

## Research Article

# Reproductive Biology, Mating Behavior, and Vibratory Communication of the Brown-Winged Stink Bug, *Edessa meditabunda* (Fabr.) (Heteroptera: Pentatomidae)

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We describe different aspects of the reproductive biology, mating behavior, and vibratory communication of the pentatomid *Edessa meditabunda* (Fabr.). This species shows lower copulation frequency and reproductive potential with longer sexual maturation period compared to other species of pentatomids. Females with multiple mating show increased fecundity when compared with single-mated females and both increased fecundity and reduced longevity when compared with virgin females. Courtship and mating behavior and vibratory signals are typical and similar to what was observed in other species of pentatomids, except that males started the courtship. These results constitute the first paper on biology, behavior, and vibratory communication among species of the subfamily Edessinae.

## 1. Introduction

The brown-winged stink bug, *Edessa meditabunda* (Fabr.) (Heteroptera: Pentatomidae), has been reported as a minor component of the stink bug species complex that is economically important pest in many crops mainly of families Solanaceae and Leguminosae [1]. In Brazil, *E. meditabunda* is present in the central-west and southern states [2–4]. Although *E. meditabunda* has been reported on a large number of plant families [5–7], soybean plants (*Glycine max* L.) seem to be a preferred host for adult feeding and reproduction [2].

The life cycle of *E. meditabunda* has been reported by several authors [8–11], however, with no detailed information about the reproductive and sexual behavior of the species.

Reproductive behavior of pentatomids is mediated by communication signals of different modalities among which sex pheromones are involved in female-male encounters, courtship, and mating [12–16]. Sex pheromones of stink bugs show high variability in the chemical structure, but

in some cases, as in *Nezara viridula* (L.) and *Chinavia* spp. the pheromones consist of isomers of the same compound (trans and cis (*Z*)-bisabolene epoxide) in different ratios [16]. Communication with vibratory signals transmitted through the plant is used among others for mate location and recognition [17–20]. Communication with species-specific substrate-borne vibratory signals as the key element of mating behavior has been demonstrated in different species of Heteroptera, for example, in *Nezara viridula* [21], *Acrosternum hilare* (Say) [22], *Lygocoris pabulinus* (L.) [23], *Chinavia impicticornis* (Stål) (= *Acrosternum impicticorne*), *Euschistus heros* (Fabr.), *Piezodorus guildinii* (Westwood), *Thyanta perditor* (Fabr.) (Heteroptera: Pentatomidae) [17], and many other species [19].

No data on sex pheromones or communication signals of any other modality and general mating behavior among species of Edessinae have been described. Therefore, we investigated different aspects of *E. meditabunda* reproductive biology, mating behavior, and communication signals.

## 2. Material and Methods

**2.1. Insect Rearing.** Adults of *E. meditabunda* were collected from soybean fields in Brasilia, DF, Brazil, and maintained in a colony in the Semiochemicals Laboratory at Embrapa Genetic Resources and Biotechnology (Brasilia, DF). The insects were reared in plastic cages (8 L) and fed on bean pods (*Phaseolus vulgaris*), soybean pods, and stalks of “boldo-brasileiro,” *Plectranthus barbatus* (Lamiaceae). Stalks of *boldo* were placed in 7.5 cm high  $\times$  7.5 cm diameter plastic containers, half-filled with vermiculite (sterilized at 120°C for one hour), and sprayed with tap water to simulate a wet soil conditions to the plants and provide humidity.

The containers were placed inside the rearing cages (one vial/cage), which were kept in a climatic room at  $26 \pm 1^\circ\text{C}$ ,  $60 \pm 10\%$  RH, and photophase of 14L:10D. Twice a week the containers were sprayed with tap water to keep the vermiculite moist and the plants replaced every week. Most of the eggs were oviposited on leaves of *boldo*, and, after oviposition the eggs mass attached to the leaves were transferred to new rearing cages. Egg masses were examined daily for hatching and molting, and nymphs were fed in the same way as adults. After completing the immature development (<24 h after the final molt), adults were collected and managed as described above for the experiments and for colony maintenance.

