Vibrational Communication of *Metcalfa pruinosa* (Hemiptera: Fulgoroidea: Flatidae)

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ABSTRACT Vibrational communication of the Nearctic flatid planthopper *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) was investigated to determine the role of substrate-borne vibrational signals in mating behavior. Signaling activity changes throughout the day and vibrational communication in this planthopper begins at night. All recorded male and female signals consisted of a series of single pulses. Most of these signals were classified as calling signals. They were emitted spontaneously or were evoked by playback stimulation with prerecorded conspecific signals. Male calling signals are simple, and the main difference between male and female calls was in the pulse repetition time. Pulse repetition time in the middle section of the calling signal was the least variable property of the vibrational signals. During later stages of pair formation, males emit a phrase that is a sequence of pulse trains that has a distinct time and amplitude pattern, whereas females produce continuing monotonous substrate vibrations that could last for several hours. Males participated in continued exchange of vibrational signals. Signals exchanged during male–male interaction did not differ structurally from male calling signals and males never overlapped their signals.

KEY WORDS Auchenorrhyncha, mating behavior, vibrational communication, chorus, nocturnal activity

In recent years, many insects have been inadvertently introduced into new areas, mostly as a result of increased human trade and travel. The flatid planthopper *Metcalfa pruinosa* (Say) (Hemiptera: Auchenorrhyncha: Fulgoroidea) was introduced into northern Italy from the United States in the 1970s (Zangheri and Donadini 1980, Dlabola 1981). It has expanded throughout southern Europe and is now established also in Spain, France, Switzerland, Austria, The Czech Republic, Slovenia, Croatia, and Greece (Lauterer and Malenovsky 2002, Lucchi and Wilson 2003, Drosopoulos et al. 2004). Although *M. pruinosa* is of little economic importance in the United States, it attracts the attention of economic entomologists in Europe because of its potential to reach extremely high population densities on a wide range of host plants, including those of agricultural importance (Wilson et al. 1994, Bagnoli and Lucchi 2000, Wilson and Lucchi 2001).

M. pruinosa has been the subject of many studies regarding its morphology, distribution, ecology, economic importance, and control (Bagnoli and Lucchi 2000; Olmi 2000; Wilson and Lucchi 2000, 2001; Lucchi and Santini 2001; Lucchi and Wilson 2003; Lucchi and Mazzoni 2004), but only preliminary observations of its mating behavior have been carried out (Santini and Lucchi 1994, 2000). Mate recognition and location in Auchenorrhyncha (with the exception of most cicadas) are mediated via acoustic signals transmitted through the substrate (for review, see Claridge 1985a,b; Čokl and Virant-Doberlet 2003, Virant-Doberlet and Čokl 2004). Vibrational communication in this group has been extensively studied (for review, see Claridge 1985a,b; Claridge and de Vrijer 1994; Čokl and Virant-Doberlet 2003; Virant-Doberlet and Čokl 2004; Cocroft and McNett 2006; Tishechkin 2006). However, in the family Flatidae only the air-borne component of common sounds of *Ormenaria rufifascia* (Walker) has been recorded (Moore 1961).

In this study, we investigated the vibrational signaling behavior of *M. pruinosa* to increase our understanding of its mating behavior. We reveal new information on signaling activity and describe male and female vibrational signals.

Materials and Methods

Insects. *M. pruinosa* used in this study were collected in June and July as fourth or fifth instars in an urban landscape in Nova Gorica (Slovenia). Nymphs were taken from several host plants: dogwood, *Cornus sanguinea* L.; black locust, *Robinia pseudacacia* L.; bramble (*Rubus* spp.); nettle, *Urtica dioica* L.; and bishop's goutweed, *Aegopodium podagraria* L. They were reared to adults in the laboratory on clippings of these plant species. Insects were kept in mesh cages

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(20 by 50 by 30 cm) at 23–28°C, 75% humidity, and with a photoperiod of 16:8 (L:D) h. Males and females were removed from the nymphal culture on the day of emergence and kept separately until they were used for recordings. Adults were housed and fed in the same way as nymphs. Preliminary observations indicated that adults of *M. pruinosa* reach sexual maturity 25–30 d after emergence (Santini and Lucchi 1994), so experiments were made with virgin planthoppers that were at least 4 wk old.

