Transmission of the vibratory song of the bug *Nezara viridula* (Pentatomidae, Heteroptera) on the *Hedera helix* plant

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ABSTRACT The green stink bug Nezara viridula uses substrate vibration for communication with other members of the species. The dominant frequency of the song is around 100Hz. It has already been shown that the bug is able to find its singing sexual partner only by means of vibrations the partner emits on the host plant. The aim of this investigation was to find out the possible clues which help the bug to locate the origin of the vibration and to see how the vibration produced by the singing animal propagates on the plant. The male Nezara viridula with implanted steel wire electrodes in both bilateral main singing muscles was left to move freely on one of its host plants - *Hedera helix*. An artificial female song was produced by means of a vibrator. The male responded to the song and often proceeded to sing spontaneously. The vibration of the plant due to the singing animal was recorded with a laser vibrometer on previously chosen fixed measuring points. The spectra of the vibrations on the measuring points were calculated. The vibrations the bug emits do not fade uniformly with distance but rather they show amplitude fluctuations with peaks being most prominent on the edges of the leaves. We couldn't find any significant difference in spectral and amplitude characteristics on the stem just above and under the stalk of the leaf where the animal sings. So the best proposed strategy for the animal searching for its singing partner, would be to test the edges of the leaves, the tips of the branches and the stalks of the leaves because (i) the amplitude of vibration there is relatively high in comparison with other parts on the plant and (ii) the stalk of the leaf on which the animal sings has the greatest amplitude of vibration; the vibration amplitudes on stalks in the vicinity diminish with distance.

POVZETEK PREVAJANJE VIBRACIJSKIH SIGNALOV STENICE NEZARA VIRIDULA PO BRŠLJANU (HEDERA HELIX) Stenica vrste Nezara viridula lahko trese podlago, na kateri stoji, z osnovno frekvenco okoli 100 Hz. Tako komunicira z drugimi osebki iste vrste, zlasti kadar išče partnerja za parjenje. Dokazano je, da se samec in samica lahko najdeta že samo na osnovi teh vibracij. S to raziskavo sva želela ugotoviti, po kakšnem ključu stenica določi položaj spolnega partnerja, ki s petjem oddaja vibracije, in kako se le-te širijo po rastlini okoli pojoče živali. Samčku stenice sva vsadila v mišici, sodelujoči pri produkciji vibracij, elektrode (20um debele žičke iz nerjavečega jekla) za registracijo miograma med petjem. Stenice te tanke žičke niso ovirale pri hoji po steblu in listih bršljana. Bršljan sva tresla z vibratorjem, ki je oddajal umetni napev samice. Samček se je odzival na umetni napev in pogosto spontano nadaljeval svojo pesem še nekaj časa. Vibracije, ki jih je

oddajal, sva merila z laserskim vibrometrom na izbranih merilnih točkah. Enkrat je samček pel na istem mestu na bršljanu dovolj dolgo, da sva izmerila vibracije skoraj vseh merilnih točk na rastlini. Izračunala sva spektre teh vibracij. Pri vsaki frekvenci v spektru sva tudi opazovala spreminjanje njene intenzitete po površini bršljana. Pokazalo se je, da intenziteta vibracije, ki jih je samček oddajal, ni padala po rastlini sorazmerno z oddaljenostjo vira tresenja, ampak so se pojavili intenzitetni vrhovi na sredini listnih pecljev in na listnih robovih. Proksimalno in distalno od mesta, kjer je bil na steblo priraščen list, na katerem je samček ves čas pel, ni bilo možno opaziti nobenih signifikantnih spektralnih ali amplitudnih razlik. Zato bi bila za žival iščočo pojočega spolnega partnerja najboljša strategija "preizkusiti" vibracije vseh listnih pecljev v bližini, saj je amplituda vibracij na listnem peclju relativno velika v primerjavi z tresljaji drugih delov rastline in se pecelj lista, na katerem žival poje, najbolj trese, tresenje sosednjih pecljev pa pojema z naraščajočo oddaljenostjo.

