Pattern recognition and call preferences in treefrogs (Anura: Hylidae): a quantitative analysis using a no-choice paradigm

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Studies of mate attraction have traditionally employed one of two experimental methods: choice tests in which female preferences from among two or more signals are tabulated, or single-stimulus tests in which the female response to one signal is quantified. Choice tests have long been preferred for examining mate attraction in anurans. Inspired by Wagner's (1998, *Animal Behaviour*, 55, 1029–1042) discussion of the strengths and weaknesses of the two experimental approaches, we used a single-stimulus design to examine mate attraction in two sibling species of treefrogs (*Hyla versicolor* and *H. chrysoscelis*). We quantified female responses based upon the relative time required to approach signals varying in pulse rate, pulse rise time and pulse number. The data were used to generate response functions providing a quantitative measure of female attraction to the stimuli. Comparisons with data from choice experiments reveal broad similarities, as well as properties of female responses that had not been detected with choice tests. The results are discussed with regard to female selectivity for call parameters that are likely to mediate sexual selection and homospecific pairing.

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The preferences of females during phonotaxis have been studied extensively in insects and frogs in the context of mate attraction. Two methods are used to assess such preferences: (1) choice tests, in which responses to one of two or more alternative signals are tabulated, or (2) single-stimulus tests, in which female responses to one signal are measured.

Single-stimulus designs are commonly used in insects, with the magnitude of female responses measured either on a walking compensator (e.g. Weber et al. 1981; Doherty 1985; Schul 1998) or during tethered flight (Pollack & Hoy 1981; Pollack et al. 1984). Depending on the apparatus employed, the experimental set-up is described as either closed-loop or open-loop. Under closed-loop conditions, the insect is able to orient its body towards the signal, and response magnitude is quantified based on the speed and direction of phonotaxis (e.g. Schul 1998). Under open-loop conditions, the body-axis of the insect is fixed, and a directional vector is calculated based on the insect's steering movements (e.g. Doolan & Pollack 1985; Stabel et al. 1989). Although these methods have proven highly successful for quantifying female responses in insects, to our knowledge no one has used these methods with anurans, whose mode of locomotion may be unsuitable for such an apparatus.

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Female anurans are normally tested in an arena and given a choice of alternative signals. Although several authors have used single-stimulus presentations to assess call recognition in anurans (e.g. Gerhardt 1982; Ryan & Rand 1993, 1999), the percentage of females that responded (i.e. 'recognized' the signal in Ryan & Rand's terminology) was tabulated rather than a quantitative measure of response strength. As a result, single-stimulus designs have had limited power in describing female responses in anurans, and researchers have thus relied almost exclusively on choice experiments. As described in detail by Wagner (1998), the dichotomous scoring of choice experiments has various pitfalls. For example, only directional preferences can be inferred if females prefer one of a single pair of stimuli. In addition, variation in preferences among females may be difficult to detect using choice experiments. Differences among individuals or taxa in sampling behaviour can also confound the interpretation of choice tests; females that engage in repeated sampling may appear to display weaker preferences than females that sample each male only once. For these reasons, an additional quantitative assessment of female responses during single-stimulus situations is desirable.

We describe here a method for quantitative scoring of female responses in anurans using a single-stimulus design. The analysis relies on the relative response time of the frog as she approaches the loudspeaker. The results

obtained with this method are compared with preference functions derived from choice experiments.

We studied two sibling species of treefrogs, *Hyla chrysoscelis* and *H. versicolor*, the phonotactic behaviour of which has been described in detail (review in Gerhardt 2000). These species occur syntopically in many localities, and females use acoustic signals alone to identify conspecific males. *Hyla chrysoscelis* relies on the differences in pulse rates to discriminate conspecific from heterospecifics calls (*H. chrysoscelis*, 50 pulses/s; *H. versicolor*, 20 pulses/s at 20 °C, for the populations used in this study): calls with the conspecific pulse rate are strongly preferred to calls with higher or lower pulse rate (Gerhardt & Doherty 1988). In *H. versicolor*, the pulse rate also constitutes an important cue enabling females to distinguish between conspecific and heterospecific calls. In this species, however, the pulse rise time, which is slower in *H. versicolor* than in *H. chrysoscelis*, is also an important call parameter for mate recognition (Gerhardt & Doherty 1988; Diekamp & Gerhardt 1995; Gerhardt & Schul 1999). Intraspecific choice has been studied intensively in this species. Females prefer longer calls (i.e. calls with a higher pulse number) to shorter calls (Gerhardt et al. 2000), and there is evidence that call duration is indicative of the genetic quality of the male (Welch et al. 1998).

