Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 81(1) :1-5, jan./mar. 1986

BEHAVIOR OF TRIATOMINES (HEMIPTERA : REDUVIIDAE) VECTORS OF CHAGAS' DISEASE. I COURTSHIP AND COPULATION OF *PANSTRONGYLUS MEGISTUS* (BURM., 1835) IN THE LABORATORY

MARLI MARIA LIMA* PEDRO JURBERG* & JOSIMAR RIBEIRO DE ALMEIDA**

A study of the courtship and copulation behavior of Panstrongylus megistus *was carried out in the laboratory. Fifty-five newly-fed virgin couples were used. Experiments were performed during the day (9:00 to 12:00 a.m.) and at night (7:00 to 10:00 p.m.). Behavior was recorded by direct observation and was found to consist of the following sequence of behavioral patterns: the male approached the female and jumped on her or mounted her; he took on a dorsolateral position and immobilized the female dorsally and ventrally with his three pairs of legs; the male genital was placed below those of the female; the paramers of the male immobilized the female's genitals; copulation started.*

The couple joined by the iniciative of the male. The female could be receptive and accept copulation, or nonreceptive and reject the male. Copulation occurred more often on the occasion of the first attempt by the male. Duration of copulation was $\bar{X} = 29.3 \pm 9.3$ *min (CV = 83%).*

No behavioral differences were observed between couples tested during the day or at night.

Key words: courtship – mating – reproduction – triatomines – *Panstrongylus megistus* – behavior

Males of different species frequently show specific courtship behavior to which only females of the same species normally respond. "Specific recognition, which is based on the production and reception of stimuli by individuals of the opposite sex, is fundamental in behavioral isolation and, consequently, in reproductive isolation" (Mayr, 1977).

Richards (1927), Koref-Santibañez (1963) and Chianotis (1967) pointed out that "species recognition" simply functions on the basis of the exchange of appropriate stimuli between the two sexes. Reciprocity of adequate stimuli strengthens the guarantee of intraspecific crosses and consequently helps to prevent hybridization with individuals of other species.

In this respect, it is important to investigate the sexual behavior of triatomines, since specific "reproductive success" is intimately associated with the potential for colonizing of new species.

MATERIAL AND METHODS

Fifty-five pairs of *Panstrongylus megistus* (Burm., 1835) consisting of virgin adults born from 5th-instar nymphs were used. The insects were provided by the Department of Entomology of the Oswaldo Cruz Institute. After sex-screening according to the techniques of Espínola (1966) and Lent & Jurberg (1969), the nymphs were maintained in 24 x 17cm jars. The triatomines were allowed to feed on the blood of a pigeon once a week, the meal lasting until the insects were fully gorged. After imaginal ecdysis, adults were marked individually by the technique of Mac Cord, Jurberg & Lima (1983), and males and females were placed in separate jars.

At the time of the experiment, a pair was placed in a 24 x 18 x 6 cm experimental chamber with a glass front that permitted visualisation and internally lined with filter paper to favor insect locomotion. The upper part of the chamber was covered with a movable transparent and perforated acrylic plate which facilitated aeration of the chamber and permitted insect placement and removal.

Behavior was recorded by direct observation on standardized (protocol) sheets used to notate items selected from previous observations. The (+) sign was used to indicate the occurrence of a behavior, and the (–) sign to indicate no occurrence. The items marked were: time (min) for

Supported by CNPq and FIPEC.

*Departamento de Biologia, Instituto Oswaldo Cruz, Caixa Postal 926, 20000 Rio de Janeiro, RJ, Brasil.

**Fundação Oswaldo Cruz and Setor de Ecologia, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 520, 20550 Rio de Janeiro, RJ, Brasil.

Received for publication March, 19th and accepted December 4th, 1985.

male/female contact to occur; male/female (M/F) contact; male jumping onto or mounting the female (ju/mo); attempts of copulation by the male (total number); receptivity of the female (+ / –); copulation on the right or left side of the female (L/R); approximate angle of the male's antenna; approximate angle of the female's antenna; time (min) of copulation without dislocation of the pair; rythmic motion by the male (+ / –); rythmic motion by the female (+ / –); time (min) during which the female moved carrying the male in a dorsoventral position; time (min) during which the female moved dragging the male joined to her by the genitals; total duration (min) of copulation; indifference of the male towards copulation (+ / –). The protocol also contained the identification number of each insect, light intensity (lux), any possible observations, and the date of the experiment.

