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Copulation duration and fertilization success in a damselfly: an example of cryptic female choice?

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Copulation duration is highly variable (0.5–3 h) in the damselfly, *Ceriagrion tenellum* (Coenagrionidae). Using laboratory experiments, we tested four adaptive hypotheses to explain this variation: the effect of time constraints, in-copula mate guarding, sperm displacement and cryptic female choice. Copulation duration was negatively correlated with time of day, as predicted by the first two hypotheses, and positively correlated with male density, as predicted by the mate-guarding hypothesis. Males prolonged copulation in response to the volume of sperm stored by females, suggesting they were able to detect and quantify the amount of sperm stored. This behaviour is not explained by mate guarding or time constraint effects. Males removed all the sperm from the bursa copulatrix in just 10 min. Our results also suggest that, because the duct is too narrow to allow male genitalia to enter, males do not remove spermathecal sperm. Therefore, direct sperm removal could not explain long copulations. Prolonged copulations could also have evolved as a result of cryptic female choice if they increase male fertilization success by female-mediated processes. Our results support this idea: male fertilization success was greater after long copulations. Apparently, male copulatory behaviour elicits female responses that increase male fertilization success.

There are many reasons why natural selection on both sexes should favour brief matings (Daly 1978). Long copulations can be energetically expensive, and may increase the risk of predation and the likelihood of an interruption before the transfer of sperm is finished. Moreover, the probability of disease transmission increases with the time spent in copulation, and time is lost that could be devoted to other activities (feeding, oviposition, vigilance behaviour, etc.). Despite these selective factors, copulation duration is very long in many damselflies, reaching 5–6 h in some *Ischnura* species (Robertson 1985; Miller 1987a; Cordero 1990; Sawada 1995; Cordero & Andrés 1999).

Long copulations might be the consequence of sexual selection favouring male adaptations, for instance, to avoid sperm competition (Parker 1970). From the male's point of view, prolonged copulations could reduce sperm competition with future ejaculates if his body acts as a mating plug that prevents the female from remating before oviposition ('in-copula guarding' hypothesis, Table 1; reviewed by Alcock 1994). Long copulations could also reduce competition with previous ejaculates if males inseminate more sperm (Thornhill 1984; Cordero 1990) or spend longer removing their rivals' sperm ('sperm displacement' hypothesis, Table 1). These two mechanisms (sperm competition hypotheses) assume that copulation duration is at least partly under male control, which seems true in damselflies (Miller 1987a), and are based on the benefits that males obtain by prolonging copulations. Under these two hypotheses long copulations have evolved by direct male–male competition for fertilizations.

However, females might also influence male copulatory behaviour. A recent hypothesis postulates that females exert a form of postcopulatory sexual selection ('cryptic female choice' hypothesis, sensu Eberhard 1996; Table 1), by favouring some males over others. Thus, long copulations could serve to influence cryptic female choice mechanisms (Eberhard 1996). Distinguishing between 'sperm competition' and 'cryptic female choice' might be difficult in some cases. For instance, Córdoba-Aguilar (1999) documented a novel mechanism by which males gain access to the sperm stored in the spermatheca in the damselfly *Calopteryx haemorrhoidalis*. During copulation, the aedeagus distorts the cuticular plates in the female genital tract that bear mechanoreceptive sensilla, and this stimulation results in sperm ejection from the

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Table 1. The assumptions and predictions of four adaptive hypotheses to explain variation in copulation duration in damselflies

Assumptions		Predictions	
Time constraints			
There is an optimum time for oviposition owing to temperature constraints and/or predation risk	Y*	Copulation duration is inversely related to time of start	Y
		Copulation duration is independent of male density	N
		Copulation duration is independent of female mating status	N
In-copula guarding			
The last male to copulate fertilizes most of the eggs laid immediately after copulation	Y	Copulation duration is inversely related to time of start	Y
		Copulation duration is positively related to male density	Y
		Copulation duration is independent of female mating status	N
Sperm displacement			
Males are able to quantify the amount of sperm stored by females	Y	Differences in copulation duration should primarily be due to differences in stage I of copulation (when sperm removal occurs)	Y
Males mechanically remove sperm during the first stage of copulation	Y	Sperm removal gradually increases with copulation duration	N
		Stage I of copulation should tend to end near the time when all the accessible sperm stored have been removed	N
		As a consequence of more efficient sperm removal or greater insemination, male reproductive success increases with copulation duration	N
Cryptic female choice			
Males are able to assess female mating status	Y	Males stimulate mated females more than virgins	Y
Males do not remove all the sperm stored in the female tract	Y	Stage I of copulation continues after all the accessible sperm stored have been removed	Y
		As a consequence of female-mediated processes, experimental prolongation of copulation should increase fertilization success	Y

Y/N denotes whether our experiments did or did not support the assumption or prediction.
*Not tested but true in some species.

spermatheca. Males also differed in their ability to stimulate females. Therefore, sperm removal could result directly from mechanical actions of the male (sperm displacement) but also indirectly by a female-mediated process that might be a potential mechanism of cryptic female choice. If during long copulations males produce a more intense stimulation, then prolonged copulations could be the result of selective female cooperation (Eberhard 1998).

