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Copulation duration and sperm precedence in the stalk-eyed fly *Cyrtodiopsis whitei* (Diptera: Diopsidae)

Patrick D. Lorch[1], **Gerald S. Wilkinson**[1], **and Paul R. Reillo**[2]

[1] Department of Zoology, University of Maryland, College Park, MD 20742 USA
[2] Rare Species Conservatory, 1222 E Road, Loxahatchee, FL 33470, USA

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Summary. By means of field observations and laboratory experiments on the Malaysian stalk-eyed fly *Cyrtodiopsis whitei* we examined the consequences of variation in copulation duration for sperm competition. In this sexually dimorphic species over 90% of all copulations occur in nocturnal aggregations with from one to four males and up to 24 females. Copulation duration observed in both the field and the laboratory exhibited a bimodal distribution with peaks at 10 and 50 s. In the field short copulations less than 30 s long occurred frequently when more than one male was present in an aggregation but most were not the direct result of male interference. Sperm counts from female spermathecae after artificial interruptions indicated sperm are not transferred during the first 40 s of a copulation. When solitary males mated up to five times in succession to virgin females, short copulations did not occur, nor was the number of sperm transferred reduced. However, short copulations did occur when we mated isolated females within 6 min of a previous copulation. By mating irradiated and non-irradiated males in reciprocal pairs we discovered that *C. whitei* exhibits both first-male sperm precedence and sperm mixing. More than half of the females mated first to sterile and then to fertile males failed to produce offspring. Such variation in copulation duration and sperm precedence is consistent with male placement and detection of a spermatophore that acts as a temporary mating plug. Our data suggest that those male *C. whitei* which successfully defend large aggregations of females reduce sperm waste and competition by preferentially transferring sperm to females that have not mated recently.

Introduction

Parker (1970a) pointed out that multiple mating by females can produce a selective pressure on males to avoid sperm competition. Sperm displacement and sperm removal can be viewed as male behaviors that have evolved to preempt previous mates' sperm, while mate guarding and mating plug placement presumably reflect behaviors that have evolved to avoid sperm displacement or removal. Both types of behavior act to reduce competition for fertilizations between a male's own sperm and the sperm of other males (Knowlton and Greenwell 1984) and can lead to variation in copulation duration and variation in sperm precedence.

Species in which sperm competition is possible often exhibit non-normal distributions of copulation duration. Several insects exhibit particular male behaviors for reducing sperm competition that correspond to different portions of bimodal or multimodal distributions of copulation durations (Clark 1988; Miller 1983; Sillén-Tullberg 1981; Siva-Jothy 1987; Siva-Jothy and Tsubaki 1989b; Wolf et al. 1989). For example, the libellulid dragonfly *Orthetrum cancellatum* exhibits a bimodal distribution of copulation durations in which the shorter copulations occur near oviposition sites with little sperm removal, while the longer copulations occur near feeding sites with almost complete removal of preceding males' sperm (Siva-Jothy 1987). In a similar way, sperm precedence is influenced directly by male behaviors that reduce sperm competition. In the dragonfly *Mnais pruinosa pruinosa* the percentage of offspring sired by the second of two males to mate (P_2) correlated positively with the degree of sperm removal (Siva-Jothy and Tsubaki 1989a), and males in different behavioral situations employ different degrees of sperm removal. Likewise, in species where males place a mating plug (see Parker 1970a; Gwynne 1984), if the female oviposits immediately after expelling a plug, the sperm of the male that placed the plug will have priority. However, if females expel the plug some time before oviposition and subsequent mating occurs, sperm from the male that placed the original mating plug is likely not to have precedence.

Sperm competition can occur in the sexually dimorphic Malaysian stalk-eyed fly, *Cyrtodiopsis whitei*, because multiple mating by females occurs in nocturnal roosting aggregations on exposed roots (Burkhardt and

Correspondence to: G.S. Wilkinson

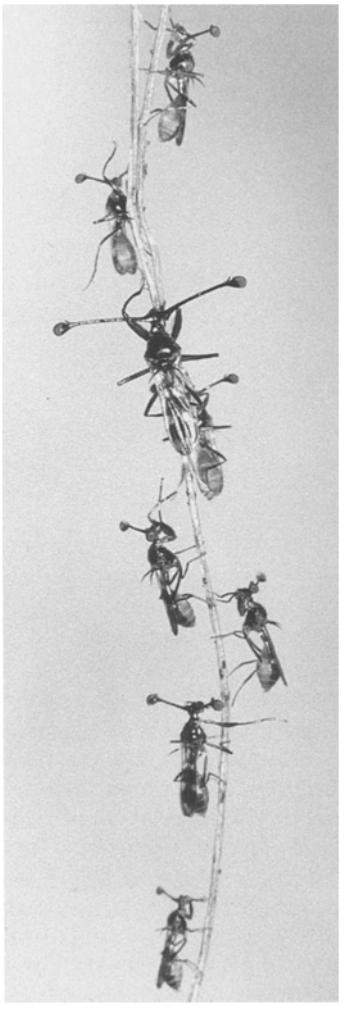

Fig. 1. Nocturnal aggregation of *Cyrtodiopsis whitei* on a root hair in peninsular Malaysia. The central fly with long eye-stalks is a male and the remaining flies are females. For scale, the male's eye span, i.e. the distance between the outside edges of his eyes, is 10.0 mm

