considerably shorter than the oligosynaptic visual projections onto the oculomotor nuclei reported to originate from cortical or brainstem regions. Our findings confirm earlier claims from study of normal material from birds, reptiles, and amphibians of the existence of a projection onto the oculomotor nuclear complex arising from the accessory optic nuclei (*17*). Recent retrograde studies have also confirmed the existence of an oculomotor projection in a teleost (*8*), further emphasizing the probable occurrence of this projection in several vertebrate classes. This bisynaptic retinal projection onto the oculomotor nuclei could result in rapid adjustments of the oculomotor muscles in response to a visual stimulus. Furthermore, our recent studies have demonstrated that the contralateral dorsolateral division and the ipsilateral ventral division of the oculomotor nuclear complex innervate the inferior and superior rectus of the same eye, which strongly suggests that the nBOR complex plays a significant role in vertical eye movements.

The recent demonstration that the input to the nBOR is derived exclusively from displaced retinal ganglion cells suggests that displaced retinal ganglion cells may be specifically involved in initiating rapid oculomotor responses to peripheral moving stimuli. This hypothesis is further supported by the finding that displaced retinal ganglion cells are distributed predominantly within peripheral retinal regions (*3*) and the accessory optic nuclei project directly onto oculomotor nuclei and folia IXc,d, and paraflocculus of the vestibulocerebellum (Fig. 2A). The lemniscal nature of a bisynaptic retinal pathway via the accessory optic nuclei indicates that this system has a specific functional role in the control of oculomotor reflexes in response to peripheral retinal stimulation.

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Dual Function of the Damselfly Penis: Sperm Removal and Transfer

Abstract. *The male of* Calopteryx maculata *(Beauvois) (Odonata) uses its penis not only to transfer sperm to the female but also to remove sperm deposited in the female's sperm storage organs from previous matings. Apparently, no such sperm removal function has previously been attributed to the intromittent organ of any animal.*

In most insects eggs are fertilized at oviposition, with sperm stored by the female from previous matings. When two or more males mate with a female before she oviposits, their sperms do not necessarily have equal chances to fertilize her eggs (*1*). Experiments with genetic markers or irradiated males have generally revealed a precedence of the sperm from the last male to mate (*2*); but lack of precedence (*3*) and mixing of sperm from successive matings (*4*) also occur. The mechanism of sperm precedence is poorly understood (*1*, *5*). In some species previous sperm may be forced to the rear of the female's sperm storage organs, resulting in a last in, first out phenomenon (*6*).

Males of the damselfly *Calopteryx maculata* defend ovipositing mates from disturbance and take-over attempts by other males (*7*). Multiple mating (*7*) and sperm storage (see Table 1) occur in females. Because these factors indicate the likelihood of sperm competition, I have investigated the fate of sperm deposited by males successively mating with the same female. No genetic markers are known for this species and females cannot be induced to oviposit under controlled conditions (*8*). Hence, evidence for sperm displacement is indirect and in my experiment restricted to three kinds.

1) I compared amounts of sperm in females after one and two matings, respectively, without oviposition to determine whether sperm from a second mating was simply added to that from the first. Females were collected on the stream, tethered, and presented to territorial males (*9*). Durations of the resulting copulations were timed, and the female abdomens were removed and immediately preserved in 70 percent ethanol. In the laboratory, the bursa copulatrix and spermatheca (Fig. 1A, *bc* and *st*) were removed from each female and the external dimensions of the sperm mass within them were measured with an ocular grid (×25 magnification). These measurements were used to calculate an index of sperm volume stored by a female.

The mean (± 95 percent confidence limits) volume index for females mated once (4.20 ± 0.59; $N = 14$) did not differ from that for females mated to two males within 10 minutes (4.38 ± 0.73; $N = 16$). Dissections of male sperm vesicles revealed that they had transferred sperm to the female (*10*). The duration (mean ± 95 confidence limits) of copulation for first and second matings also did not differ (79.0 ± 15.7 seconds; $N = 13$; 70.0 ± 11.6 seconds; $N = 16$). These results

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indicated that second matings were normal and sperm was not added to that of previous matings, but they did not establish the source of the sperm in the storage organs of females mated twice.

