

Vibrational communication of the brown marmorated stink bug (*Halyomorpha halys*)

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Abstract. Communication with substrate-borne vibrational signals is common in the family Pentatomidae, although this aspect of biology of the invasive pest *Halyomorpha halys* Stål remains unexplored so far. In the present study, the behaviour of single animals and pairs is observed on a bean plant and a loudspeaker membrane at the same time as recording substrate vibrations with a laser vibrometer, with the aim of adding to the existing description of mating behaviour. The male *H. halys* emit long, narrow-band vibrational signals spontaneously to which the nearby females reply with their own vibrational signals, triggering male searching. During this phase, the insects emit two (in females) or three song types (in males) in various combinations, until they come into physical contact, after which the final male song type, characterized by tremulation, is the only kind of vibratory emission. Females never start singing spontaneously and the mating sequence does not proceed if either partner is silent. Male signals do not attract males or females and so vibrations are unlikely to play a role in maintaining the aggregations that are characteristic of this species, whereas female signals show promise for developing behavioural manipulation methods against this invasive pest.

Key words. Biotremology, Heteroptera, mating sequence, signal repertoire, substrate-borne vibrational communication.

Introduction

Although several techniques are being explored to optimize pesticide use against the invasive brown marmorated stink bug *Halyomorpha halys* Stål (Heteroptera: Pentatomidae), little effort is being made with respect to biorational control, with the exception of research on biological control using native parasitoids (Haye *et al.*, 2015). The strategy of behavioural manipulation is used mainly for monitoring, in the form of black light traps and pheromone traps, with the latter using chemically synthesized aggregation pheromones that are characteristically produced by males (Harris *et al.*, 2015). However, one major aspect of *H. halys* biology that has been ignored up to now is vibrational communication, despite its potential use in integrated and biorational pest management, which is slowly gaining recognition (Čokl & Millar, 2009; Polajnar *et al.*, 2015).

Communication with vibrational signals is common in species of the family Pentatomidae (Čokl & Virant-Doberlet, 2003;

Virant-Doberlet & Čokl, 2004). As a general pattern, male pheromones trigger females to emit low-frequency vibrational signals (Zgonik & Čokl, 2014). In turn, this stimulates males to increase pheromone production (Miklas *et al.*, 2003), respond with their own vibrational signals (Čokl *et al.*, 2000; Čokl & Virant-Doberlet, 2003) and then start to search for the stationary female (Ota & Čokl, 1991; Čokl *et al.*, 1999). Vibrations are used both for precise source localization and mate recognition (Čokl *et al.*, 1999; Virant-Doberlet & Čokl, 2004). When the animals arrive in the immediate vicinity, vibrational courtship ensues, involving a different set of signals, which is followed by copulation itself (Borges *et al.*, 1987; Čokl *et al.*, 1999; Čokl & Virant-Doberlet, 2003). The fact that localization is mediated by vibrations may explain the observation that individuals of *Nezara viridula* and related stink bug species (including *H. halys*) tend to linger in the vicinity of pheromone traps but rarely enter (James *et al.*, 1996; Aldrich *et al.*, 2009).

In their pioneering study on mating behaviour in *H. halys*, Kawada & Kitamura (1983) describe several phases of close-range courtship, starting with the male chasing the female, tapping on the substrate and directly on the female's body, followed by antennation, then the 'crab-walk' (i.e. male

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walking sideways towards the female's abdomen), then the male lifting the female's abdomen with his head and, finally, genital coupling. The tapping by the male is the only described behaviour that may be understood as involving substrate-borne vibrational signals, although the study by Kawada & Kitamura (1983) is limited to visual observation and so the signals themselves are not recorded with suitable transducers.

Another characteristic feature of *H. halys* is the pronounced tendency to form aggregations not only during overwintering, but also during breaks in daily activities (Rice *et al.*, 2014). Toyama *et al.* (2006) demonstrate that group cohesion is attained and maintained by touch, although vibrational signals might still facilitate orienting towards groups of conspecifics. If this is the case, such signals could be exploited to design more efficient implementations of the 'attract and kill' strategy as proposed by Rice *et al.* (2014), such as the use of playback (Polajnar *et al.*, 2015).

The present study attempts to fill the knowledge gap about the biology of this important Pentatomid pest and to describe its vibrational communication. According to data concerning closely-related species, it is hypothesized that both reproductively mature males and females of *H. halys* emit vibrational signals as an important component of mating behaviour. Second, the hypothesis that vibrational signals also play a role in the pronounced aggregation behaviour in *H. halys* is tested by observing whether males and females are attracted to the signals of other males.

Materials and methods

Insect collecting and rearing

Halyomorpha halys were collected in the fields around Reggio Emilia in northern Italy (44°41'50" N, 10°37'53" E), in the spring and summer of 2014 and 2015, and reared at the Laboratory of Entomology, Department of Life Sciences, University of Modena and Reggio Emilia (Modena, Italy). The insects were kept in transparent plastic boxes under a 16:8 h photocycle at $23 \pm 0.5^\circ\text{C}$ and $70 \pm 10\%$ relative humidity. Both nymphs and adults were kept on a diet of fresh beans, carrots and raw peanuts, with water available *ad libitum*, which was changed at least twice weekly to avoid problems with mould. Rearing containers were changed and cleaned once per week for the same reason. Males and females were sexed at emergence and kept separately. Only virgin animals were used in the experiments at least 7 days after emergence to ensure reproductive maturity.

