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The adaptive significance of the prolonged copulations of the damselfly, *Ischnura graellsii* (Odonata: Coenagrionidae)

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Abstract. Copulation in the damselfly *Ischnura graellsii* lasted 1–5 h, and took place in the afternoon and evening at the study site in north-west Spain. Copula duration was measured in the laboratory under controlled temperature, humidity, photoperiod and density. At high density (15–20 males/insectary), copulations that started early in the day were always long, while at low density (two males/insectary) they could be short or long. At both densities, copulations with previously mated females were longer than with unmated females. The duration of stage II of copulation, when the male transfers sperm to the female, was constant and independent of density and time of day. A study of egg production in females mated only once indicated that copula duration is not correlated with the proportion of fertile eggs laid, and that females start running out of sperm after about 15 days. These results indicate that prolonged copulations of *I. graellsii* have a guarding function, but the existence of more sperm displacement in long copulations cannot be rejected. Guarding takes place during stage I of copulation, before the males invest sperm in the female, which is unusual. The relation between copula duration and postcopulatory behaviour in *Ischnura* species so far studied is discussed.

Dragonflies may be separated into species with short (less than 1 min), medium (1–5 min) and long (more than 5 min to several hours) copulations (Corbet 1962). Longer copulas are specific to some *Ischnura* damselflies, sometimes lasting for 6–7 h as in *I. elegans* (Miller 1987b).

In some damselflies, copulatory activity can be divided into three stages (Miller 1987a), all of which are recognizable in *I. graellsii* (Cordero 1989). In stage I, which takes up the greater part of copulation, the male displaces the sperm of his rivals; in stage II he transfers his own sperm, and stage III is a phase without movements (Miller 1987a).

In *I. graellsii*, stage I shows great variation in duration, being interrupted by long pauses without movements. Stage II lasts for 2–8 min and takes place 12·6 ± 0·84 min (± SE) before the end (Cordero 1989). In the field, copulatory activity takes place between 1330 and 1700–1800 hours (solar time) at the study area (in north-west Spain), and the earlier the copulation starts the longer it lasts, sometimes for even more than 4 h (Cordero 1989).

Prolonged copulation could be a male's in-copula guarding strategy: his body acts as a nuptial plug, and impedes additional copulations of the female. This occurs in some insects (Sillén-Tullberg 1981; Svärd & Wiklund 1988), and was proposed for *I. ramburi* (Robertson 1985), *I. elegans* (Miller 1987a) and *I. graellsii* (Cordero 1989). This hypothesis predicts that under intense competition for females, copulations should be prolonged until the end of the population's reproductive activity each day and so terminate synchronously. At low density, females have a low probability of remating so the copulations need not terminate synchronously.

Alternatively, copula duration may be proportional to the amount of sperm transferred by the male, and therefore be correlated with the proportion of fertile eggs laid by the female. A third explanation for prolonged copulations is that they allow a male to displace more of the sperm of rivals, as occurs in *Orthetrum cancellatum* (Siva-Jothy 1987). Sperm displacement occurs in *I. ramburi* (Waage 1986), *I. elegans* (Miller 1987b) and *I. graellsii* (A. Cordero, unpublished data). This hypothesis predicts that copulations with previously mated females should be longer than copulations with virgin females.

My aim in the present study was to investigate why ischnurans have much longer copulations than related species of the same family. I measured the copula duration of *I. graellsii* males at high and low densities, and the egg production of females

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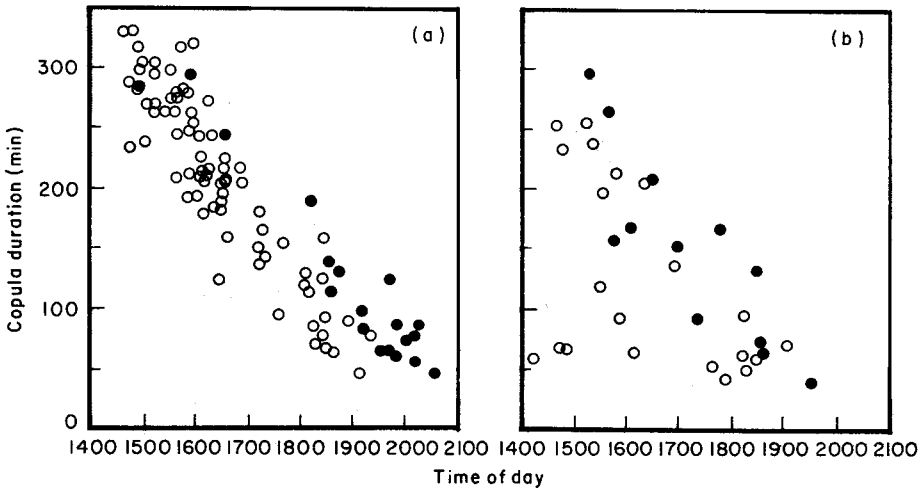

Figure 1. The relationship between the time of day when copulation started and copula duration in *Ischnura graellsii*, in insectaries at (a) high density (15–20 males/insectary) and (b) low density (two males/insectary). The insectaries were illuminated from 0700 to 2200 hours. ○: virgin females; ●: already mated females.

after short and long copulations in order to evaluate the alternative explanations for the prolonged copulations of this species.

