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SPERM DISPLACEMENT ABILITY IN THE DAMSELFLY *CALOPTERYX HAEMORRHOIDALIS ASTURICA* OCHARAN: NO EFFECT OF MALE AGE, TERRITORIAL STATUS, COPULATION DURATION AND SYN-COPULATORY BEHAVIOUR (ZYGOPTERA: CALOPTERYGIDAE)

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During copulation and before sperm transfer, odon. ♂♂ are able to manipulate rival sperm stored in the ♀ sperm storage organs (usually the bursa copulatrix and spermathecae). ♂♂ of the territorial *C. h. asturica* use 2 mechanisms for this. Bursal sperm is removed physically whilst spermathecal sperm is displaced via aedeagal stimulation (through a series of abdominal flexions) of the ♀ sensory system that controls spermathecal sperm ejection. Most bursal sperm is removed but there is individual variation in spermathecal sperm displacement. Previous results have found that this variation is related to aedeagal width. In this paper 4 variables that may also explain variation in spermathecal sperm displacement ability are investigated: ♂ age and status (territorial and nonterritorial), duration of the sperm displacement stage and the number of aedeagal stimulatory flexions. Variation in the ability to displace spermathecal sperm, however, was not related to these variables. This suggests that variation in this ability is reliant only on ♂ genitalic attributes, aedeagal width. These results are briefly discussed in terms of current theory of sexual selection as the process propelling genitalic evolution.

INTRODUCTION

Animal genitalia show a great diversity in morphology in males of internally-fertilising species (EBERHARD, 1985, 1996) but the causes of this are unclear. Recent claims have suggested that sexual selection might be the operating force propelling genitalic evolution (EBERHARD, 1985, 1996; ARNQVIST, 1998).

During copulation, but prior to sperm transfer, odonate males have the ability to manipulate the stored sperm of rivals using their specialised genitalia (e.g. WAAGE, 1979; MILLER, 1987; CORDERO & MILLER, 1992). There is, however, little evidence of male variation in sperm displacement ability, based on genital morphology, to suggest that sexual selection might be acting on the male genitalia. Recently, however, experimental evidence has suggested that sexual selection is the process responsible for genitalic evolution in the territorial damselfly *Calopteryx haemorrhoidalis asturica* (CÓRDOBA-AGUILAR, 1999). Males of this species defend riverine spaces which contain resources that females use during oviposition (CÓRDOBA-AGUILAR, 2000). Not all males are able to defend territories and, consequently, some males adopt a nonterritorial behaviour. However, a nonterritorial tactic does not prevent a male from obtaining copulations (CÓRDOBA-AGUILAR, 2000).

The behavioural processes that occur during copulation in *C. h. asturica* are similar to those of other odonates (e.g. MILLER & MILLER, 1981): males perform a number of abdominal flexions which are related to the mechanisms that occur in the female genitalia. During stage I (sensu MILLER & MILLER, 1981) males, through a series of 50.2 ± 7.2 abdominal flexions which lasts 104.2 ± 10.2 seconds (CÓRDOBA-AGUILAR, 2000), displace the sperm stored in the female's sperm storage organs (the bursa copulatrix and spermathecae). During stage II (sensu MILLER & MILLER, 1981) males transfer their sperm to the sperm storage organs (CÓRDOBA-AGUILAR, 1999). Sperm displacement occurs via two different mechanisms (CÓRDOBA-AGUILAR, 1999). Bursal sperm is removed physically whilst spermathecal sperm is displaced via male aedeagal stimulation, mediated by the abdominal flexions, of the female sensory system that controls sperm ejection during fertilisation. Most bursal sperm is displaced but there is considerable variation in spermathecal sperm displacement. The most likely explanation for this variation is that there are individual differences in the aedeagal morphology which result in differences in stimulation (CÓRDOBA-AGUILAR, 1999; for a similar suggestion in another damselfly species see ANDRÉS & CORDERO, 2000).