**2.2. Sexual Maturity and Longevity of Males and Females.** To determine the sexual maturation age and longevity for each sex separately, one virgin male (1 to 2 days after the final molt) was placed together with three 15-day-old females in plastic cup (500 mL) and fed as previously described. Subsequently, one virgin female (1 to 2 days after the final molt) was placed together with three 15-day-old males. The groups ( $N = 10$  for each adult combination) were observed daily every 30 min between 13:00 and 18:30 h that was the time interval when major frequency of copulation was previously observed. When a female or a male of each group (3 females or 3 males) dies, it was replaced by a new insect at the same age.

### 2.3. Mating Frequency Experiments

**(1) Multiple-Mated Females.** One virgin female and one virgin male (<24 h after final moult) were placed into plastic pots of 500 mL ( $N = 20$ ) to record the mating sequence. The insects were observed daily every 30 min during the period of higher frequency of copula (determined in previous observations) during the photophase (from 8:30 to 18:30 hours). The couples were allowed to mate throughout their lifetime. For this group of females, only those observed in copula at least two times ( $N = 18$ ) during their life span were considered for data analysis.

**(2) Single-Mated Females.** One virgin female and one virgin male (<24 h after final moult) ( $N = 20$ ) were placed into plastic pots of 500 mL ( $N = 20$ ) and observed every 30 min for the first mating (from 8:30 to 18:30 hours). Once mating

had finished males were removed and females were observed daily until they died. Only females observed in copula ( $N = 15$ ) were included in data analyses.

**(3) Virgin Females.** Single virgin females (<24 h after final moult) were isolated in plastic pots of 500 mL ( $N = 20$ ) and observed for egg production and longevity.

Insects of the different treatments were fed as described above. Reproductive parameters (number of egg mass/female, number of eggs/mass, number of eggs/female, and number of nymphs/number of eggs (fertility)) were recorded for each tested female. The duration of copula was estimated from couples that start and finish the mating during the observation period (from 8:30 to 18:30 hours). We measured the time to start oviposition (i.e., the preoviposition period), the intervals between the first mating and first oviposition, the time between consecutive ovipositions, the total reproductive period (time between first and last oviposition), and the females longevity.

**2.4. Courtship Behavior and Vibratory Communication.** The behavioral sequences of courtship and mating in *E. meditabunda* were observed simultaneously with the records of male and female vibratory signals (Figure 3). Insects were separated by sex in the first 24 hours after the final moult and maintained in different rooms until they reach the sexual maturation. A pair of virgins and sexual mature male and female (15–20 days old) was placed on the membrane of a 10 cm diameter low-midrange loudspeaker (40–6000 Hz frequency response,  $8' \Omega$  impedance; RadioShack, Taiwan). An acrylic box (9 cm diameter  $\times$  4 cm high) was placed over the speaker without contacting the loudspeaker membrane to prevent the insects moving away from the membrane surface. The loudspeaker was placed into a sound-insulated room to decrease environmental noise. All observations were conducted between 13:00 and 18:30 when most of the mating activities have been previously observed. If the insects did not display any courtship behavior within 20 minutes of the observations, they were classified as failed courtship. In this experiment, 62 couples were observed. To describe the courtship and mating behavior, the previously determined behavior categories in pentatomids were used [24, 25] (Table 1). The sequence of behavioral categories was registered in each observed couple.

The vibratory signals captured by the loudspeaker were amplified by a home-made operational amplifier TL081CN, digitized (Aardvark-Direct Pro 24/96 (Aardvark Computer Systems, Washington, USA), and stored on a computer using Cool Edit Pro software (Syntrillium Software 2001—Fort Wayne, Indiana, USA). Signals were followed in real time with headphones and recorded until the insects stopped singing.

