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On Recognition of Sound Communication Signals in Bush Crickets (Orthoptera, Tettigoniidae)

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Abstract—Two hypotheses have been proposed to explain the mechanisms of calling signal recognition in orthopterans: the filtration and resonance ones. To test these hypotheses, conspecific male calling songs and their models with modified temporal parameters were presented to females of bush crickets in ethological experiments. The models with a double pulse rate evoked positive phonotaxis of females while phase shift significantly complicated the recognition process. These data fit the resonance hypothesis.

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The results of numerous ethological experiments indicate that insects using acoustic signals for intraspecific communication recognize them largely by their temporal parameters. Two main hypotheses have been proposed to explain the neuronal mechanisms of signal recognition (Hoy, 1978; Zhantiev, 1981; Popov, 1985; Weber and Thorson, 1989).

According to one hypothesis, the acoustic system includes several filters which extract certain parameters of the conspecific signal and convey information to the higher association centers. The other hypothesis assumes that acoustic information, after filtration in the acoustic system, is matched against a certain rhythmic process in the central nervous system.

The electrophysiological data obtained in our previous research on katydids and true crickets supported the second hypothesis (Zhantiev et al., 2004, Chukanov and Zhantiev, 2007).

In this work, we used ethological methods to analyze the effect of changes in the rhythmic and phase parameters of acoustic signals on their recognition by the bush crickets *Metrioptera roeselii* Hag. and *Rhacocleis germanica* H.-Sch.

MATERIALS AND METHODS

Females of *Metrioptera roeselii* Hag. were collected as last-instar larvae or adults in Moscow (Krylatskoe) and the environs of Zvenigorod; young larvae of *Rhacocleis germanica* H.-Sch. were collected in the environs of Odessa and kept in the laboratory where they molted to adults. Only receptive females were used in the experiments. The insects were marked with colored nitrocellulose varnish. Altogether, more than 200 tests were carried out with 16 females.

Studies of phonotaxis were performed in a mesh cage measuring 30 × 30 × 150 cm, subdivided by removable partitions into three chambers 60, 30, and 60 cm long. The insects were initially placed in the middle chamber (30 × 30 × 30 cm) containing food and a bunch of herbaceous plants which served as shelter. Conspecific signals (CS) and their models were transmitted via a speaker installed near one end of the cage. After the partitions were removed, the insects could move along the longitudinal axis of the cage. Each sound signal was presented for 4 min, and the mobile response of the bush crickets was monitored by recording the times at which the insects became active, left the central chamber, and reached the end of the cage closest to the speaker. Video recording of phonotaxis was used in some tests. The experiments were carried out at 22–25°C, during the period of acoustic activity of the insects.

When studying the phonotaxis of female bush crickets induced by model signals, we recorded the total time of phonotaxis (T_Σ) which included the period of activation of the insect and the time of movement from the shelter to the signal source (T). These values were compared with the corresponding parameters obtained during stimulation with CS. The data were statistically processed using the Wilcoxon signed-rank test.

