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Male calling, mating and oviposition in *Isoperla curtata* (Plecoptera: Perlodidae)

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Abstract. The reproductive biology (drumming call, mating behaviour, fecundity and egg structure) of *Isoperla curtata*, an endemic species from the Southern Iberian Peninsula, is described. The male's mating call has a diphasic pattern, with a mean of 17.3 beats per call (range = 8–27; SD = 4.7) and a duration of 792.9 ms per call (range = 228–1312; SD = 307.9). This call differs from that of other species of *Isoperla* in having two distinct phases with different millisecond intervals, and is species-specific. Mating lasts between 131 and 3864 seconds (mean = 2180.9 s and SD = 1027.8). Since males and females mate more than once (mean number of matings per female was 1.85 and per male 2.25), the species is polyandric and polygynic. The position adopted by the male during mating is different from that described for other stonefly species. Other mating behaviours are interpreted as displacement manoeuvers, tactile stimulation and possibly sexual selection by cryptic female choice. There was a statistically significant correlation between size and the number of matings in females ($r = 0.849$; $p = 0.016$), but not in males. Each female laid between one to four egg masses composed of an average of 88.7 eggs. Maximum fecundity was 319 eggs. The mean egg volume was 80.5×10^5 μm^3 which is very similar to that of other *Isoperla* species. An outstanding morphological characteristic of the egg is the lobed outline of the chorion cells.

INTRODUCTION

Insects display a diversity of reproductive strategies unparalleled among animals. The evolution of insect mating systems was reviewed by Brown et al. (1997), but little is known about stonefly reproductive biology (Stewart, 1994). Moreover, the few studies published in the last decade indicate that stoneflies show a great diversity of reproductive behaviour (Zeigler, 1990, 1991; Hanada et al., 1994; Stewart, 1994; Alexander & Stewart, 1996a, b; Tierno de Figueroa et al., 1998). In this study the reproductive biology (vibrational communication, mating behaviour and oviposition) of *Isoperla curtata*, Navás, 1921, an endemic species of the Iberian Peninsula (Sánchez-Ortega & Tierno, 1996), is described.

MATERIAL AND METHODS

Collecting

Adults were collected on 1 May 1998 at the confluence of the Río Despeñaperros and Arroyo del Rey (Sierra Morena, Jaén, Spain) by sweeping riparian vegetation and collecting them directly from under stones. Probably, some of those collected from under stones were newly emerged, as they were light in colour. The specimens were placed in empty bottles, which were placed in an ice chest to keep them alive for laboratory studies. These methods are similar to those employed by Tierno de Figueroa et al. (1998).

Observations on calling

The male drumming signals were recorded two days after collection between 16:00 and 18.00 h at a temperature between 23 and 25°C, and a light intensity that varied between 60 and 1380 lux. They were recorded by placing a male in a 2.5 × 2.5 × 2.5 cm bottomless plastic box resting directly on a 7.5 cm diameter, 8 ohm, 0.8 W speaker connected to a Philips AZ8052 model cassette-recorder. Sometimes a female was placed close to the male in a separate bottomless plastic box similar to the one containing the male which permitted the female to receive the call of the male. However, no female answered, possibly indicating the females tested were not virgins and only virgin females respond to male signals (Szczytko & Stewart, 1979). Recorded calls were displayed and measured using the IBM-compatible computer program Creative Wave Studio for Windows 4.0 (1993). Fig. 2 was drawn using the STATISTICA package for Windows 4.0 (1993).

Male and female stoneflies were kept in 7 × 5 × 5 cm plastic boxes and their behaviour observed. Mating duration, number of copulations by males and females, and copulations occurring before or between ovipositions were recorded.

