

POST-COPULATORY BEHAVIOUR IN *CALOPTERYX* FEMALES (INSECTA, ODONATA, CALOPTERYGIDAE).

Martin Lindeboom

Institut für systematische Zoologie, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany, e-mail: Lindeboom@aol.com

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Abstract

The post-copulatory behaviour of *Calopteryx splendens* females was studied under field and laboratory conditions. After termination of copulation females usually perch and bend the abdomen so that its apex touches the ground (post-copulatory posture). The post-copulatory posture is a consequence of sperm removal by males. Male and female microstructures (spines and scales) interact to move previous sperm from the female sperm storage organs to the outside during copulation stage I, after which moved sperm is located on the ovipositor. After termination of copulation females require an average of 45 seconds to brush off this sperm (N=21). The post-copulatory behaviour of females may also allow males to chase rival males before the females start to oviposit (prevention of disturbances). The present study shows no evidence of cryptic female choice in *C. splendens*.

Introduction

Sperm competition in Odonata has resulted in the evolution of direct sperm removal in Zygoptera and Anisoptera. Sperm removal resulting in last male sperm precedence seems to be widespread in Zygoptera (Waage 1979a, 1984, 1986; Miller & Miller 1981; Fincke 1984; Siva-Jothy & Tsubaki 1989; Cordero & Miller 1992; Siva-Jothy & Hooper 1995). Sperm removal is also widespread in Anisoptera (Miller 1984; McVey & Smittle 1984; Waage 1984; Siva-Jothy 1984, 1987; Michiels & Dhondt 1988; Wolf et al. 1989). However, there is good morphological evidence to suggest that at least some libellulids achieve sperm precedence by packing down sperm in the female sperm storage organs before inseminating the female (Miller 1982, 1984; Waage 1984).

With the exception of Miller & Miller (1989), Rehfeldt (1989) and Lee (1994) few workers have studied the post-copulatory female behaviour - the behaviour between copulation and oviposition. Miller & Miller (1989) termed the post-copulatory female behaviour in Libellulidae "post-copulatory rest" and suggested some possible proximate causes, for example sperm handling by the female. Rehfeldt (1989) and Lee (1994) studied male interference in *Orthetrum coerulescens* and drew different inferences. However, usually the biological significance of female behaviour between copulation and

oviposition is not discussed. The three last-mentioned reports described libellulid Anisoptera. The present paper describes the post-copulatory female behaviour in the calopterygid zygopteran *Calopteryx splendens*. Heymer (1973: 51f.) and Waage (1979b: 149, 1988: 370) described short latent periods for *Calopteryx* females after copulations. Functional correlates of such post-copulatory rests in *Calopteryx* females have not been studied up to now.

Copulatory activity in *Calopteryx* is divisible into two stages: (1) sperm removal and (2) sperm transfer. Stage I, which usually occupies about 95% of the total copulation duration, entails physical removal of previous sperm from the female's sperm storage organs (bursa copulatrix and two spermathecae); stage II occupies about 5% of the total copulation duration and is associated with sperm transfer (Waage 1979a, Lindeboom 1996). The main aim of this study is to present and discuss results, which show that the post-copulatory behaviour in *Calopteryx* females is directly connected with sperm removal during copulation stage I.

Material & Methods

Post-copulatory behaviour of *Calopteryx splendens* females was investigated under field and laboratory conditions. A natural population of *C. splendens* was studied along Hanfreezen Creek in Freiburg, Germany (48°02'N, 7°49'E). In the study area the width and the depth of the stream was about 1-2 m and 10-50 cm, respectively. The duration of post-copulatory rests was measured as the period between the splitting up of the tandem and the end of the typical post-copulatory posture or the take-off. Further observations on post-copulatory behaviour were carried out under laboratory conditions.

