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REPRODUCTIVE BEHAVIOUR OF *ISCHNURA GRAELLSII* (RAMBUR) (ZYGOPTERA: COENAGRIONIDAE)

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The reproductive behaviour of *I. graellsii* in a high-density population at Lourizán (Pontevedra, Spain) and under laboratory conditions was studied. Copulation duration varies between 74 and more than 250 min. Stage II lasts for 2-8 min and takes place 12.6+0.84 min before the end. Oviposition is made by unaccompanied females, usually in the morning, before the initiation of reproductive activity (94% of copulations take place between 1:30 and 5:30 h after solar noon). It has been shown that andromorph females are not recognized as females by males. Such females mimic the behaviour of males and, in the insectary, rarely copulate twice in the same day, as is usual for heteromorph females. The high morphological and behavioural similarity between *I. graellsii* and *I. elegans*, and the fact that copulation between male *I. elegans* and female *I. graellsii* is possible indicate that they may be sibling species.

INTRODUCTION

Reproductive behaviour in the genus *Ischnura* is very variable. For example, copulation duration varies between 40 min and 5-6 hours according to the species (Tab. I); after copulation the male does not remain in tandem with the female during oviposition, except in *I. gemina* (HAFERNIK & GARRISON, 1986); females copulate several times during their life (MILLER, 1987b; CORDERO, 1987), but *I. verticalis* is clearly monogamic (FINCKE, 1987); females of *I. aurora* copulate a few hours after emergence (ROWE, 1978).

Here I describe the reproductive behaviour of *I. graellsii*, unknown until now, thus extending our knowledge of this species (CORDERO, 1987; 1989). As in other species of the genus, there is female polymorphism, including an andromorph and two heteromorphs: the normal heteromorph female (now called *infuscans*, by analogy with *I. elegans*) and the heteromorph *aurantiaca* (COR-

DERO, 1987). The aim of this work is to determine how female polymorphism affects the sexual behaviour of both sexes.

METHODS

The reproductive behaviour of *I. graellsii* was studied at a marsh situated at Lourizán (Pontevedra, Spain, latitude: 42° 25', longitude: 8° 39'), where the species is very abundant and coexists with *I. pumilio* and a very small number of *I. elegans*.

The population was sampled daily between 15 August and 20 September 1987. Specimens were marked with a number on the wings with a permanent marker pen, and their behaviour was recorded and photographed. Mature adults were recognised by age-related thoracic colour changes (CORDERO, 1987).

Several live individuals were used as models and presented to mature males in order to assess the capacity of males to recognize visually the different female types. To this end the individual used as a model was tethered by its thorax with a thread less than 0.1 mm in diameter, thus permitting great freedom of movement. The model was presented flying at a distance of about 10 cm in front of the male, and after then allowed to perch. The response of the male was recorded under 4 categories: no response; investigation or circling; tandem intent; and tandem (or, exceptionally, copulation). It is assumed that these 4 categories correspond to progressively more intense phases of sexual behaviour.

Additional observations were made in the laboratory, maintaining adults in insectaries (50x50x50 [or 70] cm), at 21-23° C, with a relative humidity of 60-80% and a photoperiod of 15:9 hours of light: darkness.

The results presented here refer to field observations unless otherwise indicated. All times mentioned for field observations are solar time such that solar noon is designated 12:00 h. The measures are presented as means ± SE.