Oviposition

Eggs were obtained from stoneflies that oviposited in the laboratory. To encourage oviposition, females with eggs on the end of their abdomen were enclosed over a Petri dish of water (see Khoo, 1964; Snellen & Stewart, 1979) or, in the case of dead females, the clutch of eggs on the end of the abdomen was removed. In some cases, females deposited the clutch directly onto a solid substrate. To determine the number of eggs that a female can produce, the number of eggs laid by the same female in the laboratory, plus those that remained inside the insect, were summed. However, as these specimens were collected from the field, it is possible that they had previously laid some eggs, and therefore the total number of eggs recorded could be less than the maximum number. However, as many of the insects were newly emerged when collected the number of eggs is likely to be close to the real fecundity for this species. In all cases, the eggs were counted under a Zeiss binocular microscope and preserved in 70% alcohol. Both optical microscopy and scanning electron microscopy (SEM) were used to describe the eggs. Egg size was determined by measuring 20 eggs using the ocular micrometer of a Zeiss binocular microscope. Egg volumes (V) were calculated using the formula: $V = ab^2/6$, a = egg length (μm) and b = egg width (μm).

Measurements

The forewing of every male and female was measured and used as an indicator of size. The lengths of both wings, from the base of the wing to the radial sector (rs) vein, were measured and the mean for each individual calculated. These measurements were made using the ocular micrometer of an Olympus binocular microscope.

The percentage measurement error (% ME) and the repeatability (R) of such measurements is known for another morphologically similar congeneric species *I. nevada* ($R = 0.99$, $F_{9,10} = 191.8$; $P < 0.01$; % $ME = 1.0$), and the same procedure for determining R + ME as used by Møller & Zamora-Muñoz (1997) was used in this study. In the case of the egg measurement, the $R = 0.97$; $F_{9,10} = 70.4$; $P < 0.01$, and % $ME = 2.8$. For both egg and wing measurements, the repeatability was very high and the % ME low. The data were analysed using the STATISTICA package for Windows 4.0 (1993).

RESULTS AND DISCUSION

Forty nine individuals (19 males and 30 females), were mainly collected from under stones along the sides and in the middle of the river. Some of the individuals were captured on vegetation, principally on Poaceae, oleander (*Nerium oleander*), ash (*Fraxinus* sp.), Umbeliferae and buckthorn (*Rhamnus* sp.). Whether found under stones or on vegetation the individuals showed a contagious distribution, which supports the idea of the existence of aggregation centres or encounter sites in some stonefly species (Stewart, 1994). Similarly, the existence of stones with a high density of exuviae per surface area (0.75 exuviae per cm^2), close to others with few exuviae indicates a preference for particular emergence sites (in general, places where the river flows fastest).

Drumming

A total of 26 drumming calls produced by 6 males were recorded in the laboratory. They were diphasic consisting of an average of 17.3 beats per call (range = 8–27; SD = 4.7) and had a duration of 792.9 ms (range = 228–1312; SD = 307.9) (Figs 1 and 2). The interval between beats decreased substantially initially (phase I; N = 23; mean beat number = 9.5; range = 5–17; SD = 3.3; mean time = 649.9 ms; range = 149–1140 ms; SD = 296.0), and then was relatively constant for the remaining beats (phase II; N = 23; mean beat number = 8.1; range = 3–12; SD = 2.4; mean time = 118.2 ms; range = 37–185; SD = 36.9). The interval between phases was 17.0 ms (N = 23; range = 13–24; SD = 2.1) (Fig. 2).

To determine whether the calls of the same male differed, we performed an analysis of variance (One-way ANOVA), after showing that the variables (beat number per call and call duration) were distributed normally (K–S d = 0.102, $p > 0.20$ for the call duration and K–S d = 0.122, $p > 0.20$ for the beat number). The ANOVA results were not significant [F (df = 5, 20) = 1.840, $p > 0.05$ for call duration; F (df = 5, 20) = 1.973, $p > 0.05$ for the beats number], therefore the difference in the call characteristics between individuals is not greater than the differences shown by an individual, and therefore we consider all calls as independent events.

