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Sexual Behaviour and Stridulation during Mating in *Triatoma infestans* (Hemiptera: Reduviidae)

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Factors affecting mating behaviour in the bug Triatoma infestans were quantitatively studied in the laboratory. Experimental conditions were established so that the probability of copulation increased with the time elapsed since the first adult meal. Copulatory attempts by males did not vary as a function of time, but rejections by females became significantly less frequent with the post feeding time. Non-receptive females displayed four types of rejection behaviour, i.e. flattening, stridulation, evasion and abdominal movements. The occurrence of stridulation performed by females in a sexual context was observed in a regular fashion and was quantified for the first time in this species.

Key words: Triatominae - sexual behaviour - stridulation - Chagas disease

Triatoma infestans is the main vector of Chagas disease in South America. There are very few reports about mating behaviour of this species and other Triatominae. Regis et al. (1985) studied the age at which males of *T. infestans* consummate their first mating, and Malo et al. (1993) analyzed the influence of age and feeding on first mating in *T. mazzottii*. However, the role of female Triatominae in the success of the copula has been relatively neglected. Only occasional observations of rejection responses from females to the copulatory attempts of the males were mentioned by Lima et al. (1986a) for *Panstrongylus megistus* and by Rojas et al. (1990) for *T. mazzottii*, but these were not analyzed in detail.

Lima et al. (1986a) described courtship and copulation in *P. megistus* and also considered some factors related to the mating behaviour, i.e. feeding, duration of copula, fecundity, fertility and longevity (Lima et al. 1986b, 1987a, b). The mating behaviour of *T. mazzottii* was described by Rojas et al. (1990), and more recently Rojas and Cruz-Lopez (1992) compared the sexual behaviour of two species of the *T. phyllosoma* complex.

In the present work we analyze the mating behaviour of *T. infestans* and the role of the females in conditioning the occurrence of the copula. We search for quantifiable behavioural signs as tools for the further analysis of different factors affecting mating, as well as sex recognition and

intraspecific communication in this species. Factors affecting the probability of copulation, the relation between the copulatory attempts by the male and the rejections by the female, and the probability of occurrence of stridulation in the sexual context were also studied.

MATERIALS AND METHODS

The insects - Fifth-instar nymphs of *T. infestans*, reared in the laboratory at 28°C and 75% RH were sorted by sex in separate recipients until imaginal ecdysis. Adults were fed *ad libitum* only once on goat blood using an artificial feeder (Núñez & Lazzari 1990). After feeding, virgin adults were individually isolated in plastic containers (4.5cm high x 4.5cm diameter), with a piece of folded cardboard as substrate and a nylon mesh as cover and floor.

The time elapsed between the imaginal moult of the first and last individual did not exceed 20 days in each experimental group.

Experimental design - The experimental arena consisted of a PVC ring (1.5 cm high x 11 cm diameter) covered by a translucent glass with an entrance hole in the centre. The substrate was a piece of cardboard.

Experiments began always at 6:00 pm, i.e. at the beginning of the scotophase, close to the main activity burst in this species (Lazzari 1992). Room temperature was maintained at 25±1°C and diffuse illumination of 100 lux intensity was provided by a tungsten lamp.

Experimental procedure - Only virgin adults were used. The bugs were placed in the experimental room one hour prior to the experiments. One female and one male were introduced to the

Dedicated to Prof. JA Núñez in occasion of his 70th birthday.

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experimental arena through the entrance hole. The hole was then covered by a piece of glass and the behaviour of the couple was recorded by direct observation.

Each assay lasted 10 min unless the male protruded its genitalia before this time. This observation time was chosen taking into account preliminary assays showing that under identical conditions, mature pairs mate within a couple of minutes.

During the observation we recorded the number of couples that "mated", the number of copulatory attempts by the male and the occurrence of the different types of rejection behaviour displayed by the female. It was considered that bugs "mated", when the males extruded their copulatory organ and contacted the female's genitalia, at this point the pair was immediately separated. In this way, the bugs remained virgin for subsequent assays. Experimental series started from the first post feeding day and lasted until an asymptotic value was reached. Four experimental series, using a total of 33 pairs of insects, were conducted.

Fourteen other pairs were allowed to consummate copulations, in order to quantify the behavioural components of mating in this species.