de la Motte 1983). Most aggregations have one large male with 1–24 females (Fig. 1) and may have one or more small males that are visually indistinguishable from small females (Wilkinson and Reillo in preparation). Large males defend sections of the roots from other males and mate multiple times the following morning (de la Motte and Burkhardt 1983). After leaving aggregations at dawn females forage and oviposit in leaf litter on the forest floor (de la Motte and Burkhardt 1983). Females return nightly to aggregations and in the laboratory often mate multiple times every morning (personal observation). Males transfer sperm in a spermatophore that fills the upper portion of the female's reproductive tract, blocking the entrance to the paired spermathecal ducts (Kotrba 1991). The remains of the spermatophore are expelled within 1 h after copulation (Kotrba 1990, 1991).

Here we investigate whether copulation duration influences sperm competition in *C. whitei*. To this end, we examine behavioral correlates of the bimodal distribution of copulation durations. We also measure sperm precedence and investigate possible explanations for its variability. Because both males and females sometimes mate multiple times during 30-min periods, we examine the effects of multiply mating flies of each sex on copulation duration and assess whether male sperm depletion or female spermatheca filling can alter copulation duration. Finally, we discuss several other alternative explanations for bimodal distributions of copulation duration.

Methods

Copulation observations in the field. In October 1989, copulation durations were recorded during 24 h of systematic observations at 12 different *C. whitei* aggregation sites along a stream 36 km north of Kuala Lumpur near the village of Ulu Gombak (350 m elevation, 3° 19′ latitude, 101° 43′ longitude). At each aggregation site observations began before any flies had alighted on roots, typically at 1815 hours, and continued until all movements had ceased, usually by 1915 hours, at which time very little light remained. The following morning observations resumed at 0645 hours and continued until all flies had departed from the roots, usually by 0745 hours. To determine if copulations occurred only in aggregations we conducted a total of 24 h of observations on foraging flies between 1100 and 1600 hours on the same days that aggregations were observed at night. For all copulations we recorded the duration to the nearest 10 s, the relative eye span of the mating male (small, medium or large) and the presence of other males and females on the same root hair at the time of the copulation. Large males had eye spans which exceeded their body length, medium males had eye spans that were comparable to body length, and small males resembled females in having eye spans much shorter than body length. Small males could be distinguished from small females when they attempted or succeeded in copulating.

General laboratory methods. We simulated a 30-min dawn and dusk period on either side of a 12-h dark period by using a 40-W incandescent light reflected off a wall at least 1 m from the cages. We suspended strings from the roofs of cages to mimic roots. All copulations were observed during and just after the 30-min dawn period in two types of cages. The first type, referred to hereafter as "large cages," consisted of ventilated Nalgene mouse cages ($13 \times 13 \times 23$ cm) that were inverted into a pan containing moistened cotton and blotter paper with two strings suspended from the roof. The second type, referred to hereafter as "small cages," were made by cutting the spout off of 250-ml flat rectangular plastic tissue-culture bottles, lining them with moistened blotting paper and closing them with a foam plug. We allowed flies kept in large cages to feed and oviposit *ad libitum* on pureed corn that was changed twice weekly. The corn was prepared by grinding whole ears in a food processor, adding 5 ml of a mold-inhibitor solution (10 g methylparaben in 90 ml 95% ethanol) to each liter of puree, adding enough water to make a pulp and autoclaving at 235° C for 30 min. In the lab, *C. whitei* become sexually mature 11–13 days after eclosion (de la Motte and Burkhardt 1983); we used only flies that were at least 21 days post-eclosion. Flies used in these experiments had been in the laboratory environment for at least five generations and were chosen without regard for size because preliminary data showed that in the absence of larval competition, flies varied little in size (CV for body length in both males and females $<4\%$), all males fell into the large category used in the field, and copulation duration showed no correlation ($r=0.04$, $n=57$) with body size in the laboratory.

Copulation observations in the laboratory. We created a 2:1 sex ratio to mimic the field situation (de la Motte and Burkhardt 1983; Wilkinson and Reillo in preparation) by placing ten females and five males in each of four large cages. We recorded copulations for the first 30 min of daylight on video tape and measured copulation duration to the nearest second from the video tapes. Using this technique, we observed and recorded the four cages simultaneously with one camera on 6 consecutive days.