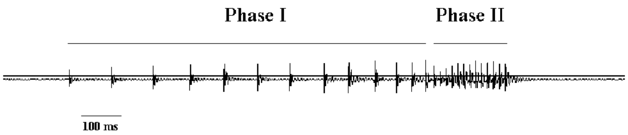

Fig. 1. Drumming call of male *Isoperla curtata*.

The *I. curtata* male call can be considered as a derived character (Zeigler & Stewart, 1987) or as a modified call pattern (Stewart, 1997) because it is diphasic with two distinct interval groups (ranging approximately from 20 to 225 ms in phase I and from 10 to 37 ms in phase II) (Figs 1 and 2). Nevertheless, the method of producing the signal, percussion or drumming, is ancestral to the tremulation or rubbing used by other stoneflies (Stewart, 1997). The call is notably different from that of other *Isoperla* species in Spain, *I. grammatica* (Poda, 1761) recorded by Membiela & Vidal (1998) and *I. nevada* Aubert, 1952 recorded by Tierno de Figueroa & Sánchez-Ortega (1999b), in its duration and intervals, and is a species-specific behavioural pattern, as is common for stonefly calls (Szczytko & Stewart, 1979; Stewart, 1997).

We did not record the response of the female, but did observe a body vibration in females when males called.

Mating behaviour

We observed 36 matings between 16 males and 20 females. The mating lasted between 131 and 3864 seconds (mean = 2180.9 s and SD = 1027.8). The mean number of matings per female was 1.85 (range = 1–4 and SD = 1.0) and per males was 2.25 (range = 1–4 and SD = 1.1). Since both males and females mated more than once, the species is polyandric and polygynic, as is *Hemimelaena flaviventris* (Tierno de Figueroa et al., 1998). As observed in other stoneflies (Berthélemy, 1979; Bottorff et al., 1990; Tierno de Figueroa et al., 1998) drumming is not a necessary prerequisite of mating.

During mating, a male partially mounts a female, with three of his legs on one side are placed on her thorax and wings, and the other three legs on the substrate, and together they take up a "V" position (Fig. 3), rather than the parallel position typical of other stoneflies (including other *Isoperla* species) or the "X" or "head-in-the-air" position (Brinck, 1956; own data). As is general in stoneflies, the male bends his abdomen ventrally and underneath the abdomen of the female in characteristic "S-form", inserts his aedeagus, and transmits sperm into

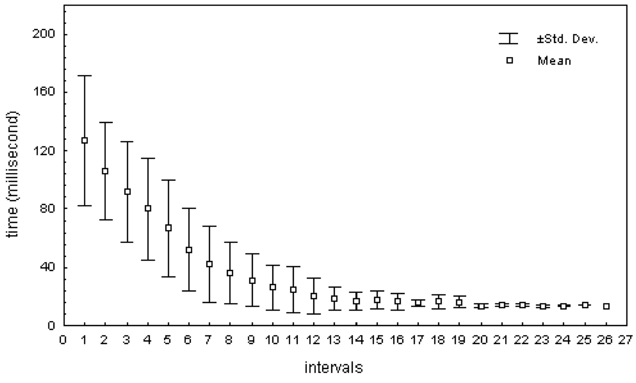

Fig. 2. Time intervals between beats in *Isoperla curtata* male drumming call.

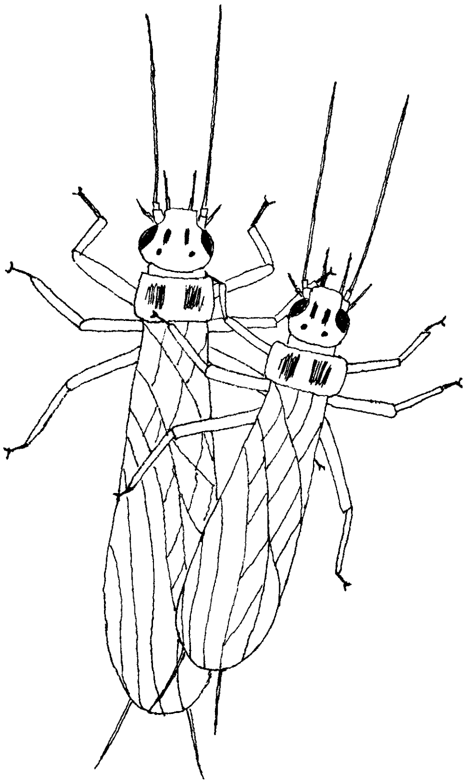

Fig. 3. Mating position adopted by *Isoperla curtata*.

