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Spectral selectivity during phonotaxis: a comparative study in *Neoconocephalus* (Orthoptera: Tettigoniidae)

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Summary

The calls of male *Neoconocephalus* have most energy concentrated in a relatively narrow low-frequency band. In *N. robustus* this low-frequency band is centered around 7 kHz, whereas calls of *N. nebrascensis* and *N. bivocatus* have center frequencies close to 10 kHz. The importance of the position of the low-frequency band for female phonotaxis in these three species was determined using a walking compensator. Female *N. robustus* showed significant phonotaxis towards call frequencies from 5 to 10 kHz, and spectral selectivity towards higher frequencies did not change with stimulus amplitude. Significant responses in *N. nebrascensis* and *N. bivocatus* occurred at significantly higher frequency ranges than in *N. robustus*. In these species, spectral selectivity changed with stimulus amplitude; at 68 dB sound pressure level (SPL), upper cut-off frequency was significantly lower than at 80 dB SPL in both species. Adding a higher harmonic to the conspecific carrier frequency had a strong inhibitory effect on phonotaxis in *N. robustus:* at higher relative amplitudes of the harmonic, phonotaxis was completely suppressed. Adding a higher harmonic to the conspecific carrier frequency had a much weaker but significant inhibitory effect in *N. nebrascensis* and little, if any, effect in *N. bivocatus*. The processing of song spectrum in the sensory system is discussed with regard to the differences in spectral selectivity among the three species. The sharp spectral selectivity of *N. robustus* is interpreted as an adaptation for species isolation.

Key words: acoustic communication, spectral processing, carrier frequency, call recognition, hearing, phonotaxis, *Neoconocephalus*.

Introduction

The calls of most groups of tettigoniids (katydids) have broadband spectral content that commonly extends well into the ultrasonic range (Gerhardt and Huber, 2002; Heller, 1988); only a few groups have narrow-band spectra in audible (e.g. Suga, 1966; Bailey, 1970) or ultrasonic (Morris et al., 1994) frequency ranges. In the genus *Neoconocephalus*, most of the call energy is concentrated in a narrow low-frequency band, with ultrasonic frequency components at least 20 dB softer than the low-frequency band (Fig. 1A) (Greenfield, 1990). The characteristic frequency (center frequency) of the low-frequency band within the genus ranges from 7 to 16 kHz (Greenfield, 1990; Schul and Patterson, 2003). Based on measurements of hearing thresholds and the sound transmission properties of the habitats (tall grasslands and marshes), center frequencies close to 10 kHz are calculated to be most advantageous in this genus (Schul and Patterson, 2003).

Male calls of *N. robustus* have the lowest center frequency in the genus at 7 kHz (Schul and Patterson, 2003). This frequency is surprisingly low given the disadvantages of calling at 7 kHz: the hearing sensitivity in *N. robustus* is about 7 dB lower at 7 kHz than at 10 kHz, and there is no improvement in signal transmission between 10 kHz and 7 kHz to justify the use of a frequency that is mismatched with female sensitivity (Schul and Patterson, 2003). A potential explanation, however, is that calling in the low-frequency band provides this species with a 'private channel' (Narins, 1995) that is free of interfering calls of sympatric congeners. Alternatively, the low-frequency band may provide female *N. robustus* with an additional cue for call recognition beyond the temporal pattern, which is similar to the temporal pattern of several congeners (Deily and Schul, 2004).

In tettigoniids, the hearing organ provides fine spectral resolution at the level of receptor cells (Kalmring et al., 1978; Römer, 1983). However, at the level of primary auditory interneurons, this frequency resolution is largely discarded as receptor cells converge on just a few interneurons with broad spectral sensitivity (e.g. Schul, 1997; Stumpner, 1999) (reviewed by Gerhardt and Huber, 2002). Accordingly, spectral selectivity of katydids is generally limited to detecting the absence or presence of energy in broad frequency bands (e.g. Latimer and Sippel, 1987; Bailey and Yeoh, 1988; Dobler et al., 1994; Jatho, 1995). Preferences based on fine-scale

differences in call spectra (e.g. Bailey and Yeoh, 1988; Schul et al., 1998) are most probably based on differences in the perceived call amplitudes (Schul, 1999) (reviewed by Gerhardt and Huber, 2002). The more detailed spectral processing found in other groups of insects (e.g. Doolan and Young, 1989; Fonseca et al., 2000; Fonseca and Revez, 2002) has not been described in katydids.

The small spectral difference between *N. robustus* and most of its congeners in the position of the low-frequency band (7 kHz *versus* 10 kHz) appears unlikely to be resolved by the spectral selectivity of the ascending pathway in katydids, and thus appears unlikely to serve an important function. However, the disadvantages that the use of the low carrier frequency entails for *N. robustus* (see above) suggest an adaptive function in this species, possibly for call recognition or masking avoidance. Here, we examine the spectral selectivity in *N. robustus* and two closely related species with sympatric occurrence (*N. nebrascensis* and *N. bivocatus*). We determine the importance of the position of the low-frequency band for female phonotaxis in *N. robustus*, and explore differences in spectral processing among the three species. Furthermore, we investigate potential mechanisms females may use to discriminate the carrier frequency of *N. robustus* from the higher carrier frequencies of its congeners.

