Sound communication in *Nezara viridula* (L.) (Heteroptera:Pentatomidae): further evidence that signal transmission is substrate-borne

M. A. Ryan and G. H. Walter

The Department of Entomology, University of Queensland, Brisbane, 4072, Queensland (Australia)

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Abstract. We investigated the sound communication associated with mating in *Nezara viridula* (L.) to clarify conflicting reports on the medium through which acoustic signals are borne. Recordings made from virgin pairs of *N. viridula* during precopulatory and copulatory behaviour have revealed that signals transmitted as vibrations through the substrate are a more likely means of communication than signals transmitted by a radiation of sound pressure waves through the air (at least in Australian populations). We present evidence (additional to that already in the literature) that air-borne acoustics are an unlikely mechanism of sexual communication in this species. Specifically, the recorded pulse train patterns showed no consistency or repeatability in relation to different stages of mating behaviour. In contrast, frequency spectrograms of substrate-borne signals reveal repeatable patterns that do correspond with the stage of mating behaviour.

Key words. Nezara viridula; substrate-borne signals; air-borne signals; sound communication; frequency spectrogram.

Conflicting opinions exist regarding the medium of sound communication in *Nezara viridula*. Based on work conducted on American populations, air-borne signals have been stated to be the primary means of communication[12]. Originally, European populations of this species, were also thought to communicate in this way[3], but subsequent work has indicated that air-borne signals would be an inefficient and unlikely means of communication in *N. viridula* and that substrate-borne communication is more likely[5, 9–11]. The evidence presented in favour of the latter claim has been derived from neurophysiological investigation of responses to auditory signals detected by vibroreceptors in the legs[8] of *N. viridula*, combined with an analysis of *N. viridula* vibratory emissions[9].

We investigated an Australian population of *N. viridula* and repeated the air-borne sound recording techniques used by Harris et al.[12]. Substrate-borne signals were also recorded from mating pairs of *N. viridula*. Although our results do not allow us to resolve the issue entirely (because we used bugs from a different population), they do allow us to draw strong inferences. These are based on certain technical difficulties we encountered in identifying putative air-borne signals and on the consistency with which substrate-borne signals were detected.

Materials and methods

N. viridula nymphs were collected off soybean crops at Kingaroy, Queensland, and reared on a diet of green beans (*Phaseolus vulgaris*), raw peanuts (*Arachis hypoaea*) and sunflower (*Helianthus annuus*) seeds. The subsequent F_1 generation was reared solely on rape seed plants (*Brassica rapa* var. *silvestris*) and it was from this population that all recordings were obtained.

Air-borne acoustics. Using equipment with sensitivity (100 mV/pascal) appropriate to the low frequency, low decibel sounds reported by Harris et al.[12], recordings were made with a microphone positioned to within 1 cm of a pair of virgin bugs exhibiting mating behaviour (leading to copulation) in an anechoic chamber. Although an anechoic chamber is a sound-dampening chamber, it inevitably has continual low-level background noise (fig. 1). For this reason 10 control recordings were made (with no bugs or observer in the chamber) to determine the background noise level. Recordings were then made from a total of 97 virgin pairs between 10 and 40 days after the imaginal moult. Initially, signals (n = 37 pairs) were recorded onto an instrumentation tape recorder (Sony TC-D5 PRO) via a condenser microphone (Bruel and Kjaer – Type 4179) connected to a measuring pre-amplifier (Bruel and Kjaer – Type 2660). These were instruments of the type used by Harris et al.[11]. The data were then digitised by a computer spectrum analyser program (Ultrasound – Version 1.1) enabling a breakdown of the signal to be displayed as an

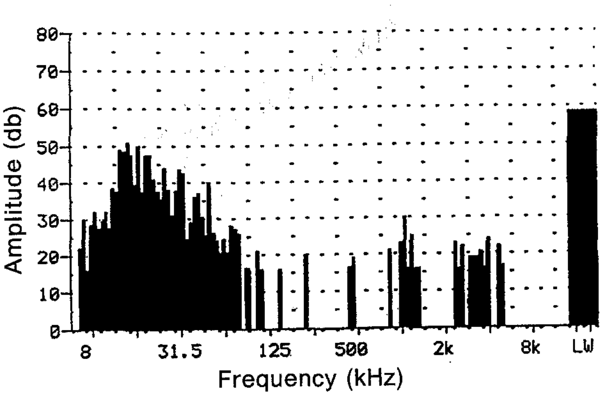

Figure 1. Typical control frequency spectrogram of an air-borne recording from within an anechoic chamber. There were no bugs or observers within the chamber during the recording period. The 'L' bar on the X-axis of the frequency spectrogram represents the broadband unweighted (linear) level of the input signal and the 'W' represents digital spectrum weighting. Amplitude of sound waves is often measured in decibels (db) where 0 db equals a particle velocity in a plane wave of 0.5×10^{-7} ms^{-1} (Bennett-Clark[1]).

oscillogram, with amplitude plotted against frequency. The time factor could be scaled up or down which permitted expansion of very short time sequences for closer scrutiny. Alternatively, the data could be plotted in a three-dimensional representation of amplitude, frequency and time.