Vibratory signals were analyzed by the Sound Forge 4.5 software (Sonic Foundry <http://www.sonicfoundry.com/>). A pulse was defined as a unitary homogeneous parcel of vibration of finite duration [26]; pulse trains as repeatable and temporally distinct groups of pulses and a song as a sequence of pulses and/or pulse trains with distinct beginning and end.

TABLE 1: Courtship sequence performed by *Edessa medidabunda* described in the ethogram.

Code of the behavior	Description of the behaviors
MFRG	Males and females at rest and grooming
MS1	Males spontaneously emit song type 1
MS2	Males spontaneously emit song type 2
MstS1	Males interrupt the emission of MS1
Mw	Males walk around the arena
FS1	Females emit song 1 in response to MS2
D	MS2/FS1 duet
MApFf	Male approaches the female from the front
MApFb	Male approaches the female from the back
MAnFAn	Male antennates female's antennae
MAnFt + p	Male antennates female's thorax and moves to the posterior side of her abdomen
Mb180° + RT	Male puts the head behind the female abdominal tip and butt her abdomen until she adopts the copulatory position At the time turning 180° from the female posterior, the insects are oriented end to end in copulatory position
Pr + 180°	Male rotates its pygophore 180° so that it is inverted
PM	Pair mate
MRF	Male rejects female and walks away from her
FRM	Female rejects male and runs away from male
NM	Pairs not mate

Spectra were described by the dominant, first harmonic, and other subdominant frequency peaks, by the spectral width 20 dB below the amplitude of dominant frequency value and by frequency modulation described as downward or upward-orientated frequency sweeps quantified by the frequency difference per signal duration (Hz/s). Songs were classified, according to their order of appearance in a duetting couple [22].

**2.5. Statistical Analyses.** Reproductive parameters were analyzed by generalized linear models (GLM) and deviance analyses (ANODEV). The models have a factor for treatments and Gaussian distribution of errors for time variables, Poisson distribution of errors for fecundity parameters (number of egg masses/female number of eggs/mass and number of eggs/female), and binomial distribution of errors for fertility (number of nymphs/number of eggs). Contrast analyses were used to multiple comparisons of means. To test the relation between successive mating and cumulate fecundity, a linear model was used, with the cumulate fecundity (cumulate numbers of eggs/female) as dependent variable.

Data from observations of all courtship sequences were used to create a first-order Markovian behavioral transition matrix of total frequency of transitions (i.e., moving from one behavioral step to the next). The repetition of a single behavior (self-transition) was not included in the records to avoid the possible influence in the relative weight of transitions between behaviors. Transition probabilities were calculated from the observed frequency of a transition between two events divided by the total number of occurrences of the first event [27]. The expected values of the matrix cells were found using the iterative proportional

fitting method of [28], and the statistical significance of the individual transitions were evaluated using a log-likelihood ratio test (*G* test) and the results presented graphically in the ethogram. Data are shown as means  $\pm$  SD, together with the number of signals analyzed (*N*) and the numbers of individuals (*n*).

### 3. Results

The age at which females reached the sexual maturity ( $18.08 \pm 1.26$  days) was estimated from the first copulation of virgin females maintained with old males. It was significantly different from the age at which males reached the sexual maturity ( $15.92 \pm 0.86$ ) (estimated from first copulation of virgin males maintained with old females) (ANODEV  $\chi^2_1 = 30.15, P < 0.001$ ).

Multiple mating with the same male increased female's fecundity of *E. medidabunda* (ANODEV  $\chi^2_2 = 51.95 P < 0.001$ ) but not its longevity (Table 2). The reproductive period also increased ( $\chi^2_2 = 651.1 P < 0.001$ ) with the increase of the number of copulations (Table 2). For multiple-mated females, we observed  $3.92 \pm 0.79$  copula ( $N = 20$ ) with duration of  $222.55 \pm 60.02$  min ( $N = 11$ ) and intervals between copulations of  $6.35 \pm 3.8$  days. The pre-oviposition period of virgin- and single-mated females was significantly longer when compared with multiple-mated females (ANODEV  $\chi^2_2 = 81.54 P < 0.001$ ) (Table 2). However, the interval between the first mating and first oviposition was similar for both groups of mated females and females with multiple mating showed shorter intervals between consecutive ovipositions (Table 2). Females with multiple mating laid a higher number of egg mass than did virgin and once-mated females (ANODEV  $\chi^2_2 = 86.51 P < 0.001$ )

TABLE 2: Effect of mating frequency on the reproductive biology and longevity of *Edessa mediatubunda* females in the laboratory.