Recording Vibrational Signals. All recordings were conducted in an anechoic and sound insulated chamber (Amplifon Fa., Amplaid, Italy) at 21–26°C and 70–75% RH. Vibrational signals were recorded from plant stems with a laser vibrometer (OFV 353 sensor head, OFV-2200 vibrometer controller, Polytec GmbH, Waldbronn, Germany), digitized, and stored directly onto a hard drive of a PC computer with an internal sound card (Sound Blaster, AWE GOLD 64, Creative Technology, Singapore) and Cool Edit Pro 2.0 (Syntrillium Software 2002) or Raven 1.2 (Charif et al. 2004). Recordings were analyzed using the computer software programs Sound Forge 6.0 (Sonic Foundry 2002) and Raven 1.2. (Charif et al. 2004). Mating of *M. pruinosa* has been previously found to take place usually during the night (Santini and Lucchi 1994). We made some preliminary 24-h recordings to determine the optimal time for bioacoustic experiments, and accordingly all subsequent recording sessions included the overnight period between 1600 and 0800 hours. Observations also showed that *M. pruinosa* was very sensitive to light, and recordings had to be done in the dark. Planthoppers (single insects, a pair or several animals of one or both genders) were placed on a freshly cut dogwood stem ($\approx$30 cm in height) with two to three leaves. The bottom of the stem was put into a vial filled with water to prevent withering and placed upright in a jar filled with moist artificial substrate. To prevent insects from escaping during the night a cylindrical mesh cage (30 cm in diameter) with a small opening for a laser beam was put over the jar, taking care that the leaves were not in contact with the mesh. Recording was started immediately after planthoppers were put on a plant and was terminated the next day. We conducted 40 overnight recording sessions with 50 males and 50 females. Each individual was used only once.

For playback experiments, the bottom of the stem was vibrated with the conical tip of a 5-cm metal rod (4 mm in diameter) screwed firmly into a Brüel & Kjaer 4810 mini shaker head (Naerum, Denmark), driven from the computer with prerecorded and amplified (Philips 5175, Silicon Valley, CA) male and female signals. Signals were recorded with a laser vibrometer as described above, and the amplitude of stimulation was adjusted to the level of the recorded responses at the point of recording. We tested five males and five females.

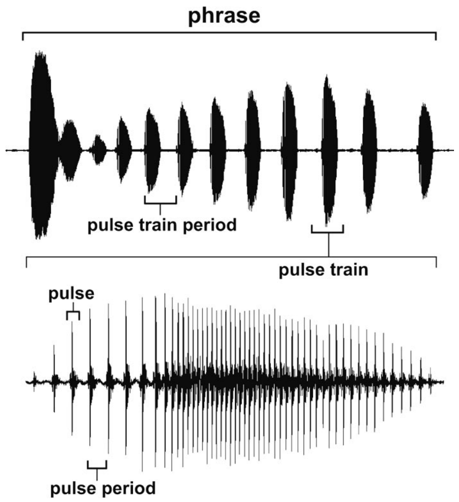

Fig. 1. Oscillogram of a male phrase of *M. pruinosa* showing principal signal parameters discussed in the text.

Terminology and Statistical Procedures. Vibrational signals were labeled according to the behavioral context. Calling signals (calls) are defined as signals that are produced spontaneously by isolated males and females (Booij 1982) or in response to playback stimulation with prerecorded conspecific signals (Claridge and de Vrijer 1994). Pulse was defined as a unitary homogenous parcel of sound of finite duration (Broughton 1963). Pulses arranged into repeatable and temporally distinct groups were termed pulse trains and sequences of more or less regularly repeated pulse trains were termed phrases (Fig. 1). We measured the following parameters: duration of the pulse train, number of pulses in the pulse train, pulse period, dominant frequency of the pulse train, number of pulse trains in the phrase, and pulse train period. Period was defined as the time between onset of two consecutive pulses or pulse trains. Data are presented as means, ranges, and SD together with the number of signals analyzed (*N*) and the number of planthoppers (*n*) from which the signals were obtained. Differences between means were determined with two-tailed Student's *t*-test. We also analyzed within- and between-individual variation (coefficient of variation$_{\mathrm{intra}}$ and $\mathrm{CV}_{\mathrm{inter}}$, respectively) (Gerhardt 1991). A Mann–Whitney test was used to test differences in pulse period between males and females. Differences in pulse period were tested at each pulse. Pearson's correlation coefficient (e.g., Triola 2006) was used to relate the mean pulse period of female calling and continuous signals.

For quantifying signaling activity throughout the day, we analyzed nine 24-h recordings in which planthoppers were emitting vibrational signals for $>$60 min; 15 males and 10 females were used for these recordings, and each animal was used only once. Time spent signaling was taken as a measure of signaling activity, and activity in each hour was expressed as the absolute amount of time spent signaling.