Description of mating behaviour

Experiments were designed to replicate the observations of pre-copulatory behaviour of *H. halys* as reported by Kawada & Kitamura (1983) and to complement them with recordings of vibratory emissions by the insects. Two different substrates were used: the membrane of a mid-range cone loudspeaker (diameter 10 cm) and a common bean plant (*Phaseolus vulgaris* L.) (Fig. 1). Bean plants were grown in small pots and used when

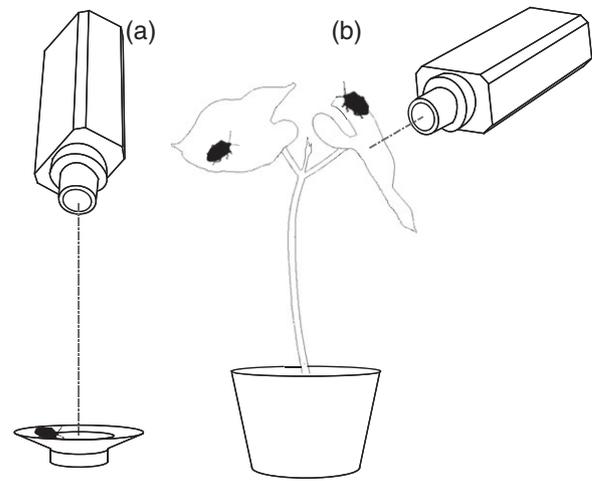


Fig. 1. Diagram of the experimental set-up; laser vibrometer recording from the surface of a loudspeaker (a) and a bean plant (b). A small piece of reflective foil was glued to the substrate where the laser was focused to improve reflection.

two leaves were developed, with the main shoot cut off at the stem/petiole crossing.

Throughout the present study, the number of signals is denoted by '*n*', whereas the number of trials is denoted by '*N*'. Different experimental scenarios were set up: single-male ($N_{\text{loudspeaker}} = 45$), double-male ($N_{\text{loudspeaker}} = 22$; $N_{\text{plant}} = 27$), single-female ($N_{\text{loudspeaker}} = 11$), double-female ($N_{\text{loudspeaker}} = 10$; $N_{\text{plant}} = 2$) and male/female ($N_{\text{loudspeaker}} = 41$; $N_{\text{plant}} = 43$). Pairs were initially placed with each individual on its own side of the loudspeaker membrane (i.e. not in direct contact) or each on its own leaf, then left to interact freely and observed for 20 min. If no signalling activity was detected in the first 10 min, the trial was stopped and zero activity was noted. Similarly, the trial was stopped if any of the animals walked or flew off the bean plant more than once.

All experiments were conducted at daytime between 08.15 and 17.15 h in the spring of 2014 and 2015, on an anti-vibration table (Astel s.a.s., Italy). The temperature during the trials was $21 \pm 1^\circ\text{C}$.

Signal recording and analysis

Vibrational signals were recorded using laser vibrometer (VQ-500-D-V, Ometron Ltd, U.K.). The vibrometer's output was acquired with a calibrated data acquisition device (LAN XI type 3050-B-040; Brüel and Kjær Sound & Vibration A/S, Denmark) and stored on a computer hard drive using PULSE, version 14.0 (Brüel and Kjær) for subsequent analysis. The laser beam was directed at a point on the substrate, either the middle of the loudspeaker or the middle of one of the leaves, perpendicular to the leaf lamina. Laser reflection was improved by a small piece of reflective tape glued to the substrate. Amplitude of vibrations was measured as velocity (expressed as mm s^{-1} or smaller units). The recording was monitored in real-time with the real-time oscillogram and playback functions in PULSE. At

the same time, behaviour was observed visually and noted. The signal terminology employed in the present study matches that used by Čokl *et al.* (2001).

To facilitate interpretation of recordings, trials in 2015 were recorded using an additional laser vibrometer (Polytec PDV 100; Polytec GmbH, Germany) plugged into another channel of the same data acquisition device. The second laser was pointed at the pronotum of the female, also equipped with a piece of reflective tape. Using this set-up, it was possible to distinguish male- and female-produced signals reliably by amplitude. Stereo recordings were also used to compare the frequencies of signals recorded directly from the female's body and from the substrate some distance away from the female.

The native file format produced by PULSE was used for amplitude measurements after applying fast Fourier transform (FFT) with a window length of 400 samples and 66.7% overlap, and with the Blackman–Harris window. Only signals emitted by the animal on the same leaf as the laser beam were measured to reduce the effect of transmission through the substrate.

The recordings were converted to uncompressed WAV format for analyzing temporal and spectral parameters with RAVEN, version 1.4 (Cornell Laboratory of Ornithology, Ithaca, New York). Frequency and time resolution of the spectrograms were balanced at an FFT window length of 1489 samples (Hann window) and 50% overlap. Accuracy of peak frequency measurement was improved by reading it from the spectrogram slices of each selection (Hann window, 10240 samples, 50% overlap). Peak frequency is defined as the frequency of the highest peak in a signal's frequency spectrum, excluding low-frequency noise below 40 Hz.

Statistical analysis

For the analysis of mating behaviour, a first-order Markovian behavioral transition matrix was created using the data from plant substrate trials with male/female couples. 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 (Haccou & Meelis, 1992), where each transition was counted only once in case of repeated transitions. The expected values were calculated using the iterative proportional fitting method of Goodman (1968) and then the *G*-test was performed to identify significant transitions, with the results presented graphically in an ethogram.

For the acoustic analysis, summary statistics were computed using a subset of signals from each trial to prevent highly-active animals from having disproportionate weight when pooling the measurements. For types of signals that are emitted as individual pulses, 5–15 signals were measured from each trial and trials with fewer than five signals of that type were discarded. For types of signals emitted in pulse trains, one to five trains were measured and trials with no signals of that type were discarded. Pulses within trains were measured from recordings of loudspeaker trials only. Ten trials were selected with well visible trains with the noise level sufficiently low to allow precise delimiting of the pulse envelope. Ten pulses were measured from 1 train per trial, totalling 100 pulses from 10 trains.

The signals were classified according to characteristics and the observed context in three male and two female types: Male Song 1 (MS-1), Male Song 2 (MS-2), Male Courtship Song (MCRS), Female Song 1 (FS-1) and Female Song 2 (FS-2). One additional signal type, the 'coo', could not be sensibly classified and so it is reported separately. Distribution tended to be skewed (Shapiro–Wilk test for normality, $P < 0.05$) and so nonparametric tests were used for hypothesis testing.