Copulations and post-copulatory behaviour under laboratory conditions

Hand-pairings for obtaining controlled matings in *Calopteryx* under field conditions were described by Oppenheimer & Waage (1987). The modification of this technique for laboratory experiments needs only a strong source of "cold" light in front of the male's head. To obtain a mating, a female is held by her folded wings in one hand, and the male is held in the other hand. The superior anal appendages of the male are moved along the dorsal midline of the female's thorax towards her prothorax where male's appendages are usually spread and grasp the female. After tandem formation the pair is released in front of a point light source (about 15 to 20 cm away), which usually prevents flight. Thus a cage is not necessary. After about one minute the pair usually starts copulation. After termination of copulation the female usually shows typical post-copulatory behaviour. In order to study the function of external genital structures, copulations were shockfrozen with liquid N₂ (during stage I), and transferred to absolute acetone at -20 °C until dissection. Genital structures were dissected in ethanol for scanning electron microscopy. Finally all preparations were critical point dried, sputter-coated with gold and examined in a Hitachi S-800 scanning electron microscope.

Measuring sperm volumes

Sperm volumes were measured in mm^3 by compressing sperm masses from the ovipositor of females or from the sperm vesicles of males to a uniform thickness of $100\ \mu\text{m}$ (using a Fuchs-Rosenthal counter slide; cf. Miller 1987 and Cordero et al. 1995). Scale drawings of the sperm mass were made using a stereomicroscope. The area of the mass was then measured precisely, three times, with a planimeter and the mean value was taken. The volume was estimated as the product of the mean area multiplied by the thickness.

Results

After termination of copulation, the pair separates and the male usually defends his territory or shows the oviposition site to the female. After copulation and before oviposition all females observed ($N=21$) adopted the typical post-copulatory posture (Fig. 1) in which the female bends the abdomen, so that its apex touches the substrate. Females seldom omit this behaviour. The duration of post-copulatory rests ranged between 24 and 160 seconds ($N=21$; mean duration 60 seconds, s.d. 36.5; median 45 seconds). The data were not normally distributed.

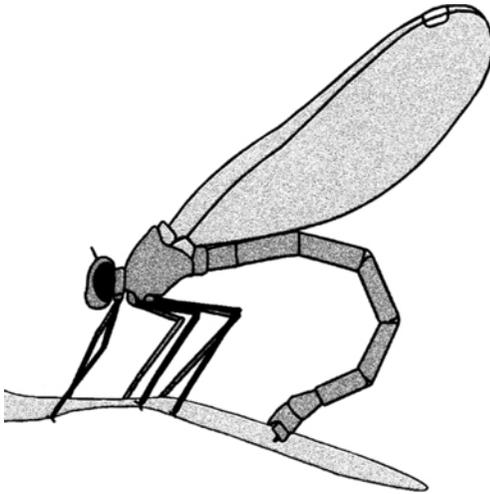


Figure 1. Schematic view of the post-copulatory posture in *Calopteryx* females.

Valvulae I and II (of the ovipositor) are connected by a longitudinal ledge of valvula II and a longitudinal groove of valvula I. This interlocking mechanism allows the valvulae II to slide back and forth on the valvulae I. The movements of the valvulae II represent the functional basis for piercing plant tissue to place eggs into small slits (endophytic oviposition). During the post-copulatory posture the valvulae II move to and fro and a sperm mass falls off or is wiped off onto a substrate. Volume measurements of four twice mated females gave values of 0.15, 0.17, 0.19 and $0.22\ \text{mm}^3$ sperm. These values correspond very

closely to the sperm volume in the sperm vesicle of males (maximum approx. 0.25 - 0.30 mm³ sperm; N=14, after precopula). After copulations with virgin females (N=2) there was no sperm mass at the valvulae, although the females nevertheless adopted the typical post-copulatory posture, including movements of the valvulae.

These findings strongly suggest that the sperm mass at the valvulae is the sperm received during the penultimate copulation. During copulation stage I the male uses the distal structures of the ligula (the copulatory organ in Zygoptera) to remove the sperm already present from the female sperm storage organs. Proximal structures of the ligula (Fig. 2A, B) transport the sperm to the outside (Fig. 3A) and brush it off on cuticular structures of the valvulae (Fig. 3B). *Calopteryx* females possess three different types of posteriorly orientated scales that are found in different locations on the inner side of all valvulae and on the proximal outer side of valvulae I and II. The scales are single and spine-like, lobed or comb-like; the scales vary in length from 1 to 16 μm. Preparations of copulating pairs, shockfrozen during copulation stage I, show that removed sperm is brushed off, especially on the proximal cuticular structures of valvulae II (Fig. 2C). During copulation stage II the male uses the dorsal sperm channel of the ligula to transfer the own sperm to the female sperm storages without contact between sperm and ovipositor scales.

