

**POST-COPULATORY "RESTING" IN *ORTHETRUM COERULESCENS*  
(FABRICIUS) AND SOME OTHER LIBELLULIDAE:  
TIME FOR "SPERM HANDLING"? (ANISOPTERA)**

P.L. MILLER and A.K. MILLER

Department of Zoology, University of Oxford, South Parks Road,  
Oxford OX1 3PS, United Kingdom

*Received October 14, 1988 / Accepted November 7, 1988*

Mated female *Orthetrum* spp. carry very small volumes of stored sperm compared to many other libellulids. In several *Orthetrum* spp. and in a few other species, most copulations are followed by periods of "rest" (PCRs) lasting from one to several minutes during which the male and female perch close together before the start of oviposition. During PCRs males sometimes show "aggressive" behaviour towards their mates, apparently attempting to induce them to oviposit. Experimental and natural disturbances of female *O. coerulescens* during PCRs were found to excite at least brief oviposition bouts in 10 out of 48 cases. Females captured during or after copulation could be induced to oviposit large egg clutches. Several possible explanations for PCRs are considered: they may allow females to assess predator pressure at the oviposition site, to judge a male's guarding capacity, or to "handle" sperm either mobilising it for fertilisation or selecting it according to the quality of their mates. PCRs do not seem to be needed for egg preparation.

**INTRODUCTION**

Many species of libellulid dragonflies copulate briefly in the air after which oviposition usually follows with minimal delay. However in others copulation may be longer-lasting and is normally completed after perching (e.g. *Orthetrum* spp., *Sympetrum* spp., *Celithemis* spp., *Tramea* spp., *Indothemis carnatica* (Fabr.), *Neurothemis t. tullia* (Drury), *Acisoma panorpoides* (Ramb.), *Diplacodes* spp., *Nannophya pygmaea* (Rambur)). In such species the pair then either takes off in tandem (*Sympetrum*, *Celithemis*, *Tramea*, *I. carnatica*) or separates and may then remain perched for some time before oviposition commences (e.g. *Orthetrum*). Reasons for the occurrence of such post-copulatory "rests" (PCRs) are not known.

The females of *Orthetrum* possess very small sperm-storage organs (bursae and spermathecae) and store much less sperm compared to many other libellulids (MILLER, 1984; SIVA-JOTHY, 1987b). Small volumes of stored sperm might be explained by different methods of sperm competition, the more efficient use of sperm in egg fertilisation, more frequent matings, or the production of fewer eggs, but there is no evidence from dragonflies for any of these suggestions at present. Males in different species may remove the sperm of rivals from females with large or small stores (WAAGE, 1986; SIVA-JOTHY, 1987a; MICHIELS & DHONDT, 1988).

In *Drosophila*, females accept few mates, store sperm for long periods and use it efficiently (TREVITT et al., 1988). In some dragonflies at high population densities, however, females may mate several times during a short visit to the water (WAAGE, 1984; SIVA-JOTHY, 1988) and are likely to have abundant sperm. At low densities, however, when matings are fewer, sperm should be used more sparingly, particularly in species in which the females have small sperm-storage organs. Additionally females may require time to handle sperm after copulation if they place it in a particular store, or if sperm cannot be instantly mobilised for fertilisation. Such limitations are more likely in species with small sperm stores. The possibility that post-copulatory resting may allow some species to handle sperm more efficiently is considered here. Efficiency is taken to be a measure of the number of sperm used for each egg fertilised.

#### MATERIAL

Observations on *Orthetrum coerulescens* (Fabr.) were made at a stream in southern England during July and August, 1986 and 1988. *O. sabina* (Dru.), *O. pruinosum* (Burm.) and *O. triangulare* (Sel.) were observed in southern India, and *O. chrysostigma* (Burm.) and *Nesiothemis farinosa* (Foerst.) in Kenya.

#### OBSERVATIONS ON *ORTHETRUM COERULESCENS*

Copulations were observed between 11.30 and 15.00 h either in a male's territory on the stream bank, or 5-10 m from the stream. The duration of copulation is given in Table I. As in some other *Orthetrum* species (MILLER, 1983; SIVA-JOTHY, 1987) it is highly variable, the longest observed value being over 16 min. Long and short copulations both occurred on the stream bank and inland. After copulation the female either immediately perched or hovered for a few s and then resettled within 10 cm of where copulation had occurred. In only 1 out of 61 observations did she commence oviposition without resettling.