Introduction

Most vibrational communication signals are produced by mechanisms which apply mechanical energy to the transmission medium in more or less short impulses containing rapid transients, thus producing broadband Fourier spectra of energy distribution which rarely have prominent harmonic bands (MARKL 1983). The vibration and sound producing organ in *Nezara viridula* is the tymbal, composed of the fused terga 1 and 2 (JORDAN 1958, LESTON and PRINGLE 1963, GOGALA 1984). The vibration of the body of the animal produced by the tymbal is a rather pure sound with the dominant frequency around 100 Hz. This frequency is in 1:1 correlation to the muscle spikes of at least one of the two singing muscles TL1. The aim of this investigation was to find out the possible clues which help the bug to locate the origin of the vibration and to see how the vibration produced by the singing animal propagates on the plant. It is interesting that the relatively sharp harmonic peak and the 1:1 correlation (phase-lock) of vibrations can be recorded also on relatively distant parts of the plant (Fig. 2).

Materials and methods

1. Animals and Experimental Conditions. Adult males of the species *Nežara viridula* were caught on the northern Adriatic coast in September 1987 and kept in the laboratory at low temperatures (around 5°C) in darkness to sustain the imaginal diapause. After two months the animals were exposed to higher temperatures (around 25°C) and to a photoperiod of 16 hours light and 8 hours of darkness. In this way the relatively inactive brown individuals became green and active again and in approximately three weeks were ready to mate.

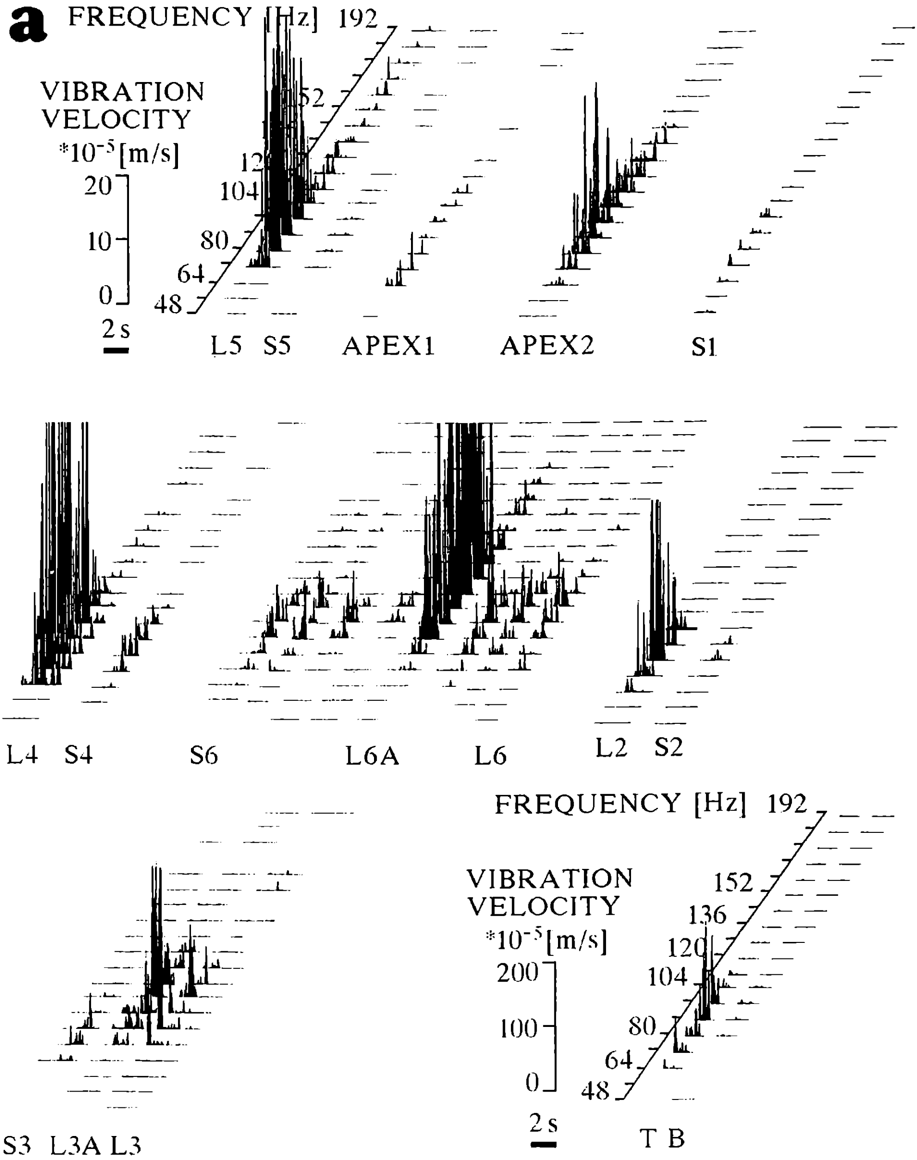
2. Vibration Measuring Device: The vibrations of the plant or of the animal were measured with a Laser-Doppler-vibrometer, which is a non-contact, non-invasive measuring system (BUCHAVE, 1975, MICHELSEN and LARSEN, 1978). It is sensitive to the component of velocity, coaxial with the laser beam. The laser system calibration is traceable to the wavelength of the laser light and is linear over the range of frequency and velocity of biological interest. The measuring spots on the plant and on the dorsi of the animals were marked with diluted, water-soluble and non-toxic white paint (Tippex) to increase the amount of reflected laser light.

