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The Diversity of Call Recognition: Selective Phonotaxis in *Neoconocephalus ensiger*

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Abstract In many insect and anuran species, the temporal pattern of male calls encodes the species identity of the signaler and females use the temporal pattern to identify and approach conspecific mates. We studied the call recognition mechanism of *Neoconocephalus ensiger* in phonotaxis experiments conducted on a walking compensator. Stimuli were presented in a no-choice paradigm and female response strength quantified. The calls of *N. ensiger* have an unusually slow pulse rate (approximately 12 Hz) for this genus, which is a derived trait. Call models were attractive when pulse durations were between 25 and 55 ms and interval durations were between 19 and 99 ms. An amplitude attenuation depth of 6 dB was sufficient for females to detect the conspecific temporal pattern. The call recognition mechanism of *N. ensiger* differs strikingly from four other temporal recognition mechanisms previously described in *Neoconocephalus*, but is similar to call recognition in more distantly related taxa (including anurans) that have male calls with similar pulse period. This suggests that the evolution of call recognition mechanisms is more strongly influenced by signal parameters and/or neural constraints than by phylogenetic constraints.

Keywords Acoustic communication · temporal pattern · call recognition · phonotaxis

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Introduction

In many acoustic communication systems of insects and anurans the calls encode the species identity of the signaler (review in Gerhardt and Huber 2002). The receivers (typically the females) use the call pattern to selectively respond to conspecific signals. Commonly the signals of closely related species with overlapping ranges differ in their temporal pattern, and females recognize the conspecific temporal pattern of the calls (e.g. Helversen and Helversen 1994; Schul 1998; Gerhardt 2005).

Interestingly, females of closely related species commonly evaluate different call parameters, even if the calls are similar and differ only in the value of one parameter: for example, the calls of the two gray treefrogs *Hyla chrysoscelis* and *H. versicolor* differ mostly in their pulse rate (50 and 20 Hz, respectively). While females of *H. chrysoscelis* evaluate the pulse rate (independent of pulse duration), females of *H. versicolor* evaluate the durations of pulses and inter-pulse intervals (Schul and Bush 2002). Similar situations occur in other groups of treefrogs (Gerhardt 1982), crickets (Hennig 2003), grasshoppers (Helversen and Helversen 1994), and katydids (Schul 1998; Deily and Schul 2004).

Male calls in the Tettigoniid genus *Neoconocephalus* are unusually fast. Of the 25 species with described calls, 21 have pulse rates well above 100 Hz (Greenfield 1990). Calls with a continuous fast pulse rate represent the ancestral call pattern within the genus (Fig. 1a, Frederick 2013). Females of species with this ancestral call pattern respond to stimuli that do not contain intervals longer than a few ms (Deily and Schul 2004, 2009); in these species, continuous sine waves without amplitude modulation are attractive. This preference for continuous, fast calls represents the ancestral call recognition mechanism in the genus (Frederick 2013).

Derived temporal call patterns in *Neoconocephalus* include variations of the pulse pattern (= double pulses; e.g. Walker et al. 1973; Walker 1975) and the presence of second order time structures (= chirps or verses; e.g. Meixner and Shaw 1986; Walker and Greenfield 1983). Each of these derived call patterns evolved multiple times in this genus (Frederick 2013). A diversity of derived call recognition mechanisms occurs among the species with derived call patterns, both for the derived pulse patterns and the verse structure. Four different recognition mechanisms have been described to date (see discussion, Bush et al. 2009; Schul et al. 2013).

The call of *N. ensiger* (Harris 1841) is unusual among *Neoconocephalus* for having an extremely slow pulse rate of approximately 15 Hz at 25 °C (Libersat and Hoy 1991). These pulses are separated by silent intervals lasting about 40 ms, an order of magnitude longer than the 2–5 ms intervals found in species with the ancestral fast call. The only other species with a similarly slow pulse rate is *N. affinis*, which produces about 22 pulses/s (grouped into 11 pulse pairs/s.); as is typical of *Neoconocephalus*, however, the intervals between pulses in *N. affinis* are only a few ms in duration (Walker and Greenfield 1983; Bush et al. 2009). Given that the ancestral recognition mechanism rejects calls with long intervals (Deily and Schul 2004; Frederick 2013), *N. ensiger* females must use a different mechanism to recognize male calls. Here we test which temporal parameters are used by *N. ensiger* females to recognize calls, and how call recognition in this species compares to that of congeners.