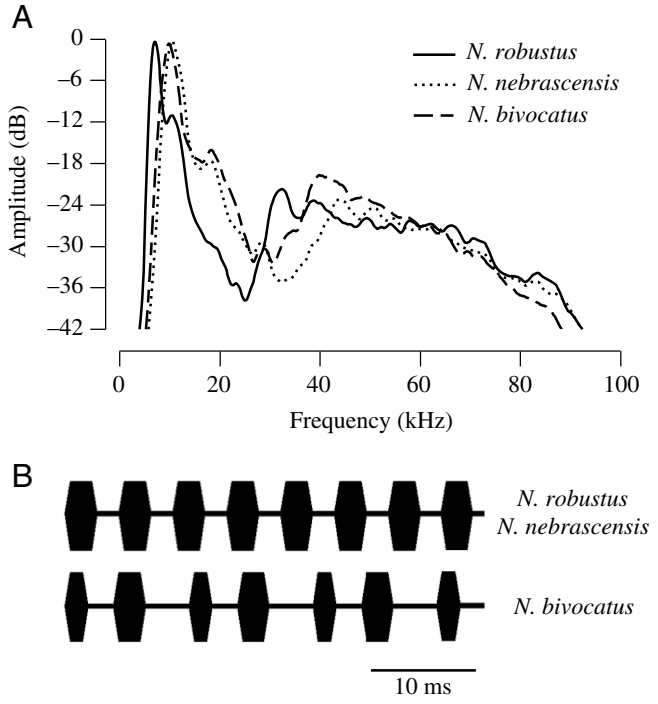

Fig. 1. (A) Averaged spectra of male calls of *N. robustus* (solid line, *N*=10), *N. nebrascensis* (dotted line, *N*=10) and *N. bivocatus* (broken line, *N*=8) at 25°C. Adapted from Schul and Patterson (Schul and Patterson, 2003). (B) Pulse patterns of the conspecific call models used for *N. robustus* and *N. nebrascensis* (top trace), and *N. bivocatus* (bottom trace).

Materials and methods

Animals

We collected female *Neoconocephalus robustus* (Scudder, 1862), *N. nebrascensis* (Bruner, 1891) and *N. bivocatus* Walker, Whitesell and Alexander, 1973 from the field as nymphs in Boone County, Missouri (USA), and identified them after Froeschner (Froeschner, 1954) and Walker et al. (Walker et al., 1973). *N. robustus* and *N. bivocatus* are considered sibling species (Walker et al., 1973; Greenfield, 1990). Preliminary results of a molecular phylogenetic analysis based on a mitochondrial locus support this assumption (R. L. Snyder and J. Schul, unpublished data), and indicate that these species, together with *N. nebrascensis* and *N. ensiger*, form a distinct clade within the genus *Neoconocephalus*.

The insects were kept at 20–25°C and a light:dark cycle of 14 h:10 h. The females were held for at least 2 weeks after their adult molt before they were used in experiments. Females were tested for up to 5 weeks, during which we detected no changes in their selectivity.

Phonotaxis experiments

We conducted behavioral tests on a walking compensator [Kramer treadmill (Weber et al., 1981)] in an anechoic chamber at 25±1°C. In short, the insects were placed on top of a sphere, free to walk but kept in place by compensatory sphere rotations, while acoustic signals were presented from loudspeakers located in the insect's horizontal plane. The intended direction and speed of the animal were read out from the control circuitry. The experiments were performed in the dark except for an infrared light used to monitor the movements of the animal on the sphere. For details see Weber et al. (Weber et al., 1981) and Schul (Schul, 1998).

Stimulation

We generated synthetic signals using a custom developed DA-converter/amplifier system (16 bit resolution, 250 kHz sampling rate). The signals were attenuated using a computer-controlled attenuator and delivered *via* one of two loudspeakers (EAS 10TH400C) mounted at a distance of 150 cm in the horizontal plane of the insect and separated by an angle of 115°. We adjusted signal amplitude using a 1/4″ condenser microphone (G.R.A.S. 40BF, Holte, Denmark) positioned 1 cm above the top of the sphere, and a Bruel and Kjaer sound level meter (B&K 2231, Naerum, Denmark). All sound pressure levels are given as dB peak sound pressure level (SPL; re 20 μPa).

The temporal patterns of the call models used in this study were based on population mean values determined by Büttner (Büttner, 2002) at 25°C. All pulses used in the three call models had 0.5 ms rise and fall times, which are included in the durations of the pulses. Call models of *N. robustus* and *N. bivocatus* were identical to the control stimuli used by Deily and Schul (Deily and Schul, 2004).