MATING BEHAVIOUR

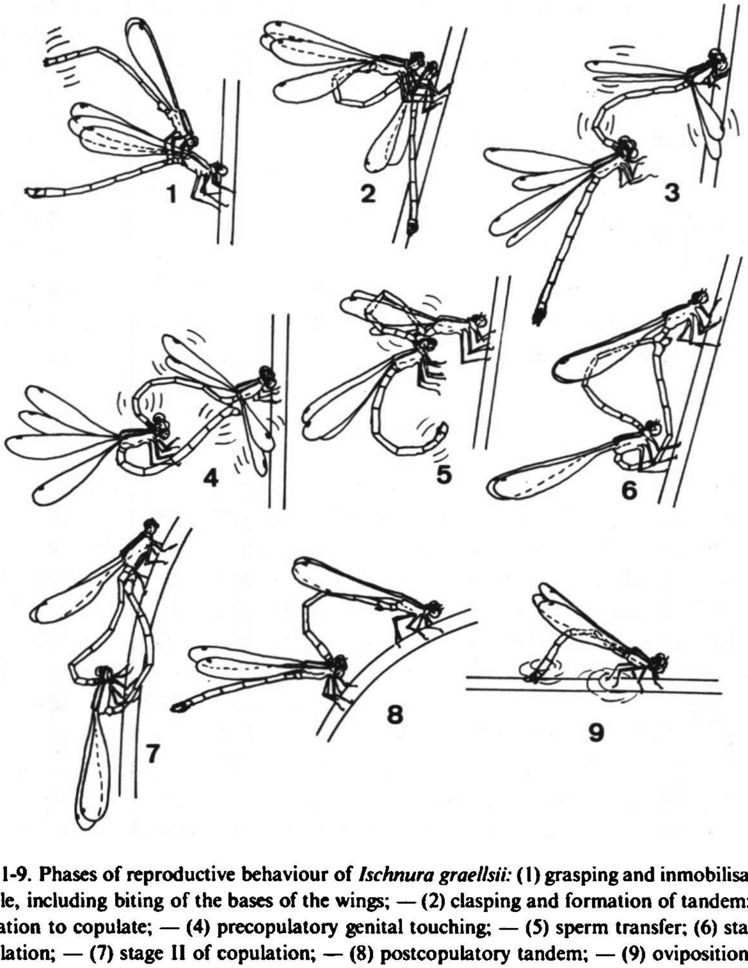
Males stay perched on the vegetation at the water's edge and in adjacent meadows, making short flights searching for females. During morning, very few mature females are observed at water and copulation does not take place. When a male detects a conspecific it rushes at it. When two males meet, in 77% of cases (20/26) the second male faces the first; occasionally (4/26) the second male escapes or a tandem is formed briefly between the males (2/26).

When a female is detected in flight or perched, the male rushes at her thorax and grasps the bases of her wings. Simultaneously (Fig. 1) he beats the female with his abdomen and moves his wings, generating a vibration clearly perceivable when the female is being used as a model. Thereafter he is suitably placed to be able to grasp the prothorax of the female with his anal appendages (Fig. 2).

Precopulatory tandem lasts 2-3 s, whereupon the male invites copulation (Fig. 3) by moving his wings and abdomen. If the female is receptive, she responds by swinging her abdomen forward and upward, resulting in 4-5 precopulatory contacts (Fig. 4; "genital touching"; ROBERTSON & TENNESSEN, 1984), sperm transfer (approximately 1 s; Fig. 5) and copulation (Figs 6, 7).

During the first minute of copulation there are quick movements of the wings and the first two abdominal segments of the male, probably associated with sperm displacement (MILLER, 1987a). As in *I. elegans* (MILLER, 1987a, 1987b), copulation features a stage I (Fig. 6) of a very variable duration, containing long pauses in which no movements are observed, and a stage II (Fig. 7), which lasts 2-8 min and takes place 12.6±0.84 min (n=7) prior to the end of copulation. Stage II is preceded by high frequency thrusting movements of the penis, which in total last less than 1 min. After copulation, the pair perch in postcopulatory tandem (Fig. 8) for 27±17.2 s (n=17).

Copulations observed since initiation lasted 88±6.1 min (n= 6); they began



Figs 1-9. Phases of reproductive behaviour of *Ischnura graellsii*: (1) grasping and inmobilisation of female, including biting of the bases of the wings; — (2) clasping and formation of tandem; — (3) invitation to copulate; — (4) precopulatory genital touching; — (5) sperm transfer; (6) stage I of copulation; — (7) stage II of copulation; — (8) postcopulatory tandem; — (9) oviposition.

