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SHORT COMMUNICATION

Measuring the sperm competition successes of field males of the yellow dung fly

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Abstract. 1. Sperm competition is extremely common in many species but conclusions about the relative successes of males, measured as the proportion of a female's offspring fertilised by the second male to mate with it, are based largely on laboratory studies using two laboratory-bred males or on interrupted last copulations.

2. A study was conducted in which virgin female yellow dung flies *Scathophaga stercoraria* mated uninterrupted with two field-captured males.

3. The results confirmed two previous conclusions: the proportion of a female's offspring fertilised by the second male was related strongly to the copula duration of the second male, and the proportion of a female's offspring fertilised by the second male was lower in larger females, though this effect was weak.

4. The results indicate that previous experiments on laboratory populations of this species are likely to be relevant to wild situations, but researchers are urged to conduct studies that will improve the confidence with which laboratory measures of the proportion of a female's offspring fertilised by the second male can be assumed to reflect the sperm precedence of the last male to mate in natural situations.

Key words. Cryptic female choice, Diptera, mating system, quasireplication, *Scathophaga*, *Scatophaga*.

Introduction

Sperm competition occurs when the sperm from more than one male are present within a female's reproductive tract during a single reproductive bout (Parker, 1970; Birkhead & Møller, 1998). Most of the information about relative male success in sperm competition comes from laboratory experiments that involve males and females bred in the laboratory. These animals may not behave completely naturally or may not be completely normal physiologically, perhaps because they may be better fed (Harschman & Hoffmann, 2000). For example, this variation could largely obscure differences due to male size variation, which is a major focus of laboratory studies, raising the concern that simply extrapolating from laboratory to field may be misleading. This concern was addressed in the work presented here by mating field-captured males with laboratory-raised females. Laboratory-raised females were used because it is extremely difficult to collect virgin adult females of the yellow dung fly *Scathophaga stercoraria* (L.) from the field (see Parker *et al.*, 1993).

The yellow dung fly has been used extensively to examine sperm competition and cryptic female choice, the female influence on the outcome of sperm competition (e.g. Parker, 1978; Simmons & Parker, 1992; Parker *et al.*, 1999; Seal, 2000; Ward, 2000; Hosken *et al.*, 2001). Males wait for females arriving to lay eggs in fresh dung. An arriving female typically has sperm stored from previous matings (Parker *et al.*, 1993) but must nevertheless copulate with another male before laying eggs (Parker, 1978).

The aim of this study was to check two findings from mainly laboratory-based studies: (1) that copula duration, which is male-determined, affects a male's fertilisation success, particularly for the last male to mate with a female (Simmons & Parker, 1992). Simmons and Parker (1992) interrupted second copulations to establish this point; the goal of this study was to check the conclusion in uninterrupted copulations; the last male to mate with a female in

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the field does not have its copulation interrupted; (2) that a female's body size can, at least sometimes, influence male fertilisation success (Ward, 2000). Palmer (2000) has recently stressed the importance of replicating studies in the original study animal, to ensure that conclusions can be properly critically evaluated. There is otherwise the risk that the phenomenon of interest cannot be probed deeply with scattered pieces of information on different species, which Palmer (2000) termed quasireplication.

Methods

Freshly emerged females from a laboratory stock were maintained under standard conditions (Ward, 2000). These females were used because wild virgins could only be collected as pupae and allowed to emerge in the laboratory, where they would have to be fed to maturity. They would thus not have experienced the natural variation in feeding conditions, making the comparison with laboratory reared flies of limited value, as all would experience the laboratory regime in the important maturation phase (Jann, 1997).

Wild males were collected from the field site at Fehraltorf, near Zurich, Switzerland (47°23′N, 8°45′E) from which the laboratory stock originated. Females mated with two males using the standard schedule described by Ward (2000). On the day after the males had been collected, a female and male were placed together in a tube containing a small quantity of cow dung. Copula duration was measured to the nearest minute. After copula, the females were removed and returned to their maintenance bottles. Males were killed by freezing and stored. The next day, the female was placed with a second male and the second copula duration was measured. The female was then allowed to lay a full batch of eggs into the dung. The male and female were then killed by freezing and the clutch size was counted. The entire clutch was then transferred to a large portion of dung, which was incubated until the adult offspring emerged. The offspring were frozen on the day of emergence. The hind tibia lengths of the female and the two males were measured. The phosphoglucomutase genotype of the possible parents and all offspring was determined by gel electrophoresis. Phosphoglucomutase genotypes have been used successfully to analyse paternity in this species (Ward, 2000). The proportion of a female's offspring fertilised by the second male was calculated from these data.

Of the 36 pairs of males used, there were only seven cases where the males were of different phosphoglucomutase genotypes. This is because the most common allele is much commoner than the others, so most flies were common homozygotes (Jann, 1997). The methods thus reflect the natural situation, in which males that are homozygous for the common allele are normally in competition with males that are heterozygous for the common allele and another less common one. The females were all common homozygotes.