Results

General Observations. Most vibrational signals were recorded between midnight and 0400 hours with

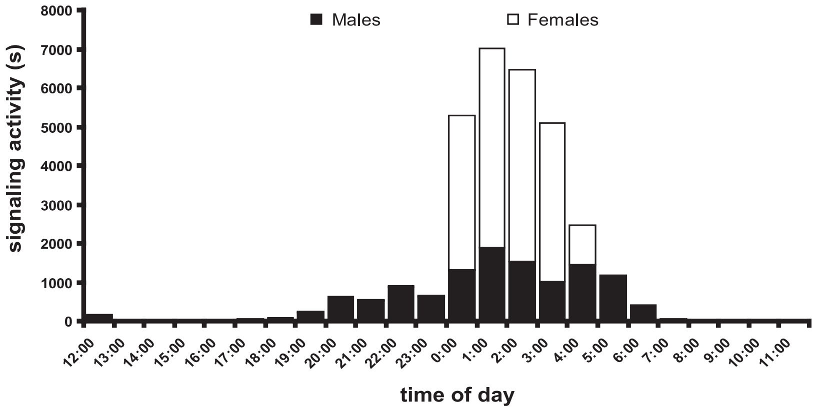

Fig. 2. Daily pattern of vibrational activity of *M. pruinosa* as determined by continuous 24-h recordings. Absolute amount of time spent signaling is shown.

a peak activity between 0100 and 0300 hours (Fig. 2). Males were the more active gender because they initiated most of the vibrational exchanges, responded readily to female signals, and emitted more signals over a longer period. Females emitted fewer signals and over a shorter period; however, their high signaling activity was a result of nearly continuous signals that were recorded for hours (see below). *M. pruinosa* proved to be very sensitive to light and under our experimental conditions even red light illumination reduced vibrational activity. Thus, we were not able to closely observe the animals and to correlate unequivocally vibrational signals emitted during the later stages of mating with actual behavior. Most of the recorded vibrational signals can be classified as male and female calling signals. To avoid possible misunderstanding, other signals recorded during male–female interaction were grouped as "signals associated with later stages of pair formation" and labeled as male phrase and female continuous signal. We also observed male–male vibrational interaction.

All recorded signals consisted of a series of single pulses and the main difference between male and female signals was in the pulse period (Table 1; Figs. 3 and 5).

Calling Signals. Although male calls were regularly emitted also by solitary males, we rarely recorded female signals in the absence of males. In the majority of recording sessions male calling signal was the first one recorded; however, in several cases the first identified vibrational signal was emitted by a female. Initially, males emitted a few signals, and if they received no response or perceived no signals from conspecifics, they usually jumped off the plant. Males readily responded to female signals (also in playback experiments), but females did not respond regularly either to a live male or a male playback signal and usually alternation (duetting) sequences (m-f-m-f) were short and few signals (four to six) were exchanged.

Male Calling Signal. Initially males produced single, irregularly spaced calls. In each recording, we

Table 1. Temporal and spectral properties of male and female signals of *M. pruinosa*

Property	Mean	SD	Min.	Max
Duration (s)				
Male call	2.35	0.47	1.38	3.44
Female call	2.34	0.52	1.07	3.64
Male phrase pulse train, S1	4.31	1.36	2.36	8.64
Male phrase pulse train, S2	2.39	0.30	1.98	3.08
Female continuous signal	1,044.00	311.50	60.00	13,050.00
No. of pulses				
Male call	94	17	49	136
Female call	30	11	14	64
Male phrase pulse train, S1	154	44	82	260
Male phrase pulse train, S2	68	12	44	91
Pulse period$^{1\text{-}10}$ (ms)[a]				
Male call	33	26	11	204
Female call	94	26	21	333
Male phrase pulse train, S1	29	7	15	56
Male phrase pulse train, S2	61	27	18	158
Pulse period$^{10\text{-}25}$ (ms)[a]				
Male call	18	2	13	33
Female call	74	13	42	108
Male phrase pulse train, S1	23	6	15	56
Male phrase pulse train, S2	23	4	17	67
Pulse period (ms)[b]				
Female continuous signal	92	29	40	255
No. of pulse trains				
Male phrase	21	14	6	66
Pulse train period$^{1\text{-}15}$ (s)[c]				
Male phrase	6.60	3.61	1.69	34.13
Dominant frequency (Hz)				
Male call	442	122		
Female call	463	107		
Male phrase pulse train, S1	414	112		
Male phrase pulse train, S2	447	112		
Female continuous signal	402	155		

Means with ± SD together with maximal and minimal measured values are shown. Male calling signal: N (number of signals analyzed) = 150, n (number of animals) = 18; female calling signal: N = 141, n = 21; male phrase signal, S1: N = 43, n = 7; male phrase signal, S2: N = 109, n = 7; and female continuous signal: N = 47, n = 9).

[a] Due to marked changes in the pulse pulse over a call, only pulses 1–25 have been included in detailed analyses, and pulse period for pulses 1–10 and 10–25 is shown separately.

[b] Pulse period of 40 consecutive pulses in the signal (duration of ≈3.5 s) has been measured at several positions during the signal.

[c] Because the number of signals in a phrase is very variable and pulse train period increases over the phrase, only first 15 pulse trains have been included in detailed analysis.