Some experiments were recorded in video films and audio records, for the posterior analysis of behaviour and sounds produced by mating pairs. The occurrence of true stridulation was confirmed by simultaneous video and audio records.

The different behavioural stages of the copula are here described using the same nomenclature employed by previous authors (Lima et al. 1986a, Rojas et al. 1990), in order to make our description of *T. infestans* mating comparable to those of other species.

RESULTS

The mating behaviour - The couple joins by the initiative of the male in 100% of cases, i.e. the male manifests the first behavioural signs of perceiving the other sex (see below). The mating pattern consisted essentially of seven behavioural steps performed by the male. After a short period (up to 2 min), during which the insects walk around in the arena, they display a quite consistent behaviour: (a) *vigilant position*: the male stops walking and moves its antennae, pointing them to the female. If the female moves, the male follows the direction of her movement with the antennae; (b) *jumping onto or mounting*: the male approaches the female and suddenly jumps onto or mounts her slowly; (c) *rotation or gyration*: once on top of the female, the male performs a rotation of 360°. This behaviour was infrequent; (d) *dorsolateral position*: the male places itself at a dorsolateral position

respectively to the female, either on the right or the left side of her. At this position the female remains immobile, grasped dorsally and ventrally by the male's three pairs of legs. The male sometimes extends the rostrum and places it on the female's head at an angle of approximately 90°. From this *dorsolateral position*, the end of the male's abdomen is placed below the end of the female's abdomen, exposing the masculine genitalia; (e) *copulation*: the male pulls the end of the female's abdomen towards it with its hindlegs. The male's paramers are released and assist in the immobilization of the female's genitalia, allowing introduction of the aedeagus. The copula lasts between 2 and 10 min ($6'24'' \pm 1'57''$, N=14); (f) *subjection*: after the separation of the genitalia, the male returns over the female's top and remains on her for some minutes, fixed by his legs. At this time, the female can walk with the male on her; (g) *separation*: the male gets down from the top of the female and thereby leaves her.

Copulation probability and female receptivity - Fig. 1a depicts the variation of mating probability as a function of the post feeding time. Under