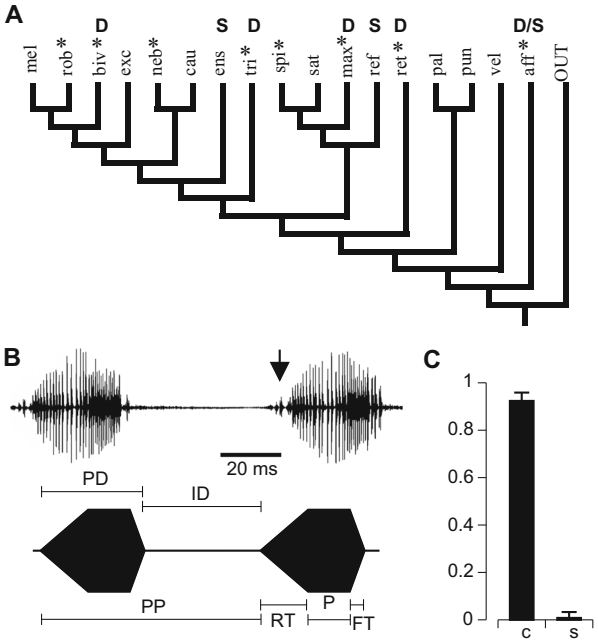

Fig. 1 **a** Total evidence tree of *Neoconocephalus* (Snyder et al. 2009; Frederick 2013). Species with derived pulse patterns are indicated by D (double pulse) or S (slow pulse rate) (see Greenfield 1990); the calls of unmarked species are in the ancestral state. *Asterisks* denote species for which the female call recognition mechanism has been previously identified. **b** Calls and call models of *N. ensiger*. *Top trace*: Oscillogram of two pulses of a male call. The sound pulses were generated both during the opening movement (small pulses, *arrow*) and closing movement (large pulses) of the forewings (Walker 1975). *Bottom trace*: call model (=control stimulus) used in this study. The temporal parameters are indicated. (*PD* pulse duration, *ID* interval duration, *PP* pulse period, *RT* rise time, *P* plateau, *FT* fall time) **c** Female phonotaxis score (mean ± SEM; $n = 7$) in response to the control stimulus (*c*) and a continuous sinusoid without amplitude modulation (*s*). Abbreviations of species names in A: mel *N. melanorhinus*; rob *N. robustus*; biv *N. bivocatus*; exc *N. exciliscanorus*; neb *N. nebrascensis*; cau *N. caudelianus*; ens *N. ensiger*; tri *N. triops*; spi *N. spiza*; sat *N. saturatus*; max *N. maxillosus*; ref *N. retusiformis*; pal *N. palustris*; pun *N. punctipes*; vel *N. velox*; aff *N. affinis*; OUT outgroup *Belocephalus davis*

Materials and Methods

We collected female *Neoconocephalus ensiger* as nymphs in Macon and Adair Counties, Missouri (USA) during July 2009. Males were collected as adults in Boone and Calloway County during August and September 2009. The population genetics of several *Neoconocephalus* species strongly suggests that no genetic divergence exists between these two localities (G. Ney, J. Schul unpublished). We identified this species after Froeschner (1954) and Walker (2008). The insects were maintained in same-sex groups in the laboratory on a diet of grasses cut in their habitat, wheat seedlings, and apples, at 20–25 °C and a light/dark cycle of 14/10 h. Females were monitored weekly for their final molt. Virgin females started to respond with phonotaxis 2 to 3 weeks after their final molt and were used in experiments after this time. Experiments were conducted in August and September of 2009.

The natural history of *Neoconocephalus* in general is given by Greenfield (1990) and of *N. ensiger* by (Faure and Hoy 2000).