The mean duration of settling, the post-copulatory rest (PCR), is also given in Table I. Copulations and PCRs occurred low on vegetation (*Juncus*, *Phragmites*, *Sphagnum*, etc.) or on the ground, usually exposed to the sun. The duration of a

Table I  
The duration of copulation and post-copulatory rests (PCRs) in *Orthetrum coerulescens*

| No. of days of obs. | Temp. (°C) | Copulation (seconds) |            |     |     |    | PCR (seconds) |            |     |     |    |
|---------------------|------------|----------------------|------------|-----|-----|----|---------------|------------|-----|-----|----|
|                     |            | Mean                 | s.e. $\pm$ | min | max | N  | Mean          | s.e. $\pm$ | min | max | N  |
| July<br>4 days      | 20-22      | 291                  | 49         | 40  | 945 | 35 | 138           | 20         | 5   | 565 | 39 |
| Aug.<br>1 day       | 25-27      | 120                  | 44         | 21  | 967 | 22 | 56            | 11         | 9   | 109 | 22 |

PCR was not correlated with the duration of the preceding copulation for observations made at approximately the same temperatures (Product-moment correlation coefficient,  $r = 0.197$  ( $n=22$ ) N.S.  $P > 5\%$ ). Nor was it related to the site where copulation had occurred, or to the time of day. The longest PCR in Table I of 565 s was fully in the sun, but a longer PCR of 1387 s, throughout which the male remained close to the female, is not included in Table I since it coincided with a period when the sun was hidden by cloud except for the last 120 s. Cloud cover greatly reduced all dragonfly activity.

A male normally guarded his female closely during a PCR by perching within 5-10 cm and taking off in pursuit of other males. He might make occasional short patrolling flights if close to the stream (cf. PARR, 1983) after which he hovered immediately over the female and then resettled close to her. In 49 out of 62 observations the male remained with the female until she commenced oviposition, although towards the end of a PCR, he sometimes perched 0.5-1.0 m from her. Even when the male had not remained with the female throughout the PCR, he usually reappeared and guarded her closely as soon as she commenced oviposition.

Figure 1 shows separate records made on one day at 27° C from 3 females each of which copulated with 3 different males during a brief visit to the water. Thus during a single visit these females spent 41-56% of the time in copulation, 17-35% in PCR and only 24-27% in oviposition. Male interference may thus sometimes considerably impede oviposition inspite of guarding, as is known in some other species (WAAGE, 1984).

A female herself sometimes interrupted oviposition to perch for periods of up to 70 s, but usually shorter, during which the male perched near her. Such additional rests have not been included in the totals in Table I. At the end of oviposition the female elevated her abdomen and flew rapidly to a height of 20-30 m, usually pursued for a short distance by the guarding male.

On six occasions a male was seen to hover over a female at the start of a PCR

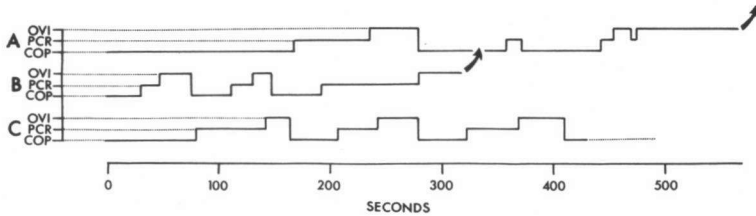


Fig. 1. The time spent ovipositing (OVI), in post-copulatory rest (PCR) and in copulation (COP) by each of three female *O. coeruleus*, A, B and C, during a visit to the stream on 7th August, 1988 (27° C). The arrows indicate times when oviposition finally ceased and females flew vertically and then left the water.

and then to ram her several times. On two occasions such "attacks" caused the female to take off and commence oviposition but she made only a few dips before again settling; on the other four occasions the female did not respond. In another case a male landed on the female's abdomen twice after a PCR of 152 s, which initiated a prolonged bout of oviposition.

In order to examine their readiness to oviposit, females in PCR were gently disturbed with a stick until they took off. Eleven females were repeatedly disturbed in this way between 5 and 20 s after the start of a PCR. On 34 of 42 occasions the female immediately re-settled usually within 5-10 cm of her original perch. On 8 occasions she commenced oviposition with the male guarding, but after a few dips returned to her perch. Thus of all experimental and natural disturbances which occurred soon after the start of PCRs, 21 % (10/48) provoked the female to oviposit briefly and none initiated a prolonged bout of oviposition.

On two occasions a male was seen to guard two ovipositing females simultaneously. In another case a female, after showing no oviposition in response to two attacks by her guard and four experimental disturbances, and having then been abandoned by her guard, took off after a 4-min PCR and flew 10 m along the stream where she then joined a second female already ovipositing. She oviposited for about 90 s sharing the second female's guard.

