphology and female storage organs are needed to elucidate the mechanism(s) of displacement.

The major difference in morphology between *Celithemis elisa* and *Erythemis simplicicollis* is that the distal penis segment of *E. simplicicollis* has fewer elements, larger inflatable sacs, and the female stores most of the sperm in her bursa. MILLER (1982, 1984), SIVA-JOTHY (1984) and WAAGE (1984) suggested that simplified penis structure and large inflatable structures could quickly enter and reposition sperm in easily accessible, simple female sperm storage organs (e.g. single sac-like bursa or spermathecae). In contrast, they argued that removal of sperm should correlate with more complex penis and sperm storage organ morphologies. Although apparently true for some species (MILLER 1982, 1984; SIVA-JOTHY, 1984), simplified morphology and sperm repositioning (packing) rather than removal is not supported by either *Erythemis*

or *Perithemis* (E. Routman, unpubl.). In addition, *Sympetrum rubicundulum* males have penis morphology that appears to pack sperm into large spermathecae; but the duration of copulation is 5-10 min or more (pers. observation). Thus the relationship among male and female morphology, copulation duration and the extent of sperm removal or repositioning remains delightfully complex (cf. also WAAGE 1986).

E. simplicicollis and *C. elisa* represent but two variations in reproductive patterns for common pond libellulid species. They differ in the location of pair forming and copulation, the frequency and duration of mating and oviposition, and the nature of postcopulatory interactions. They may reflect, at an interspecific level, the kind of trade-off between many short copulations with relatively little removal of sperm (*Erythemis*) and longer, less frequent copulations with more complete sperm removal (*Celithemis*) that SIVA-JOTHY (1986a, 1986b) has found within a single species. Thus there seem to be a variety of morphological and behavioral ways to maximize fertilization rates via sperm displacement, copulation frequency and post- or precopula guarding (WAAGE, 1984; MILLER et. al, 1984; SIVA-JOTHY, 1986a, 1986b).

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