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HIGH LAST-MALE SPERM PRECEDENCE DESPITE UNFAVOURABLE POSITIONING OF SPERM IN THE BURSA COPULATRIX OF *SYMPETRUM DANAE* (SULZER) (ANISOPTERA: LIBELLULIDAE)

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The data from *S. danae* (cf. N.K. MICHIELS, 1992, *Behav. Ecol. Sociobiol.* 29: 429-435) showed that the last male's fertilization precedence, P_2, changes irregularly with time between copulation and oviposition, but no systematic trend was found at that time. These data were now re-analyzed with a more appropriate statistical tool (GLIM), and it was found that P_2 increases with time between copulation and oviposition. This suggests that the first male's sperm initially has a positional advantage to fertilize the eggs. This advantage decreases, probably due to slow sperm mixing, and the precedence of the second male increases. This is the first odon. sp. for which the second male's sperm is shown to have a positional advantage.

INTRODUCTION

Sperm competition mechanisms have been studied extensively in many odonates. Generally, the last male achieves a high fertilization precedence P_2 (McVEY & SMITTLE, 1984; WAAGE, 1984; SIVA-JOTHY, 1987; MICHIELS, 1989). Yet, this does not show that the last male's sperm actually occupies the best place to fertilize the eggs. The last male can also get a high precedence if he removes a sufficient amount of the sperm of his predecessors. If almost all rival sperm is removed, it is very difficult to trace back directly the location of the rival sperm that is left. But there is an indirect way to solve this problem. If P_2 decreases with time between the last copulation and oviposition, the last male's sperm initially

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had a positional advantage. There are two main reasons why P_2 can decrease. (1) If the last male's sperm had a positional advantage, it may loose this position due to slow mixing of both males' sperm in the female's bursa copulatrix. (2) P_2 can decrease if the sperm of the last male becomes relatively more rapidly depleted than the sperm of the first male. Both arguments imply that the last male's sperm had an initial advantage. On the other hand, if P_2 increases with time after copulation, this shows that the first male initially had a positional advantage.

In *Sympetrum danae* P_2 immediately after copulation equals on average 0.95 ± 0.054 (MICHIELS, 1992). After the last copulation P_2 changes very irregularly with time, even by up to 90% in two consecutive days (MICHIELS, 1992). This irregular change can be caused by clumping of a male's sperm: if the first male's sperm fertilizes an egg, there is, due to clustering, a high chance that the next egg will also be fertilized by the first male, and the same for the next, resulting in an abrupt decrease in P_2. It was unclear if P_2 overall tended to increase or to decrease (MICHIELS, 1992), and hence it was unknown which male has a positional advantage. Since there is some evidence that the high P_2 can be due to almost complete removal of the first male's sperm, it might be hard to obtain direct evidence on this matter. But indirect evidence can be found by re-analyzing the data of MICHIELS (1992) with a more appropriate statistical tool, GLIM (PAYNE, 1985).

METHODS

In 1989, females were mated with a normal and an irradiated male (not necessarily in that order), to identify P_2. For a description of the study site, observational techniques and identification of the last male's fertilisation precedence, see MICHIELS (1992). Eggs from those double-mated females were obtained for as long as the females survived. All analyses were performed with copulation durations corrected for temperature (NUYTS, 1994).

Due to a short-cut technique used in the double-mating experiments (MICHIELS, 1992), only for a few copulations were all the variables from the first males available. Yet, for all copulations all variables from the last male were available. A preliminary analysis showed that the variables in the second male influenced P_2 much more than those of the first male. Thus we included those copulations for which no data on the first male were available.

Due to clustering, fertilization of two consecutive eggs is not independent, and a common logistic regression gives false results (NUYTS, 1994). But the Williams procedure (WILLIAMS, 1982; COLLETT, 1991) takes such a dependence into account. The statistical package GLIM (PAYNE, 1985; FRANCIS et al., 1993) allows us to perform a logistic regression in combination with the Williams-procedure (COLLETT, 1991). Therefore, we used GLIM to re-analyze the data from *S. danae*. In this paper, we present the main results. More technical details can be found in NUYTS (1994).

GLIM measures the significance of a variable in a model by the deviance. The deviance is the lack of fit between the model and the data (PAYNE, 1985; FRANCIS et al., 1993). An independent variable explains the variance of the dependent variable significantly better if the deviance decreases significantly after adding the variable to the model. Under conditions that hold for our data set, the decrease in deviance has approximately a Chi-squared distribution (COLLETT, 1991).

RESULTS

We performed a logistic regression in combination with the Williams-procedure on P_2, using the maximum number of variables available (i.e. copulation duration of the last male, time between the last copulation and oviposition, size of the female and the last male, day time of the last copulation, last male irradiated or not). P_2 depended only on the copulation duration, and on the time between oviposition and the last copulation:

Logit (P_2) = a_0+a_1*(cop.dur.)+a_2*(time cop.-ovi.), with

$a_1 = 0.1287 \pm 0.0238$ min, decrease in deviance = 41.02, df=1, $p<0.001$;

$a_2 = 0.1668 \pm 0.0655$ days, decrease in deviance = 6.123, df=1, $0.01<p<0.025$. A positive estimate of a coefficient means that P_2 increases with the variable under consideration. So P_2 increases both with copulation duration and with the time between copulation and oviposition.