3. Data Evaluation. The laser vibrometer recordings were digitized (resolution 8 bits) with the transient recorder (CED) with the sampling speed 2000/s to enable accurate measurement of 200 Hz the highest frequency of interest. The digitized recording was segmented into 250 ms long overlaping segments. The time between the onsets of segments was 25 ms. The segments were long enough to enable the accurate calculation of the lowest frequency of interest of the Fourier transform 50 Hz. The Fourier transform of these segments was calculated, and the amplitudes of the vibration velocities of the frequencies 48, 56, 64, 72, 80, 88, 96, 104, 112, 120, 128, 136, 144, 152, 160, 168, 176, 184, 192 Hz were plotted as a linear function of time. This three dimensional diagram (X axis = time, Y axis = vibration velocity (linear scale), Z axis = frequency) represented the 3D-spectrogram of a single measuring point on the plant. To better show the vibration velocity oscillations in predefined frequency bands, a graph with the maxima of vibration velocities (connected by spline-curve) was plotted for each frequency band. The number at the ordinate of each graph denotes the magnification factor relative to the vibration velocity scale of the spectrogram. The abscissa shows the distance between the measuring points on the same scale as the drawing of the leaf.

Results

The male *Nezara viridula* with implanted steel wire electrodes in both bilateral main singing muscles TL1 was left to move freely on one of its host plants *Hedera helix*. An artificial vibratory female song was produced by means of a rod attached to a vibration exciter. The male responded to the artificial song with its courtship song (ČOKL et al., 1972) and often proceeded to sing spontaneously. The vibration of the *Hedera helix* plant due to the singing animal was recorded with a laser vibrometer on previously chosen fixed measuring points the circles in the fig. 1.

The first position of the singing male on the plant (fig. 1: a) was in the upper part of a higher branch of the plant (VIBR. in the fig.1a). The vibrations recorded from the surface of the stalk just beneath the feet of the animal (fig.1a: B) show an attenuation of velocity of vibrations by a factor of about ten in comparison with the vibrations as recorded from the dorsum of the animal (fig.1a: T). Let us now observe the distribution of the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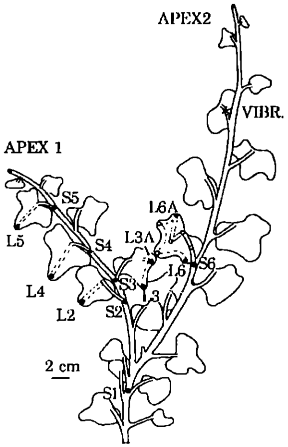

Fig. 1. a 3D-spectrograms of vibrational calls of the male singing on the point marked with VIBR. on the drawing of the plant and measured on various parts of this plant. The scale in the upper left corner holds for all spectrograms except the one in the lower right corner of the opposite page, which has its own scale.