The temporal pattern for *N. robustus* (Fig. 1B) consisted of a continuous train of pulses of 3.0 ms duration, separated by silent intervals of 2.0 ms duration (i.e. a single-pulse pattern).

The temporal pattern for *N. bivocatus* consisted of a continuous train of paired pulses: the duration of these pulses was 2.2 ms and 3.0 ms, with an interval of 2.3 ms in between. These paired pulses were repeated after an interval of 4.0 ms (Fig. 1B). The call models of both *N. robustus* and *N. bivocatus* were presented as continuous signals, without a second order time pattern modulating the pulse pattern.

The call model of *N. nebrascensis* had the same pulse pattern as the *N. robustus* model (pulse duration of 3.0 ms and interval duration of 2.0 ms). However these pulses were not presented continuously, but grouped into verses of 1000 ms duration, which were repeated after a silent pause of 800 ms.

The calls of *N. robustus*, *N. nebrascensis* and *N. bivocatus* had similar spectral composition (see Introduction) but differed in the center frequency of the low-frequency band (Fig. 1A). The center frequency was at 7.0 kHz in *N. robustus*, 10.4 kHz in *N. nebrascensis* and 10.1 kHz in *N. bivocatus* (Schul and Patterson, 2003). We used pure tone carriers of 7 kHz (*N. robustus*) and 10 kHz (*N. nebrascensis* and *N. bivocatus*) with the temporal patterns described above to construct conspecific call models for each species. This simplification of both the temporal and spectral structure did not noticeably reduce the attractiveness of these stimuli relative to natural calls (Deily and Schul, 2004). These call models were used as control stimuli throughout this study. We used the conspecific temporal pattern for each of the three species during all experiments.

Experiment 1

We tested the effects of both carrier frequency and call amplitude on attractiveness. The carrier frequency of the call models varied from 5 to 60 kHz. Stimuli were presented at amplitudes of both 68 and 80 dB SPL.

Experiment 2

We tested the effect of an additional high frequency component on the attractiveness of the call models by adding a second sinusoid to the conspecific carrier frequencies. Frequencies were chosen as integer multiples of the carrier frequencies (14, 28 and 42 kHz for *N. robustus*; 20 and 40 kHz for *N. nebrascensis* and *N. bivocatus*). Note that although up to three high frequency components were tested per species, only one high frequency component was added to the low-frequency band per trial stimulus, i.e. for *N. nebrascensis*, the three stimuli consisted of 10 kHz alone (control), 10 kHz + 20 kHz and 10 kHz + 40 kHz. The absolute amplitude of the low-frequency component was set for each individual to the lowest amplitude at which it showed consistent phonotaxis when presented alone, and was held constant within each series of an individual. Amplitudes of the low-frequency component ranged from 50 to 56 dB SPL for *N. robustus*, 44 to 56 dB SPL for *N. nebrascensis* and 44 to 62 dB SPL for *N. bivocatus*. The amplitude of the high frequency component was varied between 0 dB and +18 dB relative to the conspecific carrier. We conducted this experiment at amplitudes close to the behavioral threshold to detect weak effects of the high frequency component which would be masked by the strong excitation at higher stimulus amplitudes. Phonotaxis at these near-threshold amplitudes was comparable to that observed at 68 and 80 dB SPL.

Experimental protocol

The experimental protocol is described in detail by Schul (Schul, 1998) and Bush et al. (Bush et al., 2002). Briefly, all stimuli were presented twice for approximately 1.5 min each (3 min in total), with the position of the loudspeaker changed between the two presentations. At the beginning of each series the control stimulus was presented, then two or three test stimuli, then another control, etc. Between stimuli a 1-min period of silence was imposed. Each experimental series lasted between 30 and 90 min, during which up to nine experimental stimuli (plus four controls) were presented. We varied the sequence of stimulus presentation among the individual females tested.

Data analysis

To evaluate the relative response of a female during a test situation, we calculated a phonotaxis score (Schul, 1998) which included measures for three criteria that describe the relative strength of phonotaxis: (1) the walking speed relative to the speed during the control stimulus (describing the locomotion activity elicited); (2) the vector length, describing the accuracy of orientation; and (3) the orientation relative to the orientation during the control stimulus. Phonotaxis scores range from approximately +1 (perfect positive phonotaxis) to –1 (perfect negative phonotaxis). Phonotaxis scores close to 0 indicate either no response or random orientation [for details of the data analysis and calculation of the phonotaxis score see Schul (Schul, 1998)]. To facilitate comparison between species and between stimulus intensities, we normalized phonotaxis scores by setting the phonotaxis score to the control stimulus to 1.