Six females caught during copulation or during a PCR were persuaded to "dump" large egg clutches by dipping their abdomens into a small tube of water: egg release usually began after a few seconds and such eggs were fertile as shown by the development of eyespots (MILLER, 1987). This suggests that eggs are normally available for laying with little delay.

## OBSERVATIONS ON OTHER SPECIES

*ORTHETRUM CALEDONICUM*. — ALCOCK (1988) reports that after short copulations the female commenced oviposition immediately, but after a long copulation she remained perched for several minutes.

*ORTHETRUM CHRYSOSTIGMA*. — Ten copulations have been observed (MILLER, 1983) all of which were followed by PCR's accompanied by males: their mean duration was  $86 \pm 38$  s (s.d.). This does not include one exceptionally long PCR of 505 s, the male abandoning the female after 303 s. At the start of 4 PCR's the male flew at the female ramming and butting her a number of times, but the female did not respond. One unusually long copulation (64 min) was followed by a PCR of 126 s throughout which the male perched beside the female.

*ORTHETRUM JULIA*. — PARR (1980) reported that 5/6 times females started ovipositing immediately after copulation, but on one occasion a female first rested for 6 s.

*ORTHETRUM PRUINOSUM*. — I have observed four copulations which were all followed by PCR's lasting 1-2 min. On two occasions the male hovered over the female, butting her and landing on her abdomen or wing several times before perching close to her. When the female flew up she was again butted by the male.

*ORTHETRUM SABINA*. — I have observed 9 copulations after each of which the female perched for 1-2 min. At the start of each PCR the male flew round the female several times in a circle of about 1 m radius making continual downwards dipping movements towards the water, an activity which resembled oviposition. He then returned to perch beside her.

*ORTHETRUM TRIANGULARE*. — I have observed two copulations each followed by a PCR of 1-2 min duration.

*NESCIOTHEMIS FARINOSA*. — Of 18 copulations observed, 17 were followed by PCR's ranging in durations from 20 to 70 s. Males remained with females during PCR's and on two occasions a male was observed to perch on the female's abdomen (MILLER, 1982a, 1982b).

OTHER SPECIES in which PCR's sometimes follow perched copulations include *Orthetrum cancellatum* (KRÜNER, 1977); *Neurothemis t. tullia* in which I have observed PCR's as well as subsequent rests during oviposition with the guarding male settled nearby; and *Nannophya pygmaea* which may do the same (M.T. Siva-Jothy, pers. com.). *Palpopleura l. lucia* is unusual in that it makes aerial copulations and may then settle for a period before oviposition commences.

## DISCUSSION

### FEMALE BEHAVIOUR

By resting after copulations, females delay oviposition. The duration of PCR's does not appear to be related to the duration of the preceding copulation, and cannot be explained by situation, territoriality of the male, or time of day. Moreover females may interrupt oviposition with further rests. Several possible functions of PCR's may be considered:

- (1) The PCR may allow a female to test the current male's willingness to act as a guard. However since PCRs continue after males have departed, this seems unlikely. Moreover females were sometimes guarded during oviposition even when the male had not remained with them throughout the PCR (*O. coerulescens*).
- (2) A female may wait for the appearance of other ovipositing females and then share a male guard. This seems relevant only when a female's current guard has departed. On three occasions (2 in *O. coerulescens*; 1 in *O. chrysostigma*) one male was seen to guard two females simultaneously, a phenomenon known in some other libellulids (JACOBS, 1955; UEDA, 1979).
- (3) A female may use the presence of other ovipositing females to determine if a site is relatively predator-free (e.g. from frogs). This can occur only when the PCR takes place close to the oviposition site.
- (4) Females may require time to prepare eggs for oviposition. However this could presumably also be done during the relatively long copulations of some *Orthetrum* spp. Some females caught during or after copulation dumped large egg clutches when held and dipped into a tube of water, starting to release eggs after a delay of only a few seconds. Moreover many females were seen to commence oviposition as soon as they arrived at the water and before they encountered males (*O. coerulescens*, *O. chrysostigma*, *O. sabina*, *N. farinosa*), suggesting that eggs are ready to be laid and that egg preparation does not explain PCRs.
- (5) Females may need time to "handle" sperm. In *Orthetrum* the sperm-storage organs are unusually small (MILLER, 1984; SIVA-JOTHY, 1987b). Sperm may therefore be used more sparingly than in other species. Oviposition rates appear to be as high as in other libellulids (McVEY, 1984), but it may take time to move sperm from the spermathecae down the long narrow ducts. This could also explain why rests are sometimes taken during oviposition. *Pantala flavescens* has even longer spermathecal ducts and copulates on the wing: it subsequently flies in tandem for several minutes before oviposition, which could also provide time for sperm handling. However this hypothesis does not explain why a few species with large storage organs and wide ducts (e.g. *Nesciothemis farinosa* and *Nannophya pygmaea*) also have PCRs.