2) Comparisons of sperm volume in females caught in the field (Table 1) showed that sperm displacement occurs and revealed its approximate magnitude. Females were collected (i) perching on shore, (ii) flying in tandem with males before copulation, (iii) after copulation but before oviposition, and (iv) in copula, with copulation interrupted before completion. The mean sperm volume index for the first three groups of females (Table 1) did not differ ($F = 1.478$, $P > .10$); and no female had an empty bursa copulatrix or spermatheca. However, 18 of 24 females from interrupted matings had no sperm in their bursa copulatrix, and the rest had only small amounts (compare Fig. 1, A and B). Nineteen females from interrupted copulations had small to moderate amounts of sperm in the spermatheca and the rest had none. Males from the interrupted copulations had normal amounts of sperm in the sperm vesicle (*10*), an indication that they had not yet transferred sperm to the female. These results revealed that (i) females prior to mating carry substantial amounts of sperm, (ii) this sperm is expelled or removed prior to sperm transfer by a subsequent mate, and (iii) this sperm displacement is about 88 to 100 percent complete (*11*).

Table 1. Indices of sperm loads of *C. maculata* females before, during, and after mating.

Context	*N*	Volume of sperm* (mean ± 95 percent confidence limits)
On stream	17	3.94 ± 0.51
In tandem	16	3.49 ± 0.75
In copula	24	0.41 ± 0.29
Postcopula	14	4.20 ± 0.59

*The index of the volume of sperm mass in the bursa copulatrix and spermatheca of the female was derived from measurements with an ocular scale at ×25.

3) In order to elucidate the mechanism of displacement, I observed copulating pairs directly, dissected pairs in copula, and examined the penis and sperm storage organs with light and scanning electron microscopy. When tethered females were used, pairs in copula were induced to land on a net or my hand where the movement of the copulatory organs could be observed with a ×10 hand lens. In the five pairs so observed the male made regular undulating movements of his first three abdominal segments during all but the last few seconds of copulation. During this time the sperm vesicle was not in a position to transfer sperm to the female via the penis (*10*). In the final seconds of copulation the undulating movement stopped, and the sperm vesicle opening was moved to make contact with the sperm channel of the penis (Fig. 2A, *sc*), presumably for sperm transfer.

In 15 pairs separated after 16 to 75 seconds of copulation while the undulating movement was in progress, the male's sperm vesicle always contained sperm and in only two was there any in the sperm channel. In 12 of the males there was sperm (presumed to be from the female) on the head of the penis (Fig. 2B, *sm*) or in the cavity beneath it (Fig. 2A, *ph*). Of the 15 females, 13 had no sperm left in the bursa copulatrix and only small amounts in the tips of the spermatheca (Fig. 1B, *st*). The sperm volume index (mean ± 95 confidence limits) for these females was 0.18 ± 0.06 ($N = 14$). Clumps of sperm were found on the ovipositor valves of several females and in the cavity housing the copulatory equipment of six of the males. Thus the undulatory phase appears to involve sperm removal but not transfer.

In six pairs separated during the pause following the undulatory phase the male's sperm vesicle was partly ($N = 2$) or completely empty ($N = 4$). There was sperm in the penis sperm channel of four of these males and only one female had an empty bursa copulatrix. These results suggested that sperm transfer follows the undulatory phase.

Nine pairs were preserved in copula (at 30 to 110 seconds) and dissected (*12*). In six of these, the penis head was in the vagina and in three it was completely within the bursa copulatrix, and one of the lateral horns of the penis head (Fig. 2B, *hrn*) was in one of the spermathecal branches (Fig. 1A, *st*). These dissections suggested that the penis head moves in and out of the bursa copulatrix during

