

SPERM DISPLACEMENT BY MALE *LESTES VIGILAX* HAGEN (ZYGOPTERA: LESTIDAE)

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Male *L. vigilax* are shown to displace (remove and reposition) sperm from previous males stored in the bursa copulatrix of females they mate with. Females carry sperm from two or more matings in a large bursa copulatrix. Spermathecae do not exist in this lestid. The magnitude of sperm displacement, while difficult to estimate because of changes in sperm density, appears to be on the order of 50%. Displacement occurs primarily from the central region of the bursa adjacent to the vagina. The last male to mate appears to gain a fertilization advantage by not only displacing the sperm of previous males, but also by placing his sperm closest to the area where fertilization occurs. Male *L. vigilax* penis morphology used for sperm displacement is less complex than that used by coenagrionid and calopterygids so far studied.

INTRODUCTION

Whenever two or more males inseminate an insect female prior to her oviposition, an important evolutionary question is: which male's sperm fertilize her eggs. Competition among males and their gametes for fertilization of eggs is an important facet of sexual selection. It is believed to underly the evolution of many variations in genitalic morphology and behavior that aid males in winning and avoiding sperm competition interactions (PARKER, 1970; SMITH, 1982). Several odonate species are known or expected to be capable of sperm displacement — the removal or repositioning of some or all of the sperm of previous males prior to fertilization (MILLER & MILLER, 1981; MILLER, 1981, 1982a; WAAGE, 1979a, 1982).

To date, three of the four major temperate zone zygopteran genera (*Calopteryx*, *Argia* and *Enallagma*) have been examined¹ for their sperm

displacement ability (MILLER & MILLER, 1981; WAAGE, 1979a, 1982). This paper presents evidence that the fourth major genus (*Lestes*) and a member of a third family (Lestidae) is also capable of sperm displacement, despite having a penis morphology lacking some of the major structures used by other zygopterans for removing sperm from the bursa copulatrix and spermatheca of females.

METHODS

Indirect assessment of the sperm displacement ability of male *Lestes vigilax* Hagen was accomplished by comparing sperm volumes carried by samples of females collected before, during and after copulation (cf. WAAGE, 1979a and 1982 for details). Specimens were collected in late July and early August, 1980 at a small man-made pond in Arcadia State Park, Exeter, Rhode Island. They were immediately killed and preserved in 70% ethyl alcohol and stored at 4°C until dissection.

The female genitalia and sperm storage organs (Figs 2-3) were removed by pulling off the ventral sclerite of the 8th abdominal segment with dissecting forceps and then pulling upward and proximally on the ovipositor valves. This removes the ovipositor, vagina, bursa copulatrix and spermatheca plus surrounding tissues, leaving behind the more fragile oviducts. The surrounding tissues were then teased and pulled away from the genitalia with fine point dissecting forceps after first soaking the specimen in a 1:1 mixture of 10% acetic acid and ethyl alcohol for about three minutes. The acetic acid treatment does not appear to affect the size or shape of zygopteran sperm.

Sperm volumes were estimated by drawing side and end views of the sperm mass visible through the transparent walls of the bursa copulatrix/spermatheca using a camera lucida at 100x magnification. The volume of this sperm mass was then estimated by assuming it was a uniformly dense, irregular rectangle and estimating the surface area (side view) and depth (end view) from measurements of the camera lucida drawings. Whenever the sperm mass or some portion of it was shaped more like a sphere than a rectangle, its volume was estimated by measuring its average diameter. The assumption of uniform density of sperm was not always met for *L. vigilax* (cf. results and discussion). Using these estimates to assess the magnitude of displacement is therefore problematic. The volumes estimated by this method are only considered as relative indices and are intended to show that differences exist among females collected in different contexts.

Data from these measurements were analyzed using analysis of variance and results are presented in text and tables as mean + standard error.

RESULTS

CHANGES IN SPERM VOLUMES DURING MATING

The indirect method used in this study to detect sperm displacement involves comparison of average sperm volumes carried by samples of females collected in one of three contexts: precopula, interrupted copula and postcopula (WAAGE, 1979a, 1982). Precopula specimens included six pairs taken after sperm translocation occurred but prior to the formation of the wheel (copulatory) position; plus three females collected as they came to the water during the daily mating period. Since no unaccompanied oviposition by females was observed, I assumed that these three females would soon have mated once discovered by

males. All three females contained large numbers of mature eggs which indicated that they were not leaving the pond after ovipositing. These nine precopula specimens had the greatest range of variation in sperm volumes (Fig. 1, Tab. I) including the highest found in the study. The data indicate that female *Lestes vigilax* generally come to the water carrying sperm from previous matings and that they will mate again before oviposition.