Sperm transfer. To measure the time required for sperm transfer, we placed 15 males separately into 15 small cages, and over the course of 6 days, we allowed them to engage in the following kinds of copulations with virgin females: 20, 30 or 40 s interrupted copulations or uninterrupted copulations. We considered males that failed to transfer sperm in two full matings sterile and discarded them. We mated males only once per day, recorded the actual duration of each copulation, and isolated females after mating for at least 1 h to allow sperm to move into the triple spermathecae. We then dissected the female's spermathecae using fine forceps under a dissecting scope, placed them on a hemocytometer in 5 µl of 7.5% NaCl solution, squashed them under a cover slip fragment and counted any sperm present under a light microscope at 400×.

Sperm precedence. To study sperm precedence, we first tested all males to be used for normal sperm transfer by mating each male with a virgin female whose spermathecae were subsequently examined to verify sperm transfer. We then modified the methods of Parker (1970b) and used males sterilized by irradiation with 10 krad of gamma radiation from a ^{60}Co source. This dose effectively sterilized males but did not affect sperm motility or morphology as determined by inspection (see Gromko et al. 1984, p. 401). In two separate experiments, we mated females in small cages to irradiated males (I males) and non-irradiated males (N males) in the following combinations. IN, NI, NN and II (experiment 1) and IN, NI, NN, N and I (experiment 2). The I and II mating categories are controls intended to test the effectiveness of sterilization. We only mated females to one I male in experiment 2 because the absence of any progeny from females mated to two I males in experiment 1 indicated that the irradiation treatment had effectively sterilized males. IN and NI matings determine sperm precedence if one of these two treatments differs significantly in offspring production from the other. If all females in both the IN and NI treatments produce pupae, then sperm mixing must be occurring. If IN females but not NI females produce, then last-male sperm precedence occurs. In contrast, if NI but not IN females produce, then first-male precedence occurs. The NN control was included to determine if pupal production is limited by the number of sperm transferred by a single male and the N control provided a check on male fertility after the precedence matings. Of the 83 males tested 31% transferred no sperm in the initial sperm transfer check and were not used in either experiment. We used only females copulating for longer than 40 s in both experiments to separate the effect of copulation duration from the effect of mate order on pupa production.

In experiment I, we observed a pair of matings in each of the four combinations on 6 consecutive days and used a total of 12 I males, 12 N males, and 24 virgin females. Once all males of a given type had been mated once, we reused them, but no male was used more than once a day. In experiment 2, 16 males were kept isolated so that individuals could be followed throughout the experiment. On the first day of experiment 2, we observed eight different females mate first to an I male and then to an N male. On the next day, we observed the same pairs of males mating in the reverse order with eight new females. On day 3, single I matings were observed with eight new females. On day 4, eight new females were mated first to the N type males used on the 2 previous days and then mated each to a new N type male. On day 5, eight N males were mated singly to virgin females. Because we monitored the mating performance of individual males in all treatments, in experiment 2 we were able to verify that N males always transferred sperm. After mating, all females in both experiments were isolated in separate large cages and allowed to oviposit for 3 weeks. To measure offspring production, we counted pupae emerging from the 100-ml plastic cups containing corn medium. In order to compare females that laid eggs for different periods of time due to fecundity differences or death, we calculated the rate of offspring output by dividing the total number of pupae that a female produced by the number of days on which eggs could have been laid.

Sequential mating. In the laboratory we sequentially mated 45 males by placing them individually in small cages and allowing them to mate with virgin females. Females were replaced as soon as they had mated so that males mated with as many virgin females as possible in a 30-min dawn period. We then recorded copulation durations to the nearest second and counted sperm in the spermathecae of 25 mated females. Similarly, we sequentially mated 36 isolated females to as many males as they would accept in a 30-min dawn period. We recorded copulation durations, and at the completion of the dawn mating period, counted sperm in the spermathecae of all females.

Statistical methods. We performed contingency table analyses and logistic regressions using SYSTAT (Wilkinson 1989) and LOGIT (Steinberg 1985) packages on the Macintosh computer. To test the significance of the contribution of individual terms to a contingency analysis model, we tested the difference χ^2, calculated by finding the difference between the likelihood ratio χ^2 statistics of two models differing only in one term (Fienberg 1980).