DISCUSSION

MICHIELS (1992) had already shown that in *Sympetrum danae* the second male's precedence increases with copulation duration. Our analysis supports this result, and it adds to it that P_2 also increases with the time between copulation and oviposition. This increase is only possible if the first male initially has a positional advantage that is lost afterwards. An increasing P_2 is opposite to previous studies in odonates, where the last male seems to have the positional advantage (*Erythemis simplicicollis*, McVEY & SMITTLE, 1984; *Mnais pruinosa*, SIVA-JOTHY & TSUBAKI, 1989). A P_2 that in-

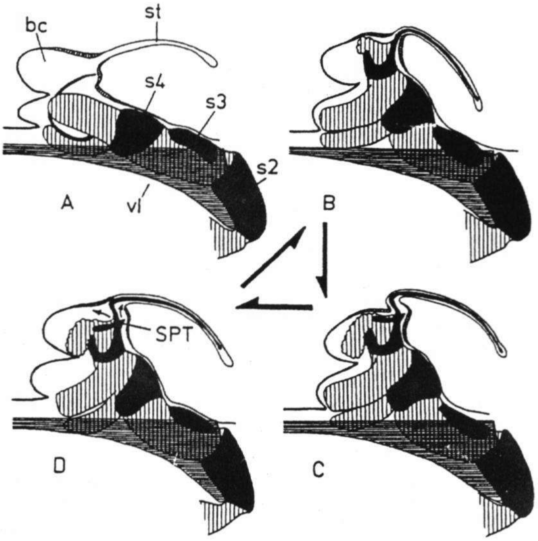

Fig. 1. Suggested mechanism of sperm transfer and removal in *S. danae*: (A) penetration phase; – (B) expansion phase; – (C-D) ejaculation phase. – [Female structures are horizontally hatched (8th sternite with valvular lamina, vl) or clear (bursa copulatrix, bc; – spermathecae, st). The penis of the male is vertically hatched (black when chitinized); – s2-s4 = penis segments 2-4; – SPT = sperm tube. Anterior = left and dorsal = up in the female, and the opposite in the male. Arrows in the middle indicate proposed cyclic process during the rhythmical copulatory movements. Small arrows in D represent sperm release. Figure from MICHIELS, 1989]

creases with time after copulation is physically possible in *S. danae* (Fig. 1). Sperm is ejaculated into the dorsal part of the bursa copulatrix and pushes the first male's sperm to the ventral part (MICHIELS, 1989). There it is removed by the second male, both by flushing with his own sperm and by collecting it on the spines of the apical lobe of the penis (MICHIELS, 1989). In this way, the "removal zone" is separated from the "ejaculation zone", preventing excessive removal of own sperm. But, as a result, any sperm of the first male that is not removed from the bursa copulatrix will initially occupy the best place to fertilize the eggs.

The present picture of the sperm competition mechanism in *S. danae* is also supported by mathematical modelling. To this end, we adapted models that were proposed by PARKER et al. (1990). As a preliminary result we found that the best fitting model assumes that (i) males remove rival sperm during the whole of copulation, but they remove hardly any of their own sperm, and (ii) the first male occupies the best place to fertilize the eggs (NUYTS, 1994).

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REFERENCES

COLLETT, D., 1991. *Modelling binary data.* Chapmann & Hall, London.

FRANCIS, B., M. GREEN & C. PAYNE, 1993. *Glim 4. The statistical system for generalized linear interactive modelling.* Oxford Univ. Press, Oxford.

McVEY, M.E. & B.J. SMITTLE, 1984. Sperm precedence in the dragonfly Erythemis simplicicollis. *J. Insect Physiol.* 30(8): 619-628.

MICHIELS, N.K., 1989. Morphology of male and female genitalia in Sympetrum danae (Sulzer) with special reference to the mechanism of sperm removal during copulation (Anisoptera: Libellulidae). *Odonatologica* 18(1): 21-31.

MICHIELS, N.K., 1992. Consequences and adaptive significance of variation in copulation duration in the dragonfly Sympetrum danae. *Behav. Ecol. Sociobiol.* 29: 429-435.

NUYTS, E., 1994. *Modelling in behavioural ecology: the optimal copulation duration in insects and the fighting strategy in the black-headed gull.* PhD thesis, Univ. Diepenbeek, Belgium.

PARKER, G.A., L.W. SIMMONS & H. KIRK, 1990. Analyzing sperm competition data: simple models for predicting mechanisms. *Behav. Ecol. Sociobiol.* 27: 55-65.

PAYNE, C., 1985. *The GLIM system release 3.77 Manual.* R. Statist. Soc., Oxford.

SIVA-JOTHY, M.T., 1987. 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. & Y. TSUBAKI, 1989. Variation in copulation duration in Mnais pruinosa Selys (Odonata: Calopterygidae) 1. Alternative mate-securing tactics and sperm precedence. *Behav. Ecol. Sociobiol.* 24: 39-45.

WAAGE, J.K., 1984. Sperm competition and the evolution of odonate mating systems. *In*: R.L. Smith, [Ed.], Sperm competition and the evolution of animal mating systems, pp. 251-290. Acad. Press, New York-London.

WILLIAMS, D.A., 1982. Extra-binomial variation in logistic linear models. *Appl. Statist.* 31: 144-148.