The experiments were carried out during the day (9:00 to 12:00 a.m.) and at night (7:00 to 10:00 p.m.). Time was recorded from the moment when each pair was placed in the experimental chamber. If no attempt of copulation occurred within 30 minutes, the pair was replaced for another one. The pairs were used seven to thirty days after the last ecdysis. Mean temperature was 25.6 ± 0.9°C and relative air humidity was 80.2 ± 7.5%. The only type of environmental illumination used was a 5 watt red lamp placed 20cm from the experimental chamber which supplied 88 lux of light intensity (LUNASIX 3 photometer). This type of illumination left the experimental room in semi-darkness, thus permitting the observation of insect behavior.

RESULTS

Recording of copulation behavior showed a sequence of behavioral patterns initiated by the male (Fig.1). The male first moved about to explore, then approached the female and jumped onto or mounted her. When on top of the female, he took on a dorsolateral position placing himself either on the right or on the left side and immobilizing her with his three pairs of legs by securing her ventrally and dorsally by the pronotum and abdomen. The male's antennae opened to an angle of approximately 180°, while his rostrum moved away from his gula at an angle of approximately 40°. The posterior end of the male's abdomen was placed below the posterior end of the female's abdomen, with exposure of the male's genitals. The male's paramers, which are normally withdrawn were released and used to immobilize the female's genitals, thus facilitating the introduction of the aedeagus into her genital opening. Copulation was then initiated and the pair did not move for $\bar{X}$ = 16.5 ± 4.9 min. During this time the male performed rythmic movements by shaking the antennae back and forth and upwards over a 180° path and by tapping lightly the pronotum, conexivum and abdomen of the female with his legs. He moved his body laterally and vertically, then returned to a resting position until the female started to move. At the beginning of this phase, the male remained on the dorsolateral part of the female for about one minute, with all his legs located on the dorsal and ventral regions of the female. He then jumped off the female and placed his legs on the substrate. The two insects placed themselves in diametrically opposite positions while attached to each other by the genitals and the female dragged the male around the chamber for $\bar{X}$ = 11.0 ± 6.6 min, usually ending up with a complete separation of the pairs. The total length of the copulation period was $\bar{X}$ = 29.3 ± 9.1 min (CV = 83%).

Receptive females lifted the end of the abdomen, thus allowing copulation to occur. Non-receptive females push the male away with their legs and placed the end of the abdomen against the wall of the chamber intensely shaking their body from side to side even when the male approached them several times.

All attempts of copulation were made by the male and were characterized by the patterns described above, such as male approaching the female jumping on top of her or mounting her, taking on a dorsolateral position and exposing the genitals. During these attempts, copulation could or could not occur. It occurred after one or more attempts, being more frequent during the first one, that was 74.8% of the cases studied here. Copulation occurred during the second attempt in 14.2% of the cases, and during the third in 7.9%. Copulation also occurred after the fourth, fifth or sixth attempts, but more rarely.

At the end of copulation the pairs separated their genitals, although one pair died during copulation and in other cases the mates died 24 and 72 hours after copulation, respectively, while their genitals were still attached.

No differences in courtship or copulation behavior were detected between pairs tested during the day and at night.