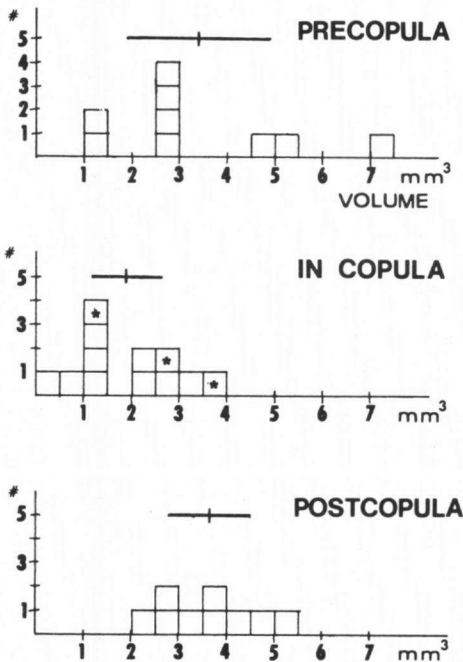


Fig. 1. Histograms of sperm volumes (mm^3) in females of *Lestes vigilax* collected (a) before, (b) during and (c) after copulation. * = Females in copula with males whose sperm vesicles were less than full. Mean and 95% confidence limits are shown for each histogram.

Interrupted copula specimens were pairs collected after sperm translocation had occurred but prior to insemination, which in Zygoptera appears to occur during the final stages of copulation when the male's abdomen is arched dorsally and anteriorly (MILLER & MILLER, 1981). Durations of these copulations ranged from 3 to 10.5 min. Because of the considerable variation in duration of in-copula pairs, the magnitude of sperm displacement is probably underestimated. Males from three of the 12 interrupted-copula pairs were lost and could not be checked to see if they had transferred sperm from their sperm vesicles. Six of the remaining 9 had full vesicles and 3 had partly empty vesicles. These latter males may thus have been transferring sperm to the females when collected. The volumes of sperm for two of the three females associated with the three males with partly empty vesicles were greater than the average for the

remaining females (cf. Fig. 1). This suggests that some of the sperm that these three females were carrying might have come from their current mate. However, since male sperm vesicle contents vary (see below) these three females are included in the interrupted-copula sample.

Postcopula females were obtained from pairs that had broken from the wheel position and were flying toward the water, presumably to oviposit. *Lestes* is known to break the wheel position one or more times during a single copulation

(BICK & BICK, 1961; BICK & HORNUFF, 1965), which makes identification of the postcopula pairs difficult. However, males from 7 of the 9 presumed postcopula pairs had no more than traces of sperm left in their sperm vesicles, indicating that these were indeed postcopula pairs. Males from the other two pairs were lost during capture.

Table I reveals that the average sperm volume carried by interrupted-copula females was significantly less ($F=9.05$, $p<0.01$) than that in precopula or postcopula females. Pre- and postcopula sperm volumes did not differ significantly ($F=0.044$, $p>0.75$). Interrupted-copula sperm volumes averaged 50% less than those carried by pre- and postcopula females, but this may not accurately indicate the degree of sperm displacement (see below). While histograms of the sperm volume data (Fig. 1) reveal considerable overlap, there is a clear trend toward decreased sperm volumes for interrupted-copula females. The greatest variability occurs, as would be expected, among precopula females. Thus while sperm displacement appears to occur in *Lestes vigilax*, it is by no means as dramatic as in *Calopteryx maculata* (WAAGE, 1979a), where nearly 100% of the sperm of previous males is removed prior to insemination.

Table I
Sperm displacement by *Lestes vigilax*

Context	N	Sperm volume (mm ³)	
		Mean	S.E.
Precopula	9	3.41	0.66
In copula	12	1.92	0.34
Postcopula	9	3.65	0.37

Analysis of variance

Source	df	SS	F	p
Among	2	19.105	4.59	<0.025
In vs Pre + Post	1	18.844	9.05	<0.01
Pre vs Post	1	0.261	0.13	NS
Within	27	56.210		
<i>Total</i>	29	75.315		

SPERM DISPLACEMENT — REMOVAL AND REPOSITIONING

Figures 2 and 3 show the basic morphology of the penis and sperm storage organ of *Lestes vigilax*. Relative to other zygopteran families, lested penes have fewer morphological features that seem designed for sperm removal. Calopterygids and coenagrionids use a spine-covered, recurved flap-like (*Calopteryx* and *Enallagma*) or hook-like (*Argia* and *Ischnura*) distal appendage

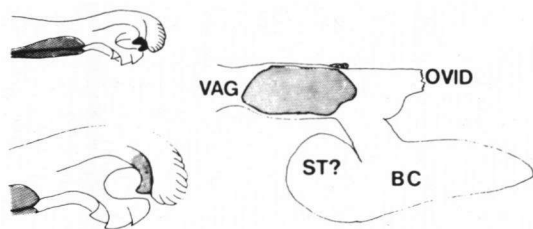


Fig. 2. Genitalic morphology of male and female *Lestes vigilax*. Distal segment of male penis (to scale and enlarged) is on the left, female genitalia on the right. OVID=oviduct, VAG = vagina, BC = bursa copulatrix, and ST? = possible homologue to spermatheca.