On the other hand, the time of day may also limit copulation duration if there is an optimum time for oviposition, because of temperature requirements and/or predation risk (time constraint hypothesis, Table 1; Rehfeldt 1985, 1992; Martens 1989, 1991).

In *Ceriagrion tenellum*, a nonterritorial damselfly that shows postcopulatory mate guarding, pairs remain in copula from 30 min to 3 h (Andrés 1998). Copulation follows the typical Coenagrionid sequence: a first stage when males remove rivals' sperm (see Results), a second stage where insemination takes place, and a third stage with no apparent activity (Miller & Miller 1981). In this paper we present the results of laboratory experiments that tested the four hypotheses (Table 1) proposed for the evolution of long copulations in *C. tenellum*.

METHODS

In all experiments we used animals of known reproductive history. The majority were obtained by rearing the offspring of laboratory crosses (Andrés & Cordero 1999), but some were collected as newly emerged adults from natural populations in northwest Spain. To obtain matings we introduced mature females (at least 9 days of age) into insectaries (50 cm each side) containing mature males (at least 6 days of age) and observed them continuously. Results are presented as $\bar{X} \pm \mathrm{SE}$. Unless otherwise stated, all tests are two-tailed.

Time Constraints and Guarding

Effect of time of day

If long copulations evolved to avoid competition with future ejaculates (in-copula guarding hypothesis) or because there is an optimum time for oviposition, earlier copulations should last longer, independently of the female's mating status (Table 1). Thus, in our first experiment we measured 55 copulations starting at different times of day, using either virgin or once-mated females. All individuals were used only once. Male density was 7–27 males/insectary.

Effect of male density

The mate-guarding hypothesis predicts that copulations should be longer when male density is high, while the time of day hypothesis predicts that copulation duration is independent of male density (Table 1). In a second experiment we measured copulation duration

under two male densities: high (22–27 males/insectary) and low (1–3 males/insectary). Individuals were maintained at the desired density from emergence and were used only once. Females (N=20) were randomly assigned to treatments and were introduced at 1300 hours into the insectaries containing males, but were removed if copulation did not take place within 1 h. The time at which copulations started was entered as a covariate in the statistical analysis. Because preliminary results indicated that copulation duration was longer with mated females, we used only virgin females in this experiment.

Sperm Displacement and Cryptic Female Choice

Both hypotheses assume that males have information about the mating status of the female (Table 1). The sperm displacement hypothesis predicts that males increase their fertilization success during long copulations by directly removing or inseminating more sperm. Copulations should thus be short if sperm removal and insemination are rapid. The cryptic female choice hypothesis also predicts higher male fertilization success with long copulations, owing to a female-mediated process that would favour the ejaculate of some males over others (Table 1). To test these predictions we carried out five laboratory experiments.

Can males remove sperm?

First, to test whether males are able to remove sperm, we compared the volume of sperm stored in: (1) once-mated females; (2) females mated twice with an interval between copulations of 24 h; and (3) females mated twice with a 24 h interval, but in this case the second copulation was interrupted at the end of stage I, when the removal of sperm had presumably finished, but insemination not yet started (Miller & Miller 1981). If males remove most of the sperm, once- and twice-mated females should store a similar quantity of sperm, and interrupted females should have few or no sperm in either sperm storage organ. Because our data meet the assumptions for ANOVA, statistical analyses were carried out using ANOVA tests with the Q test for post hoc pairwise comparisons.

Sperm removal pattern

If males prolong stage I of copulation to remove more rival sperm, this should be a gradual process. Furthermore, removal activity should end close to the time when all or almost all of the accessible sperm have been removed (Table 1). So, we carried out a second experiment in which we estimated the pattern of sperm removal with time. A group of 14 once-mated females were mated a second time the day after first mating, but the second copulation was experimentally interrupted at different times within stage I. We then measured the amount of sperm stored by these females.