	Preoviposition period (days)	Days between 1° mating and 1° oviposition	Days between 1° consecutive oviposition	Number of egg mass/female	Number of eggs/mass	Total eggs/female	Reproductive period (days)	Fertility <sup>b</sup> (%)	Female longevity (days)
Multiple-mated females	18.50 ± 2.06 <sup>a</sup> (N = 20)	1.35 ± 0.48 <sup>a</sup> (N = 20)	6.26 ± 3.02 <sup>a</sup> (N = 95)	5.70 ± 1.17 <sup>a</sup> (N = 20)	13.50 ± 0.79 <sup>a</sup> (N = 115)	77.65 ± 16.11 <sup>a</sup> (N = 20)	30.45 ± 10.22 <sup>a</sup> (N = 20)	96.11 ± 9.70 <sup>a</sup> (N = 115)	56.65 ± 7.51 <sup>a</sup> (N = 20)
Single-mated females	21.27 ± 1.53 <sup>a</sup> (N = 15)	1.53 ± 0.74 <sup>a</sup> (N = 15)	9.21 ± 2.67 <sup>b</sup> (N = 14)	2.07 ± 0.70 <sup>b</sup> (N = 15)	13.32 ± 1.88 <sup>a</sup> (N = 31)	26.87 ± 9.30 <sup>b</sup> (N = 15)	9.14 ± 5.17 <sup>b</sup> (N = 15)	95.16 ± 13.47 <sup>a</sup> (N = 31)	57.87 ± 6.32 <sup>a</sup> (N = 15)
Virgin females	21.25 ± 2.76 <sup>a</sup> (N = 9)			0.70 ± 0.77 <sup>c</sup> (N = 17)	9.45 ± 2.58 <sup>b</sup> (N = 11)	5.78 ± 6.44 <sup>c</sup> (N = 18)	1.22 ± 0.44 <sup>c</sup> (N = 19)		64.50 ± 9.53 <sup>b</sup> (N = 18)

Letters in each column followed by the same letter are not significantly different (ANODEV and contrast analyses  $P > 0.05$ ).

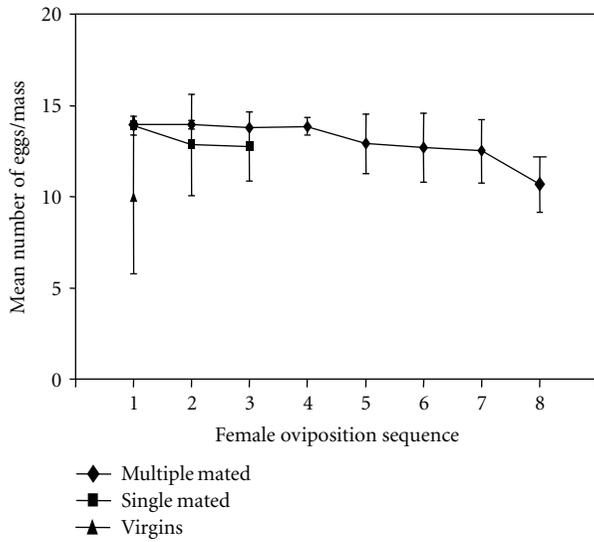


FIGURE 1: Mean number of eggs oviposited by *Edessa meditabunda* females in successive ovipositions. Multiple-mated females: females maintained with males during all their reproductive life. Single-mated females: females maintained with males until complete one mating. Virgins: females maintained isolated of males during all their reproductive life. Vertical lines indicate the standard deviation of the means.