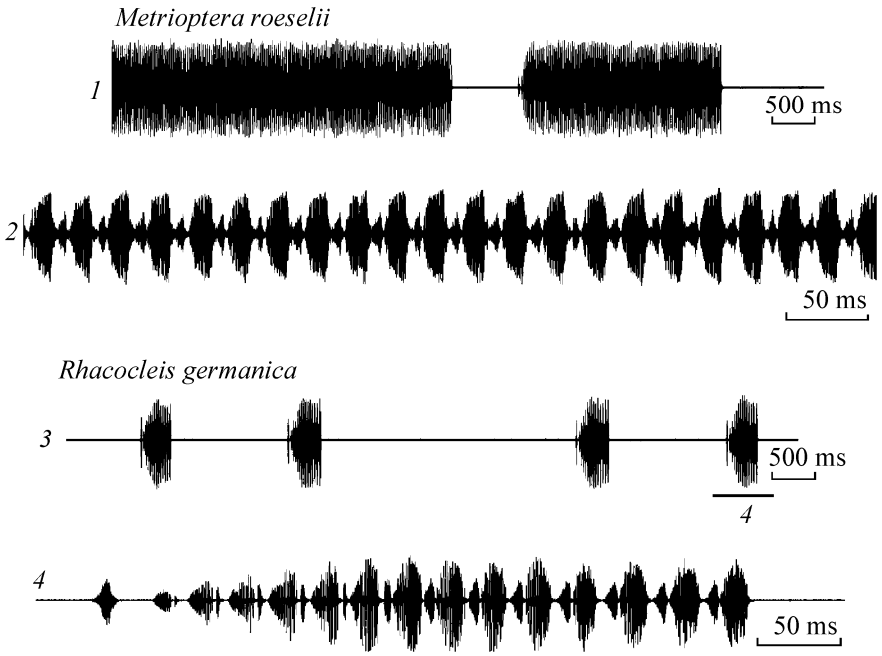

Fig. 1. Oscillograms of the calling signals at different time scales: (*1*, *2*) *Metrioptera roeselii* Hag.; (*3*, *4*) *Rhacocleis germanica* H.-Sch. The signals were recorded at 24°C.

In order to determine the possible effect of repeated stimulus presentation, a series of experiments with *Metrioptera roeselii* was carried out in which the recorded CS were played several times. The results of the first and the second tests did not show any significant differences either in the total time of phonotaxis, or in the time of movement toward the speaker.

Sounds were recorded using Type 4135 microphone and Type 2604 amplifier (Brüel & Kjær, Denmark). The microphone was positioned 5–10 cm from the insect. The conspecific acoustic signals were amplified and digitized with an E14-440 analog-digital converter (L-Card, Russia) at sampling frequency 44 100 Hz. The records were then transformed into various test models using Cool Edit Pro software. The sound intensity measured at the exit from the central chamber was 76–81 dB.

The descriptions of the signals follow the previously proposed terminology (Zhantiev, 1981).

RESULTS

Phonotaxis of Females of Metrioptera roeselii

The conspecific signal recorded at 25°C was used as control (Fig. 1, *1*, *2*). The mean duration of pulses was 9.6 ms (SD = 0.7 ms), that of interpulses, 4.9 ms (SD = 0.4 ms), the pulse period was 16.8 ms (SD = 0.4 ms), and the dominant carrier frequency, 18 kHz.

The following models were used in ethological experiments.

(1) Signals (trills) in which the duration of one interpulse interval was changed every 150 ms in such a way that the repeat period of the subsequent pulse was doubled or halved (Fig. 2*a*).

(2) A trill in which the interpulse intervals were reduced twice by 4 ms during the initial 50-ms portion of each 500-ms fragment.

Changes in the pulse period in models 1 and 2 resulted in the signal phase shifting by 180° every 150 or 500 ms.

(3) Trills with a doubled pulse repetition rate (PRR) (Fig. 3*a*).

(4) Signals with PRR 1.5 times lower than the corresponding parameter of the CS.

(5) Signals with PRR twice as low as that of the CS.

(6) An initial fragment of CS 225–230 ms long, followed by a period of white noise without any amplitude modulation.

(7) A signal similar to model 6 but including a noise fragment with a conspecific carrier frequency.

(8) An initial trill of CS 150 ms long, followed by a long fragment (4 min) of white noise without ampli-