We present all phonotaxis scores as mean ± standard error of the mean (s.e.m.). Female responses were considered significant if two criteria were met: (i) the phonotaxis scores were significantly greater [Wilcoxon paired sample test, $P<0.05$ (Zar, 1984)] than the phonotaxis scores obtained from the same females in response to silence; and (ii) the average response was at least 50% of the response to the model of the conspecific call. Both criteria agreed for most data points; in the few cases that only one was significant, the second criterion was usually more stringent than the first. Therefore, we do not present the results of the Wilcoxon paired sample tests in the text. Note that the application of significance criteria and cut-off frequencies (see below) merely emphasize the relative attractiveness of stimuli and are not meant to classify stimuli as ‘recognized’ or ‘not recognized’ (for a detailed discussion, see Bush et al., 2002).

For experiment 1, we constructed frequency response functions; each function had a distinct roll-off towards higher frequencies above the conspecific call carrier frequency (Figs 2, 3). We fitted a sigmoidal function to the phonotactic

response curve above the conspecific carrier frequency (see above) of each female by minimizing the sum of the squared errors. The frequency at which the sigmoid had an amplitude of 50% was defined as the upper cut-off frequency. We compared median upper cut-off frequencies between the three species with a Mann-Whitney test, and at different stimulus intensities within each species using a Wilcoxon paired sample test (Zar, 1984).

We tested the effect of the added high-frequency components during experiment 2 using a repeated measures analysis of variance (individual females as a random-effect), using the phonotaxis score as the measure of performance. The Tukey test was used for *post-hoc* pairwise comparisons between the call model and treatment groups (Zar, 1984). We calculated analysis of variance (ANOVA; General Linear Model) and *post-hoc* comparisons using Minitab (Release 14.12.0, Minitab Inc., USA). We used a significance criterion (α) of 0.05.

Results

Experiment 1

In the first set of experiments, we tested female responses to call models with varied carrier frequencies. Females of the three species showed significant phonotaxis in the frequency range around the center frequency of their calls (Fig. 2). At 80 dB SPL significant phonotaxis scores occurred in *N. robustus* from 5 to 10 kHz; in the two other species significant responses occurred at higher frequencies (*N. nebrascensis*: 8–15 kHz, *N. bivocatus*: 7–15 kHz). The median upper cut-off

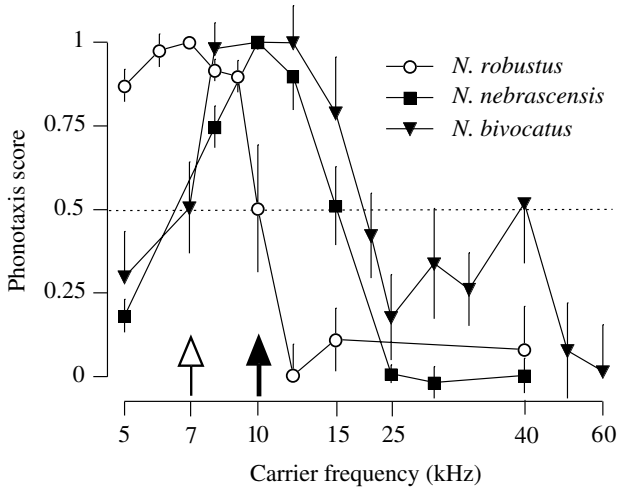

Fig. 2. Importance of call carrier frequency for female phonotaxis of *N. robustus* (circles), *N. nebrascensis* (squares) and *N. bivocatus* (triangles). Each point represents the mean phonotaxis score (± s.e.m.) of seven or eight females. Phonotaxis scores were normalized relative to the phonotaxis score at the conspecific carrier frequencies (7 kHz for *N. robustus*, 10 kHz for *N. nebrascensis* and *N. bivocatus*; arrows). All responses above 0.5 (dotted line) were significant (see Materials and methods) except for *N. bivocatus* at 40 kHz. All stimuli were presented at 80 dB SPL.

Table 1. *Median and range of cut-off frequencies in experiment 1*

Species	Amplitude (dB SPL)	Cut-off frequencies (kHz) Median	Range	*N*
N. robustus	80	10.3	9.1–11.4	7
	68	10.2	9.0–12.5	8
N. nebrascensis	80	14.9	12.7–19.0	8
	68	12.3	10.1–15.0	8
N. bivocatus	80	17.9	12.1–19.4	7
	68	11.9	10.2–12.9	7

frequency in *N. robustus* (10.3 kHz; Table 1) was significantly lower (Mann-Whitney U-test, $P<0.002$) than in both *N. nebrascensis* (14.9 kHz) and *N. bivocatus* (17.9 kHz). There was no significant difference in upper cut-off frequencies between *N. nebrascensis* and *N. bivocatus* (Mann-Whitney U-test, $P>0.2$).

For frequencies of 20 kHz or higher, mean phonotaxis scores of *N. robustus* and *N. nebrascensis* were below 0.1; in *N. bivocatus,* however, response strength remained above 0.1 for frequencies up to 40 kHz (Fig. 2). Although these responses were not significant, they suggest that frequencies between 20 and 40 kHz were somewhat attractive to *N. bivocatus* females.