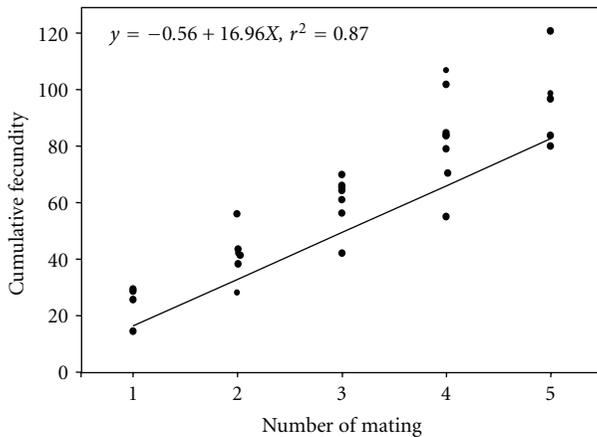


FIGURE 2: Relation between number of mating and cumulative fecundity (cumulated number of eggs/female after successive mating). A linear model was adjusted to the variables. Points indicate the cumulative number of eggs of each individual observed and line the values estimated from the adjusted model.

(Table 2). However, there was no significant difference in the number of eggs per mass in multiple- or single-mated females (Table 2) but a significant difference in the number of eggs/mass from these groups of females in comparison with the lower number determined in virgin females (Table 2).

Most eggs were laid at night in two rows of approximately seven eggs per row, generally, under the surface of *bold* leaves and never on the smooth surface of the cages. Despite the increased fecundity of multiple-mated females there was no significant difference in the fertility (number

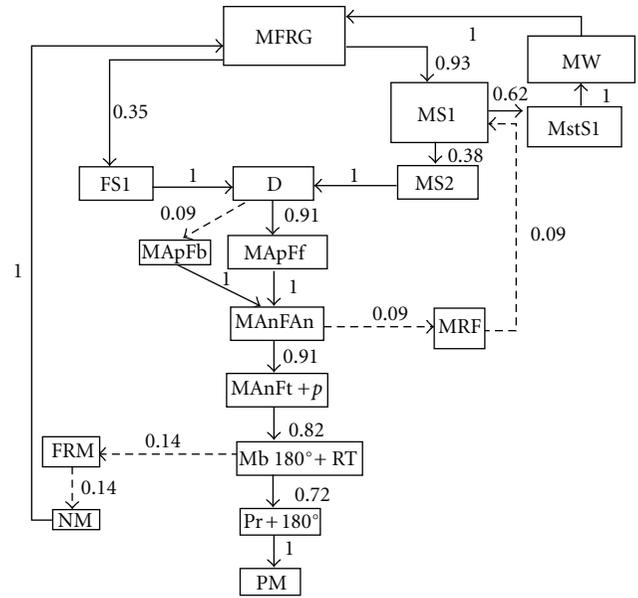


FIGURE 3: Sequence of courtship and mating behavior of *Edessa meditabunda*. Values represent the probability of transitions between behaviors. Solid-line arrows indicate the significant transitions ( $P < 0.05$ ) and dashed-line arrows not significant transitions ( $P > 0.05$ ). Boxes representing behavioral categories are in relative size to the frequency-observed behaviors from 62 pairs (male and female) observed. Codes for behavioral categories are listed in Table 1.

nymphs/number of eggs) of the single- or multiple-mated females (Table 2). Thus, multiple mating was required for fecundity but not for egg viability.

Despite of higher fecundity, the number of egg/mass was constant and no effect of female age was observed for multiple- or single-mated females; the number of eggs/mass did not show significant differences in consecutive ovipositions in these groups of females (ANODEV  $\chi^2 = 4.08$   $P = 0.77$  for multiple-mated females and  $\chi^2 = 0.64$   $P = 0.72$  for single-mated females) (Figure 1). In the same way and for multiple-mated females, the cumulative fecundity (mean number of cumulative eggs oviposited by females) after one to five mating shows a significant linear increase ( $F_{1-67} = 443.6$   $P < 0.001$   $r^2 = 0.87$ ) (Figure 2).