Table I
Duration (min) of copulation and postcopulatory tandem of *Ischnura* — [N = sample size]

Species	Copulation+SE	(N)	Tandem+SE	(N)	References
elegans	324±190 180-400	(13)	4-68 —		MILLER, 1987a KIEGER & KRIEGER-LOIBL, 1958
ramburi	202±114	(11)	—		ROBERTSON, 1985
graellsii	74-250		0.45±0.29	(17)	this paper
erratica	62-84		—		PAULSON & CANNINGS, 1980
gemina	43.6±127.4	(190)	35.7±25.4	(106)	HAFERNIK & GARRISON, 1987
verticalis	43.5±2.0	(14)	—		FINKE, 1987
aurora	~ 20		—		ROWE, 1978

after 13:00 h, except in one case. The pairs which had already initiated copulation before 13:00 h remained in copulation for 174±16.4 min (n=10), stage I lasting 162±20.8 min (n=6). Complete copulations witnessed lasted from 74 to 114 min, but two copulations whose initiation was not observed, lasted at least 249 and 250 min. Copulation in the insectary varied between less than an hour and more than five, depending on the hour of commencement. Table I compares the duration of reproductive behaviour in *Ischnura*.

OVIPOSITION

At the end of postcopulatory tandem, the male usually flies first and after a few minutes the female. Most copulations end in the field about 17-18 h (there being close synchronisation among different pairs) and only once was a female seen laying eggs three minutes after the end of copulation. Many females lay eggs (Fig. 9) at midday, usually between 11:00 and 13:00 h before the initiation of copulation.

TEMPORAL DISTRIBUTION OF COPULATION

Analysis of the 297 copulations observed between 15 and 30 August reveals a temporal pattern resembling that described for other populations of *I. graellsii* (CORDERO, 1987). Ninety-four percent of copulations were observed for the first time between 13:30 and 17:30 h. Only 4% started before 13:30 h, in contrast to *I. elegans*, in which many copulations start between 7:30 and 8:00 h (MILLER, 1987a).

VISUAL RECOGNITION OF FEMALES BY MALES

The results of tests in which live models (the different phases of coloration of females and males) were presented to mature males are shown in Table II. Use of live models is efficient; only 18 (5%) of the 329 tested males did not respond, and in one case the female copulated in spite of being tethered by her thorax with wire.

Table II

Response of mature males of *I. graellsii* to models (live conspecific individuals) — [N = number of males tested (18 males which did not respond to the model are excluded). — The first two phases of each model are mature, and the third is immature (CORDERO, 1987)]

Models in colour phase	N	Response (% of males) Approximation	Tandem trial	Tandem or copulation
Heteromorph *infuscans*				
brown	27	0.0	25.9	74.1
olive green	27	29.6	14.8	55.6
clear violaceous	27	14.8	55.6	29.6
Heteromorph *aurantiaca*				
brown	27	22.2	14.8	63.0
orange-red	27	18.5	44.4	37.0
clear orange	24	25.0	46.0	29.0
Andromorph				
blue	27	66.7	11.1	22.2
yellow-green	27	44.4	33.3	22.2
violaceous	25	48.0	52.0	0.0
Male				
blue	27	74.1	18.5	7.4
yellow-green	28	57.1	32.1	10.7
white	18	5.6	94.4	0.0

DISCUSSION

As usually described for *Ischnura,* copulation begins with the male rushing at the female and clasping her prothorax (ROBERTSON, 1985; MILLER, 1987a; FINCKE, 1987). There are complicated steps at this moment in *I. graellsii:* usually the male clasps the female, even biting the bases of her wings (Fig. 1) and quickly clasps her in tandem (Fig. 2), as happens also with *I. elegans* (KRIEGER & KRIEGER-LOIBL, 1958; personal observations). This phase can continue for a few minutes when the female is unreceptive, the male preventing her escape. Sometimes, in the insectary, no receptive females captured in tandem consented to copulate, after having been clasped in tandem for several minutes. There are similar observations from the field, particularly when models were used. This indicates that the persistence of the male can modify the behaviour of the female, and force her to copulate (at least among heteromorphs) as found also by UTZERI (1988).

Some of the phases described here do not always take place. Thus, in the insectary two copulations passed directly from precopulatory contact to copulation, without sperm transfer. In both cases it was observed that the male had previously transferred sperm to his second abdominal segment before an unsuccessful copulation.