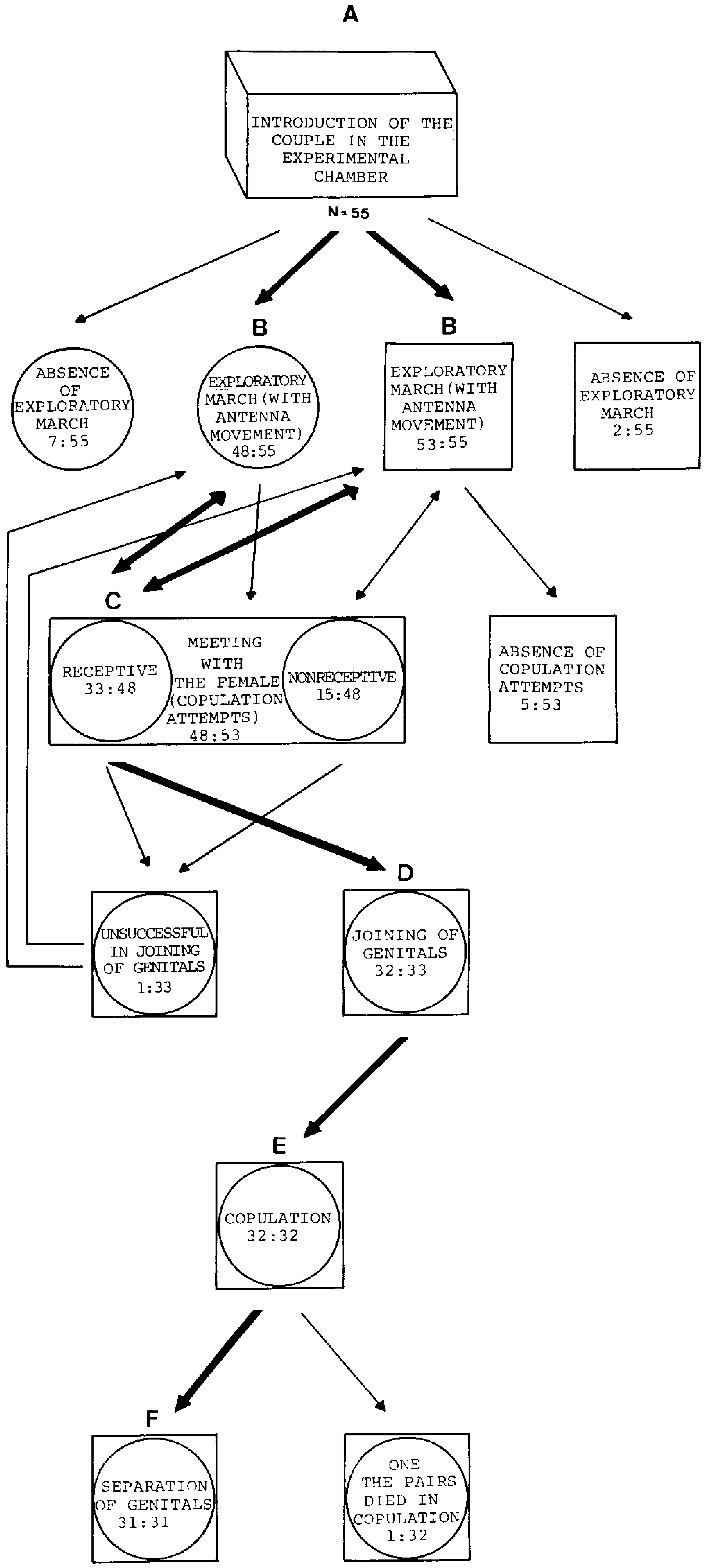

Fig. 1: sequence of the behavioral repertoire of courtship and mating of *P. megistus.* The thick arrows show the preferencial sequences. The thin arrows show the alternative sequences. The rectangles indicate the patterns performed by the male; the circles indicate the patterns performed by the female; the rectangles with the circles indicate the patterns performed by the couple. The numbers indicate the frequence ratio of each pattern and the letters A to E, the complete sequence of copulation.