To allow instantaneous results (no tape recording necessary) to be displayed as frequency versus amplitude, and also for any single spectrum throughout the recording time to be individually monitored, recordings were taken (n = 60 pairs) via the microphone and measuring preamplifier connected directly to a real time frequency analyser (Bruel and Kjaer – Type 2143). The frequency analyser has the ability to subtract the control spectrum from any source spectrum enabling a concise interpretation of any detectable signals. Results were stored automatically on computer disc.

Substrate-borne acoustics. A male and a female *N. viridula* (both virgin) were placed onto a suspended sheet of thin aluminium. A high density substrate was used to avoid degradation of any transmitted signal. Substrate-borne recordings were obtained directly from the aluminium substrate (no tape recording necessary) by conveying insect-induced vibrations directly from an accelerometer (Bruel and Kjaer – Type 4370) into a real time frequency analyser (Bruel and Kjaer – Type 2143). Controls were taken between sessions when mating bugs were recorded. Frequency spectrograms of substrate-borne communication were obtained from the 14 pairs of *N. viridula* investigated.

Results

Air-borne signals. Initial recordings of air-borne signals showed frequencies similar to those recorded in previous studies[3, 12] (table 1). However, we found no consistency in the acoustical characteristics of consecutive pulses of sound produced by any pair of bugs, i.e. pulse trains were not detected when the time scaling factor was 1000–2000 ms. Further, there was also no consistency in the pulse train structure of sounds recorded in the presence of the different pairs of bugs. These results were obtained despite having the microphone within 1 cm of the bugs all through courtship and copulation in an anechoic chamber. Only when the time scaling factor of the analyser program was reduced considerably (10 ms) did pulse trains become evident. Assignation of these brief pulse trains to a specific source signal would be tenuous. We later found, in the second method that we used for analysing air-borne acoustics, that control recordings void of background noise were unattainable even from within an anechoic chamber. 90% of control recordings returned frequency spectrograms similar to those illustrated in figure 1.

Despite being unable to record (at 1000-2000 ms) pulse trains like those illustrated in previous studies[3, 12], we did record frequencies consistent with that work. There is, however, no reason to believe that those frequencies were not derived from background noise. We therefore have no confidence that either the pulse train patterns (discernible at a time scale of 10 ms) or the associated frequencies that we recorded represent communication signals in Australian *N. viridula.*

Substrate-borne signals. Control spectrograms, void of background noise, were consistently obtained when using a real time frequency analyser (Bruel and Kjaer – Type 2143) (n = 6). The substrate-borne frequencies recorded in table 2 are listed in relation to the four different stages of mating behaviour illustrated in figure 2. While the recorded frequencies are undoubtedly higher on an aluminium sheet than would be expected from *N. viridula* on a more natural substrate (e.g. leaf, stem etc.), they do represent repeatable patterns correlated with different stages of behaviour in *N. viridula* courtship (recorded simultaneously on videotape). Since control recordings revealed an absence of background noise we are confident that the signals derive from the bugs. Figure 3 averages the frequency spectrograms associated with the four stages of mating behaviour and table 2 provides a more detailed breakdown of the sounds.

Discussion

According to our results it seems unlikely that *N. viridula* uses air-borne sound signals for sexual communication. An efficient radiation of sound pressure waves through the air is possible only in the frequency range for which the sender's diameter is not much smaller than one third of the wavelength emitted[13]. In light of this, Cokl[5] em-

Table 1. Mean (± SD) air-borne frequencies (kHz) associated with the different stages of mating behaviour (illustrated in fig. 3) in an Australian population of *N. viridula*. N_1 is the number of pairs observed to reach each stage of mating behaviour. Although sounds were recorded during the mating behaviour of all pairs (see 'Materials and methods'), it was only in association with a limited number (N_2) that sounds of the expected frequencies were recorded.

Stages of mating behaviour	N_1	N_2	$\frac{N_2}{N_1} \times 100$ (%)	Mean lowest frequency (kHz)	Mean highest frequency (kHz)	Mean peak frequency (kHz)
1	50	37	74.0	0.07 ± 0.09	0.106 ± 0.07	0.088 ± 0.16
2	31	25	80.6	0.082 ± 0.14	0.123 ± 0.22	0.115 ± 0.18
3*	23	16	69.5	0.139 ± 0.49	0.167 ± 0.57	0.151 ± 0.54
				0.231 ± 0.12	0.272 ± 0.07	0.254 ± 0.01
4	11	8	72.7	0.08 ± 0.13	0.150 ± 0.2	0.085 ± 0.12

* Two frequency peaks were recorded during stage 3.