Results

Vibrational signal description

Vibrational signals were detected in the majority of total trials, depending on the experimental scenario: 88 out of 129 trials with the insects on the loudspeaker and 60 out of 72 trials on the plant substrate. The signals were recognized most reliably as narrow frequency bands in the spectrogram.

The basic song type emitted spontaneously by male *H. halys* consisted of long pulses with a peak frequency in the range of 50 Hz, downward frequency modulation, and a gradually increasing amplitude (Fig. 2 and Table 1). They are referred to as MS-1 (putative Male Calling Song). Their frequency spectra were characterized by very narrow frequency peaks, with a bandwidth of approximately 3 Hz at –20 dB below the peak frequency value, and remarkable harmonics, including up to 20 visible peaks or more and extending beyond 1 kHz frequency on the loudspeaker. However, the number of harmonics depended on the signal's amplitude at the recording point; in most signals, only up to three harmonics were distinguishable. There was a small, but significant difference (Mann–Whitney test, $W = 8283$, $P = 0.005$, $n = 308$) in peak frequency between signals recorded on the plant and on the loudspeaker membrane, and so the values are reported separately (Table 1).

The basic female song type is referred to as FS-1 (putative Female Calling Song): a single pulse with a spectral structure similar to MS-1 and, on average, being three times shorter than MS-1 (Fig. 2 and Table 1). Sixteen out of 513 FS-1 pulses recorded on both substrates had short (approximately 0.1 s) gaps after which a pulse continued at the same amplitude as before the gap. Again, a small difference was observed in peak frequency between signals recorded on the plant and on the loudspeaker membrane (Mann–Whitney test, $W = 10136$, $P < 0.001$, $n = 268$), although the dominant frequency of FS-1 pulses recorded simultaneously from the female's body and from the plant substrate did not differ (median_{substrate} = 56 Hz, median_{female} = 57.7 Hz, Mann–Whitney test, $P > 0.05$, $n = 14$, $N = 5$).

The FS-1 pulses were accompanied by trains of short pulses with pronounced downward frequency modulation (FM), decreasing from 85 to 40 Hz and separated by approximately 0.5-s long pauses. They are termed FS-2 (putative Female Courtship Song) (Fig. 2 and Table 1). The duration and number of pulses within FS-2 trains were both highly variable (Table 1) but not significantly different between substrates (Mann–Whitney test, $P > 0.05$, $n = 156$). These trains normally followed FS-1 pulses but could also precede it or be emitted

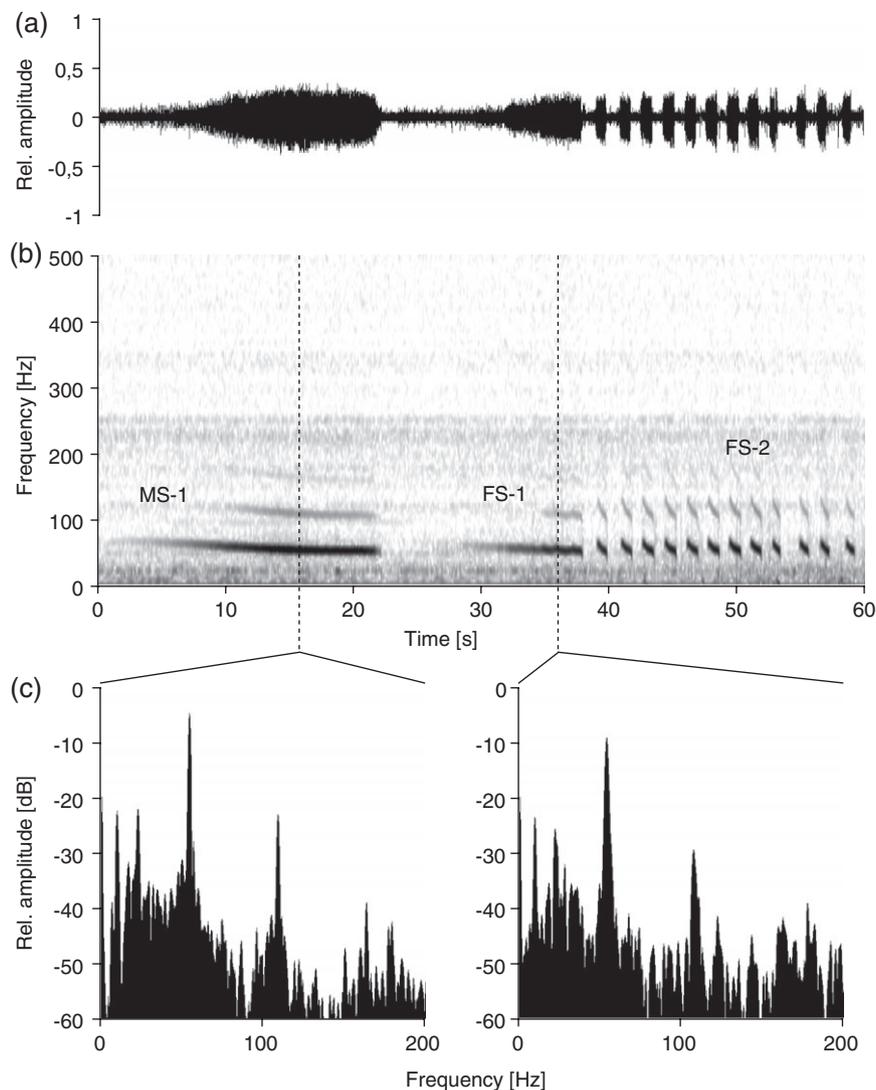


Fig. 2. A typical sequence of male call and female response in the brown marmorated stink bug *Halyomorpha halys*. A pulse of Male Song 1 (MS-1), emitted spontaneously by males, is followed by a Female Song 1 (FS-1) pulse and a short Female Song 2 (FS-2) train. (a) Oscillogram, (b) spectrogram and (c) spectrogram slice views for MS-1 and FS-1. Dashed lines denote time positions of the spectrogram slices.

alone. A transition between FS-1 and FS-2 was also observed in the form of the second structural type of FS-1. A regular, 'clean' FS-1 began with downward frequency modulation and then proceeded for most of the duration with constant frequency. A 'pulsed' type resembled an FS-2 sequence without pauses; when displayed in a spectrogram, the signals had a jagged appearance because of the rapid downward frequency modulation of individual FS-2 pulses (Fig. 3). Transitional forms that began as 'clean' signals, gradually broke down to fused pulses, and then finally to an FS-2 train, were also recorded.

