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Secondary spectral components of substrate-borne vibrational signals affect male preference

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ABSTRACT

Animal sexual signals contain information about both compatibility and quality of the signaller, but combined with influence of the signalling medium, the complexity of mate selection makes it difficult to separate different components of this process. We approached the problem of teasing apart different functions of sexual signals by using the planthopper *Hyalesthes obsoletus*, which uses unimodal vibrational communication, as a model. Vibrational signals are known to encode information about identity in their temporal pattern, while a useful cue for quality may reside in their spectral properties. In this study, we demonstrate a connection between spectral properties and attractiveness of female signals based on male behavioural response to signal playback. Artificially increasing the amplitude of high-frequency components increases signal attractiveness and vice versa, which indicates that spectral properties could function as an index of quality. Presence of high-frequency spectral components might indicate a larger or healthier individual, but direct connection with female fitness is not yet clear. In addition, we found that *H. obsoletus* males are able to exploit female pulses as directional cues and can discriminate between female signals of different attractiveness coming from spatially separated sources.

1. Introduction

The ability of animals to accurately detect and assess relevant ecological parameters allows optimal exploitation of information collected from the environment. Since multimodal communication, involving different sensory spheres, contributes decisively to survival and reproduction, only the correct interpretation of cues from relevant sources allows animals to fulfil their fundamental needs (Dall and Johnstone, 2002; Bro-Jorgensen, 2009; Schmidt et al., 2010).

Mate choice is a key factor of reproductive success and mating signals are a crucial element of intersexual communication eventually driving two potential partners to make decisions (Edward and Chapman, 2011). Constant exchange of messages between a male and a female makes mating a dynamic process, from the initial approach to the final copula via courtship during which both partners try to estimate the quality of the other and adjust their behaviour accordingly (Saetre, 2000; Smith and Harper, 2003). Therefore, signal perception is crucial for mate assessment and thus, for mate choice; on the other hand, environmental constraints may affect the perception of sexual signals (Maan and Seehausen, 2011), and may eventually contribute to sexual selection.

In many *Hemiptera* species, males stimulate females present in their vicinity (i.e. on the same host plant) with the so called "calling song". Then, if a female responds, they establish a mating duet that, from the initial identification, goes through different stages consisting of partner localization, courtship and copula (Čokl and Virant-Doberlet, 2003).

Perception of key signal components triggers the choice in a receptive individual, which is in turn expressed as a response (or lack thereof). The parameters of substrate-borne vibrations involved in recognition and in eliciting the male's search for the female (i.e. mate choice) are not sufficiently understood. It is generally accepted that temporal features of mating signals are crucial for species identification (Claridge, 1985; Čokl and Virant-Doberlet, 2003; Hill, 2009; Virant-Doberlet et al., 2014), while the role of spectral properties has been poorly investigated, despite their potential as indicators of the signaller's quality. Vibrational signals are strongly influenced by characteristics of the substrate (e.g. structure, shape), which coincides for many insects with their own host plants. For polyphagous species, which live on different hosts, or even monophagous species inhabiting different host populations, the shift to another plant may also correspond with a shift in effective signalling frequency that can contribute to species

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diversification on an evolutionary scale (Cocroft et al., 2008; McNett and Cocroft, 2008; Rebar and Rodríguez, 2015). In general, herbaceous plant parts impose a low-pass filter where higher frequencies are progressively attenuated during transmission of vibrational signals (Michelsen et al., 1982).

Hyalesthes obsoletus Signoret, 1865 (*Hemiptera*, Cixiidae) is a Palaearctic planthopper that lives on several host plants, including bindweed (*Convolvulus* spp.), lavender (*Lavandula* spp.) and stinging nettle (*Urtica* spp.) (Kessler et al., 2011). The mating behaviour of *H. obsoletus* is characterized by five distinct stages: recognition, localization, courtship, precopula and copula (Mazzoni et al., 2010, 2014). Except for the copula, each stage is associated with vibrational signalling, which involves male–female duetting during the first three stages and male-only signalling in the fourth. Other senses, vision and olfaction, do not have an important role in mating behaviour of this species, especially during early stages, when individuals are not in close proximity. Consequently, *H. obsoletus* may be considered a useful model for studying the mechanisms of mate preference in vibrational communication. *H. obsoletus* emits exclusively broadband pulses (MSy1) produced by abdomen vibration, while the harmonics and frequency modulation are absent. In this species, either partner may start calling and once a male–female duet is established, there follows a stage of initial recognition during which none of them moves, but keeps on duetting from the initial position. Recognition duet is characterized by female pulse trains and individual male replies inserted between female pulses. Afterwards, the male may choose to start searching along the plant for the female. This is the courtship stage during which a male emits a train of two types of pulses (MSy2 and MSy3) and a female occasionally replies with a single pulse (Mazzoni et al., 2010). In addition, *H. obsoletus* is a good model, because it responds well to two of the factors that are thought to induce high degree of male mate choice in a species (Bonduriansky, 2001): male–male competitiveness, often occurring in this species, and female quality variation, which could also be assessed by males through the perception of female vibrational signals (Mazzoni pers. observ.).