METHODS

I kept adult *I. graellsii* in the laboratory in insectaries of 50 × 50 (or 70) × 50 cm, with numerous twigs as perches. The sides of the insectaries were covered with aluminium foil which reflects sufficient light to produce a mirror effect and so inhibits escape (Johnson 1965). The insectaries were placed in a chamber maintained at 21–23°C and 60–80% humidity. Illumination was provided by 19 fluorescent lamps of 58 W, placed 1 m above the insectaries, and kept on between 0700 and 2200 hours, so the chamber was in darkness for 9 h. Adult *Drosophila* were added as food.

All individuals were obtained by the rearing of offspring from 20 laboratory matings and developed under these conditions. After emergence, specimens were individually marked and their total length (from head to tip of abdomen) measured. I could thus record their age and mating history. The sexes were maintained in separate insectaries and, to allow them to copulate, mature females were introduced into insectaries of males. I measured the duration of copulation and of its phases by direct observation of males which matured and lived at two densities: high (15–20 males/insectary) and low (two males/insectary).

I used 25 females that had mated once to study the relation between copula duration and proportion of fertile eggs laid. These females were placed every 2–3 days in individual oviposition chambers, with humid filter paper as an oviposition substrate. The eggs were maintained in petri dishes with water in the same conditions as the adults, until the first egg hatched. Ten days later, the eggs were preserved in 70% ethanol, and were scored as fertile or sterile. All eggs wholly or partially hatched and those with dark eye spots were scored as fertile.

Data on age and number of matings (y) were transformed as $\sqrt{[(y+1/2]}$ before being entered into the correlation analysis, and proportion of fertile eggs (p) as arcsine of $\sqrt{p}$ (Steel & Torrie 1985). Values are given as means ± 1 SE.

RESULTS

Copula Duration

At high density, copula duration was clearly dependent on the time of day when copulation started: short copulations occurred early in the afternoon at low density but not at high density (Fig. 1). The effects of time of start of copulation, age, body length and number of previous matings of both the male and the female on copula duration were investigated using a stepwise regression (Table I). At both densities, time of start had a negative effect on copula duration, and the number of previous matings of the female (which indicates the

Table I. A stepwise regression of the factors affecting mating duration of *I. graellsii* males at high and low density

Density	Variables in the model	*b*	*F*-remove	*P*	Multiple R^2
High	Time of start	−53·81	510·75	<0·0001	0·87
	Matings of female	92·35	25·05	<0·0001	
Low	Time of start	−34·68	30·72	<0·0001	0·61
	Matings of female	109·98	16·24	0·0004	
	Age of female	−49·47	5·25	0·0296	
	Age of male	38·14	4·28	0·0480	

Copula duration is the dependent variable and time of start of copulation, age, body length and number of previous matings of both the male and the female are the predictor variables. Number of matings of the male did not enter into the model.

presence of sperm in the female's reproductive tract) had a positive effect, being the second predictor variable. Thus, males prolong copulation with previously mated females, and the effect is more evident at low density (Fig. 1). At low density, the age of the female negatively affected copula duration and the age of the male positively affected it (Table I).

The duration of stage II was similar at both densities (3·38 ± 0·22 min at high density and 3·93 ± 0·14 min at low density, $F=2·64$, $P=0·11$), and stage III was slightly longer at high density (13·41 ± 0·37 min) than at low density (10·31 ± 0·62 min, $F=20·59$, $P<0·001$). Therefore the variation in copula duration is due to stage I, because stages II and III are also independent of when copulation starts (correlation between stage II duration and time of start, $r=-0·07$, $P=0·62$; stage III, $r=0·07$, $P=0·71$ at high density and $r=-0·30$, $P=0·26$ at low density). Stages II and III were also similar whether the females were mated or unmated (stage II, $F=0·61$, $P=0·44$; stage III, $F=0·25$, $P=0·62$). The duration of the postcopulatory tandem was similar at both densities (14·0 ± 2·5 s at high and 15·0 ± 2·6 s at low density, $F=0·07$, $P=0·79$) and independent of the time of start ($r=-0·10$, $P=0·49$).