During female emission of FS-2, most males [41 (or 79%) of 52 trials where FS-2 were detected] also started answering with their own short pulses, termed MS-2 in the present study. Trains of MS-2 pulses resembled FS-2 trains but with lower amplitude, usually without visible harmonics and partially overlapping the ongoing FS-2 train; each MS-2 pulse began approximately

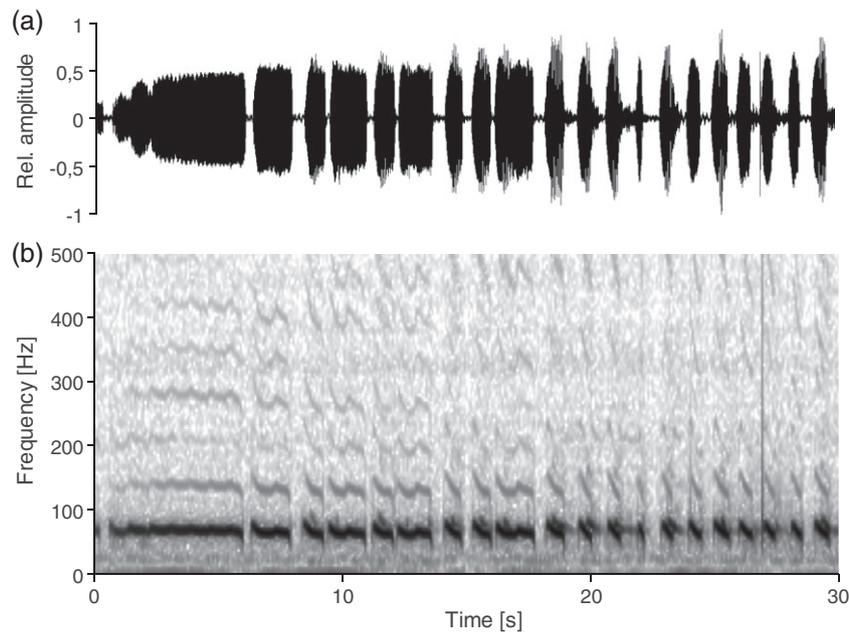
halfway during an FS-2 pulse, and then continued after the FS-2 pulse had ended, through the first half of the pause (Fig. 4 and Table 1). The weaker male pulses could be clearly distinguished only in the spectrogram because their frequency modulation paralleled the overlapping FS-2 pulse. The FS-2 trains were not the only context in which MS-2 trains appeared; they could also be emitted as standalone pulse trains leading to an MS-1 pulse, or succeeding it or as pulse trains that started within an FS-2 train, then continuing afterwards on their own (Fig. 4). Similar to FS-2, the MS-2 trains included anything from several pulses to hundreds of pulses. Fusing of MS-2 pulses into longer pulses (similar to FS-1/FS-2 fusing) was also observed, which could be followed by a proper MS-1 signal with a higher amplitude and visible harmonics (Fig. 4) or might even continue seamlessly into an MCrS train characterized by the addition of tremulation (see below). Because of high variability, only individual pulses

Table 1. Descriptive statistics of vibratory communication signal types (songs) of the brown marmorated stink bug (*Halyomorpha halys*), with median, minimum and maximum values reported for each parameter.

Song	Substrate	Duration (s)	Repetition time (s)	Number of pulses	Peak frequency (Hz)	<i>n</i> (<i>N</i>)	
MS-1	Train	Loudspeaker	18.6 (7.6–29.9)	120 (42.1–243.2) ^a	NA	61 (51.5–77.5)	207 (27)
	Train	Plant	19.1 (9.2–29.9)	NA	NA	62.5 (56.5–72)**	101 (16)
MS-2	Pulses	Loudspeaker	0.6 (0.3–1.9)	1.1 (0.7–3.8)	NA	65 (56.5–85.5)	98 (10)
MS-2	Train	Loudspeaker	23.9 (8.8–126.6)	NA	22 (9–143)	NA	43 (16)
	Train	Plant	48.7 (25.4–122.7)***	NA	43 (23–112)***	NA	16 (12)
	Pulses	Loudspeaker	0.6 (0.3–0.9)	1.3 (0.9–1.8)	NA	66.8 (42–84)	100 (10)
'coo'	Pulses	Plant	1.4 (1–2)	NA	NA	283.5 (218–357.5)	64 (7)
FS-1	Train	Loudspeaker	5.6 (0.6–22.5)	NA	NA	65 (49.5–77)	167 (17)
	Train	Plant	6.5 (1.7–14.7)***	NA	NA	65.5 (40.5–76)***	89 (10)
FS-2	Train	Loudspeaker	10.8 (0.5–174.4)	NA	8 (1–125)	NA	87 (28)
	Train	Plant	9.1 (0.7–716.8)	NA	8 (1–660)	NA	69 (21)
	Pulses	Loudspeaker	0.8 (0.5–1.1)	1.4 (0.8–2)	NA	59.5 (48–76)	100 (10)

^aMeasured only in a single-male scenario.

Statistical differences between signals of the same type emitted on loudspeaker and plant are reported (Mann–Whitney test: ** $P < 0.01$, *** $P < 0.001$). *n*, number of signals; *N*, number of trials; MS, male song; FS, female song.