Call Recordings

We recorded males in a temperature regulated chamber at an ambient temperature of 25 ± 2 °C with males placed individually in fiberglass screen cages (15 cm diameter and height). We recorded calls using a digital recorder (Marantz PM671, 16 bit, 48 kHz sampling rate) and an audio microphone (Audiotechnica ATR55, frequency response 30 to 18,000 Hz). The temporal call structure was analyzed using custom-made software with a temporal resolution of 0.125 ms.

Phonotaxis

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. Although the position of the animal on the sphere did not change as the sphere rotated under it, the intended direction and speed of the animal were recorded by a computer from the electronic circuitry controlling the sphere. 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. (1981); Schul (1998); Deily and Schul (2004).

Stimulation

Synthetic stimuli were generated using a custom-developed DA-converter/amplifier system (16 bit resolution, 250 kHz sampling rate). Signal amplitude was adjusted using a computer controlled attenuator and delivered via one of two loudspeakers (Motorola KSN1218C) mounted at a distance of 150 cm in the horizontal plane of the insect and separated by an angle of 105°. We measured signal amplitude using a ¼″ condenser microphone (G.R.A.S. 40BF) positioned 1 cm above the top of the sphere, and a sound level meter (Bruel and Kjaer 2231, Naerum, Denmark). All stimuli were presented at 80 dB peak SPL (re 20μPa).

The amplitude spectra of *N. ensiger* calls had highest amplitudes in a narrowband low-frequency component centered around 14.5 kHz, and the frequency components at ultrasonic frequencies were at least 20 dB softer than the low frequency band in the averaged spectra (Schul and Patterson 2003). To generate our stimuli, we added two sine-waves of 14 and 28 kHz (at – 18 dB relative to 14 kHz) and used the resulting sinusoid as carrier signal, to which we subsequently applied amplitude modulations.

Our standard call model (=control, see below) had the described simplified spectrum and consisted of pulses with 35 ms duration (including 15 ms rise and 5 ms fall time) repeated after silent intervals of 39 ms (Fig. 1). In preliminary experiments, this stimulus was as attractive as high quality recordings of natural calls, i.e. walking speed and orientation of female responses were comparable to those in response to the natural calls (data not shown).

In the experiments described below, we varied the temporal properties of the stimuli, while keeping the carrier signal constant. We performed four phonotaxis experiments to identify the temporal parameters used by females for call recognition.

Experiment 1 We tested whether female *N. ensiger* respond to signals without amplitude modulation (i.e. a continuous sinusoid), to determine whether *N. ensiger* uses the ancestral recognition mechanism of the genus (Deily and Schul 2004; Frederick 2013). Responses to the control stimulus (described above) were compared with responses to a continuous stimulus with same carrier frequency.

Experiment 2 We varied the pulse and interval duration independently of each other and evaluated the effect on female phonotaxis. Pulse durations were varied from 10 to 95 ms and interval durations from 2 to 179 ms. Specific combinations of pulse and interval durations were chosen to delineate the shape of the response field and to distinguish between recognition systems that attend to pulse rate, duty cycle, or absolute durations of pulses and/or intervals. For pulse durations from 25 to 95 ms, pulse rise and fall time were kept at 16 and 5 ms, respectively. Pulses with 10 and 15 ms duration had no plateau and rise times of 8 and 10 ms, respectively, with the balance covered by the fall time. We tested a total of 37 stimuli, with a sample size of 7–10 each.

Experiment 3 Because the shortest pulses in experiment 2 (10 and 15 ms) had shorter rise times than the standard signal, we tested a series of stimuli that varied in rise time to determine whether any change in responsiveness toward short pulses in experiment 2 could be explained by the shorter rise times. In this experimental series, all stimuli had the same pulse and interval duration as the standard signal (35 and 39 ms, respectively). The pulse rise time was varied from 6 to 21 ms (in steps of 5 ms) and the plateau time adjusted to keep the pulse duration constant; the fall time was 5 ms in all stimuli.