Altogether, because of these characteristics *H. obsoletus* is ideally suited to test our hypothesis, namely that spectral features of substrate-borne vibrational signals produced by females play an important role in species recognition and male mate choice, and thus, decisively condition the mating behaviour of a vibrationally communicating insect. Our working hypotheses are that (1) by manipulating secondary components of the frequency spectrum (while keeping the dominant frequency and the temporal frame stable) it would be possible either to reduce or increase male responsiveness to female calls, that means to affect species (or mate) recognition; (2) that similarly, males choose to search for females (once recognised) whose signals they find more attractive; (3) that it would be possible to synthesize ideal female signals, endowed with optimally adjusted parameters, that may compete with or even work better than the natural signals. Finally, we also aim (4) to evaluate the discriminating ability (accuracy) of males in locating the preferred source, by means of two-choice tests where signals of unequal attractiveness are transmitted from different locations on the host plant.

2. Materials and methods

2.1. Insects

Nymphs of *H. obsoletus* were collected from the roots of stinging nettle plants (*Urtica dioica*, L.) bordering a vineyard near Carpi (Northern Italy; 44°47′N, 10°53′E) in June 2011 and placed in plastic cups (Pint-sized BugDorm, vol. 720 ml) covered by net screen, together with the roots and the soil they were collected from. Rearing conditions were 25 °C, 70% HR. Newly emerged adults were collected daily and kept isolated in Falcon vials (height 10 cm, diam. 3 cm) containing a shoot of stinging nettle the stem of which was inserted into an Eppendorf vial filled with water.

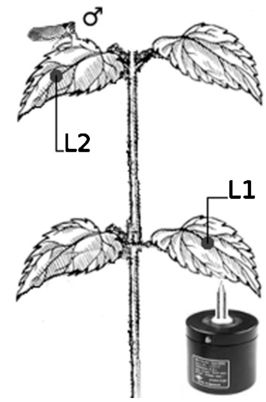

Fig. 1. Experimental setup with a nettle cutting pruned to four leaves, one male placed on an upper leaf, the minishaker applied to a basal leaf (in Sections 2.4 and 2.6; in Section 2.5 "two-choice", there was a minishaker for each basal leaf) and two lasers, one pointed to the "vibrated basal leaf" as reference (L1) and the other to the "male apical leaf" (L2).

2.2. Arena and recordings

Insects were tested on stinging nettle shoots 10–12 cm tall and pruned back to 4 leaves (2 basal + 2 apical leaves, internode ~5 cm), inserted into a vial filled with water set inside a cubic Plexiglass cage (sides of 30 cm). In all tests, males were initially placed on either of the apical leaves (Fig. 1).

Recordings of vibrational signals were made with two laser vibrometers (VQ-500-D, Ometron Ltd., UK), pointed at a piece of reflecting foil on the apical leaf lamina through an opening on the top surface of the cage. Laser output was acquired with LAN-XI data acquisition hardware and analysed (FFT window size of 200 points, overlap 66.7%) with Pulse version 15.1 software (both Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark). Playbacks were transmitted by means of a conical aluminium rod (length 2 cm, diam. 4 mm) screwed into the head of a minishaker (vibrator exciter mod. 4810, Bruel & Kjaer), and attached to one of the basal leaves with blu-tack adehesive gum (Bostik Ltd.). The minishaker was driven via the standard computer headphone output using Adobe Audition version 3.0 (Adobe Systems Inc.). For all experiments, we used adult males and females, 7–15 days after eclosion.

2.3. Characterization of playbacks of female calling songs

We chose four different female calls from our collection of *H. obsoletus* signals, previously recorded (see Mazzoni et al., 2010) with laser vibrometer pointed at the same leaf lamina of stinging nettle from which the female was calling, at a maximum distance of 2 cm. We then created a playback signal from each call, composed of a 60 s train sequence of female pulses. The signals were characterized by measuring the pulse amplitude (as substrate velocity) and dominant frequency, the spectral structure (occurrence of subdominant frequency peaks), pulse mean duration and pulse repetition time. In addition, the playback signal from the apical leaf lamina was recorded at the same time to evaluate what a male could actually perceive from his starting position. A spectral (frequency + amplitude) analysis was made from 10 randomly chosen pulses of each type of female call, replicated from 9 arbitrary positions (i.e. measurement points) of the apical leaf lamina. In total, 900 pulses (10 pulses × 9 calls × 10 types) were used for the analysis of each playback. The same approach was also adopted for the analysis of the modified signals in Section 2.6 (see below).