Several females of *O. sabina* and *Pantala flavescens* caught and dissected after oviposition were found with mature eggs remaining in the oviducts but with no sperm in the spermathecal ducts or bursae, suggesting temporary sperm shortage. Dumped eggs were normally found to be fertile, but in one female *O. sabina* and one *O. taeniolum*, less than half were fertile. Subsequent dissection showed that these females possessed very little remaining sperm with none in the bursa or the spermathecal ducts. That infertile eggs are sometimes dumped indicates either that females are incapable of detecting the volume of their remaining stored sperm, or more

likely that dumping represents abnormal oviposition. Thus sperm shortages may sometimes be limiting in species with small storage organs and PCRs may reflect the need to use sperm economically.

In addition a PCR could allow a female to spend time storing a current male's sperm or selecting the sperm of a preferred male from among the ejaculates of several. But it is not known how a female would be able to make such a choice or manipulate sperm appropriately, although this possibility has been discussed previously (cf. LLOYD, 1979; SMITH, 1984; EBERHARD, 1985).

No conclusion about the function of PCRs can be reached at present. They are probably not needed to give time for egg handling but on some occasions they may permit females to check oviposition sites for the presence of predators or to test a male's willingness to act as a guard. The observations are compatible with the suggestion that PCRs allow time for sperm handling in some species, but there is no direct evidence to support this proposal and no reason to suppose that PCRs serve the same function in all species in which they occur.

#### MALE BEHAVIOUR

In species in which females make long PCRs, males have an increased risk of losing their mates before they oviposit. If a female mates again before laying, the first male is likely to fertilise very few of her eggs (WAAGE, 1984, 1986; McVEY & SMITTLE, 1984; MICHIELS & DHONDT, 1988). It is therefore strongly in a male's interest to minimise PCRs and to guard closely while they last. Since territorial males commonly copulate in their territories, they can continue to guard a female during a PCR while maintaining an active look-out for intruding males and further females. During PCRs males normally perch close to their mates, and in 4 species at least (*O. coerulescens*, *O. chrysostigma*, *O. pruinosum*, *Nesiothemis farinosa*) they sometimes show "aggressive" behaviour by hovering immediately above them and ramming, butting or landing on them repeatedly, perhaps attempting to stimulate females to oviposit. After 2 out of 6 observed attacks by male *O. coerulescens*, delivered soon after the start of PCRs, and after 8 out of 42 experimental disturbances, females started to oviposit briefly, whereas an one attack by a male towards the end of a PCR provoked a long bout of oviposition. Thus aggressive behaviour by males is sometimes successful in initiating oviposition and can be interpreted as a measure reducing the likelihood of take-over by rival males. Post-copulatory aggressive behaviour directed at their mates has been described in a rove beetle by ALCOCK & FORSYTH (1988) who suggest that it also can be explained in the context of sperm competition.

In contrast males of *O. sabina* fly round females several times at the start of PCRs, making downwards dipping movements with the abdomen towards the water, apparently mimicking oviposition. Females are known to be attracted to

other females which are ovipositing in some Calopterygidae and Libellulidae (JACOBS, 1955; ALCOCK, 1982; WAAGE, 1987). The mimicry of oviposition by males, if that is what this behaviour represents, might sometimes induce a female in PCR to oviposit, particularly in species such as *O. sabina* in which male and female colouring are the same. Similar ovipositing movements over water have been seen commonly in single male *Tramea limbata* and in *O. coerulescens* and they might sometimes induce a female perched nearby to approach, although there is no direct evidence for either suggestion.

#### ACKNOWLEDGEMENT

We are very grateful to MICHAEL SIVA-JOTHY for his valuable comments.