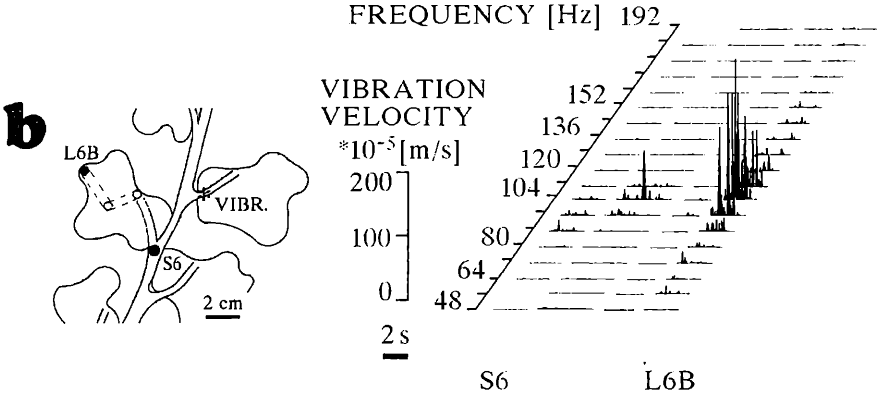

Fig. 1.b 3D-spectrogram of the same male singing on the spot marked with VIBR.. For details see text.

vibrations on the stem and the two branches. The apex of the branch where the male sings (APEX 2) vibrates with the velocity about 3x higher as the point under the feet of the animal, but the apex of the other branch (APEX 1) vibrates with a velocity of about the same amplitude as under the feet of the animal. The comparison of the points lying on the right branch of the plant (APEX 2, S6,S1) shows that the velocity of vibration towards the base of the stem declines. When the vibrations reach the left branch of the plant, its stem vibrates with the greatest velocity in the middle (points S3 and S4) and in the apex (APEX 1). On the other hand there is a very prominent vibration velocity increase when the vibrations reach the edges of the leaves (L5, L4, L3, L3A, L2, L6A).

As the vibrations travel on the S6-L6 path their velocity remains about the same on the stalk as on the stem (fig.1a: S6 and the two points afterwards). In the center of the leaf lamina, the minimum velocity occurs, but along the edge of the leaf the vibrations are much more intensive with a large peak (fig. 1a: L6A).

The second position of the singing male (the same animal as above) was on the stalk of the leaf next to the S6 point (VIBR. in the fig. 1b: b). Although the absolute intensities of velocities at all the frequencies are distinctly larger because of the vicinity of the singing male, a similar velocity distribution as on the S6-L6 path remains low in the center of the lamina and high on the edges of the leaf. Also the phase-lock (see below) between EMG and VB is clear (fig. 2: L6B).##

To study the degree of phase-locking (fig. 2) between the vibrations of the plant (VB) due to the singing animal and the muscle spikes of its singing muscle TL1 (EMG), the latter were taken as the reference point. According to these reference points, both EMG and VB traces were segmented and superimposed. Let us first observe a representation of the recording from the dorsum of the animal (fig. 2: T). The male sang in the spot marked VIBR. in fig. 1: a. The reference spike in the EMG trace is seen, followed by the next two successors, which are blurred because of the varying interspike interval. In the upper trace (VB) we note at first the close phase-lock of the body vibrations to the muscle spikes. The vibrations recorded on the apex of the branch above the singing animal (fig. 2: APEX2) show that the phase-lock between EMG and VB persists, altough it is strongly masked with noise. On the tip of the other branch (APEX1) the velocity of the basic frequency of the song remains in the order of magnitude the noise and the degree of phase-locking cannot be studied with the method of superposition of traces. The animal could make use of such noise-masked information if it posessed a kind of additive averaging system, which would enhance the signal-to-noise ratio. However, on the edges of the leaves the EMG and VB traces clearly remain phase-locked (L6A, L2, L3A, L4, L5), as also in the most distant point from the singing animal - L5.