Results

Distribution of copulation durations

Each of the 12 aggregation sites we observed in the field contained flies on one to five roots. Copulation durations were obtained for 27 aggregations each with 1–24 females and comprising a total of 150 females and 53 males. More matings occurred during the 30-min period after dawn than during the 30-min period before darkness (148 versus 51 copulations; $z=6.88$, $P<0.0001$, binomial test). Fewer copulations occurred during the dusk period because males spent most of their time in the evening chasing and fighting, presumably to ensure access to females the following dawn. In contrast, only eight copulations were observed during daytime obser-

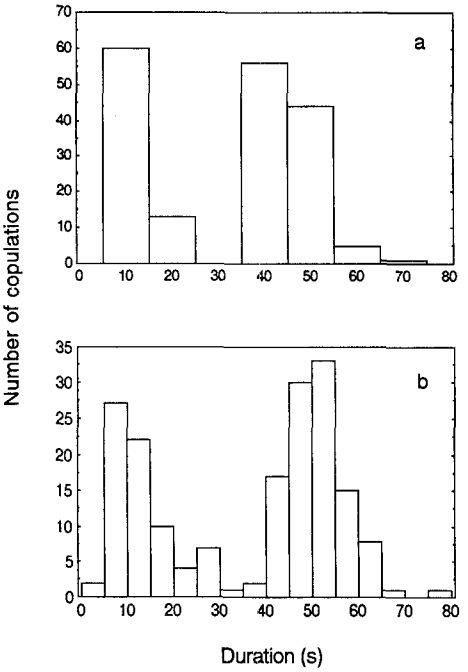

Fig. 2a, b. Frequency histograms of copulation duration from **a** field observations and **b** laboratory observations. Pooled results for 4 replicate cages each observed over 6 mornings

vations. From these observations we estimate that 94% of all copulations occur in aggregations.

The distribution of copulation durations in the field was bimodal (Fig. 2a) with a lower peak centered around 10 s and a higher peak at 40 s. The mean duration was 28 s ($n=199$). Median laboratory copulation duration did not differ between the four replicate cages (Kruskal-Wallis H, corrected for ties = 6.47, $df=3$, $P=0.09$); therefore, we pooled the durations obtained from the four cages. The resulting distribution of 180 copulation durations was also bimodal (Fig. 2b) with one mode centered around 5 s, a second at 50 s and an overall mean of 35 s.

Sperm transfer

Sperm were not stored in females' spermathecae after matings of 40 s or less (Fig. 3), indicating that males did not transfer sperm during this period. Logistic regression yielded a significant association between copulation duration and whether sperm were present or absent ($\chi^2=36.0$, $df=1$, $P<0.001$). Thus, the copulations represented by the lower peak in Fig. 1 were shorter than needed to transfer sperm. For this reason, we use 40 s as a qualitative cut-off and describe copulations lasting longer than or equal to 40 s as "long" and those lasting less than 40 s as "short". Long copulations transferred an average of 90 (SE = 12) sperm.

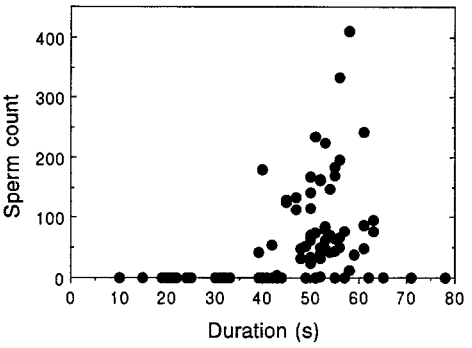

Fig. 3. Number of sperm transferred during interrupted and uninterrupted copulations

Table 1. Number of uninterrupted copulations and (number of individual males) observed 30 min before dusk or after dawn in aggregations of *C. whitei* in peninsular Malaysia

Number of males in aggregation	Copulation duration	Mating male's relative size		
		Large	Medium	Small
1	≥40 s	33 (13)	8 (5)	2 (2)
	<40 s	10 (6)	2 (1)	0 (0)
≥2	≥40 s	31 (14)	8 (6)	24 (13)
	<40 s	51 (12)	5 (4)	9 (6)

Male interference

In the field, short copulations occurred as often as long copulations during dusk and dawn periods ($\chi^2=0.004$, $df=1$, $P=0.95$). In 16 of the 199 copulations observed large males attacked and displaced smaller males from females. After displacing another male the larger male invariably mounted the female but rarely remained *in copula* for more than 10 s. The remaining 77 short copulations occurred without direct intervention by another male. To test for an association between copulation duration, size of the mating male, and number of males in the aggregation we performed a multi-way contingency analysis (Fienberg 1980) using all uninterrupted copulations (Table 1). The difference χ^2 values (Table 2) reveal that copulation duration was not independent of the number of males in the aggregation or the size of the mating male. Short copulations occurred more often when more than one male was present and when the mating male was large (Table 1). The difference χ^2 values (Table 2) also show that the size of the mating male was not independent of the number of males in an aggregation because small males obtained matings only when other males were present (Table 1). Many small male copulations appeared to go unnoticed by large males because the large males were either engaged in fights with other males or were *in copula*.