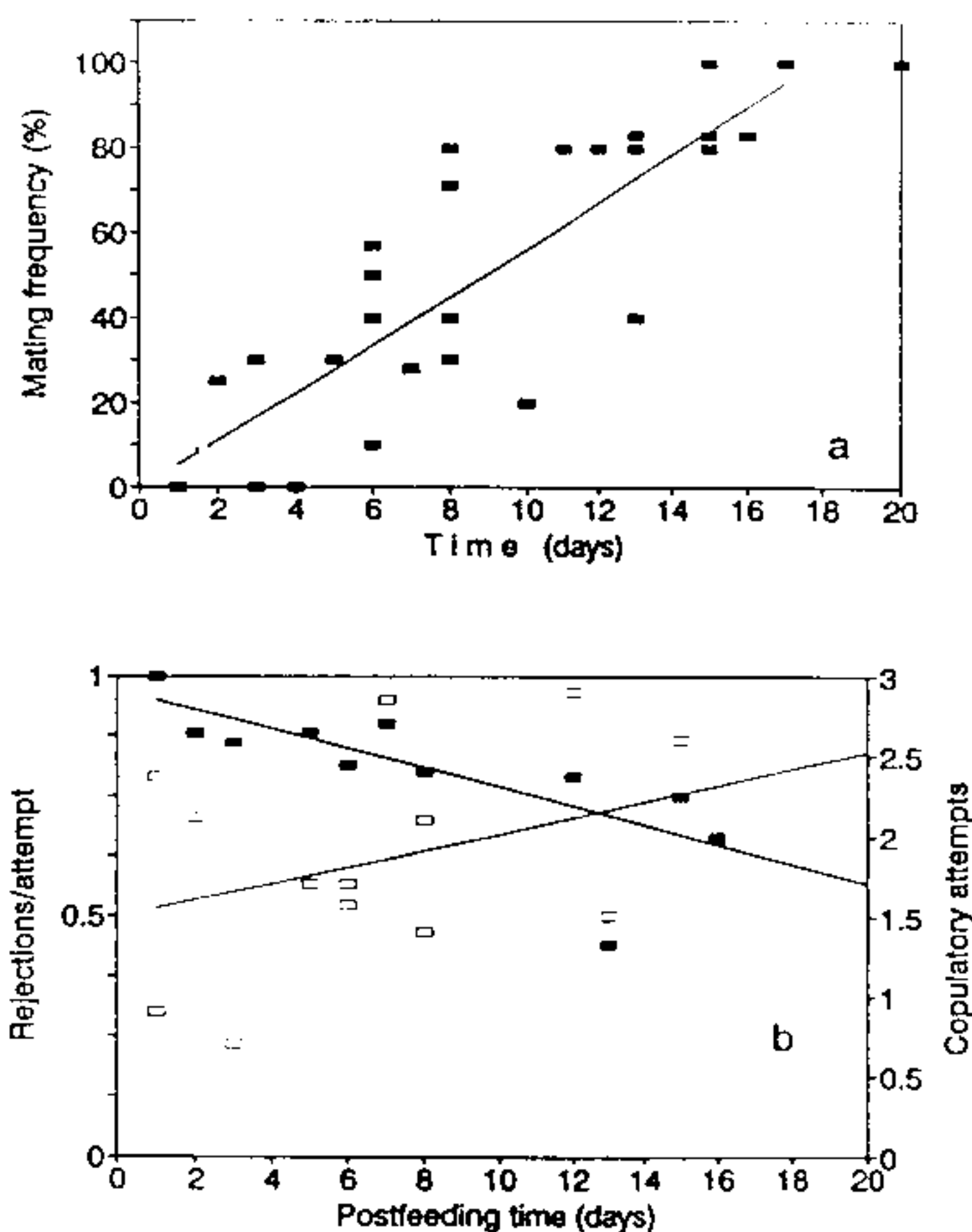


Fig. 1: a - proportion of copulating pairs as a function of the postfeeding time. Points correspond to data from four experimental series (N=33). The regression line represents the variation of mating probability as a function of the time (ANOVA, $P < 0.001$). b - Frequency of all types of female's rejections per male copulatory attempt (ANOVA $P < 0.001$) (■, left y-axis) and frequency of copulatory attempts by males (ANOVA n. s.) (□, right y-axis) as a function of the postfeeding time (two experimental series, N=18).

our experimental conditions, 100% mating probability was reached approximately 16 days after the first meal as adults. The lower probability of mating during the previous days does not mean that copulatory attempts by males did not occur. Males attempted to copulate from the first day onwards, but were rejected. From day 16 onwards, copulation always took place although most females still showed rejection behaviour before the occurrence of copula. This means that female's rejection was not always successful. The frequency of copulatory attempts by males did not vary along the experimental time (ANOVA n.s.), remaining around an average value of 1.9 attempts per male per assay (Fig. 1b). Analysis of the female's behaviour reveals that the proportion of rejections to male copulatory attempts significantly decreased with time (ANOVA $P < 0.001$) (Fig. 1b). The low copulation probability observed until the 16th post feeding day can be ascribed to the female's rejection behaviour.

The female's rejection behaviour - As a response to the males' copulatory attempts (steps 2-5), females performed four kinds of rejection behaviour, as follows: (a) *abdominal movements*: the female moves its legs and body up and down. As a result, the male leaves her; (b) *flattening*: the female flattens her body against the substrate and performs small quick jumps, so that the mounted male can not settle on her back and is unable to reach the female's genitalia; (c) *evasion*: the female avoids the male's jumps by running away; (d) *stridulation*: the female rubs the end of its rostrum against the prosternal groove, in the typical way observed in stridulating bugs (Schofield 1977). Female stridulation occurs once the male is over the female (steps b to e). When the female