Table 2. Mean (± SD) substrate-borne frequencies (kHz) and amplitudes (dB) associated with the different stages of mating behaviour (illustrated in fig. 3) in an Australian population of *N. viridula*. N_1 is the number of pairs observed to reach each stage of mating behaviour, N_2 is the number of pairs that produced sound.

Stages of mating behaviour	N_1	N_2	Mean lowest frequency (kHz)	Mean amplitude (db)	Mean highest frequency (kHz)	Mean amplitude (db)	Mean peak frequency (kHz)	Mean amplitude (db)
1	14	14	0.34 ± 0.26	17.6 ± 3.6	1.2 ± 1.0	17.2 ±2.5	0.67 ± 0.56	22.7 ± 3.5
2	6	6	0.30 ± 0.27	20.0 ± 3.0	2.7 ± 1.5	15.6 ± 1.7	1.19 ± 1.0	25.5 ± 2.3
3	4	4	0.15 ± 0.9	19.5 ± 4.5	4.9 ± 0.6	17.5 ± 5.8	1.52 ± 0.43	29.5 ± 5.2
4	2	2	0.14 ± 0.9	19.5 ± 4.9	4.6 ± 0.57	16.5 ± 6.3	2.70 ± 1.5	28.0 ± 5.6

phasizes that air-borne sound communication by an animal 1 cm in diameter would be poor at frequencies less than 10 kHz. Although our equipment could detect frequencies from 0.01 kHz to 22.4 kHz we could detect pulse train patterns only by reducing the time scaling factor to values so low (i.e. 10 ms) that the patterns would be unlikely to be important in communication.

That brief pulses of sound could not be used for sexual communication is supported by experimental results obtained from European *N. viridula*. When signals were simulated with the time scaling of the pulse train rate reduced below 1000–2000 ms, male bugs did not respond to simulated calling signals[4]. Harris et al.[12] do document their recorded pulse train rate as 1200–3000 ms; however, they direct most attention (30 out of 31 figures) to the patterns within a pulse train, reputed to be communication signals of *N. viridula*, and these have time scales as low as 8 ms in duration. Compelling evidence would be required before such an interpretation could be accepted.

A further consideration is that with sound recording equipment as sensitive as that used by ourselves, and by Harris et al.[12], for detection of air-borne signals, any movement from an observer within the chamber would inevitably trigger random frequency peaks. During their recording sessions, Harris et al.[12] relied on 'an observer in the anechoic chamber [who] monitored the insects' behaviour and made appropriate verbal observations to an engineer in the laboratory.'

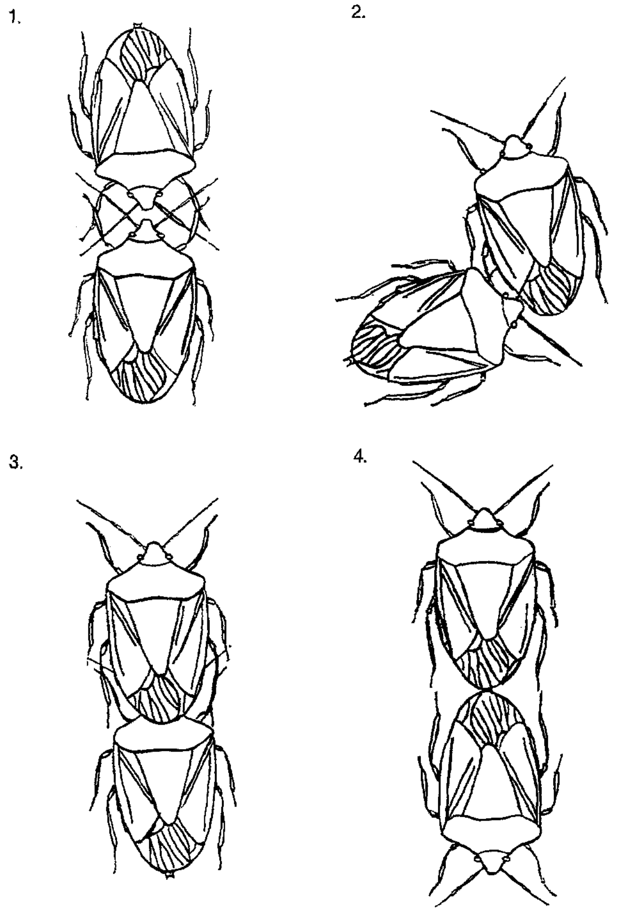

Figure 2. Stages of mating behaviour in *N. viridula* (modified from Borges et al.[2]).