Copulation duration and quantity of sperm removed

We designed a third experiment to investigate whether longer copulations allow a greater proportion of sperm to be removed. Each of 13 males was allowed to have one short and one long mating with females mated the previous day. For short copulations we introduced females into the insectaries at 1500 hours, and for long copulations at 1300 hours. All copulations were interrupted at the end of stage I. Stage I lasted a mean ± SE of 39.92 ± 4.71 min for short copulations and 109.04 ± 9.68 min for long copulations. For six of the tested males the first copulation was short while for seven it was long. We analysed the results of this experiment with the Wilcoxon test which allows a comparison of the means of two dependent groups (Sokal & Rohlf 1995).

In all the above experiments females were preserved in 70° ethanol after either complete or interrupted copulations. We measured sperm volumes stored in females as described by Cordero & Miller (1992). Sperm storage organs (bursa copulatrix and spermatheca) were dissected out and mounted under a supported cover slip on a slide. The area of the sperm mass was measured using GlobalLab 3.0 software. We calculated the volume of sperm by multiplying the surface obtained by the diameter of two copper wires located between the cover and slide. For statistical analyses we used only the samples in which the density of the sperm mass was homogeneous.

Assessment of female mating status

The sperm displacement and cryptic female choice hypotheses assume that males are able to distinguish between virgin and mated females (Table 1). To test this assumption we measured copulation duration of virgin males with virgin females (N=10) and with once-mated females (N=10). We also measured copulation duration with a group of 'pseudovirgin' females (females that copulated for 49.86 ± 4.40 min, N=9, but received no sperm because we interrupted copulation at the end of stage I). Given that in some coenagrionids copulation duration depends on time of start, the density of males, temperature and age (Cordero 1990), all these conditions were controlled. Females were 11–16 days old and were mated one, two or three times depending on the experimental treatment to which they were randomly assigned. The interval between copulations was always 24 h. All males were virgin and between 10 and 17 days of age. The number of males in each cage was 12–16, while the temperature was 20–23°C. Cages received natural indirect light. Females were introduced into the cages at 1300 hours, but if copulation did not take place within 1 h they were removed.

The sperm displacement hypothesis assumes that males could not only assess female virginity but also quantify the sperm stored in the female genital tract and therefore adjust copulation duration to the volume of rival sperm stored (Table 1). To test this assumption, we measured copulation duration in the same conditions as above but with females storing a reduced amount of sperm. We allowed 10 once-mated females to mate a second time, but their second copulation was interrupted at 50 min into stage I. Twenty-four hours after this interrupted mating, half of the females were mated again, and we measured copulation duration. The remaining females

were preserved in 70° ethanol, to quantify the reduction in the amount of sperm obtained with this method.

Variance in copulation duration was analysed using ANOVA and *Q* tests as post hoc pairwise comparisons. Data were transformed into common logarithms to meet the assumption of homogeneity of variances (Levene test after transformation: 0.302, df=3, 28, P=0.823).

Copulation duration and sperm precedence

The sperm displacement and cryptic female choice hypotheses predict higher last-male sperm precedence after long copulations (Table 1). In this experiment we measured last-male fertilization success after long (132.40 ± 11.18 min, N=8) or short copulations (86.50 ± 7.00 min, N=7). Long and short copulations were obtained as in previous experiments.

To study the effect of copulation duration on sperm precedence, we used the irradiated sterile male technique. Sterilized males received 3500 cGy from a ^{60}Co source. Virgin females were mated either once with a normal (N) or irradiated (R) male, or twice with two males (R-N and N-R matings). The interval between matings was 24 h.

After the experiment, females were maintained in glass containers with humid leaves of *Tradeschantia fluminensis* as an oviposition substratum. The eggs were maintained in petri dishes with dechlorinated water for 10 days at room temperature. After this period of incubation, fertile eggs were yellow-brown with bilateral symmetry and two black ocular spots, while the sterile eggs were whitish. Of the eggs laid by R females, 24% were fertile, while the fertility of N females was 87%. The proportion of eggs fertilized by the last male in double matings (P_2) was calculated with the algorithms proposed by Boorman & Parker (1976) and Gromko et al. (1984). Because female *C. tenellum* hardly ever lay eggs without their mate, we studied only the first clutch of each female. Females that laid fewer than 20 eggs were excluded from the analyses.

Variance in the proportion of eggs fertilized by the second male (P_2) was analysed with generalized linear models (GLM) with binomial errors of the number of eggs fertilized by the second male, with the total number of eggs laid per female as the binomial denominator and a logit link function (see Arnqvist & Danielsson 1999). To compensate for overdispersion (McCullagh & Nelder 1989) we used the method of Williams (1982) before statistical inference.