Fig. 3 compares the spectral selectivity of the three species at two stimulus amplitudes, 68 dB SPL and 80 dB SPL. In *N. robustus*, female selectivity did not change with stimulus amplitude (Fig. 3A); median cut-off frequencies (Table 1) did not differ between 68 dB SPL and 80 dB SPL (Mann–Whitney U-test, $P>0.20$).

In both *N. nebrascensis* and *N. bivocatus*, spectral selectivity changed significantly with stimulus amplitude. Significant responses occurred over a narrower frequency range at 68 dB SPL than at 80 dB SPL in both species (Fig. 3B,C). Accordingly, median upper cut-off frequencies (Table 1) were significantly lower at 68 dB SPL than at 80 dB SPL (Mann–Whitney U-test, $P<0.05$ for both species).

The amplitude independence of spectral selectivity in *N. robustus* is a typical signature of 'lateral inhibition', i.e. the spectral selectivity seems to be generated by low-frequency excitation and high frequency inhibition. Conversely, changes of spectral selectivity as seen in *N. nebrascensis* and *N. bivocatus* suggest that selectivity is generated by excitation only. We tested whether high frequencies have an inhibitory effect on female phonotaxis in the second set of experiments.

Experiment 2

In *N. robustus*, the inhibitory effect of adding a higher harmonic to the conspecific carrier frequency of 7 kHz (Fig. 4A) was highly significant (ANOVA, $P<0.001$ for all three frequencies, see Table 2 for details). *Post-hoc* pairwise comparison demonstrated that responses to all stimuli that include a high frequency component were significantly weaker than to the control stimulus (Table 2). Females failed to show

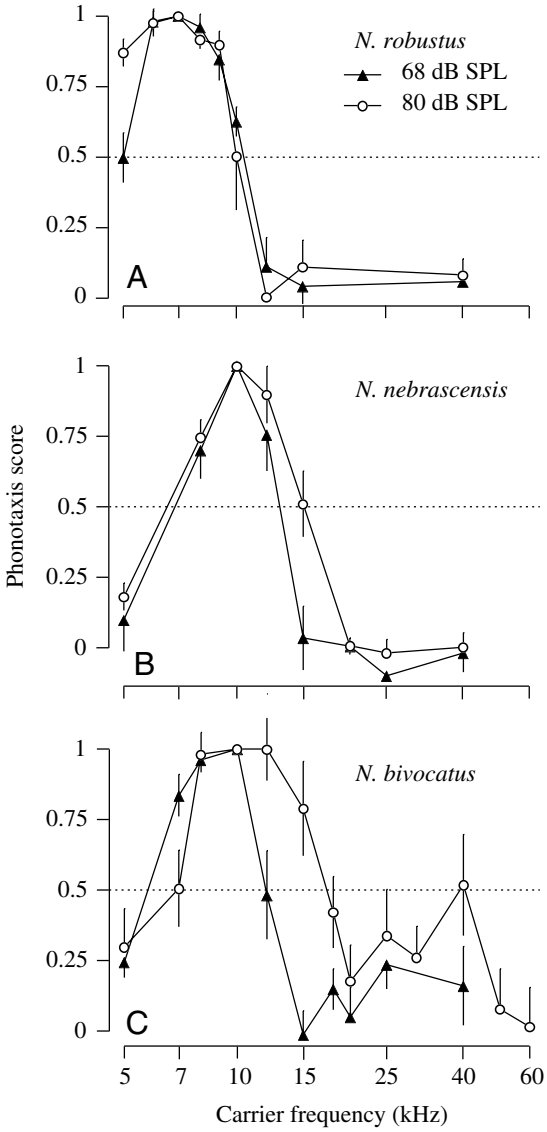

Fig. 3. Importance of call carrier frequency for female phonotaxis at stimulus amplitudes of 68 dB SPL (triangles) and 80 dB SPL (circles) in (A) *N. robustus*, (B) *N. nebrascensis* and (C) *N. bivocatus*. Each point represents the mean phonotaxis score (± s.e.m.) of 7 or 8 females. Phonotaxis scores were normalized relative to the phonotaxis score at the conspecific carrier frequencies (7 kHz for *N. robustus*, 10 kHz for *N. nebrascensis* and *N. bivocatus*). All responses above 0.5 (dotted line) were significant (see Materials and methods) except for *N. bivocatus* at 40 kHz/80 dB SPL.