At close range (i.e., below 10 cm between mates), successful copulation followed the usual behavioral steps described until now in most stink bugs as resting, grooming (i.e., rubbing the antennae, thorax, or abdomen with a pair of leg), approaching, antennation during male-female interaction, abdominal vibration, genitalia contact, and copulation [15]. Courtship was initiated by male approaching the female by emission of vibrational signals before any physical contact, indicating that at close range vibratory signals are involved in the first encounter. The courtship steps are characterized and coded in the Table 2, and their transitional probabilities are shown in the ethogram (Figure 4).

Vibratory communication started with the emission of the first male song (MS1) (Figure 4(a)). This song was produced when a male was alone in the arena, in the presence of a

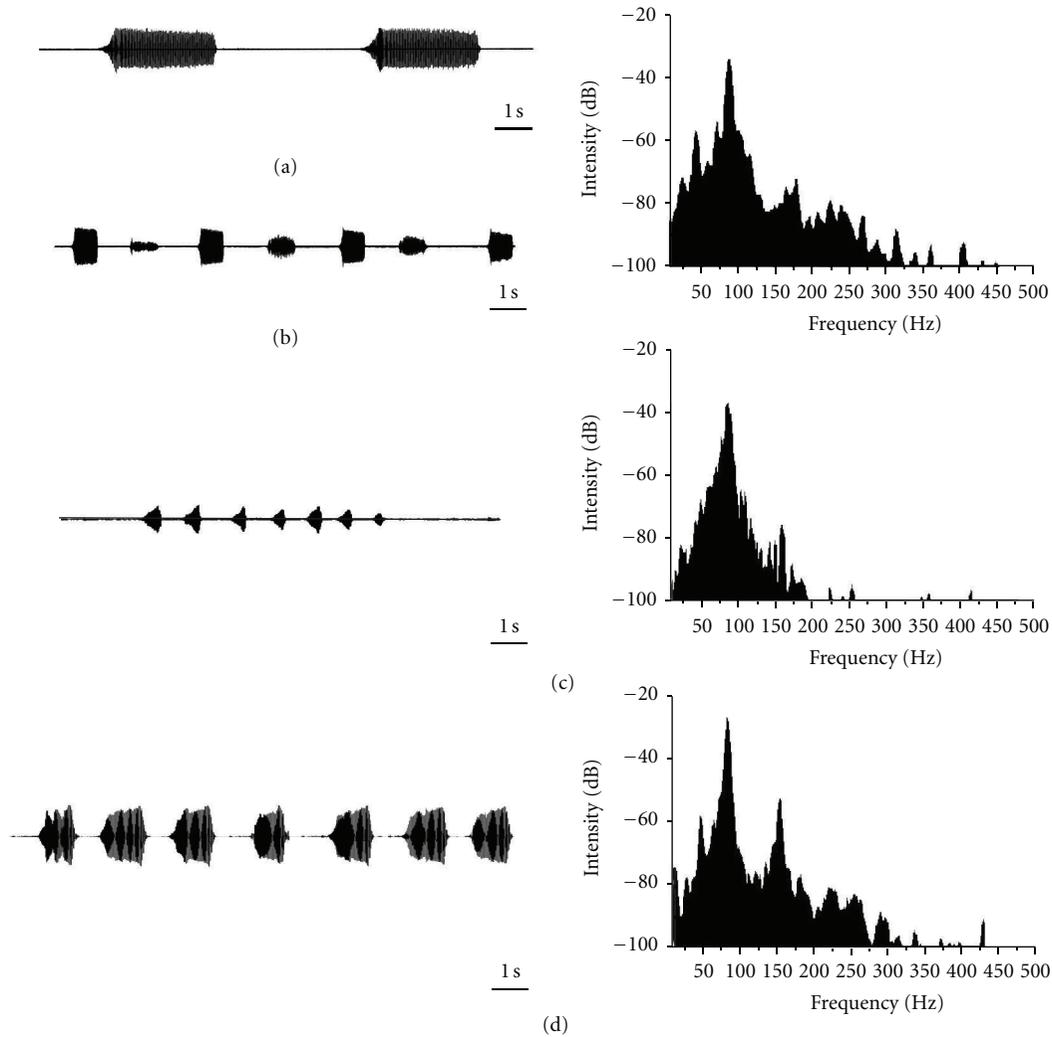


FIGURE 4: Oscillogram and frequency spectrum of one pulse of *Edessa meditabunda* vibratory songs: (a) male song 1 (MS1); (b) male rivalry song (MR); (c) male song 2 (MS2); (d) female song 1 (FS1).

female or as response to a female song. During courtship, the MS1 may be changed to MS2 or interrupt it for some minutes (Figure 4(c)). In response to MS2 females emitted their first song (FS1), and, after the emission of some pulses, the couple started to exchange FS1 and MS2 first as intercalated and later with superimposed pulses (Figure 4). After some seconds of duet song emission, the male approached the female and started to antennate her body and antennae. The female responded with a slow antennation of male's head and antennae. Subsequently, the male antennated the posterior end of female's abdomen. During this mutual stimulatory antennation phase, both proceeded with the FS1 and MS2 song emission. In the final courtship step, both mates stopped singing, and the male lifted the female with the head to get the copula position, then turned its body 180° to female's back and its phygophore, so that male and female in copula faced each other in opposite direction, and finally the copulation occurred (Figure 4). We recorded no vibratory emissions of copulated male and female. The male