the female's genital aperture in the same way as in other perlodids (Brinck, 1956; Berthélemy, 1979; Tierno de Figueroa et al., 1998). During mating the couples were usually immobile, although sometimes they walked. During mating the male touched the head and antennae of the female with his antennae (Stewart et al., 1969; Tierno de Figueroa et al., 1998). After mating, a female vibrates her abdomen possibly to repulse a new mating attempt. Nevertheless, in all cases females subsequently remated prior to oviposition, an unusual behaviour in stoneflies, and in two cases a female even remated after laying its first clutch of eggs.

The prolonged duration of some matings can be interpreted as mate guarding. Males were frequently observed attempting to displace mating males, but only in one case was a male able to displace the copulating male. In other species such attempts to displace copulating male are usually unsuccessful (Tierno de Figueroa et al., 1998; and unpublished data). In all cases, males began to drum with greater activity just after mating, as has been recorded by Brinck (1949) for other species of stoneflies. This behaviour might give females an indication of the males quality, important in terms of cryptic female choice (female choice between males can often occur when a female has allowed a male to mate her) (Eberhard, 1997).

There was a statistically significant correlation between female size (measured as wing length) and the number of matings ($r = 0.849$; $p = 0.016$) and between the total time spent mating ($r = 0.765$; $p = 0.045$) and female size, but the latter is a consequence of the higher number of matings because the mean time spent mating is not correlated with female size ($r = 0.325$; $p = 0.477$). This could be related to the need of females to fertilize all their eggs, but previous studies on another species of stonefly (Zeigler, 1990) indicate that females can fertilize all their eggs, or the majority of them, with the sperm from one male. Moreover, in the case of *I. curtata*, fecundity does not seem to be correlated with female size ($r = 0.129$; $p = 0.598$) as in other stoneflies (Brittain, 1990; Peckarsky & Cowan, 1991). Other advantages of mating more than once (higher genetic variability, a higher sperm quantity, which favours sperm competition and so compensates for the lower quality of a previous male's sperm) are similar for all females and independent of size, and since females