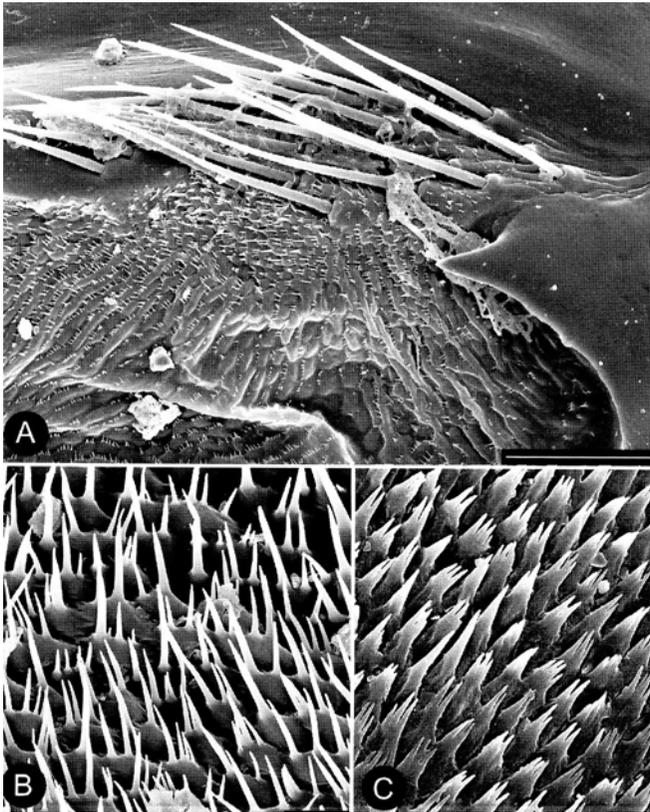


Figure 2. (A) Ventrolateral spines of the ligula. (B) Spiny area on lateral surface of the ligula. (C) Scales on the proximal inner side of the second valvulae. Scale bar for A: 100μm; - for B and C: 10μm.