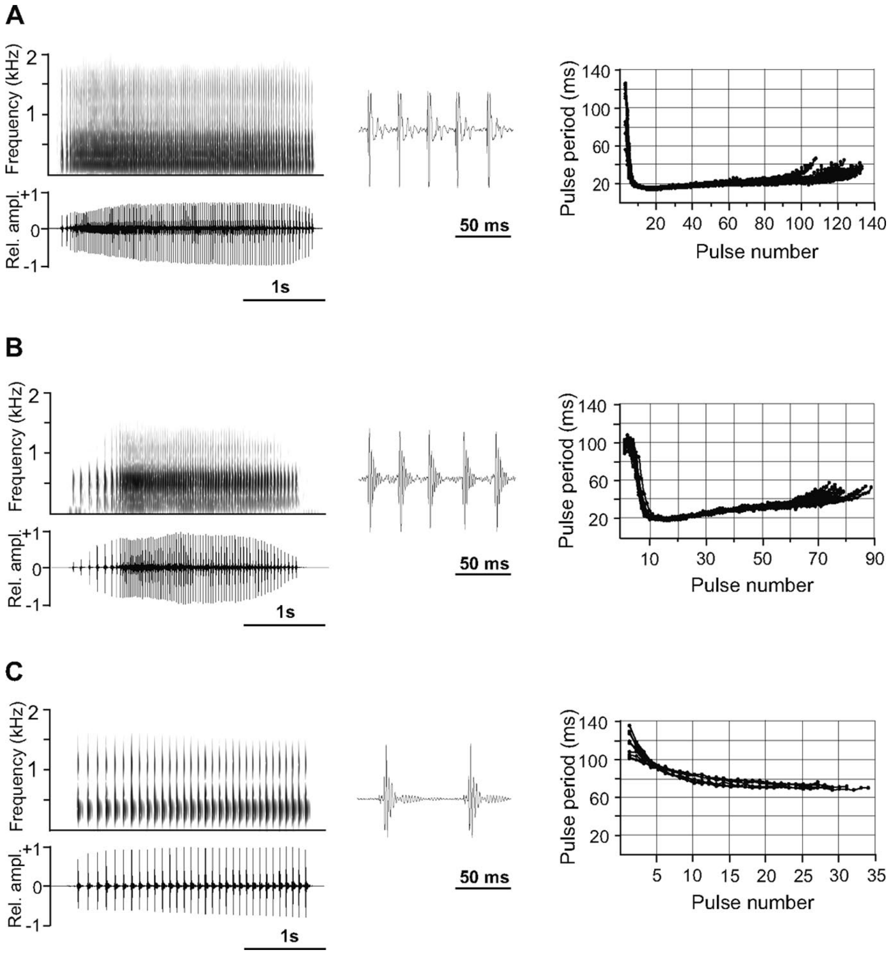

Fig. 3. Vibrational signals of *M. pruinosa.* (A) Male calling signal, (B) pulse train from section two in a male phrase, and (C) female calling signal. Sonagram (above) and oscillogram (below) of representative signals are shown in the left column. Signals in A, B, and C are composed from 121, 80, and 32 pulses, respectively. In the middle column, individual pulses from the same representative signal are shown. Graphic profiles of consecutive signals from sequences that include representative signals are shown in the right column. Graphic profiles in A, B, and C are constructed from 20, 15, and 9 signals, respectively.

evaluated as "calls" only signals that were emitted before any other conspecific vibrational signal could be perceived by the calling male. The duration of the calls ranged from 1.3 to 3.4 s (Table 1) and varied by >0.8 s among 10 consecutive calls in one male. Graphic profiles of the calls were constructed by measuring the successive pulse periods over a complete call (Fig. 3A). Calls have a marked structure, starting with a long pulse period followed by an increase and a final decrease in duration. The duration of pulse period at the beginning and at the end of the call was variable, even with the same male. However, it showed little variation among males in the middle section of the call, between the 10th and 25th pulse in the call (Tables 1 and 2; Fig. 3A). Counting the number of pulses and plotting them against the duration of the signal showed that males primarily extended the duration of the call by producing more pulses (Fig. 6).

Female Calling Signal. Female planthoppers infrequently emitted calling signals, even in response to male calls. The duration of female calls was variable, ranging from 1 to 3.6 s (Table 1), and, as with males, females extend the duration of the signal by emitting more pulses (Fig. 6). The graphic profile shows that pulse period slowly decreased over the call (Fig. 3C). The pulse period was longer than in male calls and more variable (Tables 1 and 2). Duration of the pulse period differed significantly between males and females throughout the call ($P < 0.001$ at all pulses), and after the first 10 pulses in the signals, there was no overlap in measured values for males and females.