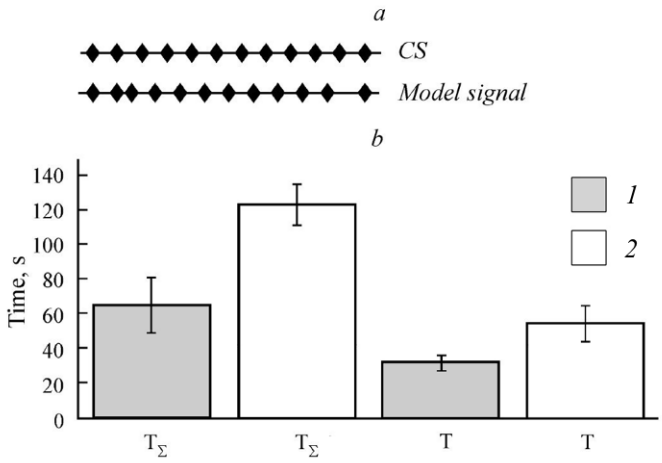

Fig. 2. Phonotaxis of females of *Metrioptera roeselii* Hag.: (*a*) temporal pattern of the presented signals (without interpulses); (*b*) time of phonotaxis (T_Σ) and time of movement to the source of the signal (T): (*1*) CS; (*2*) model signals with the phase of pulse rhythm changing every 150 ms. The data are shown as means and standard errors for 10 tests. The differences between the results of stimulation with CS and model signals are significant: $p = 0.036$ and $p = 0.019$, respectively.

tude modulation which was interrupted every minute by a 150-ms fragment of the conspecific trill.

(9) A noise fragment 4 min long without amplitude modulation.

In the first series of experiments, we determined the effect of periodical phase shifts of the acoustic signal on the phonotaxis. The responses to models 1 and 2 were very similar. The T_Σ values were significantly greater than those in the control (the mean difference being 84 and 58 s, respectively); however, the time of movement to the speaker T was significantly greater than in the control ($p = 0.022$) when the signal rhythm changed every 150 ms (Fig. 2*b*), and approximately the same as in the control ($p = 0.083$) when the signal phase shifted less frequently.

In the second series of experiments, the rhythm of the CS was modified. Models with PRR values 1.5 or 2 times lower than that of the CS did not evoke a phonotaxis of the females. By contrast, phonotaxis was observed in response to signals with PRR twice as high as that of the CS. The T values were slightly greater than those in the control but the differences in T_Σ and T between the test and the control were non-significant (Fig. 3).

In the third series of experiments, we tried to determine what time was necessary and sufficient for recognition of CS and eliciting of phonotaxis. Two model signals were used for this purpose. The first model consisted of a fragment of CS 225–230 ms long followed by a long continuous period of noise with a conspecific carrier frequency but without amplitude modulation. In the second model, the initial fragment of CS of the same length was followed by white noise interrupted by a conspecific trill fragment (150 ms) every minute. In the first case, the females became active and left the shelter but stopped early on their way; in the second case, each fragment of the conspecific trill caused the female to move closer to the signal source. The models consisting only of white noise or including shorter fragments of the trill were ineffective.

Phonotaxis of Females of Rhacocleis germanica

The male calling signal of this species consisted of irregularly repeated series of 10–16 (on average 13, SD = 1.6) pulses each (Fig. 1, *3, 4*). The pulse period increased from 25 to 29 ms during the series. The duration and period of the pulses were the most stable in the middle of a series (the mean values being 17.5 ± 0.2 and 25.7 ± 0.1 ms, respectively; SD = 1.8 and 1.4). In one of the experimental models, only the first series had a conspecific amplitude-temporal structure whereas all the remaining series presented during 5 min were replaced by noise fragments with a conspecific carrier frequency but without amplitude modulation. In the second model, series with a conspecific pattern were repeated once a minute, alternating with noise fragments. Preliminary results of our experiments showed that some females of *Rh. germanica* could locate the source of the signal even by a single series. Two out of four females reached the