Female Fecundity

The females that were maintained in the insectaries after one copulation laid a mean of 1073 ± 127 eggs over their lifetime ($N=25$ females, range = 196–2937 eggs), with a mean of 91 ± 2·2% of fertile eggs (range = 56–99%).

Copula duration does not correlate with female fecundity (lifetime egg production, $r=-0·16$, $P=0·45$; proportion of fertile eggs, $r=-0·18$, $P=0·39$) but is correlated with female body length ($r=0·42$, $P=0·04$). Nevertheless, when all available copulations (including females whose egg production was not measured) are taken into account the correlation with body length disappears (high density, $r=-0·01$, $P=0·93$; low density, $r=0·24$, $P=0·18$). As expected, female longevity is an important factor determining the lifetime egg production ($r=0·74$, $P=0·0001$). For females of equal lifespan, age at copula had a negative effect on egg production (partial $r=-0·62$, $P=0·001$), but age had no effect for females living an equal number of days after copula, indicating that number of days lived after copula is the best predictor of egg production ($r=0·85$, $P<0·0001$).

The male transfers sufficient sperm in one copulation for the fertilization of all eggs laid by the female. Only in the case of exceptional longevity of 16 or more days after copula did the proportion of fertile eggs diminish (Fig. 2). Taking into account the 5–6 days of the maturation period, and the daily survival rate (0·8046 in a natural population, Cordero 1987), less than 1% of females will survive until this age in the field.

DISCUSSION

My results indicate that stage II of copulation, during which the male transfers his sperm to the female (Miller 1987a), is constant and independent of copula duration. In the same way there is no correlation between copula duration and the proportion of fertile eggs laid. This indicates that male *Ischnura* do not transfer more sperm during a prolonged copulation than during a short one. For

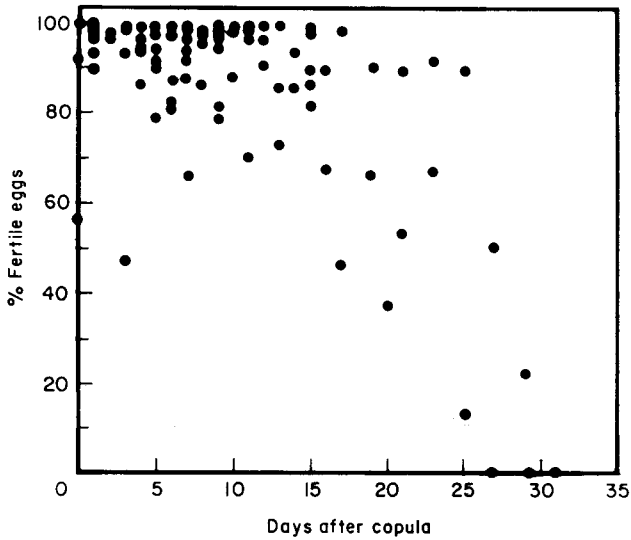

Figure 2. The relationship between the number of days passed after copula and the percentage of fertile eggs laid by *I. graellsii* females mated only once.

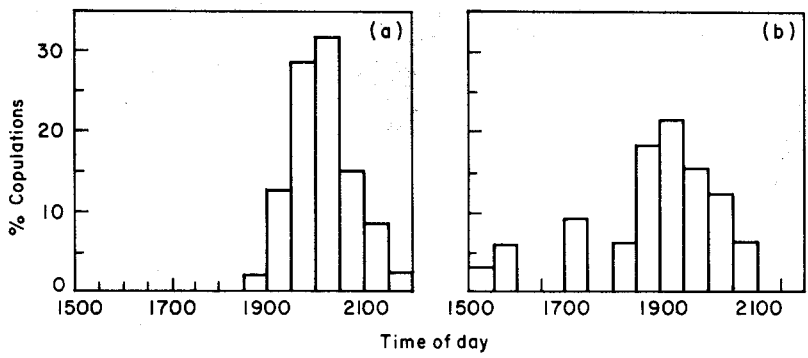

Figure 3. Distribution of termination time of copulations of *I. graellsii* in the insectaries at (a) high density (15–20 males/insectary) and (b) low density (two males/insectary).

example, a copulation of 88 min resulted in 97% of eggs being fertile ($N = 1297$ eggs) while a copulation of 262 min fertilized only 66% of eggs ($N = 902$ eggs). This is because the amount of sperm a male transfers to the female is the whole contents of the vesicle (Miller 1987b), which is filled before the start of copulation.