Sperm precedence

One of the females mated to an I category male in experiment 2 produced a single pupa (Fig. 4), but otherwise all I category males failed to father any offspring. For this reason, we did not correct the NI and IN matings

Table 2. Hierarchy of log-linear models examined in a multi-way contingency analysis of the uninterrupted copulations displayed in Table 1. The difference χ^2 tests the independence of each of the three pairs of possible associations

Model	Likelihood-ratio			Difference			Models Subtracted
	χ^2	df	P	χ^2	df	P	
1. C+S+N+C×N+N×S+C×S	0.80	2	0.747				
2. C+S+N+C×N+N×S	13.73	4	0.094	12.93	2	<0.001	2-1
3. C+S+N+C×N	29.73	6	<0.001	16.00	2	<0.001	3-2
4. C+S+N	43.69	7	<0.001	13.96	1	<0.001	4-3

C = copulation duration: <40 s or ≥40 s
S = male size: large, medium or small
N = number of males in aggregation: one or more than one

Table 3. Number of females which did or did not produce offspring in sperm precedence experiments 1 and 2

Experiment	Offspring produced	Mating categories			
		IN	NI	NN	N
1	Yes	2	5	6	
	No	4	1	0	
2	Yes	5	8	6	6
	No	4	0	1	0

Mating categories indicate the sequence of mating by irradiated (I) or non-irradiated (N) fertile males

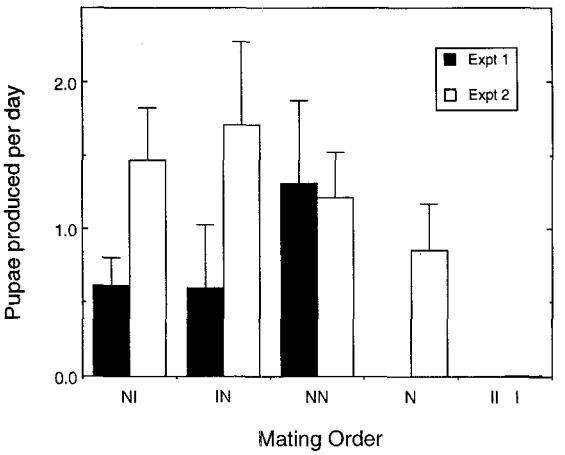

Fig. 4. Sperm precedence as measured by the mean (±1 SE) number of pupae produced per day. *Mating order* indicates the sequence of males used in each treatment. *N* refers to nonirradiated and *I* to irradiated males. Differences in the two experiments are described in the text (*solid bars*; experiment I; *black bars*: experiment 2)

for partial fertilization by the irradiated males (cf. Sillén-Tullberg 1981).

In both experiments a substantial number of females of the IN category did not produce any pupae (Table 3). We performed a multi-way contingency analysis (Fienberg 1980) on the data in Table 3 to test for an association between experiment, mating category, and presence or absence of offspring. The difference χ^2 values (Table 4) indicate that the proportion of females that produced pupae did not differ between experiments. However, a significant association between mating category and the presence or absence of pupae was detected (Table 4). The standardized residual for the cell in the IN mating category where no offspring were produced had the largest absolute value (2.34 versus −1.31 for the residual with the next largest absolute value) indicating that pupa production was not independent of mating category due to the disproportionate number of IN females that failed to produce pupae. If these females were sterile, no association would be expected between whether a female produced offspring and whether she was in the IN or NN mating category. A significant association (Fisher's exact test, $P=0.01$) was found, however, when the data from both experiments were pooled. This observation in conjunction with all but one of the NI females producing pupae is consistent with first-male precedence.

Comparison of the mean number of offspring produced by females in each treatment (Fig. 4), however, provides evidence for sperm mixing. The IN and NI treatments in each experiment show similar pupal production rates, as expected if irradiated and nonirradiated sperm mix prior to fertilization. A one-factor repeated measures ANOVA on the data in experiment 2 failed to detect a between-subjects effect ($F=1.01$; $df=5, 18$; $P=0.44$) or any difference among the four treatments ($F=0.56$; $df=3, 15$; $P=0.65$). No difference in pupal production rates between NN and IN females suggests that multiple mating did not increase female fecundity, at least over a 3-week period.

Male sequential mating

In the field copulation duration decreased within a morning for an individual male. Of 22 least squares regression slopes of copulation duration on mating order for males that mated at least three times in a morning, 17 were negative ($z=2.56$, $P=0.011$, binomial test). However, short copulations cannot be explained simply by sperm depletion. Last copulations in the field were no more likely to be short than earlier copulations (contingency $\chi^2=0.47$, $P=0.49$). One male mated 24 times in 1 h yet his 23rd and 24th copulations both lasted more than 40 s.