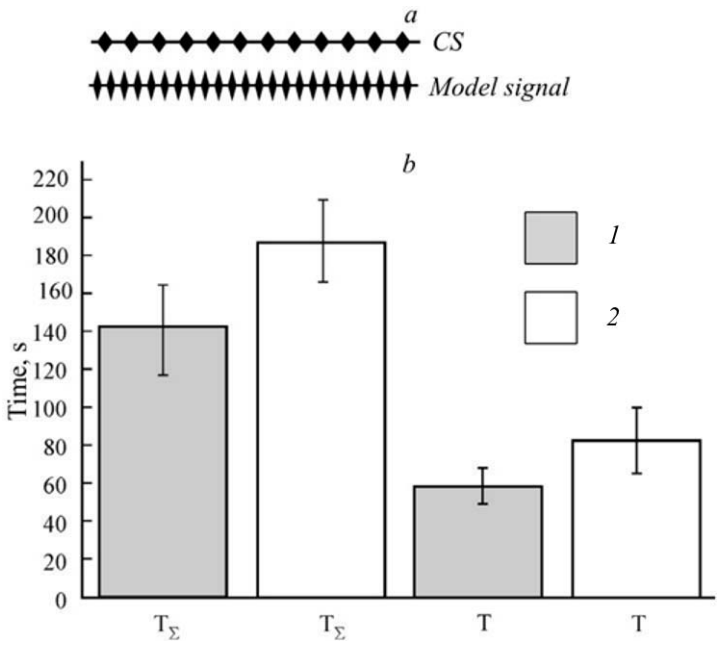

Fig. 3. Phonotaxis of females of *Metrioptera roeselii* Hag.: (*a*) temporal pattern of the presented signals (without interpulses); (*b*) time of phonotaxis (T_Σ) and time of movement to the source of the signal (T): (*1*) CS; (*2*) model signals with a doubled pulse repetition rate. The data are shown as means and standard errors for 13 tests. The differences between the results of stimulation with CS and model signals are non-significant: $p = 0.125$ and $p = 0.086$, respectively.

source of the signal which consisted of a single initial series with conspecific amplitude-temporal parameters and a long (5 min) period of discrete noise signals without amplitude modulation. The source of the sound could be more easily located in a modification of the preceding model in which the series with a conspecific pattern was repeated every minute.

DISCUSSION

In the experiments with *M. roeselii* described above, two types of signals were used besides the control ones: signals with modified PRR and signals with phase shifts.

In the first case, the doubled PRR reduced the attractiveness of the signal but did not eliminate the positive response completely. At the same time, the halved PRR totally eliminated the phonotaxis. A several-fold increase of PRR did not exclude the possibility of phase tuning of the internal rhythm generator though it could hamper this tuning. If the filtration mechanism was involved, the signal with a doubled PRR could also pass the filter. In theory, the insect's response to the signal with a halved PRR could have been preserved to a certain extent, but in reality no response was observed.

The adaptive significance of these differences may lie in the fact that *M. roeselii* in natural biotopes has the maximum PRR, so that increase of this parameter in the experiments does not suppress the phonotaxis. By contrast, the signal with a halved PRR resembles the sounds emitted by the dangerous predator *Tettigonia cantans* Fuess. (its PRR is 25 s^{-1}); therefore a complete suppression of the phonotaxis should be expected.

It is interesting to note in this connection that the opposite phenomenon is observed in *T. cantans*: the females do not respond to signals with a doubled PRR but retain their response to those with a halved PRR (Bush and Schul, 2006).

Phase shifts of the signal hamper its recognition, even though its natural PRR is preserved and its rhythmic pattern remains almost unaffected. These data indicate that during signal recognition, the rhythmic processes in the CNS are tuned to the impulse activity obtained from the acoustic system. Phase shifts of the signal hinder synchronization of these two processes.

Analysis of the impulse patterns of some spontaneously active neurons in the CNS of two species of bush crickets (*M. roeselii* and *T. cantans*) showed that

as the result of phase shifts, their spikes could mostly synchronize with conspecific signals; it was therefore concluded that these neurons could participate in signal recognition (Zhantiev et al., 2004). Similar neurons were later found in true crickets (Chukanov and Zhantiev, 2007). All these ethological and physiological data confirm the hypothesis of resonance-based mechanisms of acoustic signal recognition in Orthoptera.

In the above experiments, we tried not only to reveal the informative elements of the signals but also to determine the minimum time needed for their recognition. In experiments with *M. roeselii*, the signal fragment containing the conspecific temporal pattern had to be at least 220 ms long; for successful completion of the phonotaxis the short fragment of CS had to be repeated at least once a minute. Thus, no less than 14 pulses were required for CS recognition.

In *Rh. germanica*, emitting series of pulses, recognition of CS was possible even by a single series which included on average 13 pulses. It may therefore be assumed that CS recognition was based on the same functional mechanism in these two cases.

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