Discussion

The male *Nezara viridula* with implanted steel wire electrodes in the main singing muscle was let to move freely and sing on one of its host plants *Hedera helix*. To find out the possible clues which help the bug to locate the origin of vibrations and to see how the vibration produced by the

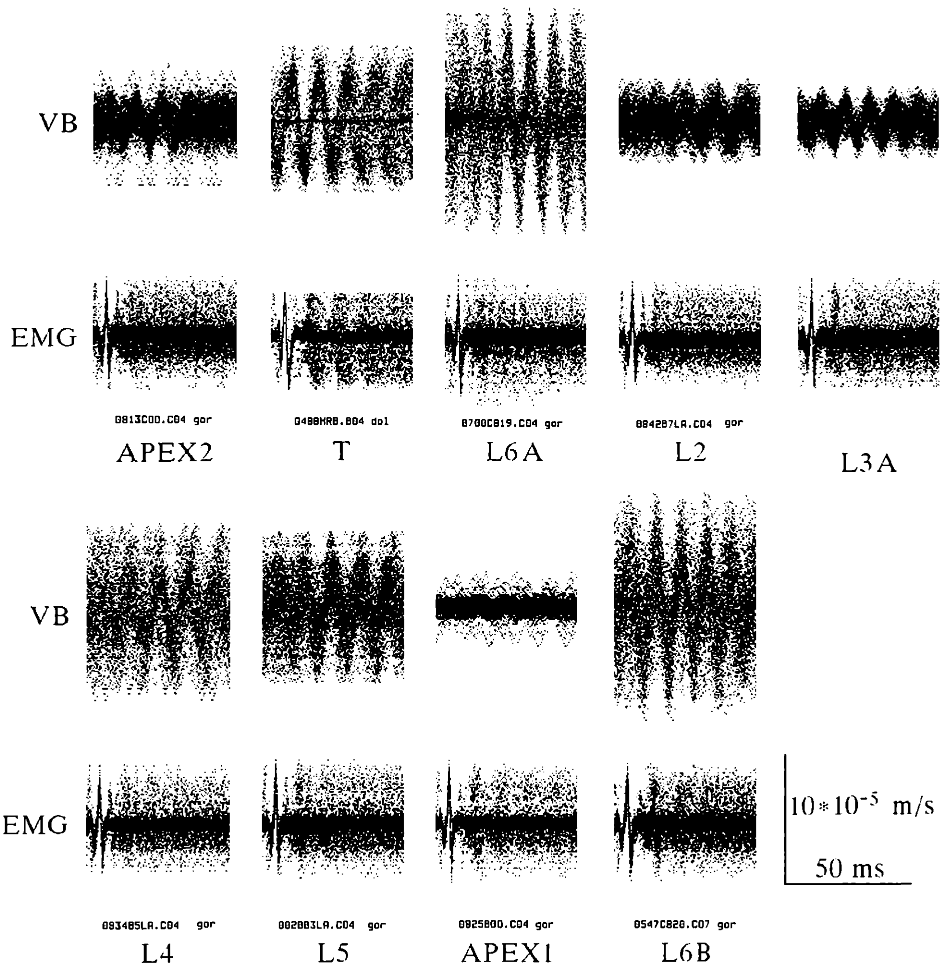

Fig. 2) The degree of phase-locking between the vibrations of the plant (VB) due to the singing animal and the muscle spikes of the singing muscle (EMG) of the animal, which are taken as the reference point. For details see text.

singing animal propagates on the plant, the laser vibrometer was used to measure the surface vibration of the plant due to the singing animal. This study demonstrates that the bug *Nezara viridula* is able to vibrate the *Hedera helix* plant with amplitudes more than ten times above the threshold of their vibration receptors. The vibrations the bug emits do not fade uniformly with distance but they rather show amplitude fluctuations with peaks being most prominent on the edges of the leaves.