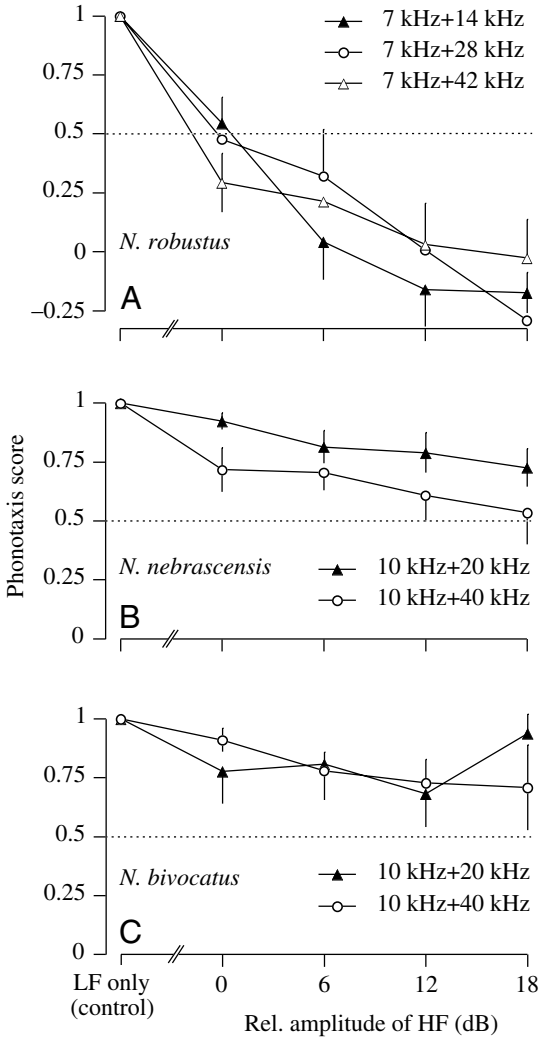

Fig. 4. Effects of adding a high frequency (HF) component to call models on female phonotaxis of (A) *N. robustus*, (B) *N. nebrascensis* and (C) *N. bivocatus*. Each point represents the mean phonotaxis score (± s.e.m.) of 7 or 8 females. Phonotaxis scores were normalized relative to the phonotaxis score to the conspecific call model (control), which consisted of only the conspecific carrier frequency (LF; 7 kHz in *N. robustus*, 10 kHz in *N. nebrascensis* and *N. bivocatus*). HF components were added at amplitudes of 0 dB to +18 dB relative to the amplitude of the LF component. All responses above 0.5 (dotted line) were significant (for further details see Materials and methods).

significant responses to any stimulus containing a high frequency component, except for 14 kHz at 0 dB relative amplitude (Fig. 4A).

In *N. nebrascensis,* adding either 20 kHz or 40 kHz to the conspecific carrier frequency (10 kHz) had significant effects on female responses (Fig. 4B; ANOVA: 20 kHz, $P<0.005$; 40 kHz, $P<0.002$; Table 2). *Post-hoc* pair-wise comparisons indicated that female responses to stimuli containing either frequency at +12 dB and +18 dB were significantly weaker than to the control stimulus (Tukey test, $P<0.05$ in all cases). However, all stimuli that included a high frequency component elicited significant responses in *N. nebrascensis* (Fig. 4B).

In *N. bivocatus*, adding a higher harmonic to the conspecific carrier frequency of 10 kHz (Fig. 4C) had marginally significant effects (ANOVA: 20 kHz, $P<0.05$; 40 kHz, $P<0.1$; Table 2). *Post-hoc* pairwise comparison to the control stimulus detected a significant reduction in response strength ($P<0.05$) only for 20 kHz at +12 dB relative amplitude, whereas for all other stimuli with high frequency components these comparisons were not significant (Table 2). All stimuli that included a high frequency component elicited significant responses in *N. bivocatus* (Fig. 4C).

Table 2. *Results of analysis of variance of experiment 2*

Species	Frequency (kHz)	d.f.	*F*	*P*	*P* values from *post-hoc* comparisons to control			
					0 dB	+6 dB	+12 dB	+18 dB
N. robustus	14	4	17.24	**<0.001**	**<0.05**	**<0.005**	**<0.001**	**<0.001**
	28	4	23.82	**<0.001**	**<0.05**	**<0.001**	**<0.001**	**<0.001**
	42	4	16.07	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
N. nebrascensis	20	4	4.91	**<0.005**	=0.809	=0.084	**<0.05**	**<0.01**
	40	4	5.92	**<0.002**	=0.091	=0.058	**<0.01**	**<0.001**
N. bivocatus	20	4	2.95	**<0.05**	=0.195	=0.375	**<0.05**	=0.960
	40	4	2.16	=0.100	=0.911	=0.343	=0.168	=0.144

$P<0.05$ indicates significant statistical results (indicated in bold type).

Discussion

In *N. robustus*, *N. nebrascensis* and *N. bivocatus* female responses were limited to a narrow frequency range around the center frequencies of their conspecific calls. The addition of higher frequencies affected the three species to different degrees, strongly inhibiting phonotaxis in *N. robustus*, decreasing response strength in *N. nebrascensis*, and having only marginally significant effects in *N. bivocatus*.

The female response curves in Figs 2 and 3 were most probably a function of attractiveness of the different frequencies, rather than of their localizability. Analyzing the walking speed alone resulted in the same pattern of response functions as using the phonotaxis score. The walking speed indicates how enthusiastically females respond to a stimulus, independent of the available directional cues (i.e. it is thus influenced only by call attractiveness).