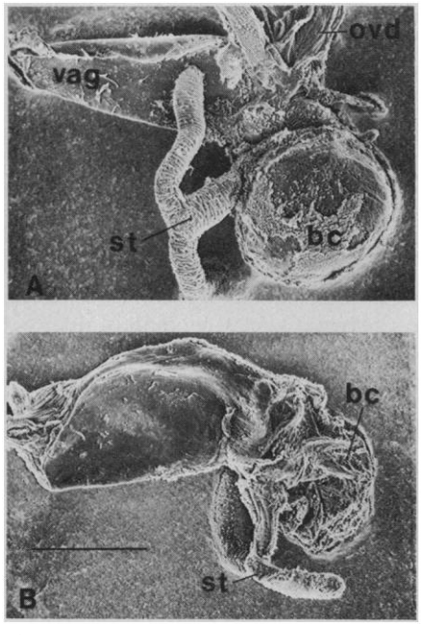

Fig. 1. Scanning electron micrographs of female internal reproductive tract and sperm storage organs. (A) Side view of postcopula female showing the bursa copulatrix (*bc*) and T-shaped spermatheca (*st*) full of sperm. The vaginal canal (*vag*) and paired oviducts (*ovd*) are also indicated. (B) Side view of female from an interrupted copulation (following sperm displacement) showing the empty bursa copulatrix (*bc*). The spermathecal tubes (*st*) contain sperm only in the distal tips. Under light microscopy, the bursa and spermatheca are transparent and the sperm mass within is easily seen. Scale bar is 0.5 mm.

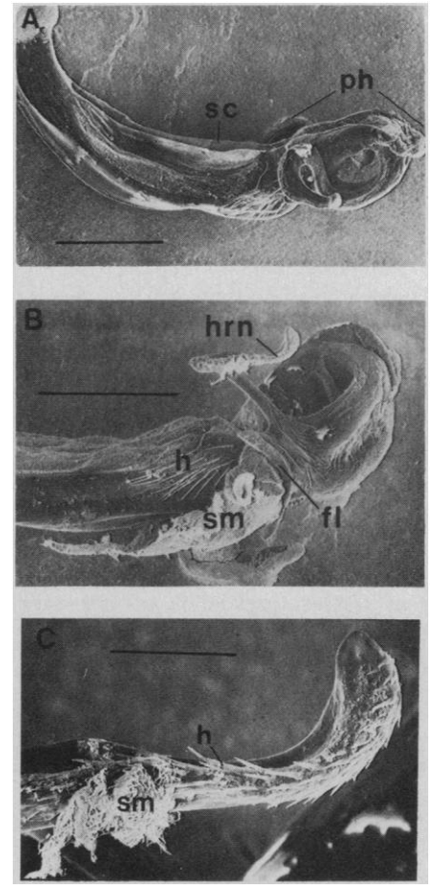

Fig. 2. Scanning electron micrographs of the male penis. (A) Side view of male penis showing sperm canal (*sc*) that runs along the dorsal side of the penis from its base (left) along the rigid shaft, and over the flexible and recurved penis head (*ph*). (B) Penis of male in copula showing a mass of sperm held on the ventral side by long, stiff hairs (*h*) and extending under the scooplike flange (*fl*) of the flexible penis head. A pair of hornlike processes (*hrn*) extend laterally from the penis head. (C) Close-up view of the tip of the lateral horn [*hrn* in (B)] used to remove sperm from the spermathecal tubes (Fig. 1A, *st*). The stiff, backward pointing hairs (*h*) of the horn are holding a sperm mass (*sm*). Scale bar is 0.5 mm for (A) and (B) and 0.1 mm for (C).

the undulatory phase of copulation and that the horns of the penis head remove sperm from the spermatheca.

Scanning electron microscopy (*13*) revealed several morphological features of the penis that could aid in sperm displacement. First, the flexible head of the penis (Fig. 2A, *ph*) is probably extensible by internal fluid pressure (*14*) and may aid in scooping or displacing sperm from the bursa copulatrix. In Fig. 2B a mass of sperm (*sm*) is held under the distal flange (*fl*) of the penis head shown folded back on the venter of the penis. The backward pointing hairs on the venterolateral surfaces of the base of the penis head (Fig. 2B, *h*) aid in removing the sperm mass. Removal of sperm from the spermathecal tubes involves backward pointing hairs on the horns of the penis head (Fig. 2B), seen at higher magnification (Fig. 2C) to be holding a clump of entangled sperm.

The penis of *Calopteryx maculata* thus serves the dual function of sperm removal and sperm transfer. It appears that such a dual function has not previously been reported for the intromittent organ of any animal.