rival song (MR) was emitted when two males were placed with a female in the arena, these males emitted pulses similar (same temporal and spectral parameters) to MS1 pulses in an alternated a-b-a-b-a-b fashion. The pulses of one of the males were emitted with higher amplitude, until the other was silenced (Figure 4(a)).

Vibratory signals showed typical temporal and spectral characteristics described until now for most Pentatominae species (Figure 4, Table 3). The male song MS1 was a typical calling song, with relatively long pulses (~1 sec in duration) that may be repeated for several minutes. The dominant frequency and band width were also in the typical range of pentatomid vibratory signals (Table 3). The second song, MS2, was composed by a pulse trains of 5 to 11 short pulses with similar frequency characteristics as those of the MS1 (Table 3). Females emitted only one song type (Figure 4(d)) with specific temporal parameters and similar dominant frequency and band width as males' songs (Table 3). FS1 showed a clear frequency modulation with the dominant

TABLE 3: Temporal and spectral characteristics of male and female songs of *Edessa meditabunda*. Mean  $\pm$  SD are show with the number of signals test ( $N$ ) and the number of insect tested ( $n$ ).

Song	Duration (ms)	Repetition time (ms)	Dominant frequency (Hz)	Bandwidth $-20$ dB (Hz)
MS1	1666,74 $\pm$ 147,38 (76/11)	4590,09 $\pm$ 1834,26 (65/11)	73,75 $\pm$ 2,60 (76/11)	20,08 $\pm$ 4,77 (76/11)
MS2	175,71 $\pm$ 83,91 (75/12)	305,97 $\pm$ 210,92 (64/12)	80,27 $\pm$ 5,68 (75/12)	57,87 $\pm$ 19,39 (75/12)
FS1	433,04 $\pm$ 78,81 (80/11)	677,19 $\pm$ 104,48 (80/11)	76,93 $\pm$ 5,82 (80/11)	17,73 $\pm$ 7,29 (80/11)

MS1: male song 1, MS2: male song 2, FS1: females song 1.

frequency decreasing throughout the duration of each pulse (Table 3). The duet emission of MS2 and FS1 shows a clear courtship function initiating all the sequential behaviors that lead to copula.

#### 4. Discussion

Results presented here are the first report on the *E. meditabunda* reproductive biology, behavior, and vibratory communication. Adults of *E. meditabunda* showed lower copulation frequencies, reproductive potential, and longer sexual maturation period if compared with other pentatomid species reared under similar [29, 30] or different [25, 31, 32] laboratory conditions. The preoviposition period (time between emergence and first egg mass) for females with one or multiple mating was also longer when compared with other pentatomids [29, 32].