Experiment 4 The final experiment tested the attenuation depth required for the recognition of the temporal pattern. We inserted a standard pulse (35 ms duration) centered into the silent interval of the control stimulus, resulting in a pulse train with 35 ms pulse duration and 2 ms silent intervals. This stimulus was not attractive in the response field representing the results of experiment 2. We attenuated the amplitude of every other pulse from 0 to −18 dB relative to the un-manipulated pulses (Fig. 4).

Experimental Protocol

The experimental protocol is described fully in Schul (1998) and Bush et al. (2002). Briefly, each stimulus was presented sequentially from the two loudspeaker positions for approximately 1.5 min each. Each insect was initially presented with the control stimulus, followed by two test stimuli, then the control, etc., until all stimuli in the series had been presented. We imposed a 1-min period of silence between the presentation of the different stimuli. Individual females were typically presented with 4–7 test stimuli and 3–4 controls per series. We used at least two different sequences of test stimuli presentation for each experiment to control for sequence effects. We could not detect systematic differences between the two series in any of the experiments.

Data Analysis

The two presentations of each stimulus were added after rotating the second presentation by 105° (= the separation between the speakers). We quantified female responses to the test stimuli relative to their responses to the two surrounding control stimuli as a "phonotaxis score," which represents the attractiveness of the stimulus (Schul 1998). In short, the relative walking speed (= speed during test/speed during control), the orientation of the response (= relative vector length) and the cosine of the deviation of the mean walking direction during the test from the direction during the control were multiplied. Scores range from approximately +1 (indicating perfect phonotaxis) to −1 (perfect negative phonotaxis), with values close to 0 indicating no response. The phonotaxis scores to each stimulus were calculated relative to the average of the controls tested immediately before and after the stimulus. A detailed description of the phonotaxis score is given in Schul (1998). We present phonotaxis scores as mean +/− standard error of the mean (SEM).

Female responses were considered significant if two criteria were met: (i) the mean phonotaxis score was significantly greater (Mann-Whitney u-test, $P < 0.05$; Zar 1984) than the mean phonotaxis score in response to silence (-0.06 ± 0.12, $n = 10$) measured in the same way as the other stimuli, and (ii) the average response was at least 50 % of the response to the standard call model. Since the second criterion was always much more stringent than the first, we do not present the results of the u-tests in the text. Note that the application of significance criteria merely emphasizes the relative attractiveness of stimuli and is not meant to classify stimuli as 'recognized' or 'not recognized' (for a detailed discussion see Bush et al. 2002). Employing a significance criterion is useful for visualizing the shape of the stimulus field that elicits strongest responses (e.g. Fig. 2).

We used the Friedman test as a non-parametric repeated-measures ANOVA (Zar 1984) using an MS-Excel macro (Pace 2013) to test the importance of pulse rise time and modulation depth on female phonotaxis scores.

Results

Temporal Pattern of Male Calls

Male calls of *N. ensiger* consist of monotonously repeated sound pulses separated by silent intervals (Fig. 1b). Each sound pulse comprises a short, low amplitude pulse produced during the opening movement of the forewings (arrow in Fig. 1b), and a longer, louder pulse produced during the closing movement (Walker 1975). It is not always possible to determine unequivocally the end of the opening pulse. The duration of the pulse (including opening pulse) was 38.9 ± 8.5 ms (mean ± SD, $n = 12$ males, all measurements at 25 ± 2 °C), of which 29.2 ± 6.13 ms consisted of the closing pulse ($n = 6$). The mean pulse period was 83.1 ± 7.8 ms, equivalent to a pulse rate of 12 Hz. The pulse period had remarkably high variability both among and within males. At 25 °C, pulse periods among males ranged from 68.9 to 92.9 ms and pulse periods within some individual males differed by a similar amount. To confirm this variability, we recorded an additional 21 males that had not heard another calling male for several