Variation in sperm displacement ability has been previously documented in odonates and the variables that have been demonstrated to have an effect on this variation are male status (territorial or nonterritorial; SIVA-JOTHY & TSUBAKI, 1989; WOLF et al., 1989), male age and copulation duration (SIVA-JOTHY, 1987). Another potential source of variation in sperm displacement ability particular to *C. h. asturica* may be the number of stimulatory abdominal flexions occurring during stage I.

In this study I investigate the effect of male status (territorial and nonterritorial), male age, the duration and number of flexions during the sperm displacement stage I in *C. h. asturica*. My aim is to explore if these variables could also explain the observed variation in male sperm displacement ability.

MATERIAL AND METHODS

Field work was carried out in a narrow stream in SW Spain (see CÓRDOBA-AGUILAR, 2000 for a description of the field site) in July and August, 1996, 1997 and 1998. Because this was part of a long-term study, most animals had been individually marked, using an indelible ink pen, by the time the manipulations were made. Two male age categories were defined according to the following criteria (see also JOHNSON, 1973; PLAISTOW, 1997 for a rationale of this aging method): (a) "young", individuals with fully developed colouration and "shiny" or "glassy" wings; and (b) "young-old", animals with a dusty appearance of the thorax and abdomen but a degree of "shininess" on the wings.

Females in the field have variable volumes of spermathecal sperm (unpub. data). For the logic of the treatments described below, however, it was necessary to have females with filled sperm storage organs previous to manipulations. This was carried out by inducing females to complete copulations using the "hand-pairing" technique (OPPENHEIMER & WAAGE, 1987).

EFFECT OF MALE AGE ON SPERM DISPLACEMENT – The volume of stored sperm was measured in females that had been paired to either a "young" or "young-old" territorial male respectively (two different sets). Previous analyses of censused individuals had suggested that the difference in age between these two categories varied from seven to ten days (8.3 ± 2.4 days, N = 53 males). Since males were observed for 15-18 days (from the first to the last observation during daily censuses), a difference of seven days was the best logistic compromise to test the effect of age. Copulations were interrupted at the 50th abdominal flexion, since this is the behavioural event at which maximum sperm displacement occurs (CÓRDOBA-AGUILAR, 1999). Sperm volume was compared for both groups of females.

EFFECT OF MALE TERRITORIAL STATUS (TERRITORIAL OR NONTERRITORIAL) ON SPERM DISPLACEMENT – Two groups of females were paired to a similar number of "young-old" territorial and "young-old" nonterritorial males respectively. Copulations were interrupted at the 50th abdominal flexion. The volume of sperm of both groups of females was compared.

EFFECT OF DURATION OF STAGE I ON SPERM DISPLACEMENT – Sixteen females were paired and duration (in sec) of stage I was recorded. Once the flexions that characterise this stage (for a detailed description of this see CÓRDOBA-AGUILAR, 2000) were completed, pairings were interrupted. The volume of sperm was measured and correlated with the duration of this stage.

EFFECT OF VARIATION IN THE NUMBER OF ABDOMINAL FLEXIONS DURING STAGE I ON SPERM DISPLACEMENT – Sixteen females were paired and the number of abdominal flexions occurring during stage I was recorded. These pairings were interrupted soon after they completed this stage. The volume of sperm was measured and correlated with the number of flexions.

Copulating pairs were preserved immediately after interruption in 75% ethanol. Calculation of sperm volumes (in mm^3) was as described by SIVA-JOTHY & HOOPER (1995). Only spermathecal sperm volume was measured since the bursa was always emptied (see also CÓRDOBA-AGUILAR, 1999). To fulfill the assumptions of the parametric statistical tests used, sperm volumes were transformed using the formula $log(x + 1)$. Means ± standard deviations are provided.

RESULTS

EFFECT OF MALE AGE – Females paired with the two groups of males ("young" males, N = 8; "young-old", N = 7) showed no difference in stored sperm volumes (females paired with "young" males = 0.050 ± 0.03 mm^3; females paired with "young-old" males = 0.047 ± 0.03 mm^3; t-test = -0.31, d.f. = 16, P = 0.76).

EFFECT OF MALE STATUS (TERRITORIAL AND NONTERRITORIAL) – The volume of sperm of females paired with territorial males (0.041 ± 0.02 mm^3, N = 22) did not differ from that of females paired with nonterritorial males (0.048 ± 0.02 mm^3, N = 22; t-test = -0.87, d.f. = 39, P = 0.39).