**Fig. 3.** An example of Female Song 1 (FS-1) pulses in the brown marmorated stink bug *Halyomorpha halys* gradually breaking down into Female Song 2 (FS-2) elements. (a) Oscillogram and (b) spectrogram.

were measured, and in the same way as in other trains (Table 1). The MS-2 pulses were more variable than FS-2 pulses because they were in various stages of fusion (i.e. the last pulses in the sequence leading to a MS-1 were longer than the previous ones).

The male courtship song (MCrS) was emitted by males when in physical contact with females, just before copulation. Trains were similar to MS-2 or FS-2 but differed in that pulses were always interspersed with tremulation (Fig. 5 and Table 1). They were emitted throughout the final phase of mating behaviour until the genital connection itself.

The last signal type was recorded only in trials where males were present: pulses with higher frequency and broader frequency peaks than the other signal types (Fig. 6 and Table 1)

resembled the cooing of pigeons when listening to the playback and so this type was labelled a 'coo'. 'Coo's were emitted by males before the final courtship phase but only in seven out of 43 trials (16%) on plant substrate. They were found overlapping both other male and female signals in various recordings and their context is unclear. The pulses, emitted individually and with unpredictable repetition rate or sometimes alone, were downward frequency modulated, with the frequency dropping from approximately 365 to 235 Hz from beginning to end.

Signal amplitude was highly variable, depending on the exact position of the singing animal and leaf geometry. The amplitude of both MS-1 and FS-1 signals was in the range of 1 mm s^{-1} peak velocity, with 1.76 mm s^{-1} maximum amplitude recorded

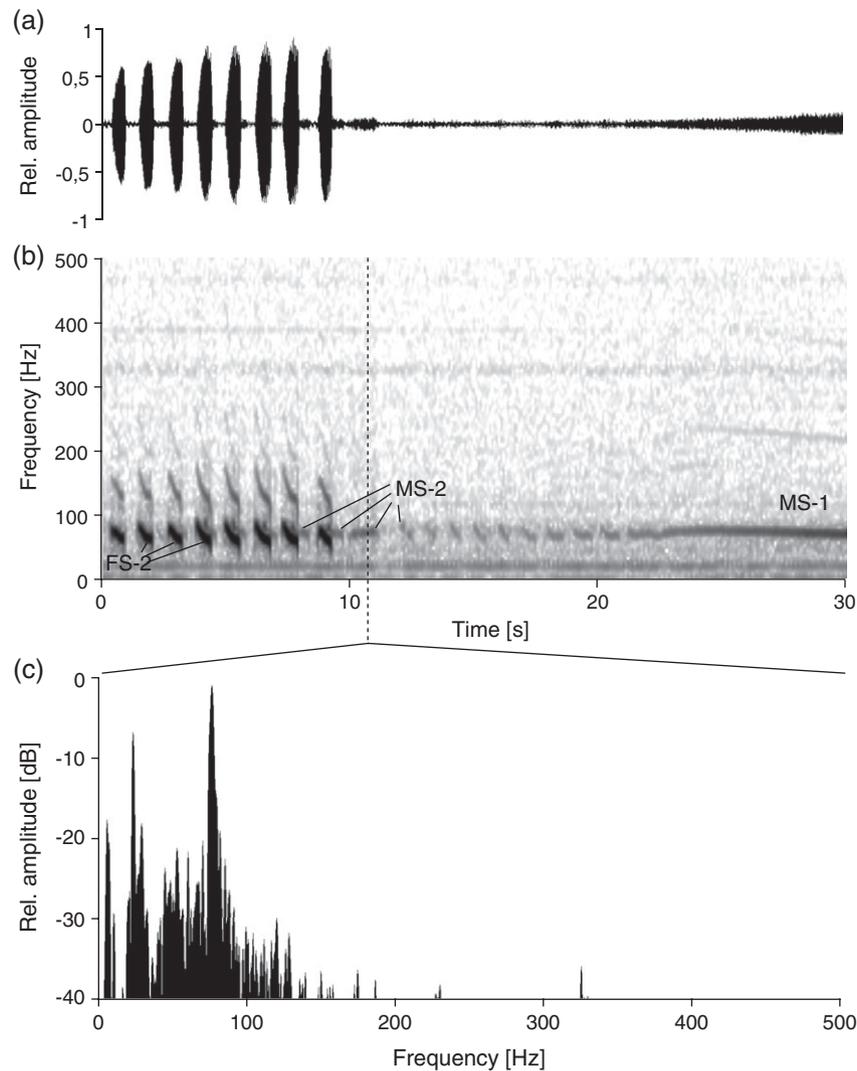


Fig. 4. An example of a Male Song 2 (MS-2) train in *Halyomorpha halys* at first overlapping a Female Song 2 (FS-2) train, then continuing on its own and finally morphing into a Male Song 1 (MS-1) train. Such transitions between song types were common. (a) Oscillogram, (b) spectrogram and (c) spectrogram slice views. Dashed line denotes the time position of the spectrogram slice.

in MS-1 signals (average $0.65 \pm 0.5 \text{ mm s}^{-1}$, $n = 20$) and 1.5 mm s^{-1} recorded in FS-1 signals (average $0.46 \pm 0.4 \text{ mm s}^{-1}$, $n = 10$).

Behavioural analysis

Mating sequences proceeded in more or less the same way in the loudspeaker and plant substrate trials (Fig. 7), although localization of the recording target on the loudspeaker was facilitated by its small area and the insects usually came into physical contact by random movement, before emitting any signals. However, no mating was observed without an exchange of vibrational signals, even if the animals found each other by chance.

In 63 out of 65 (97%) male/female trials on both substrates in which signals were detected, the males initiated communication

by spontaneously emitting MS-1 pulses or 'coos', usually within 2 min after being placed on a substrate (first signal onset after 10.4–527.5 s, median 73 s, $N = 63$). The other two trials started with FS-2 trains.