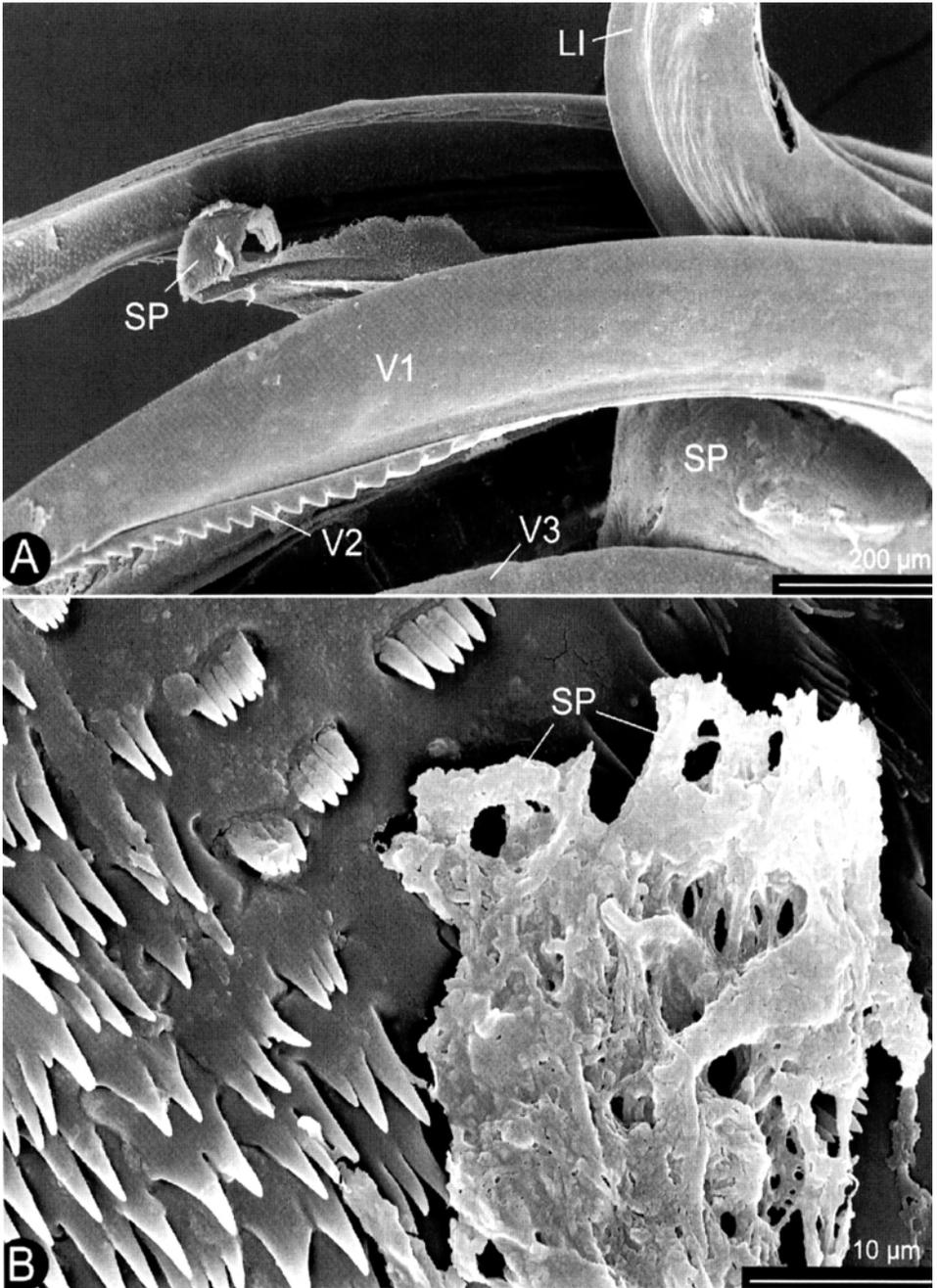


Figure 3. (A) The ligula (LI) between the left and right valvulae (V1, V2, V3 = valvula 1, 2 and 3) during copulation. Removed sperm (SP) accumulates at the base of the valvulae and along the inner side of the first and second valvulae. (B) Removed sperm (SP) at the second valvulae.

Discussion

Table 1 lists the duration of post-copulatory rests in certain libellulid and calopterygid species. In general the mean durations range between one and two minutes.

In this paper one function of the post-copulatory behaviour in *Calopteryx* is demonstrated: *Calopteryx* females use the post-copulatory rest to remove sperm located between the valvulae. This sperm has been transferred to the female sperm storage organs by the penultimate male and then removed by the next male to copulate with her. The female sperm storage organ consists of a bursa copulatrix and two spermathecae joined to the bursa by a common duct. While sperm from the bursa copulatrix is pulled out by the whole distal part of the ligula, the spermathecae will be emptied by spines on the two lateral horns (Lindeboom 1996). Sperm transfer to the outside takes place with spines of the proximal part of the ligula. Finally the removed sperm is brushed off on ovipositor microstructures. Interestingly sperm removal in *Calopteryx* also involves direct interactions between ligula structures and ovipositor structures. Up to now ovipositor scales have been discussed only in relation to the egg transport mechanism (Gorb 1996). The removed sperm on the ovipositor hardens fast and could cause obstruction during oviposition. It is possible that the passage of an egg may be blocked or impeded by hardened sperm so that the post-copulatory cleaning behaviour is of selective advantage.

Miller and Miller (1989) suggested possible functions of the female resting behaviour after copulation in libellulids (e.g. sperm handling, egg handling, time to check the oviposition site for the presence of predators).

Table 1. Duration of post-copulatory rests in certain Libellulidae and Calopterygidae.