RESULTS

Time Constraints and Guarding

Effect of time of day

Copulation duration was negatively correlated with the time of day when copulation started (overall data: r_s= − 0.250, one-tailed P=0.022; Fig. 1), but the reproductive status of females was also significant and copulations were longer with mated females (126.43 ± 9.24 min) than with virgins (56.37 ± 2.62 min; t test: t_{58}= − 10.077, P<0.001).

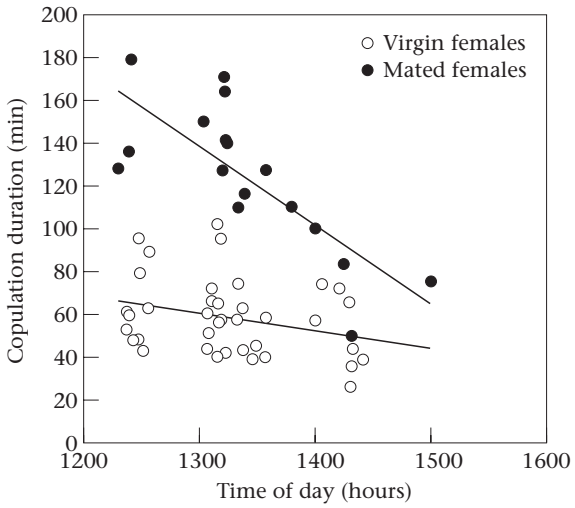

Figure 1. The relationship between the time of day when copulation started and its duration with virgin and once-mated females.

Because the density of males in the insectaries varied (see Methods), to test the relative effects of time of start, male density and female mating status we carried out an ANCOVA where female mating status was the factor and both time of start and male density were covariates. Female mating status ($F_{1,53}$=14.92, P<0.001) and time of start ($F_{1,53}$=11.57, P=0.001) had a significant effect, but male density had no effect on copulation duration ($F_{1,53}$=1.98, P=0.166). Nevertheless, interactions between male density and female mating status ($F_{1,53}$=6.94, P=0.012) or time of start ($F_{1,53}$=13.31, P=0.001) were also significant.

Effect of male density

As the interactions of the above experiment suggested, male density had a significant effect on copulation duration. At high density (22–27 males/insectary), copulation with virgin females lasted 62.82 ± 5.48 min (N=14), but only 42.03 ± 3.48 min (N=6) at low density (1–3 males/insectary; ANCOVA, with time of start as covariate: $F_{1,19}$=8.19, P= 0.01). Male density was positively correlated with copulation duration (copulations obtained at intermediate densities in the previous experiment are also included; partial correlation after controlling for the effect of the time of start: r=0.414, N=36, P<0.01; Fig. 2).

Sperm Displacement and Cryptic Female Choice

Can males remove sperm?

Figure 3 shows the volume of sperm in the bursa and spermatheca of experimental females. To test if there were significant differences in the volume of the sperm stored we carried out independent ANOVAs for each of the sperm storage organs (bursa: $F_{2,33}$=44.63, P<0.001; spermatheca: $F_{2,31}$=16.18, P<0.001).

Males were able to remove almost all the sperm in the bursa: once- and twice-mated females had almost the same volume of sperm in this organ (Q test: P=0.708), and it was empty in nine out of 10 interrupted females.

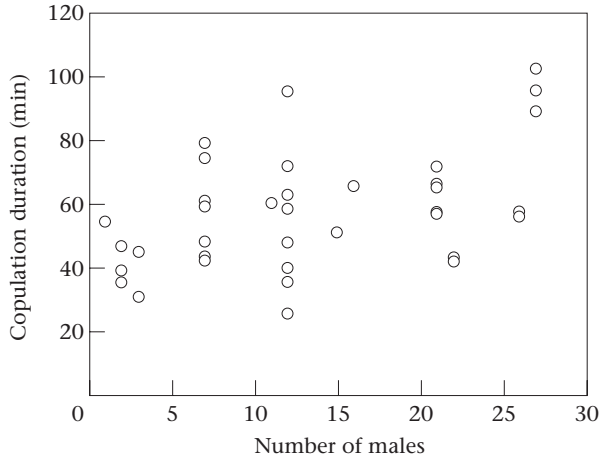

Figure 2. The relationship between male density and copulation duration with virgin females.

