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The effect of noise on sexual behaviour of the southern green stink bug *Nezara viridula*

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Abstract

Stink bugs of the pentatomid family communicate using substrate-borne vibrational signals transmitted through host plants. Specific signal length and repetition rate are critical for mate recognition and are susceptible to masking by simultaneous conspecific and congeneric signals. We tested whether overlapping conspecific signals interrupt *Nezara viridula* (L.) sexual behaviour with playback of continuous vibration (disturbance). The disturbance decreased the number of males responding to simultaneously presented female signals, but it did not influence the time they needed to find the source of female signals. *N. viridula* females proceeded with mate calling during disturbance playback, but most of them paused for one or more signal intervals and/or emitted so-called repelling signals. The proportion of females that changed the dominant frequency of their calls decreased with increasing difference between the dominant frequency of the disturbance signals and the emitted female calling song. There was no equivalent change in other call parameters, or in the proportion of females that changed rhythm or song type. This indicates that female calling frequency shift is the main method of reducing the effect of signal masking in *N. viridula* vibrational communication.

Key words: *Nezara viridula*, Pentatomidae, sexual behaviour, vibrational communication, noise.

Introduction

Stink bugs of the family Pentatomidae are phytophagous insects that communicate on short to medium ranges by producing substrate-borne vibrations of their host plants (Čokl *et al.*, 2000). Several songs have been described in *Nezara viridula* (L.), consisting of vibrational signals with specific frequency spectra, duration and repetition rate. They are used by both sexes for mate recognition and by males to find the female on the host plant. The signals are well tuned to the resonant properties of plant tissues (Čokl *et al.*, 2005) and propagate in the form of bending waves along the plant with very little attenuation (Michelsen *et al.*, 1982). The acoustic environment of *N. viridula* individuals consists of the plant they are on and possibly the adjacent plants. External sources have been shown by Barth *et al.* (1988) to produce sound frequencies which are picked up by plants and selectively amplified, with resonant spectral peaks. This phenomenon was used before by Saxena and Kumar (1980) to interrupt sexual communication and mating of a leafhopper and a planthopper with sound waves produced by a harmonium device and picked up by plants. Apart from external sources, signals from conspecific signalers can constitute noise as well (Römer *et al.*, 1998). In conditions where several animals sing at the same time, their signals may overlap and form a continuous vibration. Such overlapping signals present a significant problem for succesful detection of individuals because of the spectrum overlapse, rendering frequency filtering for improving signal-to-noise ratio ineffective (Ronacher and Hoffmann, 2003). Several strategies have been described in various animal groups to improve signal-to-noise ratio when several signalers sing at the same time: signal repetition (e.g. Ronacher *et al.*, 2000 for grasshoppers), chorusing or singing in periods of relative silence (reviewed in Greenfield, 1994), and altering of frequency (e.g. Howard and Young, 1998 for frogs). *N. viridula* relies on temporal structure of the signals to localize and recognize the mate (Miklas *et al.*, 2001), so the recognition is vulnerable to masking by conspecific signals and external noise. The aim of our study was to investigate the level and degree of interruption of the sexual behaviour of *N. viridula* by vibrations of frequencies similar to those of their songs. In such a way we simulated conditions in which several bugs sing at the same time.

Materials and methods

Males were placed onto a leaf of the host plant (*Phaseolus vulgaris* L.), which was vibrated with one shaker attached to the leaf and the other to the base of the stem. By the leaf attached one the artificial 100 Hz female calling song signals were reproduced and by the second, the plant was vibrated with continuous 100 Hz pure tone signal ('noise'). In tests with males we placed an individual on the leaf opposite to the vibrated one and analyzed his behavioural response, which consists of movement towards the vibration source and emission of vibratory signals. We measured the time males needed to arrive on specified points on the host plant and recorded their signals. The signals were recorded with a laser vibrometer (OFV 2200 (controller) & OFV 303 (measuring head), Polytec GmbH, Waldbronn, Germany) and Cool Edit Pro 2 software. In tests with females we placed an individual onto a leaf and played twice a minute of continuous vibration ('noise') when they started to sing, with a no-stimulus pause between. Their songs were recorded by the above mentioned set-up. We analysed signal duration, repetition rate, frequency, and first harmonic frequency using SoundForge 6.0 and Raven 1.0 software. Females were split into three groups (N_1=12, N_2=13, N_3=31), according to how close the dominant frequency of their signals at the start of the experiment was to the frequency of noise played to them. Finally, the signals emitted during no-stimulus periods were compared with those emitted during periods of noise for each female.

Results and discussion

When disrupted by noise, males responded less to female signals than the males listening to female signals alone; less males searched for the source of female signals and less males responded with their own song. Noise did not influence the time males needed to locate the source of female signals. Noise did not stop females from singing, but almost all of them either changed song type, or stopped singing for a short time in the presence of noise. Females changed the dominant frequency of their calls during the playback of noise according to how close the dominant frequency of their signals was to the frequency of noise – when the difference was less than 10 Hz (group 1), 90% of the females changed the dominant frequency away from the frequency of noise. In group 3, where the difference was more than 20 Hz, only around 20% of females changed the frequency of their calls. After each period of noise, some females kept the altered frequency, some changed it back to the starting value, and some changed it to a different value, with no apparent rule except that all values remained in the species' range. Two females from group 1, however, changed the dominant frequency of their signals during each period of noise and returned it to the starting value during periods of no stimulus. Such an effect was not observed in other groups or for other parameters. The first harmonic frequency, repetition rate, and pulse duration remained more constant, the number of females in which changes were observed did not correspond to the similarity of their dominant frequency to the noise frequency. In most cases both the signal duration and repetition rate decreased. The resulting decrease of duty cycle could represent an attempt to increase the possibility of signals falling in a period of silence, but our results are not significant enough to suggest that this was a response to continuous vibration.

Because noise did not stop females from singing and the dominant frequency was the only parameter reliably dependent on how similar the female signals were to noise, we assume that frequency shift is the primary strategy for improving signal-to-noise ratio in *N. viridula* females. *N. viridula* vibrational receptors are tuned to the whole range of conspecific signal spectral properties (Čokl, 1983) and their changes as observed in our experiments probably do not reduce receiver's ability to detect them. How/whether the animals detect simultaneous signals with slightly different frequencies is presently unknown. Signals with frequency equivalent to that of noise interrupt male searching. On the other hand, judging by the time males spent searching for females with or without the presence of noise, there remains a possibility that some other changes (such as in amplitude) occur during transmission of signals through plants. Those changes could enable the receivers to recognize the signals even in the presence of noise.

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