In *N. robustus* responses decreased steeply toward higher frequencies, and the upper cut-off frequency of female responses did not change with stimulus amplitude (Fig. 2, Fig. 3A). This suggests that 'lateral inhibition' (Gerhardt and Huber, 2002; Hennig et al., 2004) is involved in generating the spectral selectivity towards higher frequencies; frequencies below the upper cut-off frequency (10 kHz) have excitatory effect, while higher frequencies inhibit female responses. Experiment 2 directly demonstrates the inhibitory effect of frequencies above 10 kHz.

In *N. bivocatus* spectral selectivity changes significantly with stimulus amplitude (Fig. 3C), and the inhibitory effect of high frequencies during experiment 2 was marginal. This suggests that lateral inhibition plays only a minor role in the spectral selectivity towards higher frequencies in this species. Rather, an excitatory function alone seems to sufficiently explain the selectivity found in experiment 1. The non-significant positive responses to frequencies from 20 to 40 kHz (Fig. 2) also indicate that high frequencies have little, if any, inhibitory effect in *N. bivocatus*.

Results in *N. nebrascensis* were intermediate between the two other species. High frequencies had a highly significant inhibitory effect during experiment 2, although considerably less than in *N. robustus* (Table 2, Fig. 4). Frequency selectivity towards higher frequencies changed with stimulus amplitude, albeit less than in *N. bivocatus*, and there was no positive trend for responses in the frequency range between 20 and 40 kHz as there was for *N. bivocatus* (Fig. 2). These results suggest that lateral inhibition plays a significant role in spectral selectivity in this species, but to a much lesser extent than in *N. robustus*.

Our results indicate that the influence of lateral inhibition on the spectral selectivity towards high frequencies differs significantly among the three species: inhibition is weakest in *N. bivocatus*, somewhat stronger in *N. nebrascensis*, and by far the strongest in *N. robustus*. Additionally, the border-frequency between excitation and inhibition was lower in *N. robustus* (approximately 10 kHz) than in the other two species (approximately 15-18 kHz).

Neuronal processes underlying spectral selectivity

Among tettigoniids high hearing sensitivities occur in the broad range from below 5 kHz to above 80 kHz [(Kalmring et al., 1990) in *Neoconcocephalus* (Schul and Patterson, 2003)]. Auditory receptor cells project into the prothoracic ganglion where they converge onto a small number of auditory interneurons, which consequently have broad spectral selectivity (reviewed by Stumpner and Helversen, 2001). Sharpening of spectral selectivity through lateral inhibition occurs most prominently in one neuron: AN-1 receives excitation from frequencies below 20 kHz, but inhibition from frequencies above 20 kHz (Schul, 1997; Stumpner, 1997). Accordingly AN-1 thresholds increase steeply between 20 and 30 kHz [roll off >50–60 dB per octave (Schul, 1997; Stumpner, 1997)]. AN-1 is most probably involved in generating the spectral selectivity observed during phonotaxis in several tettigoniid species (Schul, 1997; Stumpner, 1997).

The ascending pathway of *Neoconocephalus* has not been studied in detail. However, it is likely that the differences in spectral selectivity described here result from differences in AN-1 properties among the three species. The strength of the high-frequency inhibition on AN-1 should vary dramatically among them, being strongest in *N. robustus* and weakest in *N. bivocatus*. Furthermore, the border between excitation and inhibition should be shifted towards lower frequencies in *N. robustus* compared to the two other species. Among closely

related species of the tettigoniid subfamily Phaneropterinae, differences of AN-1 properties occur in a similar order of magnitude as suggested by our experiments (Stumpner, 2002).

The sharp decline in response strength of female *N. robustus* between 9 and 12 kHz and the amplitude independence of this decline are exceptional among ensiferans. In tettigoniids, behavioral tuning is usually amplitude dependent [*N. bivocatus* and *N. nebrascensis* in this study (Hardt, 1988; Dobler et al., 1994)]; preferences based on small-scale spectral differences (within frequency ranges of a few kHz) are overridden by small changes in amplitude (Bailey and Yeoh, 1988; Schul et al., 1998). In some crickets, behavioral tuning does exhibit steep roll-offs (e.g. Hennig and Weber, 1997). However, this selectivity is caused by the tuning of the hearing organ, and in this respect is also amplitude dependent. In contrast, *N. robustus* responds to 9 kHz, but not to 12 kHz, largely independent of call amplitude.

Although spectral selectivity in *N. robustus* appears to have attained a 'new quality' among tettigoniids in steepness and amplitude independence, it is instead most probably based on quantitative changes in the sensory system: high frequency inhibition on AN-1 shifted towards lower frequencies, and its synaptic weighting increased (see above). The spectral selectivity of *N. robustus* is most probably the result of evolution from less selective ancestors. Given that 7 kHz is less suited than 10 kHz for long range communication in *Neoconocephalus* (Schul and Patterson, 2003), the question arises: What evolutionary forces caused the shift in call frequency and call processing in *N. robustus*?