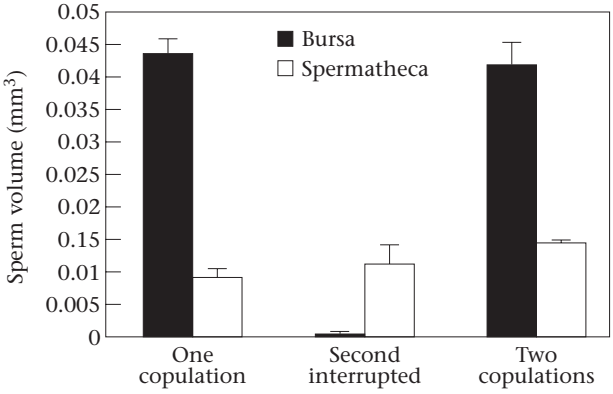

Figure 3. The volume of sperm ($\bar{X}$+SE) stored by female *C. tenellum* after one copulation, the interruption of the second copulation at the end of stage I, or two complete copulations.

On the other hand, the volume in the spermatheca increased after two copulations by 58% (*Q* test: $P<0.05$). We found no difference in spermathecal volume between once-mated and interrupted females (*Q* test: $P=0.998$), but there was a significant difference between interrupted and twice-mated females (*Q* test: $P<0.05$).

Sperm removal pattern

Figure 4 shows the volume of sperm in 14 females whose second copulation was interrupted at different times during stage I. The bursa was emptied in only 10 min whereas there was no clear pattern of variation of the sperm in the spermatheca with time.

Copulation duration and quantity of sperm removed

At the end of stage I the bursa was empty irrespective of copulation duration. We found no differences in the quantity of sperm in the spermatheca after long (0.0042 ± 0.0014 mm^3) or short copulations (0.0055 ± 0.00092 mm^3; Wilcoxon test: $T=-0.435$, $N=13$, $P=0.693$). Two females had an empty spermatheca after short copulations, and two after a long copulation.

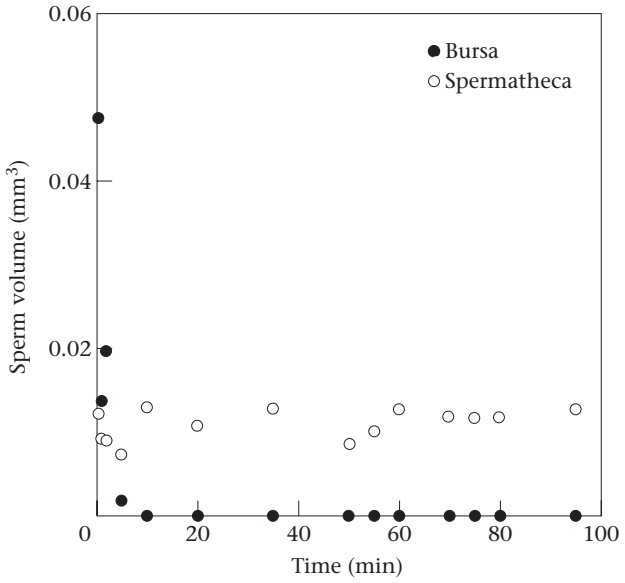

Figure 4. The pattern of sperm removal with time in *C. tenellum*. The volume of sperm stored by once-mated females whose second copulation was interrupted at different times during stage I.

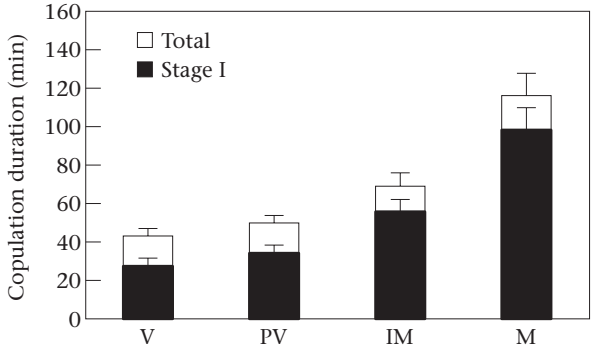

Figure 5. Time males spent in copula ($\bar{X}$+SE) with virgin females (V), females interrupted in their first mating before insemination ('pseudovirgin' females, PV), females interrupted after 50 min into stage I of their second mating (IM) and once-mated females (M).

Assessment of female mating status

The amount of sperm in the storage organs of the females had an important effect on copulation duration (ANOVA: $F_{3,28}=17.875$, $P<0.001$): males spent the same time in copula with virgin (43.15 ± 4.03 min, $N=9$) and 'pseudovirgin' females (49.86 ± 4.40 min, $N=9$; *Q* test: $P=0.518$), but copulation was much longer with previously mated females (115.55 ± 11.67 min, $N=9$) than with virgins (*Q* test: $P<0.05$; Fig. 5). Males also spent more time in copulation with mated females than with interrupted ones (68.61 ± 7.30 min, $N=5$; *Q* test: $P<0.05$), but with these females males spent more time in copulation than with virgins (*Q* test: $P<0.05$).