EFFECT OF DURATION OF STAGE I – Duration of stage I (98.1 ± 9.7 sec) was not correlated with sperm volume (0.053 ± 0.04 mm^3; N = 16, r = 0.074, P > 0.05).

EFFECT OF VARIATION IN THE NUMBER OF ABDOMINAL FLEXIONS DURING STAGE I

– No significant association was detected between the volume of sperm (0.05 ± 0.03 mm^3) and the number of stage I abdominal flexions (51.5 ± 7.8; $N = 16$, $r = -0.14$, $P > 0.05$).

DISCUSSION

In odonates, individual variation in sperm displacement ability has been related to several variables. Male age (SIVA-JOTHY, 1987), status (territorial or nonterritorial; SIVA-JOTHY & TSUBAKI, 1989; WOLF et al., 1989) and copulation duration (SIVA-JOTHY, 1987) appear to be particularly important. In these cases, relatively old (SIVA-JOTHY, 1987) and nonterritorial (SIVA-JOTHY & TSUBAKI, 1989) males copulated for longer periods and displaced more stored sperm. Experimental investigation of the role of male age and status in *C. h. asturica*, however, did not explain the variation in stored sperm volume. The same also applies for the duration and the number of abdominal flexions of the stage I of copulation. Therefore, independently of whether holding a territory, how old a male is, how long or how many abdominal flexions the stage I takes, spermathecal sperm displacement ability does not change in individual male *C. h. asturica*.

Male *C. h. asturica* displace spermathecal sperm through genitalic stimulation of the female sensory system (CÓRDOBA-AGUILAR, 1999). Males perform a series of approximately 50 abdominal flexions during the sperm displacement stage. The flexions generate in-and-out aedeagal movements that result in stimulation of the female sensory system that coordinates spermathecal sperm ejection. Experimental evidence has shown that variation in spermathecal sperm displacement is related to the width of the aedeagus (CÓRDOBA-AGUILAR, 1999). According to the results presented here, variation in sperm displacement ability seems reliant only on male aedeagal characteristics since other potential sources of variation were discarded.

Spermathecal sperm shows a reduction in volume alongside of the number of aedeagal flexions during stage I (CÓRDOBA-AGUILAR, 1999). The results show that the relatively variable number of flexions at the end of stage I did not affect the volume of spermathecal sperm displaced. Since maximum sperm displacement is achieved at about 50 abdominal flexions, one explanation for this apparently paradoxical pattern is that males keep performing stage I flexions even though maximum sperm displacement has been already achieved. The exceeding in male abdominal flexions would not elicit any more sperm displacement. This hypothesis is being currently investigated.

Recent evidence supports the hypothesis that animal genitalia have evolved by means of sexual selection (EBERHARD, 1985, 1996; SIMMONS & SIVA-JOTHY, 1998; ARNQVIST, 1998). If this true, one prediction would be that variation in fertilisation success will be related to variation in the expression of the sexually-selected genitalic trait. In the case of odonates, male genitalia might have evolved to reduce the competition with other males' ejaculates during fertilisation (WAAGE, 1984): those males with a higher sperm displacement ability would achieve a higher fertilisation success. However, although variation in sperm displacement ability has been documented in odonates (SIVA-JOTHY, 1987; SIVA-JOTHY & TSUBAKI, 1989; WOLF et al., 1989), it has not been clear whether sperm displacement ability was related to variation in male genital morphology. In fact, the most likely explanation for these cases is that variation in sperm displacement was dependent on copulation duration (however, see ANDRÉS & CORDERO, 2000 for a case in which, despite variation in sperm displacement ability, there was no relation between copulation duration and sperm displacement). The only documented case of variation in sperm displacement related to variation in genital phenotypic expression is that of *C. h. asturica* (CORDOBA-AGUILAR, 1999): the wider the aedeagus, the more sperm displaced. I suggest more attention has to be paid to similar research avenues in the Odonata since it is unlikely that this would be a unique case.

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