The MS-1 signal was the only type of vibratory emission recorded from males placed alone on the substrate. The males started singing spontaneously, again usually approximately 2 min after being placed on the loudspeaker (first signal onset after 16–559 s, median 132 s, $N = 27$) and emitted MS-1 semi-regularly every 2 min afterwards (Table 1). One to seven MS-1 signals were detected per 10-min trial (median of three signals), whereas 18 (40%) males did not start singing at all in single-male trials. MS-1 signals were completely absent in only 3 double-male trials out of 49 on both kinds of substrate (6%). Male signals in these trials almost never overlapped; only one pair of MS-1 signals in one trial overlapped by half of the duration and one male 'coo' was emitted during an MS-1

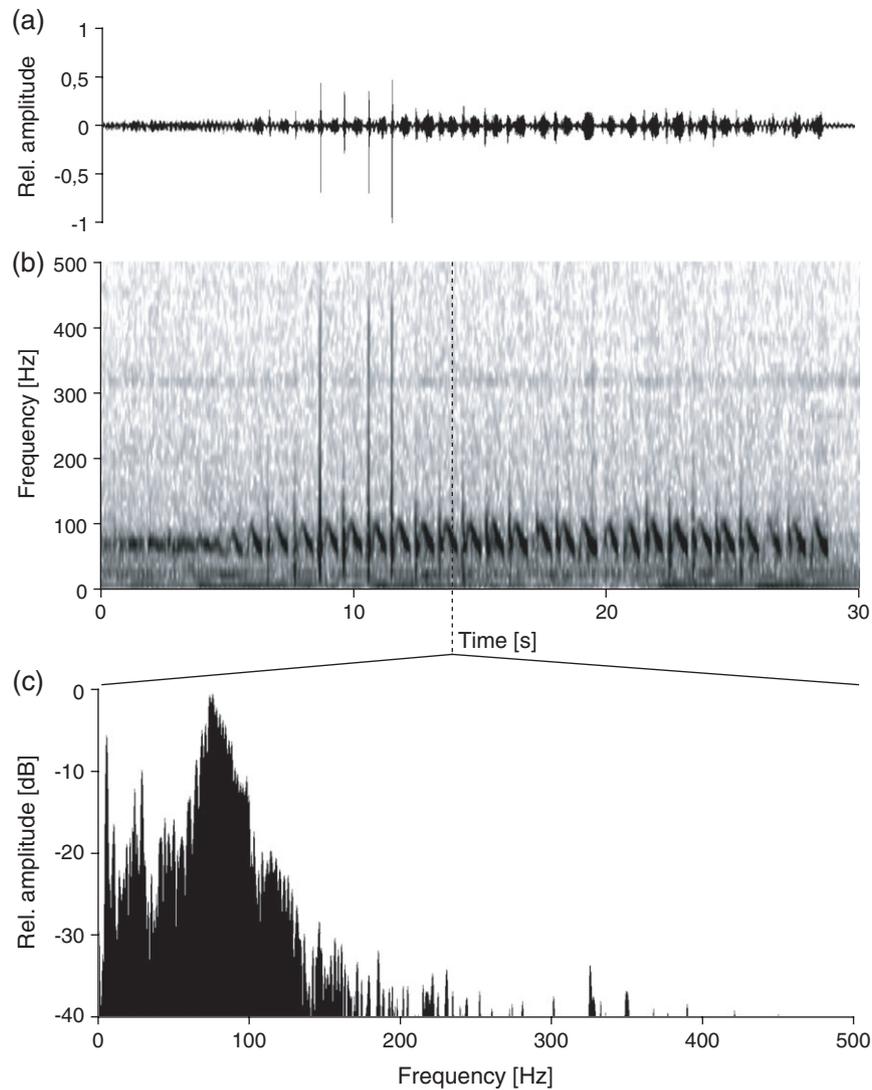


Fig. 5. A Male Courtship Song (MCRs) train in *Halyomorpha halys*. Vertical lines between vibrational pulses represent tremulation. (a) Oscillogram, (b) spectrogram and (c) spectrogram slice views. Dashed line denotes the time position of the spectrogram slice.

signal from the other male in a different trial. Additionally, an MS-1 signal by one male once started directly after an MS-1 by the other male (i.e. no pause). In all other cases, signals were separated by at least several seconds, although distinguishing the signals of one male from the other was not reliably possible. In one trial on loudspeaker and two trials on the plant, courtship behaviour by males was observed with characteristic MCRs pulse trains and tremulation after the males came in physical contact by apparently random movement. The males subsequently went through blank contact sequences (see below) (Fig. 7) or even butted each other with their heads, raised their abdomens and turned around in apparent mating attempts. However, the males did not react to conspecific male signals by moving.

Most females that were exposed to male signals [54 (or 75%) out of 72 trials on both substrates] responded with their own vibratory signals. Signal emission in this phase was variable:

typically, the sequence consisted of an MS-1 signal followed a few seconds later by an FS-1 pulse, which was then followed immediately by a short FS-2 train and then silence. Males responded to FS-2 emitted from a fixed position by searching for the source, stopping for each pulse and walking during the pauses. The whole MS-1/FS-1/FS-2 sequence could be repeated several times when the male was searching. However, many combinations of signal types were observed, ranging from one call–reply combination, to multiple male signals of which few were answered by short FS-1 pulses or, conversely, one male signal followed by a long sequence of FS-1 and FS-2 in various combinations and comprising hundreds of signals in total (Fig. 7). Male movement during longer periods of silence appeared random. If the female did not reply to the initial MS-1 signal, the male continued to emit MS-1, as when observed alone, but did not start searching or switch to other song types.