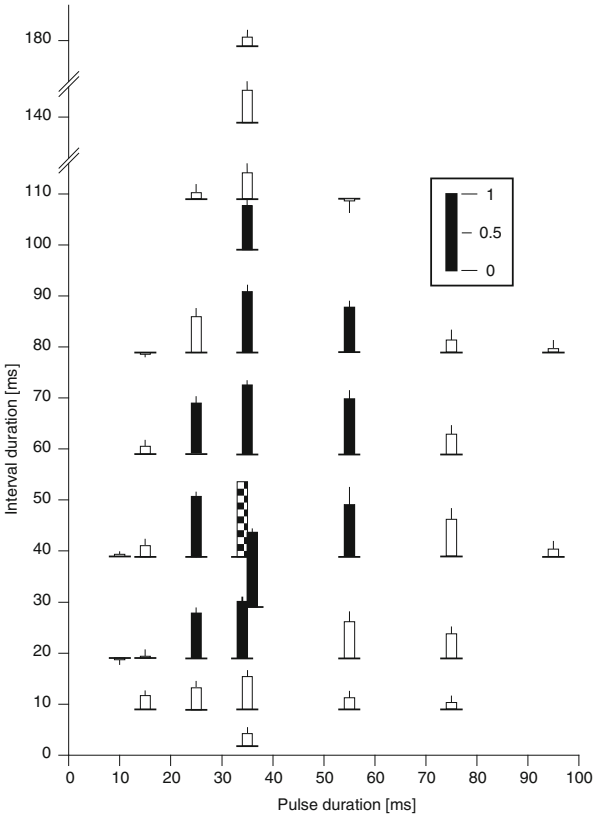

Fig. 2 Importance of pulse duration and interval duration for phonotactic responses of *N. ensiger*. The *bars* indicate the phonotaxis score (mean ± SEM, $n = 7–10$) for the respective parameter combination (see inset for the scale of the phonotaxis score). The baseline of each bar is positioned on the interval duration. *Filled bars* indicate significant responses and white bars non-significant responses (see methods for significance criteria). The *checkered bar* represents responses to the control stimulus

hours before or during the recording. We analyzed the pulse periods for 250–350 pulses for each male from a sample taken several minutes after the beginning of the calling bout. The measurements of pulse period in this larger sample (83.9 ± 5.4 ms, range 75–95 ms) were similar to those of the original sample (vs 83.1 ± 7.8 ms).

The temporal pattern of *N. ensiger* males described here was comparable to that reported by Faure and Hoy (2000).

Female Phonotaxis

In the first experiment we tested whether female *N. ensiger* respond to signals without amplitude modulation (i.e. a continuous sinusoid), to determine whether *N. ensiger* uses the ancestral recognition mechanism of the genus (Deily and Schul 2004; Snyder et al. 2009). Responses of female *N. ensiger* to the control stimulus were high with a mean phonotaxis score (PS) of 0.92 ± 0.04 (mean ± SEM; $n = 7$; Fig. 1c), but no significant responses occurred to the continuous sinusoid (PS = 0.01 ± 0.024, $n = 7$,

Fig. 1c). The difference between the responses is statistically significant (Mann-Whitney U test, U = 0, $p < 0.01$). Thus, the amplitude modulation of the male call was necessary for female call recognition.

In experiment 2, we varied the pulse and interval duration independently of each other and evaluated the effect on female phonotaxis. The resulting shape of the response field identifies the temporal parameter(s) of the stimuli evaluated during call recognition. Significant responses occurred only for pulse durations of 25–55 ms (Fig. 2), with strongest responses at 35 ms pulse duration. Towards shorter pulse durations responses decreased rapidly, while the decline towards longer pulse durations was less steep. Interval durations of less than 19 ms did not evoke significant responses. With increasing interval durations above 60 ms, female responses declined. This decline was steeper for pulse durations of 25 and 55 ms, while at 35 ms pulse duration significant responses still occurred at an interval duration of 99 ms (Fig. 2).

