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Preference of the southern green stink bug (*Nezara viridula*) males for female calling song parameters

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Abstract

Vibrational communication is widespread in insect social and ecological interactions. *Nezara viridula* L. uses vibrational signals for short range communication. Female calling song (FCS) is an important part of mate recognition and localization system. Signal recognition in nature depends on transmission properties of the medium and the ability of the sensory system to extract the information from the environmental noise. In playback experiments we measured male responsiveness to FCS stimuli varying in pulse train duration, interval duration, duty cycle, and dominant frequency. Males were clearly able to distinguish songs of different temporal parameters by responding best to stimuli values characteristic of conspecific song. Asymmetry of response was observed: decreasing of parameter values had a greater inhibitory effect than their prolongation. The results indicate that recognition functions on the basis of two temporal filters tuned to pulse train and interval duration. Males responded best to the dominant frequency characteristic of the conspecific song which is tuned to resonant properties of the green plants used for signal transmission during communication.

Key words: vibrational communication, song recognition, male preference, *Nezara viridula*.

Introduction

Vibrational communication is widespread in insect social and ecological interactions (Markl, 1983; Gogala, 1985; Čokl and Virant-Doberlet, 2003; Čokl *et al*., 2004; Cocroft and Rodriguez, 2005). Vibrational signals play an important role in mating behaviour (mate recognition and location, rivalry) as well as defensive behaviour (alarm signals) (Virant-Doberlet and Čokl, 2004).

The southern green stink bug (*Nezara viridula* L., Heteroptera Pentatomidae) uses pheromones for long-range intraspecific communication, allowing the mating partners to reach the same plant (Borges *et al*., 1987). At a shorter range, on the plant *N. viridula* communicates predominantly with vibrational signals transmitted through the substrate (substrate-borne signals) (Ryan *et al*., 1996). Female calling song (FCS) is an important part of the mate recognition and location system. It triggers the male's searching behaviour and emission of the male's courtship song (MCrS) or calling song (MCS) and enables males to find stationary females, which emit FCS with a steady repetition rate (Čokl, 1988; Ota and Čokl, 1991; Čokl *et al*., 1999; 2000).

Signal recognition in nature depends on transmission properties of the medium, which modifies signal parameters during transmission and the ability of the sensory system to extract the information from the environmental noise. *N. viridula* is one of the best models in which vibrational communication has been investigated at different levels from behaviour to underlying neurobiology. In the present study we used the stereotyped singing response behaviour of males to analyse the males' recognition and preferences for the following FCS parameters: pulse train duration, interval duration, duty cycle and dominant frequency.

Materials and methods

Males were placed on the loudspeaker membrane and stimulated with the natural and model FCS (playback experiments). Natural stimuli signal was presented to the males after the model stimuli and was used to control the daily responsiveness of males. Stimuli signals used as models of natural FCS were based on mean values determined from FCS of 5 females. The males' responses to the model song did not differ significantly from responses to pre-recorded natural FCS, therefore this song model was used as the control. In the test models songs we varied four FCS parameters independently: dominant frequency of the pulse train, duration of the pulse train, interval between pulse trains and duty cycle. Males responded to stimulation with emitting or not emitting the vibrational signals (MCS or MCrS). We determined the strength of the response by calculating the percentage of males' singing time (emitting MCS or MCrS) during the stimulation relative to the whole stimulation duration. Each male was tested once a day with a different model song stimulus. The responses of males to test model songs were recorded by laser vibrometry and compared to responses to control model song (statistical significance $p<0.05$; Mann-Whitney test, two-sided).

Results and discussion

Males were clearly able to distinguish songs of different temporal parameters. Males responded best to values characteristic of natural conspecific FCS with different roll-off towards shorter and longer values. Shortening of the pulse train and interval duration had greater inhibitory effect on male response than their prolongation. Shorter values of both parameters resemble those measured in male calling or rival song, which inhibit rival

male singing. We determined the effective range of temporal parameters of FCS. Males reacted best at pulse train duration from 600 ms to 1000 ms and at interval between 1500 to 3000 ms (outside the effective range the response significantly decreased). Males responded best when both parameters (pulse train duration and interval) corresponded to their natural values, indicating that recognition mechanism functions is based on two temporal filters which are tuned to pulse train duration and interval between pulse trains of the natural FCS.

The strength of MCrS and MCS responses differed: males emitted MCS less selectively than MCrS. In the study of Čokl *et al*. (1978) males of .*N. viridula* reacted to sympatric species only with MCS and we can conclude that emitting MCS is less specific with a less prominent function in species specific communication. *N. viridula* males responded stereotypically (searching behaviour and emission of the vibrational signals) also to *Thyanta* spp. songs, which resemble time characteristics of the conspecific call (Hrabar *et al*., 2004).

When stimulated with varying pulse train and interval duration, males exhibited trimodal and bimodal response, respectively. The primary peaks coincide with parameter values of natural FCS and the subsequent peaks coincide with temporal structure of FCrS, but are less pronounced. Compared with the temporal characteristics of FCS, those of the FCrS show higher variability and resemble temporal structure of MCrS (Čokl *et al*., 2000). Another study (Pavlovčič, 2005) shows that FCrS is rarely emitted and only at the beginning of the female – male interaction and is gradually transformed into well defined FCS.

The preferred dominant frequency range observed was between 90 to 180 Hz (outside this range the response significantly decreased) and corresponds well with the peaks of resonant spectra of green plants used for signal transmission during communication (Čokl *et al*., 2005; 2007).

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