Results and discussion

A forward stepwise multiple regression on the proportions of a female's offspring fertilised by the second male was conducted on the results from the seven males whose genotypes could be distinguished from the female's other mate using the values of the $\log_{10}$ of the copula durations of the first and second males, the size of the female, and the family size as candidate variables. First, the second male's copula duration then the female's size entered the model ($F_{2,4} = 33.92$, $r^2 = 0.94$, $P < 0.01$; log copula duration of second male $b = 1.308 \pm 0.186$, $t = 7.04$, $P < 0.01$, and female size $b = -0.062 \pm 0.021$, $t = 2.90$, $P = 0.044$). These effects are shown in Fig. 1. Clearly, the longer the second male copulated, the more successful it was (see below) and

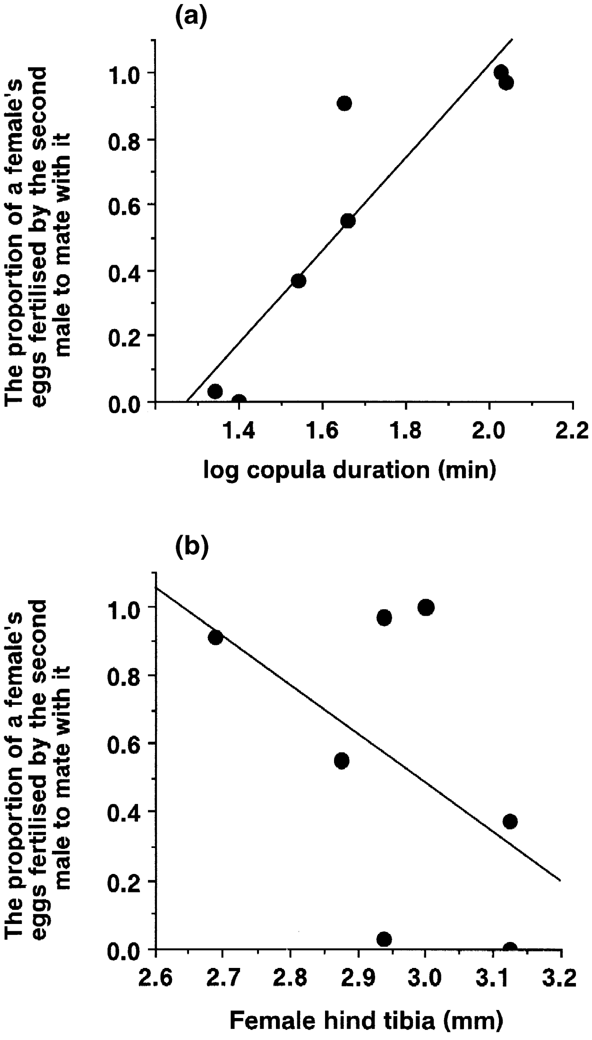

Fig. 1. (a) The relationship between the copula duration of the second male and the proportion of a female's offspring fertilised by the second male to mate with it and (b) the relationship between female body size and the proportion of a female's offspring fertilised by the second male to mate with it.

the larger the female, the lower was the proportion of a female's offspring fertilised by the second male.

These two results are consistent with previous work using mainly laboratory males and interrupted second copulations. (1) Second male copula duration determined partially the proportion of a female's offspring fertilised by the second male (Simmons & Parker, 1992). (2) The proportion of a female's offspring fertilised by the second male was lower with larger females (Ward, 2000). Indeed, the results suggest even stronger effects in field-caught males than in males from laboratory stocks. The effects were shown with a sample size of only seven and the r^2 was very high. The stepwise statistical approach used also illustrates further that male effects must be accounted for before more subtle female effects can be detected (Ward, 2000). In addition, the second result makes it extremely unlikely that the laboratory results of Ward (2000) could have been due to a locus closely linked to phosphoglucomutase, as is just conceivable using flies from lines bred in the laboratory; however there is no reason to believe that the males used here were not a completely random sample of the population and therefore of any possible linked loci.

The use of laboratory females, because of the difficulty of obtaining virgin adults that had experienced the natural variation in feeding conditions during maturation, does limit the scope of the interpretation somewhat. It remains for a future investigation to establish that this comparison is also valid for a laboratory to field comparison. Simmons and Parker (1992) used field females and interrupted second copulations, however, and concluded that there was no significant difference between field and laboratory females using their methodology.

The effect of female size on the proportion of its offspring fertilised by the second male could be due to a dilution effect on the second male's ejaculate due to the larger reproductive systems of larger females, as suggested by Parker *et al.* (1999). Alternatively, larger females may be better able to resist male attempts to manipulate them in the male–female conflict over the determination of paternity, as suggested by Ward (2000). The data presented here do not allow discrimination between these possibilities, especially because of the small sample size, which reduces the certainty of the interpretation. Larger females, however, will have larger accessory reproductive glands than smaller females. Females selected under polyandry are better able to control the proportions of their offspring fertilised by the second males than are females selected under monogamy, possibly because of their larger glands (Hosken *et al.*, 2001), perhaps making the second possibility more plausible.

Palmer (2000) suggested that failure to replicate previous studies fully makes critical evaluation of results and effects impossible. This task was undertaken here and the results validate previous conclusions. Other researchers are urged to conduct similar studies. This would improve the confidence with which laboratory measures of the proportion of a female's offspring fertilised by the second male can be assumed to reflect the sperm precedence of the last male to mate in natural situations.

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