DISCUSSION

The copulation behavior of *P. megistus* seemed to be initiated by the ambulatory movements of the female. Courtship was not as elaborate as among other insects, but basically consisted of the male perceiving, approaching and jumping on top of the female. Hase (1932) reported that *Rhodnius pictipes* and *Panstrongylus geniculatus* males performed what the author called "loving dance" ("Tantztelung") before starting copulation, but concluded that this was not a general rule. Campos (apud Galliard, 1936) observed that in *Triatoma dimidiata* copulation is sometimes preceded by nuptial movements. The male and the female intertwine their legs, performing rythmic movements and the oscillations. Silva (1982), in a study on *Triatoma infestans*, at first suggested that the male performed a "loving courtship" before starting copulation, but, after more careful observations, concluded that the phenomenon so designated only consisted of randon movements of no major significance and that "when a *T. infestans* male recognizes a female, it may or not move its antennae, proboscis and forelegs. When the last phenomenon occurs, copulation is immediate and not preceded by characteristic movements". Among other hemipterans, however, such as *Dysdercus fasciatus* and *D. maurus*, courtship is performed by the rapid touch of the male antennae of the female, while their heads look to opposite sides (Brunt, 1971; Almeida & Xerez, 1985). Amaral Filho (1981) reported for the hemiptera *Phtia picta* that antennal motion is slow and that the heads follow the same direction.

It is probable that the rythmic movements of *P. megistus* males during copulation are associated with spermatophore transfer to the female, as suggested by Almeida & Xerez (1985) for *D. maurus*, by Amaral Filho (1981) for *P. picta*, by Brunt (1971) for *D. fasciatus* and by Davey (1965, apud Brunt, 1971) for several insects.

The duration of copulation varied in *P. megistus* lasting on average 29.3 ± 9.1 minutes. Pinto (1930) stated that copulation is slow among triatomines and Borba (1972) reported that the duration of copulation of *P. megistus* is variable, but neither author specified the time. Galliard (1936), in a study on *R. prolixus* and *Triatoma vitticeps* reported duration of copulation to be three to four minutes, with one case of twenty minute duration for *T. vitticeps* and one of two hours for *R. prolixus*. In *T. infestans*, copulation, on average, lasts ten minutes (Schofield, 1979), and for most triatomine species it lasts five to fifteen minutes (Lent & Wygodzinsky, 1979). In *P. megistus*, copulation lasted 15 to 66 minutes. One pair copulated for 75 minutes and another for 8 hours and 45 minutes (data not computed in the above mean). Egg fertility could not be evaluated for the latter case, because the pair died during copulation.

In *P. megistus* the end of copulation is preceded by a period of time in which the female drags the male attached to her through the genitals. Lent & Wygodzinsky (1979) suggested that torsion of the male's genital capsule for aedeagus introduction occurs by hemostatic pressure. Thus, this period of time in which the male allows the female to drag him may be due to hemodynamic factors involving interlocking of the genitalia. The fact that the female is able to decide the direction to be followed may be linked with the fact that she is larger than the male, as previously suggested by other authors for other insects (Almeida & Xerez, 1985).

By comparing the copulation behavior of the hemipterans reported in the literature *(op. cit.)*, triatomines in particular, with the results obtained in the present paper, a few similarities were observed. It is possible that a standardized sequence of phases of reproductive behavior exists among taxonomically close triatomine species.

In some cases, reproductive isolation at the behavioral level may not be due to the nature or sequence of the ritualized phases, but rather to their duration. Quantitative differences in behaviors shared by taxonomically close groups may function as a mechanism of reproductive isolation by preventing successful synchronization of reproductive readiness.

RESUMO

O estudo do comportamento de cópula de *Panstrongylus megistus* foi feito em laboratório. Foram utilizados 55 casais virgens, recém-alimentados. As experiências efetuaram-se de dia (9-12h) e à noite (19-21h). O registro do comportamento foi feito por observação direta, constatando-se uma seqüência de padrões comportamentais caracterizados por: o macho se aproxima e salta ou sobe sobre a fêmea; assume posição dorso-lateral e imobiliza a fêmea, dorsal e ventralmente, com os três pares de patas; a genitália masculina se coloca abaixo da feminina; os parâmeros do macho atuam na imobilização da genitália da fêmea; iniciam a cópula.

A aproximação dos casais se dá por iniciativa do macho, podendo a fêmea estar receptiva, facilitando a cópula, ou não receptiva, rejeitando o macho. A cópula é mais freqüente na primeira tentativa do macho. A duração do período de cópula foi de $\bar{X}$ = 29.3 ± 9.1 min (CV = 83%).

Não houve diferenças comportamentais entre os casais testados durante o dia e à noite.