Species	Range [seconds]	N	Reference
Libellulidae (Anisoptera)			
<i>Erythemis simplicicollis</i>	3 - 120	?	McVey & Smittle 1984
<i>Nesciothemis farinosa</i>	20 - 70	17	Miller 1982
<i>Orthetrum chrysostigma</i>	? - 505	10	Miller 1983
<i>Orthetrum coerulescens</i>	9 - 109*	22	Miller & Miller 1989
	5 - 565**	39	Miller & Miller 1989
	5 - 380***	48	Rehfeldt 1989
	55 - 170***	28	Lee 1994
<i>Orthetrum pruinosum</i>	60 - 120	4	Miller & Miller 1989
<i>Orthetrum sabina</i>	60 - 120	9	Miller & Miller 1989
Calopterygidae (Zygoptera)			
<i>Calopteryx maculata</i>	30 - 60	?	Waage 1979b
<i>Calopteryx dimidiata</i>	? - 180	?	Waage 1988
<i>Calopteryx splendens</i>	24 - 160****	21	Lindeboom, this paper

* at 25-27 °C, ** at 20-22 °C, *** at 25-32 °C, **** at 27-30 °C

They ended with the statement that “no conclusion about the function of post-copulatory rests can be reached at present” (Miller & Miller 1989: 39). Rehfeldt (1989) found evidence to suggest that rival males have a significant influence on the post-copulatory behaviour of females in *Orthetrum coerulescens*. After copulation interference by rival males, females perched longer than without interference. In contrast to this result Lee (1994) found no evidence that male activity, defined as the number of rival males within a two metre radius of the copulating pair, correlated with the duration of post-copulatory rests in *Orthetrum coerulescens*. However, Rehfeldt and Lee examined different variables (interference by rival males, and density of rival males respectively), although these values are obviously correlated. Up to now there are no reports that male density or interference by rival males influences the post-copulatory female behaviour in *Calopteryx* species, although an influence of rival males cannot be excluded without adequate studies.

There is no reason to suppose that post-copulatory behaviour in different libellulid and calopterygid species serves the same function. A comparison between the well examined libellulid *Orthetrum coerulescens* and *Calopteryx splendens* shows convergent aspects (territorial males, short copulations, post-copulatory female rests) and differences (short ovipositions and a reduced exophytic ovipositor in *Orthetrum*; longer ovipositions and functional endophytic ovipositor in *Calopteryx*). Table 2 shows that copulation duration and duration of post-copulatory rests are similar in these two species. These convergences are consistent with the fact that short copulations allow territorial males to defend territories more effectively. Furthermore, it may be suggested that the post-copulatory resting behaviour of the female allows the male to chase rival males before the female starts to oviposit (thus preventing disturbances and take-overs by other males at the oviposition site).

Table 2. Duration of copulations and post-copulatory rests in *Orthetrum coerulescens* and *Calopteryx splendens*.

Species Reference	Temp. (°C)	Copulation (sec.)			Post-copulatory rest (sec.)		
		mean ± s.e.	range	N	mean	range	N
<i>O. coerulescens</i>							
Miller & Miller 1989	25-27	120 ± 44	21-967	22	56 ± 11	9-109	22
Rehfeldt 1989	25-32	437 ± 49*	10-1795	75	62 ± 7*	5-380	48
Lee 1994	25-32	-	-	-	110 ± 5	55-170	28
<i>C. splendens</i> **							
Lindeboom, this paper	27-30	133 ± 8	46-357	64	60 ± 8	24-160	21

* in round seconds, ** independent samples for copulations and post-copulatory rests

Eberhard (1996) interpreted the existence of sperm droplets at the ovipositor of *Coenagrion scitulum* (Rambur) and other species of Odonata as cryptic female choice. Eberhard (1996: 14) stated: "An even more dramatic indication of possible female influence on paternity in this order famous for supposed male control comes from the recent discovery that female odonates sometimes emit droplets of sperm during or immediately after some copulations but not others (...)", and (Eberhard 1996: 92) "In *Coenagrion scitulum*", however, sperm are not removed from the female by the male during copulation (Cordero et al. 1995), and video tapes of mating reveal apparent sperm droplets emitted from the female during pauses between the repeated intromissions (E. Gonzalez, pers. comm.)".

By contrast with this citation Cordero et al. (1995:446) point out: "Several lines of evidence suggest that sperm are displaced from the genital tract during stage I of cycle 1. ... Furthermore, the first stage I has spontaneous breaks of the copulatory wheel that could be used to expel the removed sperm from the female (Perry & Miller 1991; E. González Soriano, personal communication)". This statement and the results of the present research show that sperm droplets at the ovipositor can be explained without invoking cryptic female choice.

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