Almost all the variation in copulation duration was due to variance in stage I, as the strong correlation between the duration of stage I and total copulation duration shows ($r_s=0.991$, $N=32$, $P<0.001$).

The volume of sperm stored by once-mated females whose second copulation was interrupted at 50 min into

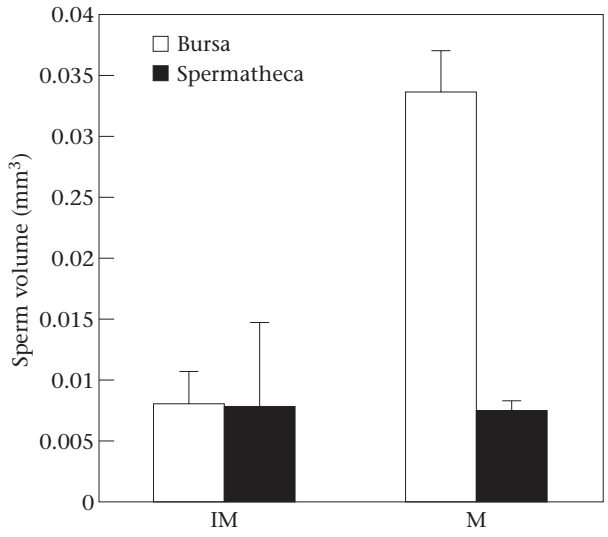

Figure 6. The volume of sperm ($\bar{X}$+SE) stored by females preserved 24 h after mating. IM: females whose second mating was interrupted after 50 min of stage I; M: females that mated once.

stage I decreased by 61% the day after mating (Mann–Whitney *U* test: $U=-2.558$, $N_1=4$, $N_2=5$, $P=0.016$). This difference was due to a reduction in bursal volume (Fig. 6). This result seems to contradict the earlier results showing that the bursa was empty after 10 min (Fig. 4). This was, however, probably due to migration/transport of spermathecal sperm to the bursa during the 24 h between the end of copulation and the preservation of the female in alcohol (see Methods).

Copulation duration and sperm precedence

Fertilization success was similar for irradiated and control males (Mann–Whitney *U* test: $U=-0.886$, $N_1=N_2=4$, $P=0.386$). For this reason we merged the results of R-N and N-R females. The experimental treatment (short versus long copulations) had a significant effect on sperm precedence ($\chi^2_1=-4.284$, $N=15$, $P=0.038$). The mean P_2 value after long copulations (1.05 ± 0.04, $N=8$) was greater than after short copulations (0.87 ± 0.08, $N=7$).We also tested whether the inclusion of copulation duration and its interaction with treatment improves the model. However, neither variable affected P_2 ($\chi^2_2=0.369$, $N=11$, $P=0.831$).

DISCUSSION

Several analyses of variation in copulation duration in damselflies have been published (e.g. Miller 1987a; Siva-Jothy 1987; Siva-Jothy & Tsubaki 1989; Cordero 1990; Cordero et al. 1995). The majority of these have shown that males either guard females in copula or prolong copulation to remove sperm, two behaviours clearly selected in a sperm competition context. Our results indicate that males detect female mating status and therefore female mating history is the main variable explaining variation in copulation duration in *C. tenellum*.

We also found a clear effect of time of start on copulation duration, as predicted by the time constraint and in-copula guarding hypotheses. This negative correlation between copulation duration and time of start seems widespread among damselflies (Conrad & Pritchard 1990; Cordero 1990; Perry & Miller 1991; Cordero et al. 1995; Cordero & Andrés 1999), and at least in some cases might be partially due to variation in temperature (Cordero 1999). However, temperature was fairly constant in our laboratory experiments and can be excluded as a key variable (see also Conrad & Pritchard 1990). Copulation might be prolonged to diminish predation risk during oviposition, an effect shown in several zygopterans (Rehfeldt 1985, 1992; Martens 1989, 1991), although not for *C. tenellum*. Nevertheless two predictions of this hypotheses are not supported by our data (Table 1).

As predicted by the sperm competition theory, copulation duration increased with male density. This result suggests that male disturbance is used as a cue to prolong copulation, a behaviour that makes sense in species whose females oviposit alone and therefore are free to remate (Robertson 1985; Miller 1987a; Cordero 1990; Sawada 1995; Cordero & Andrés 1999). However, *C. tenellum* males remain in tandem with their mate during most of the oviposition, which lasts several hours (A. Carini, personal communication). Therefore, copulatory guarding seems less likely: males could guard their mate in tandem (Conrad & Pritchard 1990; Cordero et al. 1995). On the other hand, copulations with previously mated females are longer than with virgins and this result cannot be explained by guarding or time constraints effects. If males prolong copulation to guard their mate, they should probably stay longer with virgin females, because the lack of sperm competition makes them more valuable.