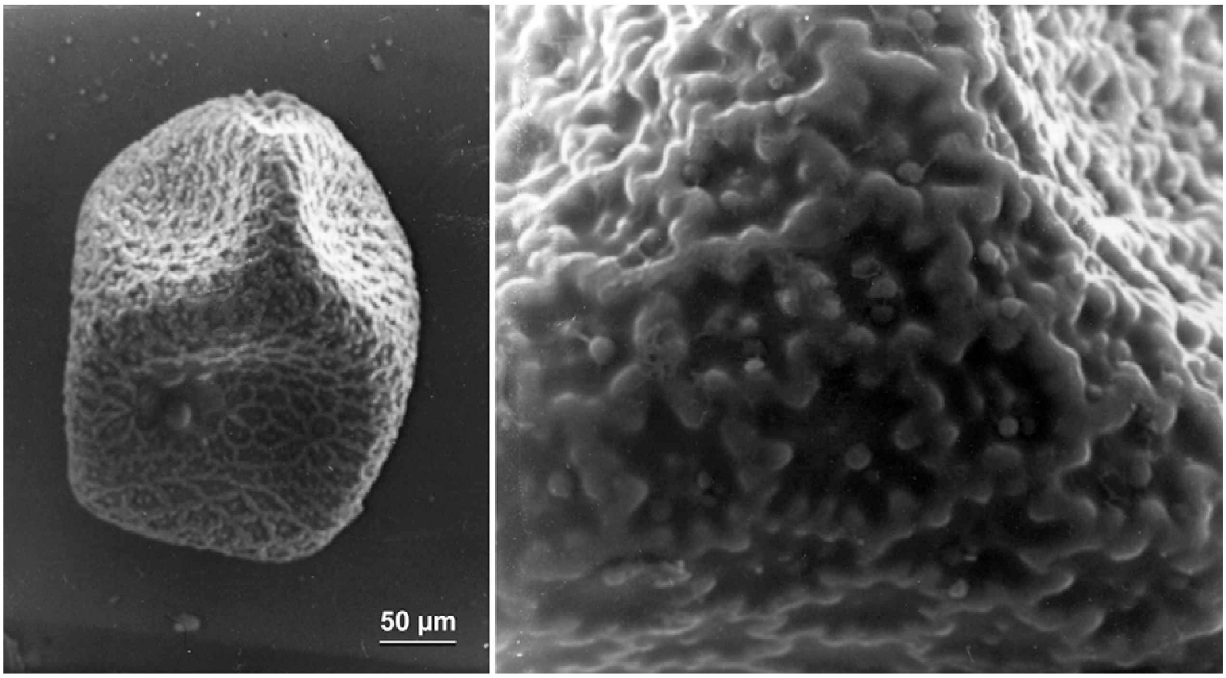

Fig. 4. SEM photographs of an *Isoperla curtata* egg. Left – egg morphology; right – chorion showing details of the follicle cell impressions.

are a scarce resource they can mate many times. There was no correlation between male size and number of matings or between male size and total time spent mating (however this may be a consequence of the small sample size).

Fecundity and eggs structure

A total of 27 clutches were obtained from 12 females. Each female laid between one to four egg masses (mean= 2.3; SD = 1.0). Mean clutch size was 88.7 eggs (range = 13–179; SD = 50.6). The maximum fecundity was 319 eggs (range = 32–319; mean = 203.0; SD = 89.9). In comparison with other *Isoperla* species (Khoo, 1964; Harper, 1973; DeWalt & Stewart, 1995), the number of eggs per clutch is moderate and slightly lower than in *I. nevada* (Tierno de Figueroa & Sánchez-Ortega, 1999a). Although the number of clutches per female is higher than in *I. nevada* (Tierno de Figueroa & Sánchez-Ortega, 1999a) and lower than in *I. grammatica* (Khoo, 1964) (3 and 5, respectively), the fecundity of *I. curtata* is lower than that of both these other species. A decrease in the number of eggs in successive clutches was not observed, as has been reported in other *Isoperla* species (Khoo, 1964) and always a few days elapse between ovipositions. Each clutch of eggs is spherical in shape and transported on the end of a female's abdomen. During oviposition, a female inserts the apex of her abdomen into the water and the eggs quickly disperse. In all the cases, females oviposited during the day, never at night.

Eggs were on average 302 μm long (range = 290–320; SD = 8.9; N = 20), and 225.5 μm wide (range = 210–250; SD = 8.9; N = 20). The mean egg volume was 80.5×10^5 μm^3 (range = 71.5×10^5–101.4×10^5; SD = 6.8×10^5). The eggs are similar in size to those of other *Isoperla* species (Tierno de Figueroa & Sánchez-Ortega, 1999a).

The egg colour varied between yellow and light brown. Each egg has the attachment disc and collar (Fig. 4), typical of other *Isoperla* species (Brinck, 1949; Lillehammer & Økland, 1987; Tierno de Figueroa & Sánchez-Ortega, 1999a). The typical hexagonal cells of the chorion observed in other *Isoperla* species are not present in *I. curtata* where the chorion cells have a lobed outline. This as an interesting and species specific taxonomic character (Fig. 4). Deformed eggs were common and may be a consequence of saving space within the oviduct, as has been suggested for other species of Perloidea (Khoo, 1964; Tierno de Figueroa et al., 1998).

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