Signals Associated with Later Stages of Pair Formation. These signals were emitted only when potential mates had established contact through calling signals. In a typical sequence, short male–female alternation of calling signals continued with a longer exchange of

Table 2. Variability of parameters of vibrational signals of *M. pruinosa* as measured by coefficient of variation (CV)

Parameter	CV_{intra}	Min.	Max	CV_{inter}
Duration				
Male call	8.3	5.4	12.0	18.4
Female call	15.9	8.0	30.6	17.0
Male phrase pulse train, S1	26.9	16.7	53.4	18.1
Male phrase pulse train, S2	6.7	4.5	7.8	13.2
Female continuous signal	105.0	52.6	161.4	121.06
No. of pulses				
Male call	8.2	3.8	15.0	16.2
Female call	15.3	7.1	24.0	34.5
Male phrase pulse train, S1	24.1	8.6	42.0	16.4
Male phrase pulse train, S2	7.8	6.6	10.7	21.1
Pulse period$^{1\text{-}10a}$				
Male call	17.9	0.0	82.4	27.5
Female call	11.5	0.0	68.8	17.6
Male phrase pulse train, S1	13.1	1.4	49.7	20.5
Male phrase pulse train, S2	14.6	3.0	45.9	34.8
Pulse period$^{10\text{-}25a}$				
Male call	6.1	0.0	35.3	7.5
Female call	5.5	0.0	31.7	14.6
Male phrase pulse train, S1	18.4	5.2	52.0	14.6
Male phrase pulse train, S2	8.3	3.2	40.2	10.9
Pulse period^{b}				
Female continuous signal	27.8	14.5	50.2	13.7
No. of pulse trains				
Male phrase	48.6	33.7	67.7	37.8
Pulse train period$^{1\text{-}15c}$				
Male phrase	21.2	3.7	67.6	33.8
Dominant frequency				
Male call	10.4	0.9	38.6	22.3
Female call	7.4	0.0	67.6	21.0
Male phrase pulse train, S1	14.9	1.2	43.9	27.3
Male phrase pulse train, S2	11.7	1.7	37.8	12.0
Female continuous signal	17.2	0.6	61.3	35.9

CV_{intra}, mean variability (%) within individual together with minimal and maximal values; CV_{inter}, variability (%) between individuals. For other details, see Table 1.

female call and a male phrase that was followed by a female continuous signal.

Male Phrase. Each phrase is a sequence of signals that has a distinct time and amplitude pattern (Fig. 4), although the number of signals in a phrase is variable (Tables 1 and 2). It was always recorded in reply to female calling signals and the first male signal in a phrase often overlapped the end of the female call. The phrase could be divided into two sections. The first section began with a pulse train that usually had a longer duration and higher amplitude than pulse trains in the second section (Fig. 4; Table 1). Marked structure typical of other male signals was not obvious in this first section. However, when the first pulse train was longer than 5 s, the pulse period oscillated over the pulse train, and it often seemed as if a marked pattern of pulse period changes, typical for other signals produced by males (as shown in Fig. 3A, B), had been repeated two to three times. In section 2, the pulse train period and amplitude increased over the phrase. Pulse trains in this section closely resemble male calling signals in their temporal and structural parameters (Table 1; Fig. 3B). The graphic profile shows slower initial decrease in duration of pulse period than in the male call and pulse period at the beginning of the signal differs significantly between the male call and pulse train in a phrase ($P < 0.001$ at pulses 2–10). As in male calling signals, the lowest variation in pulse period was found in the middle section of the pulse train (Tables 1 and 2). Pulse period in the phrase pulse train was slightly lower, and, although it did not differ significantly between these two types of signal, such a difference was found in males for which it was possible to directly compare pulse period in calling and phrase signals ($n = 5$).

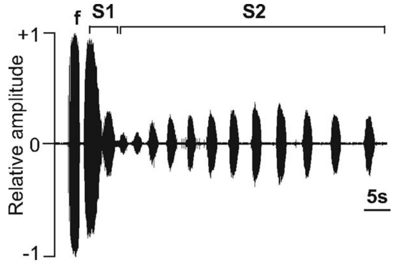

Fig. 4. Oscillogram of female call and male phrase of *M. pruinosa.* f, female call; S1 (section 1 of male phrase); and S2 (section 2 of male phrase).

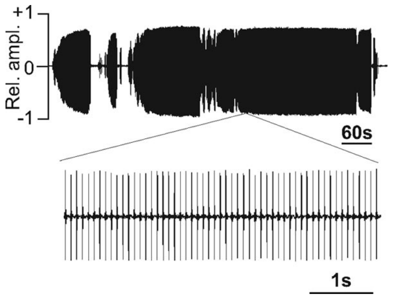

Fig. 5. Oscillograms of female continuous signal of *M. pruinosa.* Top trace, typical sequence. Middle trace, expended section of the signal.