The vibrational songs propagate most probably as bending waves with only little frictional loss in energy (MICHELSEN et al. 1982), and they can be detected by other animals on the same plant, especially at the edges of the leaves, the tips of the branches and the stalks of the leaves.

The lower frequencies, corresponding to the frequency band where most energy was emitted in the animal's song, were also the most prominent frequencies in the whole plant. The distribution of amplitudes of vibration velocities of these frequencies had the higher amplitudes on the apex and edges of the leaves, where the natural frequencies of the plant were lower (nearer to the frequency band emitted by the singing animal) and lower amplitudes on the stem, where natural frequencies are higher. This picture is unchanged even if animal changes the place where it sings (fig. 1: b).

The response of the plants resembled that expected of beams, where a vibration pulse of short duration travelled up and down the beam several times and was reflected at the ends. Reflections occur both at the root and at the top of the plant (where the impedance met by the travelling wave becomes somewhat larger and much smaller respectively). The reflections are normally larger at the top than at the root (in agreement with the larger change of impedance at the top). The occurence of reflections and the small internal dampening of the plants are likely to carry standing waves. The standing wave patterns are often very complicated and very frequency dependent, since the plants have a more complicated geometry than simple beams (MICHELSEN et al. 1982). It is interesting, however, that in spite of the above mentioned physical characteristics, the vibrations of the plant on the edges of the leaves due to the singing animal remain relatively pure tones in 1:1 correlation (phase-locked) to the myogram of its singing muscles (fig. 2: L6A, L2, L3A, L4, L5, L6B). So a proposed strategy for the bug searching for its sexual partner would be to compare the frequency of the vibrating substrate with the frequency of its own "singing generator" If both frequencies came close, then the motivation for running around would grow. The animal would test the tips of the branches, the stalks and especially the edges of the leaves because (i) the amplitude of vibrations there is relatively high in comparison with other parts of the plant (ii) the phase-lock is clearer (iii) we couldn't find any significant difference in spectral and amplitude characteristics on the stem just above and under the stalk of the leaf where the animal was singing (iv) the leaf on which the animal sang had the greatest velocity of vibration and clearest phase-lock.

References

BUCHAVE, P., 1975: Laser Doppler vibration measurements using variable frequency shift DISA Inf. 18: 15-20

ČOKL, A., M. GOGALA, M. JEŽ, 1972: Analiza zvočnih signalov stenice *Nezara viridula* (L.). Biol. vestnik (Ljubljana) 20: 47-53

GOGALA, M., 1984: Vibration producing structures and songs of terrestrial Heteroptera as systematic character. Biol. vestnik (Ljubljana) 32: 19-36

JORDAN, K.H.C., 1958: Lautäuserungen bei den Hemiptern Familien der Cydnidae, Pentatomidae und Acanthosomidae Zool. Anz. 161: 130-144

LESTON, D., J.W.S. PRINGLE, 1963: Acoustical behaviour of Hemiptera. In Acoustic behaviour of animals. (ed. R.G. BUSNEL): 391-411

MARKL, H., 1983: Vibrational communication Neuroethology and Behav. Physiol. (eds: F. HUBER & H. MARKL) Springer Verlag, Berlin Heidelberg

MICHELSEN, A., F. FINK, M. GOGALA, D. TRAUE, 1982: Plants as transmission channels for insect vibrational songs Behav. Ecol. Sociobiol. 11: 269-281

MICHELSEN, A. & O.N. LARSEN, 1978: Biophysics of the Ensiferan ear.I.Tympanal vibrations in bushcrickets (Tettigonidae) studied with laser vibrometry J. Comp. Physiol. 123: 193-203