ACKNOWLEDGEMENTS

The authors thank Dr. José Jurberg, Dr. Leonidas Deane and Dr. Virgínia T. Schall for helpful criticism.

REFERENCES

ALMEIDA, J.R. de & XEREZ, R. de, 1985. Comportamento reprodutivo de *Disdercus maurus*, Distant, 1901 (Hemiptera, Phyrrhocoridae) em condições de laboratório. *An. Soc. Ent. Brasil.*, in press.

AMARAL FILHO, B.F., 1981. Aspectos comportamentais de *Phtia picta* (Drury, 1770) em condições de laboratório (Hemiptera, Coreidae). *Rev. Brasil. Biol., 41* (2) :441-446.

BORBA, A.M., 1972. Biologia de *Panstrongylus megistus* (Burm., 1835) em condições artificiais – Aspectos ecológicos e epidemiológicos desta espécie no Estado do Paraná (Hemiptera: Reduviidae). Tese de mestrado. Depto. de Zoologia da Universidade Federal do Paraná.

BRUNT, A.M., 1971. The reproductive behaviour of *Dysdercus fasciatus*, Signoret (Hemiptera, Pyrrhocoridae) in cultura. *Ent. Monthly Mag., 107* :18-23.

CHIANOTIS, B.N., 1967. The biology of California *Phlebotomus* (Diptera: Psychodidae) under Laboratory condictions. *J. Med. Ent., 4* (2) :221-233.

DAVEY, K.G., 1965. Copulation and egg-production in *Rhodnius prolixus* the role of the spermathecae. *F. Exp. Biol., 42* :373-378.

ESPÍNOLA, H.N., 1966. Nota sobre diferenças sexuais em formas imaturas de Triatominae (Hemiptera, Reduviidae). *Rev. Brasil. Biol., 26* (3) :263-267.

GALLIARD, H., 1936. Récherches sur les reduvidés hématophages *Rhodnius et Triatoma. Ann. Parasitol. Hum. Comp., 14* :1-34.

HASE, A., 1932. Beobachtung an venezoelanischen Triatoma Arten, zur allgemeinen kanntnis der Familie der Triatomidae (hempt.- Heteropt.). *Zeits- f- Parasitenk., 4* :585-652.

KORET-SANTIBAÑEZ, S., 1963. Courtship and sexual isolation in five species of the Mesophragmatica group of the genus Drosophila. *Evolution, 17* (1) :99-106.

LENT, H. & JURBERG, J., 1969. Observações sobre o ciclo evolutivo, em laboratório, de *Panstrongylus geniculatus* (Latreille, 1831) (Hemiptera: Reduviidae: Triatominae). *Ann. Acad. Brasil. Ciências, 41* (1) :125-131, fgs. 1-7.

LENT, H. & WYGODZINSKY, P., 1979. Revision of the Triatominae (Hemiptera: Reduviidae) and their significance as vectors of Chagas' Disease. *Bull. Am. Mus. Nat. Hist. N.Y., 163* (3) :123-520.

MAC CORD, J.R.; JURBERG, P. & LIMA, M.M., 1983. Marcação individual de triatomíneos para estudos comportamentais e ecológicos. *Mem. Inst. Oswaldo Cruz, 78* (4) :473-476.

MAYR, E., 1977. Populações, Espécies e Evolução. Cia. Ed. Nacional. EDUSP, S. Paulo. 486 pp.

PINTO, C., 1930. Triatomíneos. Tratado de Parasitologia, Vol. IV: Artrópodes parasitos e transmissores de doenças (Tomo I) Ixodídeos e outros :173-234.

RICHARDS, O.W., 1927. Sexual selection and allied problems in insects. *Biol. Rev., 2* :298-364.

SCHOFIELD, C.J., 1979. The behaviour of Triatominae (Hemiptera: Reduviidae): A review. *Bull. Ent. Res., 69* :363-379.

SILVA, R.L.E., 1982. Aspectos da história natural do *Triatoma infestans*: Klug, 1834 (Hemiptera, Reduviidae, Triatominae). Tese. 200 páginas. Instituto de Ciências Biomédicas, Universidade de São Paulo – USP.