The movements of the male genitalia observed during copulation correspond with the stages described by MILLER (1987a) for *I. elegans*, the most important difference being the smaller duration of the stage II in *I. graellsii* (2-8 min in contrast to the 85±48 min in *I. elegans;* MILLER, 1987a). It is reasonable to suppose that males of *I. graellsii* are able to displace the sperm received by the female during previous matings, as occurs in *I. ramburi* (WAAGE, 1986) and *I. elegans* (MILLER, 1987b). The long duration of copulation is probably a method of "in-copula guarding" which avoids further insemination of the female before oviposition (ROBERTSON, 1985; MILLER, 1987a). Experiments are now in progress to test this hypothesis.

I. graellsii presents reproductive behaviour very similar to that of *I. elegans*. In the insectary copulations have occurred between males of *I. elegans* and females (andromorph and heteromorph) of *I. graellsii*, commencing in a manner identical to that described. However, in one case difficulties for separation were observed because the size difference between the two individuals was great (total length: 32.5 mm [male] and 26.4 mm [female]). Females of *I. elegans* also elicit sexual behaviour from males of *I. graellsii*, in the field as well as in the insectary (among the tests made with a female *I. elegans* form *infuscans* as model, 15 out of 16 males of *I. graellsii* tested in the field, made repeated but unsuccessful attempts to form tandem, and one approached the model). Such interspecific copulations are impossible because the prothoracic tubercle of *I. elegans* prevents the males from forming the tandem. Otherwise, none of the 7 females of *I. graellsii* crossed with *I. elegans* layed more than 20 eggs, of which none hatched. In the field also copulation has been seen between a male *I. pumilio* and a female *I. graellsii*, although all attempts to achieve these crossings in the laboratory have failed, and none of the two eggs laid by this female hatched. These interspecific copulations, and the high morphological and behavioural similarity of *I. graellsii* and *I. elegans*, probably indicate that these two species have diverged recently, i.e. that they are sibling species. Both species coexist in central Spain (OCHARAN, 1987), and in this study 4 individuals of *I. elegans* were found among more than 1600 of *I. graellsii;* this being the westernmost record of *I. elegans* in the Iberian Peninsula.

With regard to the female polymorphism of *I. graellsii*, the three known forms

(CORDERO, 1987) correspond to those of *I. elegans* (HINNEKINT, 1987). As happens with *I. ramburi* (ROBERTSON, 1985), the andromorphs, identical to the male in coloration, are not recognized as females by males (Tab. II). Comparing the proportion of males that approached, grasped or clasped the model (Tab.II) it is noted that: (1) the same response was observed with mature andromorphs or mature males as model (blue phase: contingency χ^2=2.6, p=0.27; — yellow-green phase: χ^2 cont=1.6, p=0.46), but different response with young specimens (χ^2 cont=7.0, p=0.008); and — (2) the two heteromorphs are distinguished by males only when the heteromorphs are mature (χ^2 cont=7.1, p=0.03) and are distinguished from the male model and the andromorph model in all phases (except in the case of the second phase of coloration of *aurantiaca* and in andromorphs; χ^2 cont=4.3, p=0.12). Therefore, males do not recognize andromorphs as females, and they do not seem to distinguish between the two heteromorphs. In contrast to heteromorphs, andromorph females usually mimic the behaviour of males (by confrontation) in their meetings with other individuals, as happens in *I. ramburi* (ROBERTSON, 1985). In the tests presenting models it was surprising that two males, after having approached the model and rushed at it (Fig 1), did not try to form the tandem, but remained in this phase. When I examined the supposed males I found that they were really andromorph females! This male-like behaviour was performed also by two andromorph females in the insectary. Therefore the andromorphs mimic with great success not only the "threat display" of males (UTZERI, 1988) but also other phases of sexual behaviour. The biological significance of this behaviour is not evident. This behaviour of andromorphs could be a means to avoid repeated, long copulations (ROBERTSON, 1985), and so allow them to exist at high population density. In fact, an andromorph female secured in tandem by a male, can seldom be forced in the insectary to copulate twice on the same day (2/12), an operation easily accomplished with heteromorphs (15/18; χ^2 cont=10.46, p=0.001). A study in preparation will analyze the mating success of each form, in an attempt to explain how this polymorphism is maintained.

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