In the laboratory male sequential mating with virgin females did not result in any short copulations, but copulation duration decreased gradually with number of mates (Fig. 5a). The least squares regression ($y=-2.04x+60.61, r^2=0.11$) explained a significant amount

Table 4. Log-linear models examined in a multi-way contingency analysis of the presence or absence of offspring in IN, NI and NN mating categories for sperm precedence experiments 1 and 2

Model	Likelihood ratio			Difference			Models subtracted
	χ^2	*df*	*P*	χ^2	*df*	*P*	
1. E+C+P+E×P+C×P	3.67	4	0.453				
2. E+C+P+E×P	14.79	6	0.022	11.12	2	<0.005	2-1
3. E+C+P	15.06	7	0.035	0.27	1	>0.900	3-2

E = experiment (1, 2)
C = mating category (IN, NI, NN)
P = presence or absence of offspring

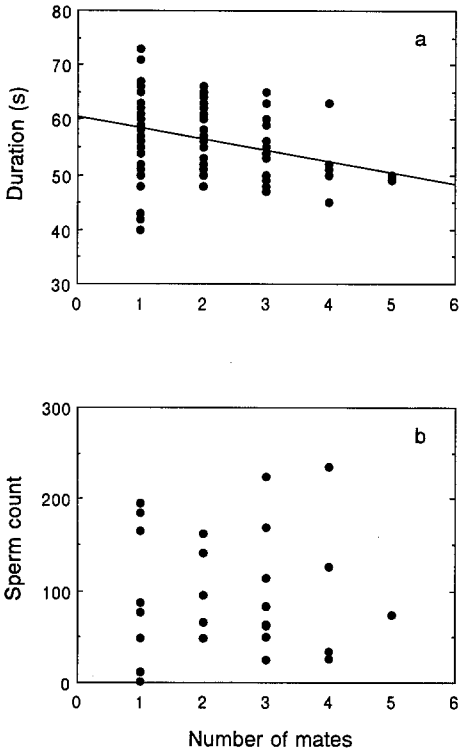

Fig. 5a, b. Results of the male sequential mating experiment shown as **a** copulation duration with least squares regression line and **b** number of sperm transferred. Number of mates represents the number of times a male mated in a morning

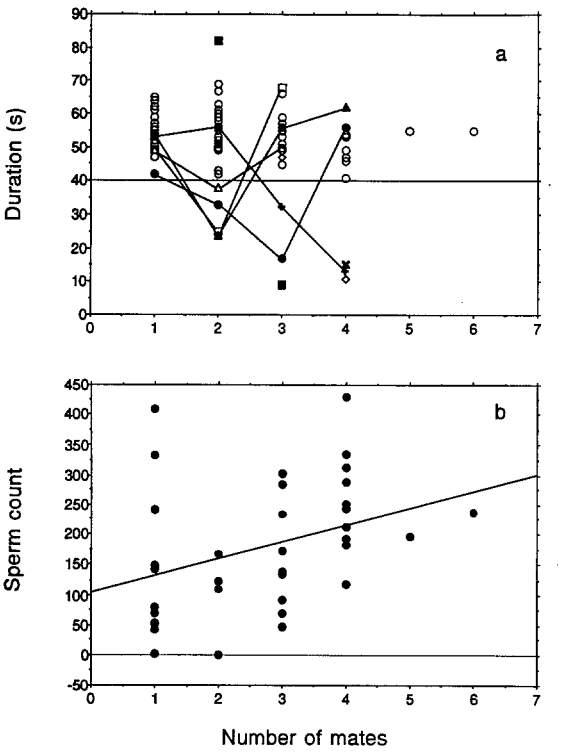

Fig. 6a, b. Results of the female sequential mating experiment shown as **a** copulation duration and **b** number of sperm transferred with least squares regression line. *Open circles* represent females that had no copulations of less than 40 s. *Other symbols* represent individual females who had at least one copulation of less than 40 s. Number of mates refers to the number of times a female mated in a morning

of the variation in copulation duration both when all observations were assumed to be independent points ($F=11.2$; $df=1.85$; $P=0.001$) and when the degrees of freedom associated with the error were reduced to the number of mating males ($F=5.6$; $df=1, 39$; $P=0.03$).

Despite a decline in copulation duration, sperm depletion could not be detected within the first four copulations. Males did not transfer fewer sperm in second, third or fourth copulations than they did in first copulations (Fig. 5b). The plot of sperm counts against the number of mates yielded a positive, but non-significant least squares regression ($y=7.44\,x+86.21$, $r^2=0.01$, $P=0.58$).