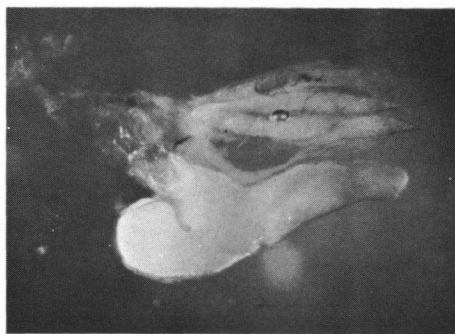


Fig. 3. Photomicrograph of postcopula *Lestes* female genitalia showing sperm in bursa and eggs in oviduct.

(mean = $0.86 \pm 0.7 \text{ mm}^3$) which is only 25% of the volume index for postcopula females. The sperm in the male's vesicles does appear denser than that in the female's storage organs (cf. discussion), and thus these volume comparisons may be misleading. However, it still seems likely that males neither remove all previous sperm from the female, nor entirely fill the female's storage organ in a single insemination.

That some sperm is removed during the pumping stage of copulation is evident from the following observations. First, in three in copula preparations there was a mass of sperm exuding from between the female's ovipositor valves or located in the distal region of the vaginal area and behind the male's penis. Since the sperm vesicles of two of these males were not completely full, it is possible, though unlikely, that this sperm might have been the male's. However, the third male's sperm vesicle was full, indicating that the extruded sperm was not his own. Dissections of in copula pairs indicate that the distal segment of the

for removing sperm from the female's bursa copulatrix. Both sperm volume and density (as judged by differences in the transparency of the sperm mass) change during copulation. This raises the question as to what extent these changes reflect (1) removal of sperm using the relatively simple lested penis morphology or (2) repositioning and compaction of previous sperm within the female's storage organ. The data presented below indicate that both removal and repositioning may occur.

The volume of sperm in postcopula females exceeded that found in the sperm vesicles of males prior to insemination. The index of sperm vesicle volumes for 6 males ranged from 0.67 to 1.08 mm^3

penis moves between the vagina and the central region of the bursa during copulation. This movement could draw sperm out of the bursa and/or push it aside from the central area of the bursa. Six of nine males from interrupted copula pairs had sperm on the distal segment and ventrally in the small cup beneath it. Since sperm is transferred to the female along the dorsal surface of the penis (WAAGE, 1979a), sperm present on the ventral surface of the distal segment probably was that of previous males.

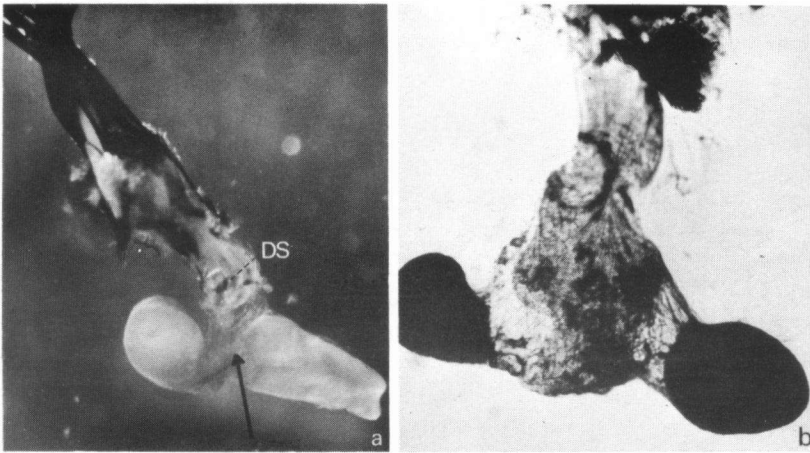


Fig. 4. Photomicrographs of two in-copula preparations: (a) Sperm has been partially removed and displaced from central area of bursa (arrow). Distal segment of male penis (DS) is in the vagina; — (b) Preparation showing nearly empty and partially collapsed central area of the bursa and two densely packed sperm masses in the ends of the bursa.

Five of the interrupted copula pairs were collected in copula by cutting first the female's and then the male's abdomen (cf. WAAGE, 1982) and preserving the joined abdomens in ethanol. Dissection of these in copula pairs revealed that the region of the bursa closest to its junction with the vagina was relatively free of sperm (Fig. 4a). The remaining sperm in at least one arm of the bursa was visibly denser (more opaque) than that found in pre- and postcopula females (Fig. 4b). This suggests at least a temporary compaction of previous sperm into the distal ends of the bursa during the pumping stage of copulation. Sperm displacement in *Lestes vigilax*, then, involves both removal and repositioning of previous sperm within the female's elongated bursa.