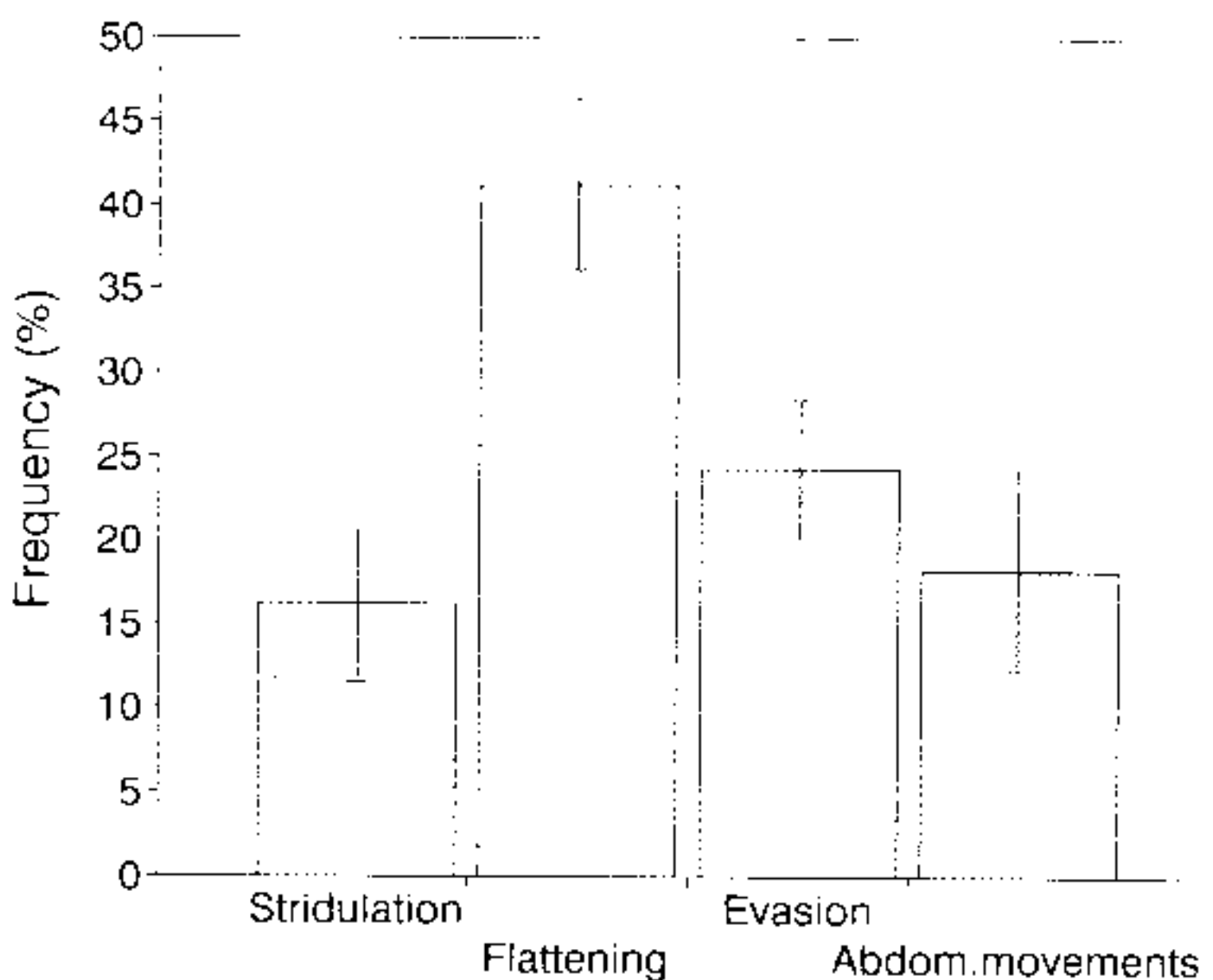


Fig. 2: relative frequency of occurrence of the different types of rejection behaviour displayed by the females (two experimental series, N=18).

stridulates, the male stops its attempt, irrespective of the mating step it was performing at this time. Then, when the female stops stridulating, the male continues with the copulation sequence. If the female stridulates again, the male stops its attempt once more. Finally, after several rejections the male leaves the female. Only on a few occasions, the male continues with his copulatory behaviour regardless of the female's stridulation.

As explained above, female rejection behaviour does not always prevent the occurrence of copulation. However, when efficient, the four types of rejection may cause interruption of the behavioural sequence performed by the male.

Mean frequencies of occurrence of the different types of rejection are depicted in Fig. 2. Our analysis did not reveal a fixed temporal sequence for these four events. While rejection by flattening and evasion exhibited a similar frequency of occurrence in all experimental series, stridulation and abdominal movements showed a considerably variability in their occurrence between series.

DISCUSSION

Mating behaviour - The mating behaviour of *T. infestans* follows the general model described for other Triatominae. However, some differences are worth comment.

Rojas et al. (1990) reported that the female of *T. mazzottii* moved slightly forward before the male jumped onto or mounted her in 100% of cases. They concluded that displacement of the female served as a trigger for continuance of the mating sequence. We also observed this movement of *T. infestans* females, but not in a regular way. In fact, the mating behaviour sequence continued, even though the female remained motionless.