Female Continuous Signal. During the later stages of vibrational interaction with a male, females emitted signals that differed from their calls primarily in their longer duration (Fig. 5; Table 1). Duration of these

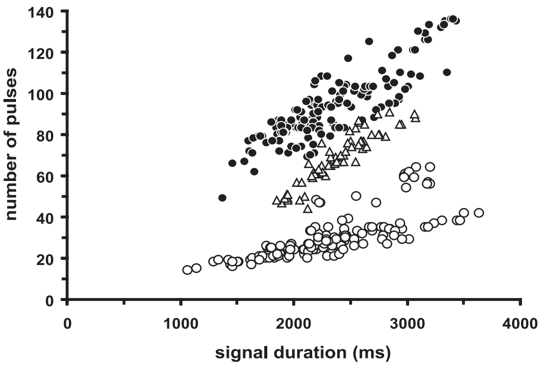

Fig. 6. Relationship of number of pulses and pulse train duration in vibrational signals of *M. pruinosa.* Filled circles, male calling signal; open triangles, pulse trains in section two of male phrase; and open circles, female calling signal.

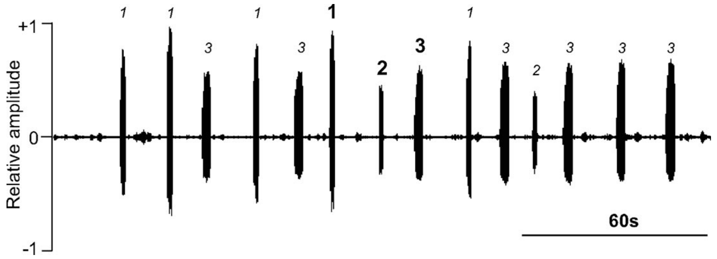

Fig. 7. Oscillogram of vibrational interaction among three males of *M. pruinosa*. Numbers indicate calling signals of the respective males.

signals was highly variable and ranged between 1 min and >3 h. In signals continuing for more than several minutes, the series of continuously emitted pulses was interrupted with short pauses (up to 5 s). Pulse period throughout the continuous signal was very variable, and it seemed as if a marked pattern of pulse period changes, typical for female calls (as shown in Fig. 3C), was continuously repeated during the signal. Analysis of mean pulse periods in a call and a continuous signal in the same female ($n = 6$) showed that they are highly correlated (Pearson's correlation coefficient $r = 0.954$).

Male–Male Interaction. When two or more males were present on a plant they often participated in a continual exchange of vibrational signals (Fig. 7). Such male–male vibrational interaction has been observed both in the absence and presence of females. There is no significant temporal or structural difference between signals emitted during male–male interaction and typical male call; however, during male–male interaction the time interval between signals was more regular. Although males did not alternate signals in a stereotypical a-b-a-b sequence, they never overlapped their signals.

Discussion

Results of the current study show that general activity and onset of vibrational signaling in *M. pruinosa* are nocturnal, which is in agreement with previous observations (Santini and Lucchi 1994). This type of behavior pattern has not been reported in other auchenorrhynchous Hemiptera (with exception of a few cicadas; Moore 1966, 1993; Sueur 2002). In Auchenorrhyncha, diurnal acoustic activity has not been studied in detail, and to our knowledge there is no available information on how widespread nocturnal activity might be. It has been reported that leafhoppers of the genus *Dalbulus* mate any time either during the day or night (Heady et al. 1986). In the leafhopper *Graminella nigrifrons* (Forbes), the peak male activity (including vibrational activity) was associated with the onset of photophase (Hunt and Nault 1991), whereas in the treehopper *Vanduzea arquata* (Say), the peak of male vibrational signaling was at midday (Cocroft 2003). The activity peak during the night also has been observed in flatid planthoppers of the genus *Cyphopterum* (D. Aguin-Pombo and M.V.-D., unpublished data). A possible explanation for such a pattern of activity could be predator avoidance, parasitoid avoidance, or both. A second possibility might be time sharing of acoustic environment (e.g., transmission medium, substrate) to increase efficiency of communication. In North America, the area of origin, *M. pruinosa* is commonly found feeding in mixed aggregations with two other flatid planthoppers, *Anormenis chloris* (Melichar) and *Ormenoides venusta* (Melichar), as well as with the acanaloniid *Acanalonica conica* (Say) (Wilson and McPherson 1980, Wilson and Lucchi 2001). Although nothing is known about mating behavior of these planthoppers, it is likely that mate recognition and mate finding in these species also is mediated by vibrational signals. Background noise due to heterospecific signaling can interfere with and mask the relevant conspecific signal. Time sharing of the acoustic environment has been described in temperate and tropical cicadas, where a large number of species may be found in the same habitat (Alexander and Moore 1962, Gogala and Riede 1995, Riede and Kroker 1995). A third explanation could be a restriction of activity to the cooler part of the day to avoid thermoregulatory problems (Sanborn et al. 1995). *M. pruinosa* as well as planthoppers of the genus *Cyphopterum* (D. Aguin-Pombo and M.V.-D., unpublished data) produce high-amplitude, long-lasting (up to several hours) continuous signals with high-pulse repetition time. Metabolic heat created by production of such signals in combination with high temperature in the environment might lead to a greatly elevated body temperature. It has been observed in some cicadas that calling activity stopped when ambient temperature was elevated (Sanborn et al. 1995).