The previous hypotheses cannot explain the variation in copulation duration with female reproductive status. This effect might have evolved by sexual selection. Copulation duration could be directly related to sperm competition, where females would play a relatively passive role, and/or might have been selected by cryptic female choice if copulation serves as an 'internal courtship'. Both hypotheses assume that males are able to differentiate between virgin and mated females (Table 1), which has been demonstrated in several other arthropods (Acari: Yasui 1994; Araneae: Suter 1990; Orthoptera: Pickford & Gillott 1972; Wedell 1998; Hemiptera: Johnson & Hubbell 1984; Lepidoptera: Wiklund & Forsberg 1985; Coleoptera: Lewis & Austad 1990; Lewis & Iannini 1995; Diptera: Lorch et al. 1993; Sivinski & Petersson 1997; Wilkinson & Dodson 1997). In some cases it is probably due to pheromone recognition, since males of some species change their precopulatory behaviour depending on whether the female is virgin. Males of *C. tenellum* remained longer in copula with mated females than with virgins. In odonates, this has been shown only for two *Ischnura* species: *I. graellsii* (Cordero 1990) and *I. elegans* (Cooper et al. 1996). However, copulation duration with 'pseudovirgin' females was similar to that with virgins, suggesting that males are detecting ejaculates rather than other cues. Furthermore, copulation duration

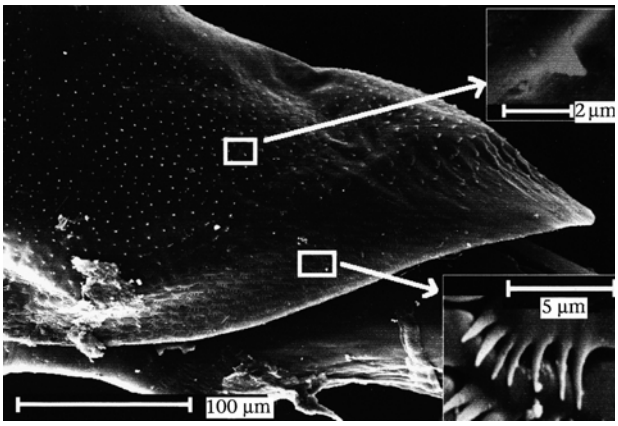

Figure 7. The head of the penis of *C. tenellum* under a scanning electron microscope, showing the spines on the exterior surface and border (bottom detail), and the conical sensilla on the main exterior part of the penis (upper detail).

with females that contained less than one ejaculate was longer than with virgins and shorter than with once-mated females, suggesting that males are not only able to differentiate between virgin and mated females, but also able to adjust the time of copulation to the quantity of sperm stored in the female, which has not previously been shown in any animal.

The most likely explanation for these results is that males are able to detect and quantify the amount of sperm stored by the female. The mechanism of sperm detection is unclear. Careful observation of male genitalia under scanning electron microscope revealed that the spoon-like head of the penis is internally and laterally covered with backwards directed spines. These spines gradually change until they appear as small conical protuberances similar to chemical sensilla (Shepherd 1985; Fig. 7), although these structures have no pores. We suggest that this might be the mechanism used to detect ejaculate. Alternatively, females might adjust their behaviour to the amount of sperm that they have stored and males detect or are limited by female behaviour, but the mechanism of how this would occur is less clear.

Earlier studies of odonates have indicated that sperm removal is slow (Cordero & Miller 1992) and that males increase copulation duration to remove more sperm (Siva-Jothy 1987; Siva-Jothy & Tsubaki 1989). However, *C. tenellum* in our study removed all the accessible (bursal) sperm in only 5–10 min. Therefore the main reason for prolonged matings is not sperm displacement.

On the other hand, we found that some mated females whose second mating was interrupted at the end of stage I had an empty spermatheca (Fig. 8). Since all females dissected after one complete copulation showed the spermatheca full of sperm (N=26) we suggest that some males were able to empty the spermatheca. Direct sperm removal is not physically possible because the spermathecal duct is too narrow for the penis to penetrate (Andrés 1998). This result suggests a female-mediated process for explaining how the spermatheca is emptied. A possible mechanism of indirect removal is via stimulation of the campaniform sensilla of the female vaginal plates by the aedeagus (Miller 1987b; Fig. 8a). When an egg is laid, it passes between the vaginal plates and stimulates these sensilla. This stimulation ejects sperm from the spermatheca (Miller 1987b) and the movements of the penis during copulation could help to remove sperm from storage organs that are otherwise inaccessible (Miller 1987b; Córdoba-Aguilar 1999). This mechanism