We obtained response functions to calls varying in pulse rate for both species. For *H. versicolor*, we also investigated the influence of pulse rise time and pulse number on female response strength. The results are largely consistent with the findings of earlier choice experiments, but also reveal properties of female responses that had not been detected previously.

METHODS

We collected *H. versicolor* of the northwestern mitochondrial lineage (Ptacek et al. 1994) from breeding sites in the Basket Wildlife Area and Three Creeks State Park in Boone County, Missouri, U.S.A. Although currently allopatric, it is likely that these populations of *H. versicolor* are descended from populations that interacted with *H. chrysoscelis*, which occur within 30 km of these two sites (Ptacek et al. 1994). *Hyla chrysoscelis* were collected from a syntopic population in Phelps County, Missouri. To ensure receptivity, we collected females in amplexus, removed the males, and held the females at approximately 2 °C until 30 min before use in an experiment, at which time they were acclimated in an incubator until the cloacal temperature reached 20 ± 1 °C. Between trials, the females were kept in the incubator to ensure a constant body temperature, which was checked periodically throughout the experiments. All individuals were released at their breeding sites at the end of the experiments. Experimental procedures were evaluated and approved by the Laboratory Animal Care and Use Committee of the University of Missouri, Columbia.

Phonotaxis experiments took place in a temperature regulated, single-walled, sound-proof chamber (Industrial Acoustics) at 20 ± 1 °C. The walls, ceiling, and other sound-reflecting objects in the chamber were covered

with anechoic foam (Illbruck, 10 cm thick), and the floor outside of the arena was covered with thick carpet or anechoic foam.

The phonotaxis arena was circular and measured 2 m diameter; the walls (height 50 cm) consisted of hardware cloth covered with black fabric and placed on a RESOPAL® floor. The loudspeaker (Analog-Digital Systems 200) was placed at floor level, on the outside of the arena wall, facing the centre of the ring. Although our measurements indicate that the sound field was uniform, we varied the position of the loudspeaker throughout the experiments. This procedure not only controlled for acoustic irregularities we might have missed, but also for any inherent directional biases in individual frogs. The acoustically transparent release box, which could be opened remotely from outside of the chamber, was positioned in the centre of the arena. An infrared light source and video camera were suspended directly above the ring, and enabled us to monitor the behaviour of the frogs from outside the chamber. Because the IR light source was centrally positioned and the walls of the arena were uniform, no optical cues were available for the frogs' orientation.

Stimulation

We generated synthetic advertisement calls using a custom made DA-converter system (12 bit resolution, 50 kHz sampling rate). The signals were amplified and their amplitude was controlled with the aid of a computer. We monitored the amplitudes of the signals with a Larson Davis sound level meter (Model 720) and adjusted fast root mean square values to 85 dB SPL (re 20 µPa) at the position of the release box. All stimuli used in this study consisted of two phase-locked sinusoids of 2.2 and 1.1 kHz (at −6 dB relative to the amplitude of the 2.2-kHz component), which simulates the spectral structure of the natural calls of both species. The computer-synthesized signals had a signal-to-noise ratio of at least 40 dB.