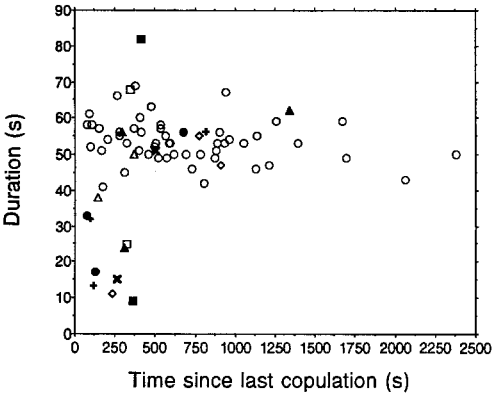

Fig. 7. Copulation duration plotted against time between copulations. Data and symbol explanation as in Fig. 6a

Female sequential mating

Virgin females engaged only in long copulations (Fig. 6a). Of 29 virgin copulations recorded, all were greater than 40 s. Short copulations occurred only when a female had already mated. There are several lines of evidence suggesting that short copulations were not a result of female spermathecae filling up with sperm. For four of the five females that copulated for short durations and copulated again, long copulations followed short ones, indicating that short copulations did not occur because spermathecae were full. Over all females, the mean slope for the regression of copulation duration on number of mates in a morning, calculated by female, did not differ significantly from zero (mean $=-3.59$, $n=33$, $t=1.39$, $P=0.17$). Finally, the plot of sperm count against number of mates (Fig. 6b) yielded a significantly positive least squares regression ($y=27.88\,x+105.28$, $r^2=0.11$, $P=0.04$), indicating that female sperm storage capacity exceeds the volume of sperm in a single insemination.

To determine if short copulations are a function of the interval between copulations, we used the data in

Fig. 6a to compare copulation duration with the time between copulations (Fig. 7). All ten of the short copulations occurred within just over 6 min of the copulation preceding them. Logistic regression yielded a significant association between the time between copulations and whether copulations were short or long ($\chi^2 = 18.18$, $df = 1$, $P < 0.001$).

Discussion

Although our data indicate that copulations observed in the field are shorter than those seen in the laboratory, we have less confidence in our field estimates of copulation duration than in the laboratory measurements. Difficulty in observing the beginning of all copulations under low light conditions in the field would lead to underestimates of copulation duration and could explain the difference between the laboratory and field data. Nevertheless, it is clear that copulation duration exhibits a bimodal distribution in both field and laboratory with long copulations lasting at least 40 s. Because sperm are not transferred during the first 40 s, the copulations in the lower peak of the copulation duration distribution transfer no sperm. Why should males initiate so many copulations but fail to transfer sperm? Our discovery of occasional first-male precedence in *C. whitei* suggests that males shorten copulations to avoid sperm waste or competition in matings with females that have recently mated.

Bimodal copulation duration in other arthropods

C. whitei is unusual in having shortened copulations that do not transfer sperm. The libellulid dragonfly *Orthetrum cancellatum* is typical of insects with variable copulation duration; copulations from both peaks of the bimodal distribution of copulation durations transfer sperm (Siva-Jothy 1987). Other studies do not report the minimum time for sperm transfer (Alcock 1988; Dickinson 1986; McLain 1980) making it difficult to conclude whether the variation in copulation duration seen was produced by shortened or prolonged copulations.

Shortened matings are seen, however, in bowl and doily spiders, where pairs engage in pseudocopulation (distinguishable from copulation only by a lack of sperm transfer) and only engage in further copulation if the female is a virgin or has mated within the past 24 h (Austad 1984; Suter 1990). Some males apparently cannot distinguish recently mated females from virgins. First males have almost complete precedence in these spiders, even if the second mating occurs within 24 h of the first (Austad 1984). The overall time females and males spend mating is therefore shortened if females are not virgins, except in those cases where females have mated within 24 h of the current mating. A bimodal distribution of mating durations would be the expected result of such behavior, although the distribution of copulation durations has not been reported.

Spermatophore/mating plug hypothesis

Support for the hypothesis that short copulations are a consequence of avoidance of spermatophores or mating plugs by males comes from the results of both the sperm precedence and the female sequential mating experiments. Only 1 of 14 females in the NI treatment failed to produce offspring in contrast with 8 of 15 females in the IN category. This effect is not the result of N males being sterile because all males were fertile when they began mating and all males checked after mating in the N treatment produced progeny. Furthermore, differences in sperm competitive ability caused by the irradiation treatment cannot explain the pattern of offspring production (Table 3). If N sperm were more competitive than I sperm, then all females in the IN matings should produce offspring. We conclude that those IN matings producing no offspring represent cases of complete first-male precedence and that NI matings producing offspring are consistent with either first-male precedence or sperm mixing. Thus, something prevents the second male's sperm from fertilizing any eggs. The most plausible mechanism is that the spermatophore of the first male acts as a mating plug, and when it is expelled, carries with it the second male's spermatophore, preventing any of the second male's sperm from being retained by the female. Because the spermatophore of *C. whitei* may remain in the female for 1 h (Kotrba 1990) and because 94% of all copulations in the field occur in aggregations, a spermatophore acting as a mating plug would be effective in ensuring that the first male to mate with a female in the morning has precedence over males mating later that morning. Although first-male precedence has rarely been reported in insects (Gwynne 1984), it has been associated with a spermatophore in *Aedes agypti* (George 1967), in *Culicoides melleus* (Linley 1975) and in *Glossina austeni* (Curtis 1968).