#### REFERENCES

- ALCOCK, J., 1982. Post-copulatory mate guarding by males of the damselfly, *Hetaerina vulnerata* Selys (Odonata: Calopterygidae). *Anim. Behav.* 30: 99-107.
- ALCOCK, J., 1988. The mating system of *Orthetrum caledonicum* (Brauer), with special reference to variation in copulation duration (Anisoptera: Libellulidae). *Odonatologica* 17: 1-8.
- ALCOCK, J. & A. FORSYTH, 1988. Post-copulatory aggression toward their mates by males of the rove beetle *Leistotrophus versicolor* (Coleoptera: Staphylinidae). *Behav. Ecol. Sociobiol.* 22: 303-308.
- EBERHARD, W.G., 1985. *Sexual selection and animal genitalia*. Harvard Univ. Press, Cambridge, Mass.
- JACOBS, M.E., 1955. Studies on territorialism and sexual selection in dragonflies. *Ecology* 36: 566-586.
- KRÜNER, U., 1977. Revier- und Fortpflanzungsverhalten von *Orthetrum cancellatum* (Linnaeus) (Anisoptera: Libellulidae). *Odonatologica* 6: 263-270.
- LLOYD, J.E., 1979. Mating behaviour and natural selection. *Fla. Ent.* 62: 498-500.
- McVEY, M.E., 1984. Egg release rates with temperature and body size in libellulid dragonflies (Anisoptera). *Odonatologica* 13: 377-385.
- McVEY, M.E. & B.J. SMITTLE, 1984. Sperm precedence in the dragonfly *Erythemis simplicicollis*. *J. Insect Physiol.* 30: 619-628.
- MICHIELS, N.K. & A.A. DHONDT, 1988. Direct and indirect estimates of sperm precedence and displacement in the dragonfly *Sympetrum danae* (Odonata: Libellulidae). *Behav. Ecol. Sociobiol.* 23: 257-263.
- MILLER, P.L., 1982a. Genital structure, sperm competition and reproductive behaviour in some African libellulid dragonflies. *Adv. Odonatol.* 1: 175-188.
- MILLER, P.L., 1982b. Post-copulatory "resting" in *Nesciothemis farinosa* Foerster. *Tombo* 25: 27-27.
- MILLER, P.L., 1983. The duration of copulation correlates with other aspects of mating behaviour in *Orthetrum chrysostigma* (Burmeister) (Anisoptera: Libellulidae). *Odonatologica* 12: 227-238.
- MILLER, P.L., 1984. The structure of the genitalia and the volumes of sperm stored in males and females of *Nesciothemis farinosa* (Foerster) and *Orthetrum chrysostigma* (Burmeister) (Anisoptera: Libellulidae). *Odonatologica* 13: 415-429.
- MILLER, P.L., 1987. Oviposition behaviour and eggshell structure in some libellulid dragonflies,



- with particular reference to *Brachythemis lacustris* (Kirby) and *Orthetrum coerulescens* (Fabricius) (Anisoptera). *Odonatologica* 16: 361-374.
- PARR, M.J., 1980. Territorial behaviour of the African libellulid, *Orthetrum julia* Kirby (Anisoptera). *Odonatologica* 9: 75-99.
- PARR, M.J., 1983. An analysis of territoriality in libellulid dragonflies (Anisoptera: Libellulidae). *Odonatologica* 12: 39-57.
- SIVA-JOTHY, M.T., 1987a. Variation in copulation duration and the resultant degree of sperm removal in *Orthetrum cancellatum* (L.) (Libellulidae: Odonata). *Behav. Ecol. Sociobiol.* 20: 147-151.
- SIVA-JOTHY, M.T., 1987b. The structure and function of the female sperm-storage organs in libellulid dragonflies. *J. Insect Physiol.* 33: 559-567.
- SIVA-JOTHY, M.T., 1988. Sperm repositioning in *Crocothemis erythraea*, a libellulid dragonfly with a brief copulation. *J. Insect Behav.* 1: 235-245.
- SMITH, R.L., [Ed.], 1984. *Sperm competition and the evolution of animal mating systems*. Academic Press, New York-London.
- TREVITT, S., K. FOWLER & L. PARTRIDGE, 1988. An effect of egg deposition on the subsequent fertility and remating frequency of female *Drosophila melanogaster*. *J. Insect Physiol.* 34: 821-828.
- UEDA, I., 1979. Plasticity of the reproductive behaviour in a dragonfly, *Sympetrum parvulum* Bartenef, with reference to the social relationship of males and the density of territories. *Res. Popul. Ecol., Tokyo* 21: 135-152.
- WAAGE, J.K., 1984. Sperm competition and the evolution of dragonfly mating systems. In: R.L. Smith, [Ed.], *Sperm competition and the evolution of animal mating systems*, pp. 251-290. Academic Press, New York-London.
- WAAGE, J.K., 1986. Sperm displacement by two libellulid dragonflies with disparate copulation durations (Anisoptera). *Odonatologica* 15: 429-444.
- WAAGE, J.K., 1987. Choice and utilization of oviposition sites by female *Calopteryx maculata* (Calopterygidae: Odonata). I. Influence of site size and the presence of other females. *Behav. Ecol. Sociobiol.* 20: 439-446.