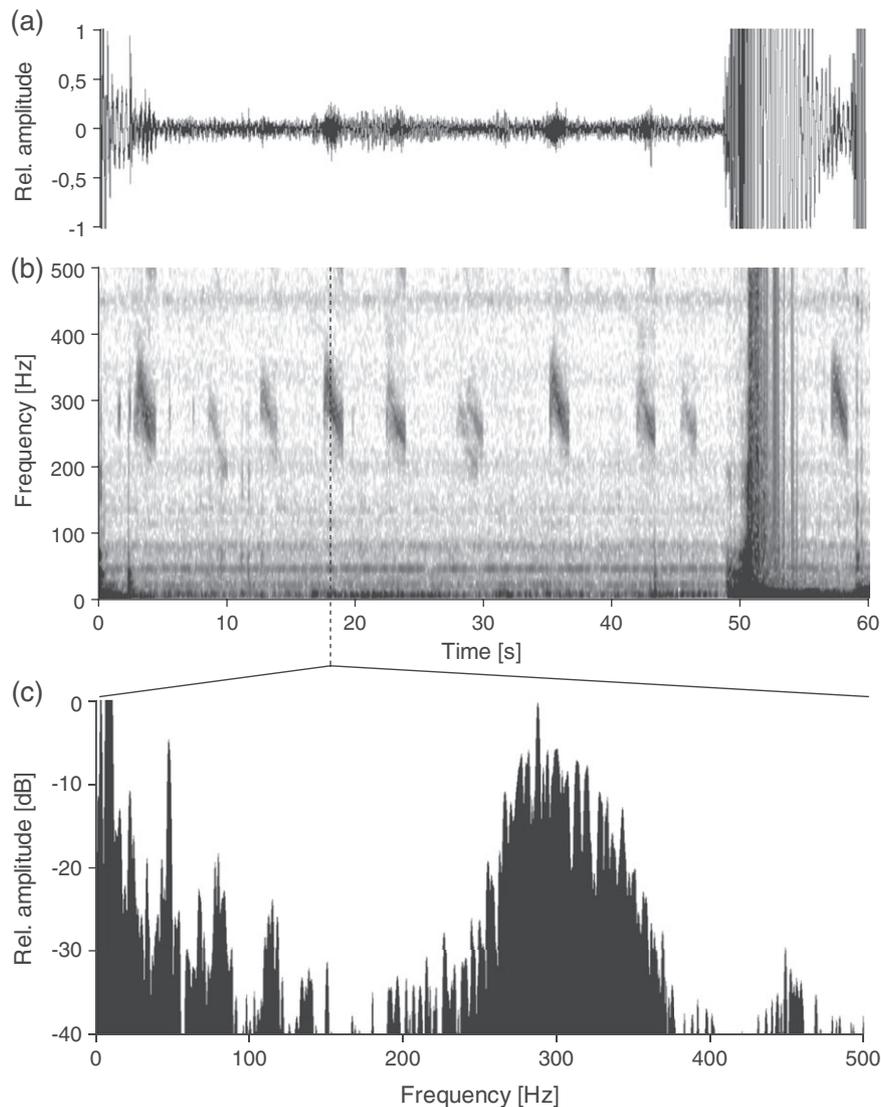


Fig. 6. A sequence of 'coos', recorded on a plant in a trial with a male-female couple of brown marmorated stink bugs *Halyomorpha halys*. (a) Oscillogram, (b) spectrogram and (c) spectrogram slice views. Dashed line denotes the time position of the spectrogram slice.

After a male came in direct contact with the female, he initiated the last phase of the mating behaviour: walking sideways with jerking motion towards the rear end of the female, butting the female's abdomen to coerce her into raising it, then turning around and establishing genital connection. Without exception, the MCrS was the only type of signal emitted in close contact, and females were silent during this phase. Some copulatory sequences, however, did not end with mating but instead with the female walking away from contact or the male crawling over the female's body when continuing with tremulation. The males continued to crawl over females for several seconds but eventually dismounted and broke contact without mating. Therefore, this sort of encounter was termed blank contact sequence (Fig. 7). A blank contact sequence could be followed by another MCrS bout and subsequently by another blank contact sequence or by mating, or if the male ended away from the female, by

restart of vibrational signalling or end of the mating attempt. Females rejected the male simply by not responding or walking away from contact. Only when the male crawled over the female for a prolonged period did she start to tremulate (which was similar to male tremulation, but without alternating vibrational signals), continuing until he dismounted and went away.

Females produced vibrational songs spontaneously in only 2 of the trials with single females or female pairs, out of 23 trials (once in a single-female trial and once in a female pair trial), and never in trials with male/female pairs. Courtship behaviour was not observed in the absence of male singing, even if the animals came in contact by random movement. Almost all female-only trials were silent, both on the loudspeaker and on the plant. A long FS-1 pulse (17.9 s) was emitted by a lone female on the loudspeaker in one trial, whereas two FS-1/FS-2 combinations were emitted by another female in one double-female trial on

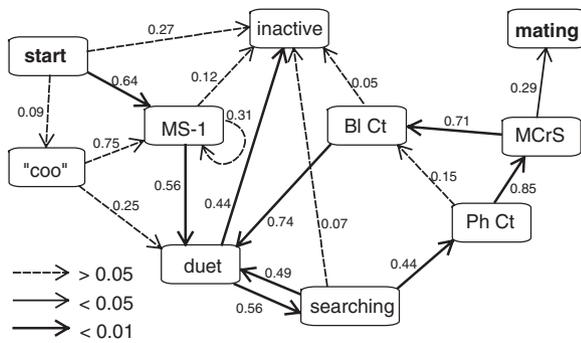


Fig. 7. Flowchart of reproductive behaviour in *Halyomorpha halys* with special reference to vibrational signals. Values represent the percentage of all transitions out of any given state (box), and the weight of the arrow denotes significance. MS-1, male song 1 (Fig. 2); MCrS, male courtship song (Fig. 5) accompanied by tremulation and sideways movement; Ph Ct, physical contact; BI Ct, 'blank contact sequence' (i.e. male crawling over the female without mating).

the loudspeaker (assuming only one of the two females was singing). In both cases, the females remained stationary for the whole duration of the trial.