Evolutionary influences on call spectrum

The three species studied here are probably sibling species (see Materials and methods); each species' call features one characteristic that distinguishes it from the calls of the other two species: In *N. bivocatus* and *N. nebrascensis* these are temporal characters (double-pulse pattern in *N. bivocatus* and verse structure in *N. nebrascensis*), while in *N. robustus* the center frequency is shifted significantly below 9 kHz (Büttner, 2002; Schul and Patterson, 2003). These three call characteristics (double-pulses, verse structure, and center frequency below 9 kHz) are uncommon in this genus: of 23 described calls, 18 have single-pulse pattern, 17 are continuous (Greenfield, 1990), and most species' calls are limited to frequencies above 9 kHz (Schul and Patterson, 2003; Greenfield, 1990). This pattern suggests that within the clade containing *N. robustus*, *N. nebrascensis* and *N. bivocatus*, the ancestral call consisted of a continuous single-pulse temporal pattern and a center-frequency of about 10 kHz. The 'unique' characteristic in each species' call are therefore probably derived call traits.

In both *N. nebrascensis* and *N. bivocatus*, the derived temporal characteristics provide cues for the females to recognize their conspecific calls: *N. bivocatus* females recognize the double pulse rate of approximately 87 Hz (Deily and Schul, 2004), and *N. nebrascensis* females require a distinct verse structure (J.A.D. and J.S., our unpublished data). Neither of these two species shows significant phonotaxis to signals with the temporal pattern of its congeners; i.e. their species-specific temporal call pattern ensures species isolation. By contrast, the presence or absence of these derived temporal characteristics is not a reliable cue for female *N. robustus*, which show significant phonotaxis to the temporal pattern of at least one congener (*N. bivocatus*, Fig. 5). Thus, temporal pattern recognition is insufficient for *N. robustus* to avoid mismatings. Furthermore, temporal selectivity in *N. robustus* could not be higher without rejecting the conspecific temporal pattern (Deily and Schul, 2004). However, in this species, the spectral difference provides a more reliable cue for species recognition, especially when combined with the temporal cues (Fig. 5).

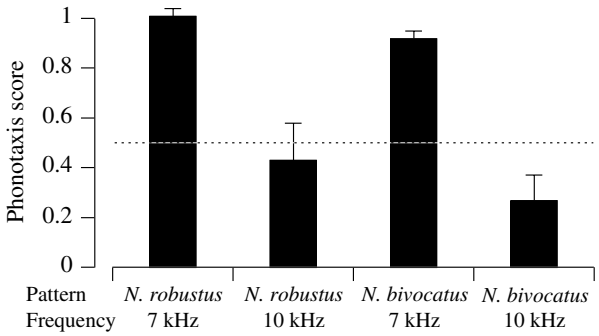

Fig. 5. Responses of female *N. robustus* to stimuli with the temporal pattern and carrier frequency of calls of *N. robustus* (7 kHz) or *N. bivocatus* (10 kHz). Bar height indicates the mean phonotaxis score (± s.e.m.) of 8 females. Stimulus amplitude: 80 dB SPL. All responses above 0.5 (dotted line) were significant (see Materials and methods).

Ancestors of *N. robustus* most probably had the same temporal call pattern (and the same temporal call recognition mechanism) as *N. robustus*, but a higher center frequency. After the appearance of species with derived temporal patterns, the temporal call recognition of this ancestral population would not have enabled reliable rejection of the 'new' calls. Thus, selection would have favored any traits that reduced the risk of hybridization. Because temporal selectivity could not be sharpened enough to reject the new temporal pattern (see above) we suggest that a lower call frequency evolved in response to the appearance of new call patterns. Thus a reinforcement-like process (Dobzhansky, 1937) could have gradually shifted the call center frequency towards lower frequencies and concomitantly sharpened spectral selectivity in *N. robustus*.

Hearing sensitivity of *N. robustus* females is considerably lower at 7 kHz than at 10 kHz and therefore the shift to the lower call center frequency resulted in a reduction of communication distance (Schul and Patterson, 2003). Also, as the ears of tettigoniids usually function as pressure receivers rather than pressure gradient receivers, 7 kHz probably provides less peripheral directionality than 10 kHz (Gerhardt and Huber, 2002). These disadvantages of the

derived center frequency in *N. robustus* support our view that *N. robustus* was 'pushed' by congeners to the lower center frequency, rather than that the low center frequency provides an advantage in itself such as a 'private' communication channel free of masking signals. Owing to the strong inhibitory effect of higher frequencies on female phonotaxis, calls of congeners should inhibit phonotaxis in *N. robustus* and therefore interfere with intraspecific communication in this species. The adaptive value of the spectral selectivity of *N. robustus* seems not to be interference avoidance, but rather species isolation.

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