Repeated mating increased female fecundity in *E. meditabunda*, but it was costly in terms of reduced longevity compared with virgin females. Studies with some pentatomids reported similar correlation as a result of mating frequency [29, 32–34]. Decreased longevity and increased reproduction associated with multiple mating have been also reported for other insect species, like *Coccinella septempunctata* (L.) and *Propylea dissecta* (Mulsant) (Coleoptera: Coccinellidae) [35]. This seems to be the general negative cost of multiple mating [36].

Arnqvist and Nilsson [36] suggested that, in many species, especially among insects, exists an optimal remating rate for females so that one or a few matings are necessary to increase the offspring production. In such a way, additional matings are not necessary. Results of works with Heteroptera showed that in some species exists an optimal number of mating [31, 32, 37, 38] but in others not [39, 40]. In *E. meditabunda*, multiple-mated females have increased fecundity but did not show any fecundity peak along successive mating.

The fecundity of multiple-mated *E. meditabunda* could be considered low when compared with previous studies [11] or with data on other stink bug species [30, 31]. The low fecundity could be a characteristic of the central Brazil populations and may be related to the reproductive biology of the insect, since both adults showed long sexual maturation period and females a short period of oviposition. This may be the one of the reasons for the low population densities of *E. meditabunda* observed in the field.

At close range, the main behavioral steps of courtship in *E. meditabunda* did not differ from those observed for other species of pentatomids [17, 23–25, 31], except that, in all couples that emitted vibratory signals, males started

the courtship by approaching females, antennate them, and emit vibratory signals. The sequence of steps was highly stereotyped, suggesting that once a male starts the courtship, the subsequent steps will most likely follow. Copulation was successful when the female remained stationary after the first contact. High percentage of courted females (53.06%) refuses copulation and run away from males during the antenation phase of courtship behavior. A similar failed courtship behavior was observed in *Murgantia histrionica* [25]. This fact could be a characteristic of the male selection behavior of females during courtship in some species of stink bugs or an effect of the artificial arenas used in the experiments.

The temporal and spectral patterns of vibratory signals of *E. meditabunda* were similar to the characteristics described previously for species of Pentatominae. However, some differences were found in the emission of signals and in the songs repertoire. In most Pentatominae species, two or three different male and female songs have been described [17–19] with calling and courtship songs of different temporal and spectral characteristics. In *E. meditabunda*, the repertoire of signals appears to be less complex with just one female and two male songs. The MS-2 has been emitted in the calling and courtship phase of the reproductive behavior. In most until now investigated Pentatominae species vibratory communication starts by female songs that trigger males to sing and move towards her [17–19]. The absence of a female song initiating the vibratory communication in *E. meditabunda* could be a characteristic of vibratory communications in Edessinae or could be related to the chemical communication in this species.

As sex pheromones of *E. meditabunda* was not identified, vibratory communication could have a central role to the sexual behavior in this species and males could use the vibratory signals to attract females.

Further observations on a plant are needed to confirm behavioral data and the role of communication signals of other modality described in this study for couples mating on a loudspeaker membrane. A possible influence of the size of arena on the vibratory communication cannot be discarded. Because the communication on stink bugs normally start on plants at distances of several cm (sometimes reaching 1 m or more) [18, 19], the reduced dimensions of the arenas used in our experiments (9 cm) could influence or inhibit the emission of some signals. In addition, the male calling song seems to act also as a rivalry song when a second male is present as reported by Shestakov [41] for Asopinae bugs.

Results here presented describe the mating biology, behavior, and vibratory signals of *E. meditabunda*. Multiple

mating showed to be advantageous for *E. meditabunda* females. During courtship, *E. meditabunda* communicates with signals produced by abdominal vibration. Songs are similar to those of other stink bugs studied with the exception that the courtship is initiated by males rather than by females as reported for other stink bugs.

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