DISCUSSION

The changes in volume and the positioning of the sperm mass during *Lestes vigilax* copulations, do suggest that significant sperm displacement does occur.

Since changes in both volume and density of the sperm mass within the bursa occur, estimates of the exact degree of removal and repositioning are not possible. Certainly the magnitude of removal is not as dramatic as that found in *Calopteryx maculata* (WAAGE, 1979a) or indicated for *Argia fummpennisi violacea* (WAAGE, 1982). In addition, comparing average volumes of interrupted and precopula females is likely to underestimate the magnitude of displacement since most interrupted copula pairs were taken before completion of the sperm displacement stage of copulation. Thus, while the data strongly suggest a combination of removal and repositioning of sperm of previous males prior to insemination, the exact consequences for the sperm precedence of the last male to mate can only be assumed. Postcopula females certainly carry sperm from at least two males, and only genetic markers or irradiated male techniques (BOORMAN & PARKER, 1976) can produce accurate information on their relative success at fertilizing eggs.

Several factors, however, seem to indicate that the last male to mate has a considerable fertilization advantage. First, his sperm are released after that of previous males has been pushed aside and/or partially removed. His sperm then occupy the area of the bursa copulatrix closest to the vagina, where fertilization most likely occurs. Second, zygopteran sperm appear to be stored in a dense, entangled mass which would retard the mixing of separate ejaculates in the bursa. This entangled nature of zygopteran sperm is indicated by the ability of males of *Calopteryx* and *Argia* to snare and remove large clumps of sperm with proximally oriented spines on their distal penis segments. MILLER (1982b) has found differences in the mobility of sperm in the bursa (immobile) and spermatheca (mobile) of female *Enallagma cyathigerum*.

Finally, immediately following copulation *Lestes vigilax* pairs fly to the water and begin tandem oviposition. Thus there is little time for mixing of ejaculates prior to fertilization. Oviposition durations for *L. vigilax* were not timed, but in *L. unguiculatus* exploration in tandem after copula averaged 28 min and oviposition in tandem averaged 99 min (BICK & HORNUFF, 1965). It is possible, of course, that females might accelerate mixing of sperm in the bursa by muscular contractions either before or during oviposition. Thus while it seems likely that the last male to mate has considerable sperm precedence during the subsequent oviposition bout, this precedence may not be complete.

An examination of Table I shows that the difference in sperm volumes between interrupted copula and post copula females (1.73 mm^3) is greater than that estimated for male sperm vesicles (0.86 mm^3). This presumably reflects one or both of the following: (1) expansion (doubling) of the volume of sperm in the vesicle upon insemination, or (2) re-expansion of the sperm already in the bursa from its compacted state during sperm displacement. The presence in five of nine postcopula females of two or more distinct sperm masses, with one or more densely packed (opaque) (Fig. 4), suggests that the second alternative is unlikely.

The volume of the less dense mass(es), presumed to be largely that of the last mate, averaged 1.83 mm^3 in these 5 females. This agrees well with the estimate of a 1.73 mm^3 difference in volume between interrupted and postcopula female sperm volumes, especially when it is remembered that this less dense sperm mass probably includes some sperm from previous males. Thus it appears that the volume of sperm in the sperm vesicle approximately doubles to fill the area of the bursa from which previous sperm has been displaced. In turn, this suggests that up to one-half of the sperm in postcopula females may belong to the last male to mate with them.

Given that there is sperm displacement and probably sperm precedence by the most recent male to mate, it seems reasonable to hypothesize that tandem oviposition in *Lestes* functions to prevent take-over and remating of a female before she has oviposited eggs probably fertilized by her last mate. (WAAGE, 1979b).

The series lestid - coenagrionid - calopterygid represents an increasing complexity of male and female genitalic morphology. This is roughly paralleled by increased behavioral complexity in the degree of territoriality, courtship and postcopulatory behavior, as well as in frequency of mating. Since sperm displacement is virtually complete in *Calopteryx* (WAAGE, 1979a) but not in *Lestes*, one can speculate that there is an important relationship, via sexual selection, between behavioral complexity and mating frequency and the complexity and function of penis morphology. Considerably more work is needed on the functional significance of variations in genitalic morphology and on the role of other factors, besides sexual selection (i.e. habitat structure and density), that influence the evolution of reproductive behavior. However, the similar morphological and behavioral trends that seem to occur in Zygoptera and Anisoptera and even within the libellulids (WAAGE, 1982) suggest that sperm competition may have played an important role in the evolution of odonate morphology and reproductive behavior.

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