All recorded signals of *M. pruinosa* consisted of a series of single pulses. Such signals have often been described in planthoppers (Ichikawa et al. 1975, de Vrijer 1984, Drosopoulos 1985, den Bieman 1986, Claridge et al. 1988, Heady and Denno 1991, Hoch and Howarth 1993, Gillham and de Vrijer 1995) and leafhoppers (Shaw et al. 1974, Inoue 1982). The detailed mechanism of the production of vibrational signals in Auchenorrhyncha has not been elucidated (Claridge and de Vrijer 1994). It is thought that substrate-borne signals in Auchenorrhyncha are produced by a mechanism that is homologous to the tymbal system used to produce air-borne sounds in cicadas (Ossiannilsson

1949; Claridge 1985a,b; but also see Mitomi et al. 1984). The morphology of the structures associated with vibration production has been best studied in delphacid planthoppers (e.g., Mitomi et al. 1984, Claridge and de Vrijer 1994, Miller and Wilson 1999). However, morphological investigations in *M. pruinosa* have not revealed any structure similar to the cicada or delphacid tymbal (S. W. Wilson, unpublished data). Moreover, in females of some planthopper species for which vibrational signals have been recorded, no obvious tymbal or specialized muscles associated with this mechanism have been found (Mitomi et al. 1984; Claridge 1985a,b). In cicadas, the airborne acoustic signals also induce substrate vibrations (Gogala et al. 1996, Stölting et al. 2002). However, the tymbal apparatus is greatly reduced in primitive cicadas from the family Tettigarctidae that communicate via substrate vibrations (Claridge et al. 1999). In Auchenorrhyncha that lack an obvious tymbal mechanism, vibrational signals are supposedly produced by vibrations of the whole abdomen (Ichikawa et al. 1975). Although it is known that such abdominal movements generate vibrational signals in the heteropteran family Pentatomidae (Čokl and VirantDoberlet 2003), it is not clear whether such a mechanism could be involved in the production of short, quickly repeated, discrete pulses that are present in substrate-borne signals of auchenorrhynchous Homoptera.

Calling signals are long-range signals associated with the initial stage of mating behavior when individuals advertise their presence and readiness to mate and should provide information about the identity (species and gender) and location of the potential partner (Virant-Doberlet and Čokl 2004). The calling signals of *M. pruinosa* are relatively simple and consist of a sequence of rhythmically repeated pulses. Male and female calls differ primarily in pulse period and consequently in the number of pulses in the signal. The pulse period in the middle section of the call also seems to be the least variable property of vibrational signals. This parameter can probably be regarded as a static property that is used for identifying conspecific mates (Gerhardt 1991). In *M. pruinosa,* this is probably the crucial parameter for species and also gender recognition. In most Auchenorrhyncha, male vibrational calls are much more complex than those of females, with an elaborate temporal pattern of repeated elements (Claridge 1985a,b; de Vrijer 1986; Heady and Denno 1991; Heady and Nault 1991; Hunt 1993; Claridge and de Vrijer 1994; Nuhardiyati and Bailey 2005). In *M. pruinosa,* similar complexity has been introduced at a later stage of mating behavior at the level of male phrase, when potential mates have already established the first vibrational contact. The number of pulse trains in a phrase and pulse train period show the highest within and between male variability and could be considered as dynamic parameters that could provide information about male quality and influence the response level of females (Gerhardt 1991). It is interesting to note that structurally simple vibrational signals consisting of homogenous pulse trains that are similar in both genders also have been found in cave-dwelling planthoppers (Hoch and Howarth 1993, Hoch and Wessel 2006). In this case, it has been argued that signals with low complexity may be sufficient to enable partner recognition in conditions where there is no competitive pressure from related species to enhance signal complexity to avoid interspecific mating (Hoch and Wessel 2006). By shifting the mating activity to scotophase when other species are not vibrationally active *M. pruinosa* might have encountered similar competition-free conditions.