Discussion

Adult stink bugs of the species *H. halys* exhibit a diverse repertoire of vibrational signals. Both males and females emit a number of context-dependent signal types, which are a key component of the mating behaviour. Pheromones are assumed to be ubiquitous for the purpose of the present study because males and females are mass-reared and not completely isolated, and so any volatiles could easily reach the inside of all of the rearing containers. Furthermore, the same substrate is repeatedly used for trials without taking steps to remove residual chemicals, and so all experimental animals are exposed continuously.

The communication system in *H. halys* appears to follow the same general pattern as that observed (with some variation) in the southern green stink bug *N. viridula* and other members of the family Pentatomidae (Borges *et al.*, 1987; Čokl & Virant-Doberlet, 2003; Blassioli-Moraes *et al.*, 2005, 2014; Čokl, 2008), where males are the more active partner in male–female interactions, emitting an aggregation pheromone that attracts all mobile life stages to the general vicinity (Khrimian *et al.*, 2014). Soon after landing on any surface, the males also start producing vibrational signals that stimulate females to respond with their own vibrational signals. The males in turn respond by actively searching for the source, in the meantime continuing to emit their own signals to stimulate female signalling. The final stage is close-range courtship where vision and tactile and/or chemical signals are added, in the form of antennation and head-butting by males, which is then followed by genital contact itself. The vibrational signals of *H. halys* in this stage notably include tremulation, which is a separate mechanism involving the production of short, broadband pulses by shaking the whole body. Such signals are described during courtship for other Pentatomid species, such

as *Podisus maculiventris* (Žunič *et al.*, 2008) and *Euschistus heros* (Kavčič *et al.*, 2013). Furthermore, numerous transitions between song types are observed, perhaps hinting at the evolution of signal repertoires from a 'basic' type. No antagonistic vibrational signals are recorded, regardless of experimental scenario, and the only antagonistic behaviour is body shaking by the female if a male crawls over her for a prolonged period.

The present study reveals several noteworthy characteristics of the vibratory signals from *H. halys*. First, the basic male signals (MS-1) are exceptionally long and, with a duration of almost 30 s, which is substantially longer than any other previously described stink bug signal produced by abdomen vibration. Male signals in other species are usually reported to be in the range of 0.1 s to several seconds, and at most slightly over 10 s in *Eurydema oleracea* (Gogala, 2006; Čokl *et al.*, 2014; Shestakov, 2015). Durations of MS-1 and FS-1 overlap substantially, and so the identity is inferred from the context or slight amplitude differences in those trials where only one laser is used (e.g. a long pulse closely succeeding another similar pulse is an FS-1 succeeding an MS-1, FS-1 pulses can be emitted in quick succession, whereas there is always at least approximately 1 min between successive MS-1 pulses, etc.). The fact that MS-1 pulses are so long, emitted spontaneously and not suppressed in male–male interactions leads to the hypothesis that they function in contexts other than reproduction, although no support for this hypothesis is found. The signal referred to in the present study as a 'coo' remains a mystery for now; the relatively high frequency and broad frequency range suggest a different mechanism of production but not even its function can be deduced from the few cases in which it is observed.

The second noteworthy property of signals is their exceptionally low frequency, which ranges between 50 and 80 Hz for most signal types, which is at the lowest end of the range observed in Heteroptera (Čokl & Virant-Doberlet, 2003; Čokl, 2008). There is a significant difference in the dominant frequency between signals emitted on the loudspeaker and plant, although the medians differ by less than 2 Hz. The biological relevance of this difference is therefore dubious, at least from the receiver's perspective; for example, preference for the peak frequency of signals in *N. viridula* is unchanged over the range of almost 100 Hz as determined by behavioural trials by Žunič *et al.* (2011), whereas Miklas *et al.* (2001) report different levels of female response to male song when perceived on a plant or a loudspeaker. The difference may either be a result of changes in the pulse envelope caused by transmission along the plant or a frequency difference of higher harmonics. Alternatively, it may reflect the emitter's response to the transmission properties of the substrate to achieve the most efficient transmission, as indicated by analysis of signals of *Palomena prasina* emitted on different substrates (Polajnar *et al.*, 2013). Such a hypothesis is supported in the present study by the fact that signals recorded from the female's thorax and from the substrate some distance away have the same dominant frequency, although this phenomenon needs to be tested more thoroughly before any conclusions can be drawn. Also notable is the extreme narrow-band character of most signals: in MS-1, the spectral width at –20 dB below the peak frequency peak is as little as 3 Hz, which is half as much as that in some signal types of *N. viridula* (Čokl *et al.*,

2001) and an order of magnitude less than in some other species producing narrow-band signals (Blassioli-Moraes *et al.*, 2005). Concentrating energy in a narrow frequency band improves the signal-to-noise ratio but carries the risk of destructive resonance, resulting in loss at certain distances from the emitter (nodes), although this may be alleviated somewhat by frequency modulation (Čokl *et al.*, 2014), as also observed in this species.

With regard to aggregating behaviour, overlapping of vibrational emissions from different individuals in a group might be expected, especially when considering the extreme duration of male signals and the fact that males sing spontaneously. However, in an experimental scenario with two males, the signals almost never overlap, which could indicate active avoidance. Partial overlapping of FS-2 and MS-2 trains is very similar to FS-1/MS-1 overlapping in the stink bug *Euschistus heros* (Čokl *et al.*, 2015) and FS-3/MS-3 overlapping in *Dichelops melacanthus* (Blassioli-Moraes *et al.*, 2014), although the function of this phenomenon is still unknown.

No attraction of males and females to male vibrational signals is observed, and so they are unlikely to play a major role in aggregation behaviour. However, the second female song (FS-2), which is highly attractive to males, does hold promise for developing monitoring and 'attract and kill' implementations, and work is underway aiming to confirm this.

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