At high density, copulations with mated females were slightly longer than copulations with virgin females, but the difference was greater at low density (Fig. 1). At low density short copulations in the first hours of the afternoon were accomplished only with virgin females. These results suggest that males are able to detect the presence of sperm in the female's reproductive tract. Sperm displacement could need long copulations, but Miller (1987b) has shown that the greater part of sperm displacement seems to occur early in stage I, and that males of *I. elegans* remove all the sperm of rivals from the bursa but none from the spermatheca. Confirmation of this hypothesis needs multiple mating experiments with genetic markers or irradiated males to estimate paternity.

The results of mating duration at high and low densities are in agreement with an in-copula guarding strategy, but a sperm displacement strategy cannot be rejected. At high density, males prolonged copulations with both mated and unmated females, using primarily an in-copula guarding strategy. At low density, copulations with mated females were

prolonged, perhaps to displace more sperm. When a male copulated with a virgin female at low density, copulations could be long or short as expected with an in-copula guarding strategy. The age of the male then had some influence on copula duration (Table I): old males tended to prolong copulation, as expected if searching for a second female on the same day were more energetically expensive than guarding the first. The age of the female had a negative effect on copula duration, which is in agreement with the fact that old copulating females produce fewer eggs than young ones, for the same lifespan.

Copulations therefore terminated more synchronously at high density than at low density (Fig. 3). Short and long copulations have been observed in a natural population of *I. ramburi* (Robertson 1985), and there is also a negative correlation between time of start and duration of both precopulatory tandem and copulation for *Coenagrion scitulum* (Utzeri & Sorce 1988).

Guarding takes place during the first phase of copulation (stage I), and before the male invests his sperm in the female. This is unusual, but is in agreement with data on *C. scitulum* (Utzeri & Sorce 1988) and the butterfly *Danaus plexippus* (Svärd & Wiklund 1988).

It is commonly assumed that female odonates cannot be forced to copulate (e.g. Waage 1984). If this is true, why do males prolong copulation? I think that the persistence of the male can sometimes modify the behaviour of the female, and force the copulation, as Utzeri (1988) also suggested. Thus, in the insectaries, normal females of *I. graellsii* accept a second copulation on the same day, particularly at high density, when females are grasped by one male after another, whereas androchromatypic females (i.e. male-like coloured females) do not usually cooperate in a second mating (Cordero 1989), showing therefore a different mating strategy. Notwithstanding, females sometimes refuse vigorously all mating attempts. I have seen a male *I. pumilio* retaining a female in tandem for more than 60 min in the field, without obtaining a mating, and there are similar observations on *I. graellsii* in the insectaries. During the morning males show some mating attempts, but females also refuse at that time: only 4% of copulations start before 1330 hours in the field (Cordero 1989).

In this context the relation between copulation duration and male postcopulatory behaviour in *Ischnura* species studied so far is of interest. In species with long copulations (more than 60 min), the male does not guard the female during the oviposition (*I. erratica*, Paulson & Cannings 1980; *I. ramburi*, Robertson 1985; *I. elegans*, Miller 1987a; *I. graellsii*, Cordero 1989; *I. pumilio*, personal observation) while in species with short copulations either the male guards the female in tandem during oviposition (*I. gemina*, Hafernik & Garrison 1986) or the female is monogamous and therefore a postcopulatory guarding strategy is not advantageous for the male (*I. verticalis*, Fincke 1987). Female *I. aurora* oviposit alone after a short copulation which takes place during the first hours after emergence, and older females refuse copulation (Rowe 1978).

In the Odonata there are therefore three types of male strategy to prevent sperm competition: (1) contact guarding in tandem during oviposition, as in Coenagrionidae (Fincke 1982, 1986; Banks & Thompson 1985; Hafernik & Garrison 1986), Lestidae (Utzeri et al. 1976; Ito & Eda 1977; Cordero 1988), Platycnemidae (Aguesse 1968), Aeshnidae (Waage 1984) and Libellulidae (Siva-Jothy 1987); (2) non-contact guarding, as in Calopterygidae (Johnson 1962; Heymer 1973; Waage 1979; Higashi 1981; Alcock 1982, 1983) and Libellulidae (Waage 1984; Tsubaki & Ono 1985); and (3) prolonged copulation (in-copula guarding), which is present in some *Ischnura* species (Robertson 1985; Miller 1987a; this study) and *C. scitulum* (Utzeri & Sorce 1988), which also shows prolonged precopulatory tandem. There are also some species of Odonata in which males do not guard mates after copulation (Corbet 1980; Waage 1984).

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