The postcopulatory behavior of the Odonata falls into three general classes: (i) no postcopulatory association of mates, (ii) oviposition in tandem, and (iii) noncontact guarding of an ovipositing mate (*15*). The first class involves oviposition by females in hidden locations, or away from male activity, or after the daily or seasonal period of male activity. These behaviors can be viewed as means of avoiding disturbance during oviposition and, especially in the second and third classes, prevention of take-over and sperm displacement by other males (*1*, *16*).

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9. Females were tethered on monofilament (No. 6 test) fishing line, with a drop of Duco cement on the thorax or between the front wings. Tethered females placed on or moved near the oviposition site in a male's territory elicited courtship and tandem formation. The tandem pair was then allowed to fly up to nearby bushes or the observer's hand for completion of copulation.
10. In damselflies (Odonata), sperm transfer to the female is indirect. The male first transfers sperm from a storage reservoir associated with his testes in abdominal segment 8 to the bulbous sperm vesicle of the copulatory complex on the venter of abdominal segment 2. The organs of this complex are not homologous with copulatory organs of other insects. Sperm is transferred from the sperm vesicle to the female's bursa copulatrix by injecting it into the sperm channel (Fig. 2A, *sc*) on the dorsal surface of the penis (*14*).
11. The estimate of 88 percent was derived by dividing the average index of sperm volume for females in tandem (at premating) by that of females from interrupted copulation (at postdisplacement). Since the interrupted pairs were still in the process of sperm removal, it should be considered a minimum. In 5 of 24 females, 100 percent of the sperm was removed from the bursa copulatrix and spermatheca.
12. Males copulating with tethered females were kept in copula by decapitation with dissecting scissors. The female's abdomen was then cut at segment 6 and the male's abdomen at segment 4 and at the thorax. The remaining abdomens and engaged genitalia were immediately placed in alcohol for storage until dissection.
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Hormone Changes Triggered by Aggression in a Natural Population of Blackbirds

Abstract. *The concentrations of hormones in the plasma of male red-winged blackbirds caught at the height of an aggressive encounter are significantly different from those in males that have not recently engaged in aggressive behavior. Concentrations of luteinizing hormone in the plasma are decreased in the aggressive males, whereas androgen concentrations are affected in a more complex manner. Concentrations of corticoids do not appear to be affected by aggressive behavior.*

Aside from seasonal variation in reproductive function, circulating steroid and gonadotropin concentrations in male vertebrates were once thought to be relatively stable or tonic. However, recent research has shown that circulating hormones can be rapidly and profoundly affected by an animal's social interactions. In a variety of species in captivity, copulation or mere exposure to a conspecific female increases the concentration of testosterone in the male's plasma (*1–4*). Increases in plasma luteinizing hormone (LH) after copulation have also been reported (*5*). Other workers (*6–7*) have found striking changes in circulating hormones in animals that have participated in aggressive interactions, the general pattern being that the adrenal corticoids increase, whereas LH and testosterone decrease. The rapidity with which these hormonal changes can occur during a social interaction suggests that the endocrine system may have a more important role in the individual's minute-to-minute response to critical social stimuli than was previously realized. This possibility is strengthened by the finding that preventing the normal changes in circulating hormone concentrations during an aggressive encounter significantly altered the behavior of the individual that was being attacked (*8*).

How applicable these laboratory findings are to animals living under natural conditions is unclear. In studies of aggression it is not usually possible to separate the effects of aggression from the effects of social confinement, thus the changes in endocrine function after an aggressive encounter under laboratory conditions may be a normal response to social stimulation or a "stress" response to abnormal social conditions that the animal cannot escape. We present evidence from a natural population of red-winged blackbirds (*Agelaius phoenecius*), demonstrating that hormone concentrations change rapidly as a function of the animal's ongoing behavior.

Because repeated blood sampling in small animals can obscure the effects of behavior on hormone concentrations, (*9*), our study was designed to compare the concentrations of circulating hormones in two different groups of birds rather than one group examined twice. Males from a breeding population of red-winged blackbirds were caught in the town of Washington, Dutchess County, New York, between 15 April and 21 May 1976 under one of two conditions—that is, when the birds were either at the height of an aggressive interaction or foraging. To elicit aggression, a live decoy male was placed in the central

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