Because the shortest pulses in Fig. 2 (10 and 15 ms) had shorter rise times than the standard signal, experiment 3 included a series of stimuli that varied in rise time to determine whether the drop in responsiveness toward short pulses in Fig. 2 could be explained by the shorter rise times. Phonotaxis scores (Fig. 3) were close to 1 for rise times of 16 and 21 ms and the responses declined towards shorter rise times (0.74 ± 0.06 at 11 ms, 0.54 ± 0.12 at 6 ms). Note the variability of the responses at short rise times, with phonotaxis scores ranging from approximately 0 to 1 (Fig. 3). Non-parametric repeated measures ANOVA (Friedman test) indicated that pulse rise time had a significant effect on female responses ($n = 8$, $\chi^2(5) = 10.95$, $P < 0.02$). However, the mean phonotaxis score for each of the stimuli was above 0.5. Taken together, these data indicate that although short rise times decrease the attractiveness of the stimuli, the effect is not strong enough to account for the low attractiveness of the short pulses in Fig. 2.

The final experiment tested the attenuation depth required for the recognition of the temporal pattern (Fig. 4). When the attenuation was 3 dB or less, female responses were not significant with mean phonotaxis scores below 0.3. Attenuations of 6 dB and higher

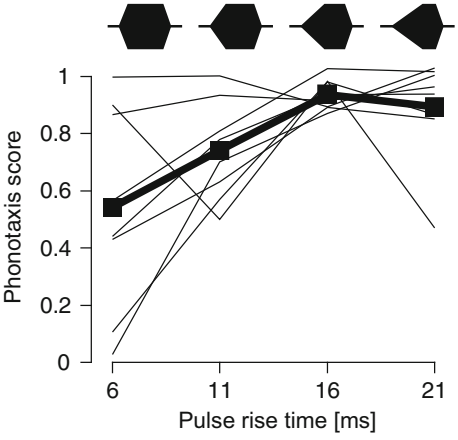

Fig. 3 Phonotaxis score of seven females (*thin lines*) and mean phonotaxis score ($n = 8$, *thick line*, *filled squares*) in response to stimuli with variable pulse rise time. The pulse duration was 35 ms and the interval duration 39 ms for all stimuli. The plateau time was adjusted to keep the pulse duration constant (*top trace*)

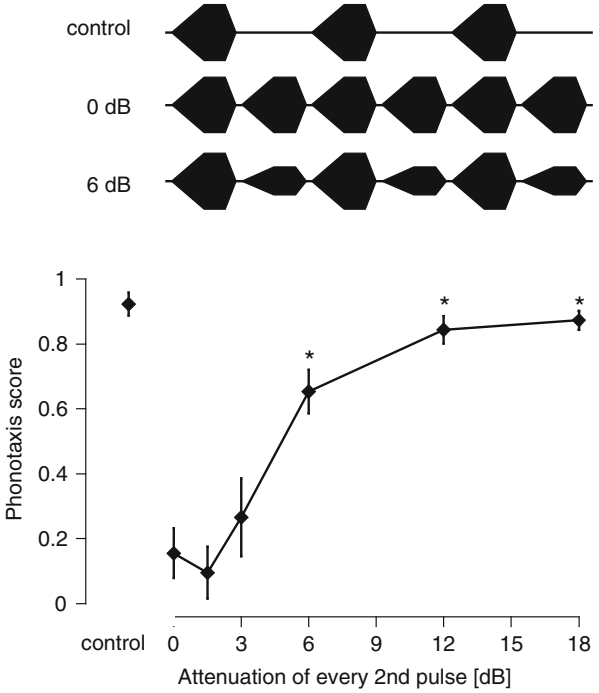

Fig. 4 Amplitude modulation depth required for female phonotaxis. (*top*) Oscillograms illustrating the control stimulus (35 ms pulse duration, 39 ms interval duration) and test stimuli with 0 and 6 dB attenuation of every second pulse. (*bottom*) Phonotaxis scores (mean ± SEM, $n = 7$ each) of female *N. ensiger* to the control stimulus and call models in which the attenuation of every other pulse was varied from 0 to 18 dB. *Asterisks* indicate significant responses. For further description of the stimuli, see text

resulted in significant responses; at 12 and 18 dB phonotaxis scores were comparable to the control stimulus. Non-parametric repeated measure ANOVA indicated a significant effect of the attenuation on female responses ($n = 7$, $\chi^2(5) = 29.94$, $P < 0.001$).