The technical problems outlined above lead us to believe that air-borne signals are not the means of acoustic communication in *N. viridula*. To date, receptors adapted for the detection of air-borne sound communication have not been found in this species[6] (trichobothria do exist, however their role in *N. viridula* is yet to be determined). It has been suggested that the air-borne signals that have been recorded so far for *N. viridula* derive indirectly from substrate vibrations[9, 11]. Given our inability to detect repeatable air-borne signals, we must concur with this conclusion, but add that some of the recordings attributed to the insects may, in fact, be artefacts of the methods used.

The consistency in the sound patterns recorded via the substrate, in the presence of *N. viridula* pairs undergoing mating behaviour, provides strong evidence that this is the mechanism of acoustic communication in the *N. viridula* population that we examined. Behavioural experiments by Cokl[5] have shown that mating pairs (from European populations) can communicate successfully only when there is substrate contact between them. Frequency patterns that we related to various stages of courtship do exist and we believe that the amplitude and frequency patterns of stages 1–3 are of importance in determining whether or not copulation occurs (the broad frequency spectra seen in stage 4 may be attributed to increased movement of the mating pair). Furthermore, the frequencies of the sounds illustrated in figure 3 show consistencies with the four vibratory interneuron types of

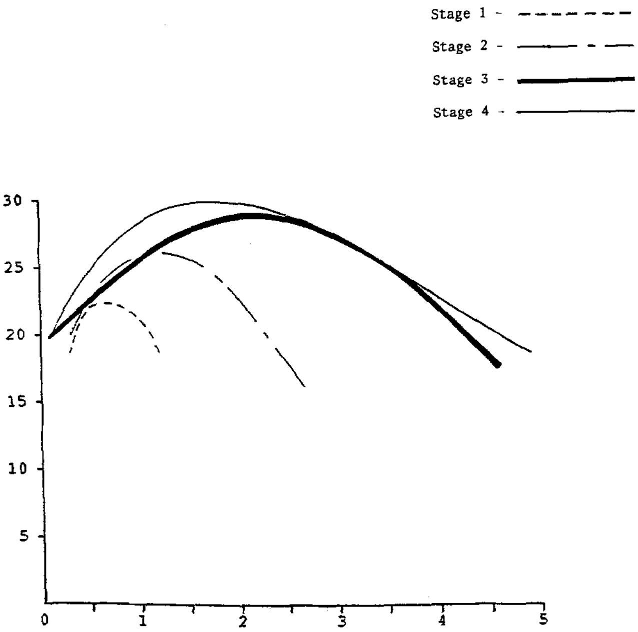

Figure 3. Frequency range patterns of associated stages (substrate-borne) of mating behaviour.

N. viridula that were electrophysiologically identified and classified by Cokl and Amon[8], in that the frequency bands represented in the figure are consistent with the frequencies detectable by the various interneurones.

We conclude that our results from an Australian population of *N. viridula* support an interpretation that substrate-borne rather than air-borne signals are the primary means of auditory sexual communication in this species.

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Amiloride impairs the cholinergic regulation of potassium permeability in the human sweat gland but not in the rat submandibular gland

S. M. Wilson, J. D. Pediani, D. McE. Jenkinson and H. Y. Elder

The Institute of Physiology, University of Glasgow, Glasgow G12 8QQ (Scotland)
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Abstract. Potassium permeability was monitored in human sweat glands and rat submandibular glands. Acetylcholine increased permeability in both tissues and the responses consisted of transient, calcium-independent and sustained, calcium-dependent components. Amiloride, a drug which inhibits Na^+-H^+ countertransport, impaired the regulation of potassium permeability in sweat glands but not in the submandibular gland. It is suggested that the stimulus-permeability coupling process in the sweat gland may be sensitive to the lowering of internal pH.
Key words. Rat salivary gland; human sweat gland; amiloride; potassium permeability; $^{86}Rb^+$-efflux; stimulus-response coupling.

Acetylcholine increases intracellular free calcium ($[Ca^{2+}]_i$) in rodent salivary acini and primate sweat gland epithelia. This response, which is initiated by the release of calcium from cytoplasmic stores and is subsequently sustained by calcium influx, causes an elevation of cellular potassium permeability which is an important part of the secretory mechanism[1-5].

In the sweat gland the initiation of this permeability increase is acutely dependent upon external sodium ($[Na^+]_0$), suggesting that the receptor-regulated mobilisation of cytoplasmic calcium is $[Na^+]_0$-dependent[6]. In the submandibular gland, however, removal of sodium primarily inhibits the sustained component of the response indicating that, in this tissue, calcium influx may be sodium-dependent[7-9].

Removal of $[Na^+]_0$ thus exerts quite different effects upon the cholinergic regulation of potassium permeability in these two exocrine organs[6-9] and it has been sug-