The observation that males leave the plant when there is no response to their vibrational signals indicates that under natural conditions males might move from plant to plant and call to determine the presence of a female on the plant. Such behavioral "call-fly" or "call-walk" strategy has been described in other planthoppers (de Vrijer 1986) and leafhoppers (Hunt and Nault 1991). Observations also showed it is the *Metcalfa* male that approaches the female (Santini and Lucchi 1994, 2000), in agreement with the behavioral pattern observed in other planthoppers (Claridge and de Vrijer 1994), leafhoppers (Hunt and Nault 1991), and treehoppers (Hunt 1993). It has been demonstrated that mate location in planthoppers (de Winter and Rollenhagen 1990), treehoppers (Hunt 1993), and at least to some extent also in leafhoppers (Heady and Nault 1991, Hunt and Nault 1991, Downham et al. 1997) is mediated by vibrational signals. When vibrational signals are used for locating a potential mate, the first step usually is to establish a duet. Searching individuals alternate periods of emitting signals, and/or waiting for signals from conspecifics, with periods of walking (Virant-Doberlet et al. 2006). In this respect, it is surprising that recorded duetting sequences of calling signals in *M. pruinosa* were usually short. Planthopper and treehopper mating systems may be shaped in part also by population density (Ott 1994, Cocroft 2003). It also should be noted that population densities of *M. pruinosa* in Europe can be extremely high, and males and females are grouped together in closely aggregated feeding associations; therefore, males usually do not "need" to walk long distances in search of a female. In the area of origin (United States) the abundance of this planthopper is much lower (Wilson and Lucchi 2000, 2001). Mating behavior of *M. pruinosa* probably evolved in different ecological conditions and population densities than seen today in Europe.

Male–male vibrational interactions have been described in delphacid planthoppers (Claridge and de Vrijer 1994, Ott 1994), leafhoppers (Heady et al. 1986, Hunt and Morton 2001, Nuhardiyati and Bailey 2005), and treehoppers (Cocroft and McNett 2006, Miranda 2006). In most cases, these signals differed from signals used in male–female alternation and were associated with aggressive behavior. Male–male alternation in *M. pruinosa* resembles in some respects the alternation choruses in the leafhopper *G. nigrifrons* (Hunt and Morton 2001). Signals exchanged during male–male interaction did not differ structurally from male calls and males never overlapped their signals with calls from other males. Our results do not provide information on the function of male–male alternation; however, chorusing in *G. nigrifrons* has been described as a competitive strategy in courtship disruption (Hunt and Morton 2001). In many insects, males coordinate the emission of their sexual communication signals, and such behavior may be an incidental by-product or may result either from cooperative or competitive interactions (for reviews, see Greenfield 1994, 2005). Although chorusing has been extensively studied in insects in which males emit air-borne acoustic signals and are approached by mute females, much less is known about chorusing in phaneropterinae katydids that show behavior similar to pattern found in insects communicating via substrate-borne vibrations, i.e., in which potential partners form a duet and males approach the female. In *Phaneroptera nana* Fieber, females replied to specific males and nonpreferred males did not approach the female (Tauber 2001, Tauber et al. 2001). In contrast, observations indicate that other *Metcalfa* males move toward and locate the courting and mating couple (Santini and Lucchi 1994, 2000). This indicates that probably all males present on the same plant perceive (and recognize) the female response (signal) and start searching. Although substrate vibrations have been traditionally regarded as an inherently private channel that is free of potential competitors (e.g., Henry 1994), it has been argued that substrate vibrations are a less private communication channel than previously thought (Cocroft and Rodríguez 2005). In addition, that spectral and temporal properties of vibrational signals can substantially change during transmission through the substrate, particularly through plants (e.g., Michelsen et al. 1982, Miklas et al. 2001, Čokl et al. 2005, Cocroft et al. 2006), also could have important implications for the development and structure of vibrational chorusing. One of the main factors involved in the evolution of a chorus is female preference, i.e., choice (e.g., Greenfield 2005). However, very little is known about how females respond to vibrational choruses (Hunt and Morton 2001, Kotiaho et al. 2004). An important question is also whether females assess males at long range by their vibrational signals, because most properties of male calling signals as perceived by females depend on the relative positions of male and female and not necessarily on the quality of the male. Females of *Aphrodes* leafhoppers often responded to a male at a long range; however, they stopped responding when a male approached them and refused to copulate (M.V.-D., unpublished data). This indicates that the final assessment of male quality and mate choice might be done during the close-range courting phase. However, detailed further studies are needed to provide reliable information how in species relying on vibrational communication females chose males under natural conditions and how the unique features of vibrational communication might influence the evolution of particular mating system, including chorusing.

The current study provides the first information about vibrational communication in flatid planthoppers. It would be very interesting to study the mating behavior in those species of flatid planthoppers that live in sympatry on the same host plants. Furthermore, inasmuch vibrational signals play an important role in social behavior of many insects (e.g., Cocroft 2001, 2005), future studies also should determine whether the aggregation of nymphs is mediated, at least in part, by vibrational signals.

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