Other minor differences were observed between the behaviour of *P. megistus* (Lima et al. 1986a.), *T. mazzottii* (Rojas et al. 1990) and *T. infestans* (this study). The duration of the copula, the angle of the extended male's rostrum or the frequency of occurrence of gyration seems not to be the same. The observed variations among species remain, at present, as descriptive inter-specific differences.

Female receptivity and mating success - Under our experimental conditions, the mating probability in *T. infestans* depended on the time elapsed since the first meal as adult. Low copulation probabilities were mainly due to the female's rejection behaviour. All previous studies on this subject report an important role of the age of males in conditioning the occurrence of the first mating (Regis et al. 1985, Malo et al. 1993). In our experiments however, the behaviour of males was not the key factor in the variation in mating probability. We present evidence here on the importance of female

receptivity, as a factor affecting copulation success in *T. infestans*.

Our results do not agree, at first glance, with previous reports. Regis et al. (1985) concluded that mating success in *T. infestans* depends principally on males' age, and that the females would be receptive from the 4th day after the imaginal moult. Malo et al. (1993) arrived at a similar conclusion in *T. mazzottii*. It is worth mentioning that our adults were not fed immediately after the imaginal ecdysis, but at a variable time up to a maximum of 20 days. As stated by Salomon (1988), in starved females the reabsorption of oocytes begins from the 9th day post ecdysis. To what extent the ovarian physiological state would affect the mating behaviour of females is at present unknown. However, this could account for the differences between our results and those of Regis et al. (1985). In addition, our experimental method differs from the previous ones. The above authors evaluated mating success of permanent joined pairs, through the presence of spermatophores, during several days. Our results, however, were based on short-term (up to 10 min) direct observation of mating pairs.

Previous reports only incidentally mention the occurrence of rejection behaviour in females of other species (Lima et al. 1986a, Rojas et al. 1990). With our experimental device, we could distinguish different types of rejection displayed by the female of *T. infestans* but the different rejection modalities neither followed a sequential order nor showed a clear relationship between contextual parameters (such as male's mating steps). Therefore we cannot yet deduce if they occur entirely at random.

Stridulation in a sexual context - It is known that Triatominae bugs stridulate by rubbing the tip of the proboscis against the striated prosternal groove (Schofield 1977, Di Luciano 1981, Gogala 1985) although the biological significance of stridulation is not clear. Stridulation of Triatominae was usually observed and recorded when bugs were prevented from moving freely, as a response to mechanical disturbance, e.g. by clasp-ing them with forceps (Schofield 1977). We report here the spontaneous (unforced) occurrence of stridulation, in contrast to disturbance stridulation mentioned in the literature. For the first time in Triatominae, the occurrence of stridulation was regularly observed in the context of sexual behaviour.

Schofield (1977) analyzed the air-borne waves produced by the disturbance stridulation of several species of Triatominae. This author interpreted the signal as "sound production", i.e. pressure waves propagating through the air, measuring frequencies that reached up to 100 kHz. He deduced

that bugs could receive the signal via the antennae (Johnston's organ), and that this corresponded to airborne waves. However, other interpretations are possible. In particular, it has been demonstrated that some land bugs utilize the vibratory channel for communication (Gogala & Cokl 1983, Gogala 1984, 1985). This involves waves propagating through the substratum or the bodies of individuals in contact, as well as nearfield medium motion. Thus, vibrational communication in reduviids may be also involved in the sexual context. Vibrational waves could be here perceived by bugs throughout cordotonal organs, such as the subgenual or the Johnston's organs of the antennae in contact with an stridulating female. Vibrations propagating as nearfield medium motion are transmitted as air-borne waves, but have physical properties different to sound pressure waves (Markl 1983, Michelsen 1983). This means that perception of the signal via the antennae (Schofield 1977) may not necessarily correspond to sound perception. Further experiments are necessary to elucidate whether vibrational communication plays actually a role in the sexual behaviour of reduviids.

We bring here some evidence supporting a communicative role of stridulation between sexes during mating, related to rejection of the copula by females. This function has been first suggested by Readio (1927), who ascribed a defensive role to stridulation in reduviids and argued that females stridulate during copulation as a "protest".

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