Discussion

We tested here the call recognition mechanism of *N. ensiger* females, a species with an exceptionally slow pulse rate for *Neoconocephalus*. We found that females require the temporal structure of the call, evaluating both the pulse and interval duration. A modulation depth of 6 dB of the pulse pattern was sufficient for females to recognize the conspecific pattern.

In the response field that resulted from independently varying pulse and interval durations (Fig. 2), the limits of the attractive range (i.e. the range with significant responses) were largely parallel to the X- and Y-axis, indicating that both the duration of pulse and interval are important temporal cues. Note that the response at 55 ms/19 ms pulse/interval duration approached significance (PS = 0.45 ± 0.12). Neither pulse rate nor pulse duty cycle, which follow diagonals in the response field, were important parameters for female selectivity in this species. Responses dropped sharply when pulse duration fell below 25 ms or when interval duration fell below 19 ms. The short pulses

(10 and 15 ms duration) tested in Fig. 2 also had shorter rise-times than the standard pulses. Although attractiveness declined with decreasing rise time (Fig. 3), even the shortest rise-time tested (6 ms), which was shorter than that of the 10 ms pulses, resulted in a significant response with an average phonotaxis score of 0.54. The short rise times, therefore, cannot fully account for the low attractiveness of stimuli with pulse durations of 10 and 15 ms and phonotaxis scores near zero (Fig. 2). Thus short pulse duration itself appears to render the stimulus unattractive.

The drop in response strength toward long interval durations was considerably more gradual than the drop toward short pulse or interval durations. The lack of a sharp border between attractive and unattractive stimuli in this part of the field suggests that the gradual decrease in responses is not caused by a negative influence of long intervals per se, but rather by the decline in stimulation (i.e., fewer attractive pulses) as intervals are elongated.

The temporal pattern of insect calls typically functions to encode the species identity of the caller (Searcy and Anderson 1986). The selectivity of the receiver then puts pressure on the callers to maintain low variability in the temporal pattern, both within and between males (Gerhardt and Huber 2002). As a result, call characters used for species identification are classified as static parameters (Gerhardt 1991) with low within male variation. Call recognition in *N. ensiger* was surprisingly permissive, particularly for variation in interval duration (Fig. 2). Phonotaxis scores in response to call models with 35 ms pulse duration were uniformly high for interval durations between 29 and 79 ms. Accordingly, the temporal pattern of male calls is unusually variable both among and within males. Females responded to pulse rates >22 Hz (Fig. 2, pulse/interval duration of 25/19 ms) The permissiveness of female preferences and the variability of male calls are unlikely to result in heterospecific matings, however, given that the pulse rate of the *N. ensiger* call is so much slower than that of congeners with overlapping distribution (Walker 2008; Whitesell 1969), and syntopic insects of other genera call during the day instead of at night.

The permissiveness of female preferences potentially provides male *N. ensiger* the opportunity to adjust their call timing to be in a favorable position relative to other males' calls (Greenfield and Schul 2008). In *N. spiza*, females prefer the leading of two overlapping male calls, and males are competing for this leader position (Greenfield and Roizen 1993). Recent studies of male calling behavior in *N. ensiger* revealed short and long term influences of interactions with other calling males; in addition, pulse rates of individually calling males often increased or decrease over a time span of 5–15 min. A subsequent publication (M.A. Murphy, N. Thompson, J. Schul, in prep.) will address the complex factors underlying the high variability of male calls in *N. ensiger*.