With this reasoning, females in the IN category that produced progeny must have expelled the spermatophore of the first mate prior to mating a second time, thereby permitting sperm mixing. Sperm precedence in *C. whitei* appears to change from first-male precedence to sperm mixing depending on whether or not a spermatophore is in place during the second copulation. Consequently, we have not expressed sperm precedence as a species-wide mean value to avoid masking this intraspecific variation (*sensu* Lewis and Austad 1990).

If males are indeed under selection to allocate sperm efficiently, then short copulations could represent aborted copulations of males who, after detecting the presence of a spermatophore, avoid wasting sperm and seminal fluid that might be expelled with a previous male's spermatophore. Because males can mount 24 females in 30 min and copulation duration decreases with number of mates, sperm may be limiting within a morning mating session, making such reproductive restraint highly adaptive.

Female sequential mating is clearly important in producing short copulations. Short copulations never occur with virgins, which is consistent with the hypothesis that spermatophores function as mating plugs. Short copula-

tions also do not appear to be a result of females having full spermathecae. The strong association between whether a copulation is long or short and the time between copulations supports the spermatophore/mating plug hypothesis. Such an association is expected because as more time elapses between copulations, a female is more likely to have expelled the last spermatophore. Kotrba (1991) has shown that 2 min after a copulation, 20% of females have ejected spermatophores and by 30 min after copulation, over 50% of females lack spermatophores.

Alternative hypotheses

Male interference. In many species of insects larger males forcibly separate copulating smaller males from females (Parker 1968; Rutowski and Alcock 1980; Johnson 1982). Such takeovers result in variation in copulation duration and could account for shortened copulations occurring when multiple males are present in an aggregation. However, only 15% of the short copulations we observed in the field resulted from male displacement. Furthermore, our observations of large males usually engaging in brief copulations after displacing a smaller male are consistent with male detection of a spermatophore and subsequent avoidance of sperm transfer. Male interference also cannot explain why short copulations occurred in the female sequential-mating experiments when only one male was present.

Sperm depletion. Even though the duration of most males' copulations decreased within a morning, short copulations were not caused by males lacking sperm to transfer. If provided with virgin females, male *C. whitei* copulate for at least 40 s and show no decrease in the transfer of sperm after four successive matings a few minutes apart. In contrast, if the female has recently mated, males may perform short copulations after only one previous copulation (Fig. 6a). In the field, the last copulation by a male in a morning is as likely to be short as long. Thus, while sperm depletion is not the direct cause of a short copulation, males may vary copulation duration in order to allocate sperm efficiently.

Seminal feeding. If females control copulation duration, short copulations may represent inseminated females "stealing" nutrients in seminal fluid. For example, in the corn rootworm, *Diabrotica virgifera* males transfer seminal material before transferring spermatozoa (Lew and Ball 1980). However, our preliminary attempts (unpublished data) to measure seminal fluid transfer, using males fed ^{14}C-labeled food (Pitnick et al. 1991) and subsequently allowed to copulate for varying lengths of time, have failed to reveal any seminal fluid transfer prior to 40 s.

Female choice. The role played by female responses to male behaviors that reduce sperm competition has not received much study. Rutowski and Alcock (1980) consider the potential effects of selection on females to diminish the effectiveness of males at reducing sperm competition. They conclude that the distribution of copulation durations in *Nomadopsis puellae* is primarily a result of an increase in mate guarding *in copula* as females get closer to ovipositing. In *C. whitei*, long matings that occur shortly after previous matings (upper left of Fig. 7) could represent instances of female choice where females expel a spermatophore within minutes in order to accept the current male's spermatophore (see Sivinski 1984, p. 103).

Alternatively, long duration copulations that occurred shortly after previous copulations may represent failures of males to detect existing plugs. Male bowl and doily spiders appear to err in assessing female virginity when a female has mated within the past 24 h (Austad 1982). These copulations also may represent alternative mating strategies by males who are depending on failure of a predecessor's plug, as in the case of *C. whitei*, or are counting on the imperfection of the sperm precedence system, as in the bowl and doily spider. The reliability of mating plugs and the mechanisms that result in sperm precedence warrant further study.

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