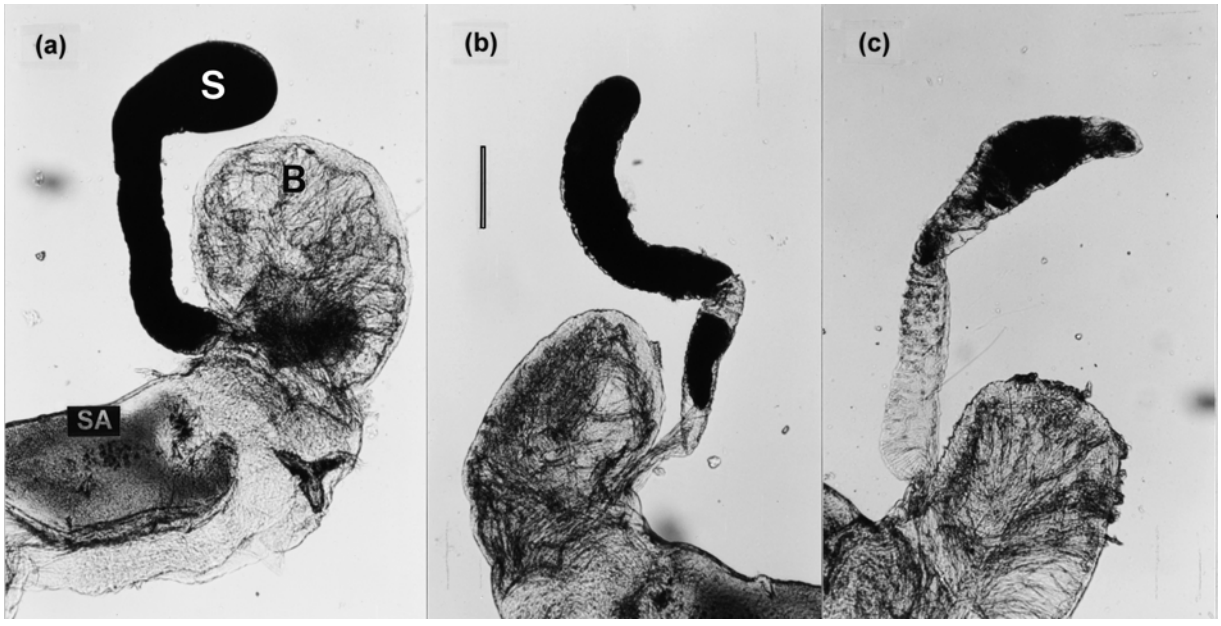

Figure 8. Three examples of the amount of sperm found in the spermatheca of females whose second mating was interrupted at the end of stage I. (a) Female with a full spermatheca (S), and some sperm in the bursa (B). Note the sensilla on the vaginal plates (SA). (b) A discontinuous sperm mass, which suggests that this cannot be the result of male removal. (c) A sperm mass that has started to disappear from the tip of the spermatheca. Scale bar is 0.01 mm.

could explain why the sperm in the spermatheca sometimes appear as discontinuous masses, or are found only at the base and not the tip of the spermatheca (see also Córdoba-Aguilar 1999; Fig. 8).

Ceriagrion tenellum males may prolong copulation after all the accessible sperm stored have been removed in an attempt to eject sperm from the spermatheca. Because this is a female-mediated process it constitutes a mechanism of cryptic female choice (sensu Eberhard 1996) if it is related to fertilization success. Females might for instance transport more sperm from the last male to the fertilization site after long copulations.

This critical prediction is supported by our results: male fertilization success was greater after long copulations but the volume of spermathecal sperm was not different after short or long copulations. These results contrast with those of Sawada (1998) who found that copulation duration is unrelated to sperm precedence in the damselfly *Ischnura senegalensis*.

Male copulatory behaviour apparently elicits female responses that increase male fertilization success. As we have discussed, this could be achieved if mechanical stimulation during long copulations elicits a greater ejection of spermathecal sperm. In contrast, we found that the volume of sperm remaining in the spermatheca did not decrease after long copulations. Nevertheless, this result is not conclusive because the great variance of spermathecal volumes could make it difficult to detect any effect of copulation duration on the volume of sperm removed in this storage organ.

In conclusion, although our sample sizes were modest, our results suggest that, even in odonates whose mating systems were traditionally interpreted as being male controlled (Fincke 1997), longer copulations could serve to influence cryptic female choice mechanisms.

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