The pulse pattern of *N. ensiger* is an order of magnitude slower than that of most *Neoconocephalus* species, which typically have pulse periods of 4–10 ms (Greenfield 1990; Deily and Schul 2004). The second order time structure (i.e. verses or chirps) used by some *Neoconocephalus* species is in turn an order of magnitude slower than the pulse pattern of *N. ensiger*, with chirp or verse periods in the order of 300–2000 ms (Meixner and Shaw 1986; Walker and Greenfield 1983). Species with versed calls use two separate call recognition mechanisms, one tuned to the pulse pattern and the other tuned to the verse pattern (e.g. *N. nebrascensis*, Deily and Schul 2009). Given that the pulse pattern in *N. ensiger* falls midway between the typical pulse rates and verse rates of the genus, it raises the question of whether pattern recognition of *N. ensiger*

corresponds to the faster pulse recognition or to the slower verse recognition of other *Neoconocephalus* species. Female *N. nebrascensis* require an attenuation depth of >18 dB to recognize the verse pattern (Deily and Schul 2009). In contrast, *N. ensiger* required less than 6 dB to detect the pulse pattern of the call (Fig. 4), which is similar to the attenuation depth required for pulse recognition in other katydids (e.g. 2–4 dB *Tettigonia cantans*, Schul and Fritsch 1999). These results align more closely with pulse pattern recognition than with verse recognition, though further exploration of neuronal mechanisms are needed to determine whether the pulse pattern recognition is homologous between *N. ensiger* and other Tettigoniids. That *N. ensiger* does not require the 18 dB attenuation required by *N. nebrascensis*, though, confirms that this surprisingly high attenuation requirement is not standard across *Neoconocephalus*.

Calling *N. ensiger* males are often close enough together for females to be in hearing distance of several males simultaneously. Females therefore likely encounter less than 6 dB separation among males. Males might adjust the timing of their calls (e.g. by synchronizing pulses) to remain attractive for females in dense choruses.

In the genus *Neoconocephalus*, male calls with a single fast pulse rate (>100 Hz) represent the ancestral state (Fig. 1; Snyder et al. 2009; Frederick 2013). Females of species with such fast pulse rates respond to calls that do not contain intervals longer than a few ms (Deily and Schul 2004, 2009). Derived call patterns and derived call recognition mechanisms have arisen multiple times within the genus. Derived call patterns include grouping the pulses into pairs, producing discontinuous (i.e., versed) calls, and significantly reducing the pulse rate (as shown here for *N. ensiger*) (Greenfield 1990). Previously described call recognition mechanisms include the ancestral mechanism described above, two forms of pulse rate recognition (*N. bivocatus*, Deily and Schul 2004, and *N. triops*, Beckers and Schul 2008) that differ in their neural basis (Triblehorn and Schul 2009; Schul et al. 2013), and recognition of two alternating pulse periods within a call (*N. affinis*, Bush et al. 2009). Our results illustrate that *N. ensiger* uses a mechanism in which both pulse and interval duration are evaluated, representing the fourth derived mechanism known in this genus. These four species appear at disparate positions in the *Neoconocephalus* phylogeny (Fig. 1; Snyder et al. 2009; Schul et al. 2013), suggesting that the derived call recognition mechanisms evolved independently in each of the four species. Theoretical analyses revealed that only small changes may be required to result in large phenotypic differences in call recognition (Hennig et al. 2014).

Although *N. ensiger* is the only *Neoconocephalus* known to evaluate the duration of pulses and intervals, this mechanism has been described in other diverse systems, e.g. katydids (*Tettigonia viridissima*, Schul 1998), crickets (*Teleogryllus commodus*, Hennig 2003 and *T. Leo*, Rothbart and Hennig 2012), and treefrogs (*Hyla versicolor*, Schul and Bush 2002). The signals recognized by these species have periods in the range of 40–100 ms. In contrast, related species with faster calls (e.g. *Tettigonia cantans*, *T. caudata*, *Gryllus bimaculatus*, *H. chrysoscelis*) use call recognition mechanisms based on pulse rate or duty cycle (Schul 1998; Schul and Bush 2002; Weber et al. 1982; Doherty 1985). The genus *Neoconocephalus* adheres to this pattern: the call period of *N. ensiger* is approximately 90 ms and females evaluate pulse and interval durations, while the species with faster calls use other recognition mechanisms. This pattern suggests that signal parameters and possibly neural constraints have a strong influence on the evolution of call recognition mechanisms, while phylogenetic

constraints and relationships may play a lesser role in determining the shape of novel recognition mechanisms.

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