For *H. chrysoscelis*, the control stimulus (i.e. the standard synthetic signal modelled after a typical conspecific call, which in choice tests was found to be as attractive as representative prerecorded calls; Gerhardt & Doherty 1988) consisted of 45 pulses with 10 ms duration, repeated at 50 Hz (=pulses/s), that is, the silent interval between the pulses was 10 ms. Each pulse had an inverse exponential rise time and an exponential fall time, each equal to 50% of the pulse duration. In this species, we tested one experimental series in which the pulse rate was varied between 19 and 120 Hz, while the pulse duty cycle was held constant at 50% and pulse shape was held constant as in the control (Fig. 1a). Pulse number per call was varied to maintain the call duration at approximately 890 ms.

For *H. versicolor*, the control stimulus (i.e. the model of the conspecific call) consisted of 18 pulses with 25 ms duration, repeated at 20 Hz, that is, the silent interval between the pulses was 25 ms. Each pulse had linear rise and fall times, with the rise time equal to 80% of the pulse
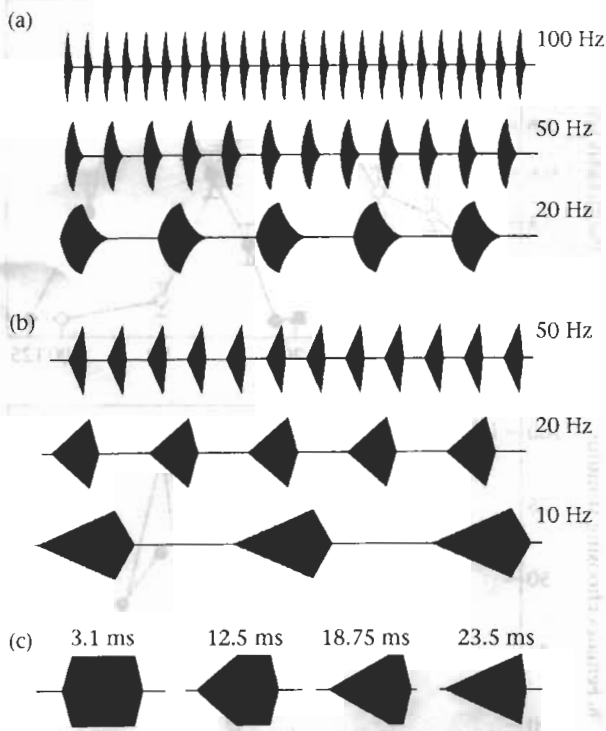
Figure 1. Oscillograms of song models used, illustrating the parameter ranges tested in the different experimental series. Variation of pulse rate in *Hyla chrysoscelis* (a) and *H. versicolor* (b) and variation of pulse rise time in *H. versicolor* (c).

duration, and the fall time, 20%. As for tests of *H. chrysoscelis*, the first experimental series consisted of signals varying in pulse rate from 5 to 100 Hz, while holding pulse duty cycle constant at 50%. In this experiment, the pulse rise time was always 80% of the pulse duration, the fall time 20% (Fig. 1b). Pulse number per call was adjusted to maintain call duration at approximately 875 ms, as in the control.

In a second series of *H. versicolor* signals, we tested the influence of pulse rise time on female responses. Here, pulse duration and pulse rate were held constant at 25 ms and 20 Hz (as in the control stimulus), but the linear pulse rise time of the pulses was varied (values used: 3.13 ms=12.5% of pulse duration, 6.25 ms=25%, 9.38 ms= 37.5%, 12.5 ms=50%, 18.75 ms=75%, 23.5 ms= 94%). The duration of the linear fall time was 2.5 ms in all stimuli except for the 23.5-ms rise time, where it was 1.5 ms (Fig. 1c). All stimuli of this series consisted of 18 pulses.

The third series of test stimuli was designed to examine the effect of call duration on female response in *H. versicolor*. The stimuli had the same pulse shape and pulse rate (20 Hz) as the control, but the number of pulses per call ranged from three to 54. Call duration therefore varied from 125 ms to 2675 ms, which is three times the duration of an average male call (Gerhardt et al. 1996).

The call period (i.e. duration from one call beginning to the next) was 5.5 s for all experimental series except when varying pulse number. In this series, call period increased from 5.1 s for the three-pulse call to 7.0 s for the 54-pulse call.

The Experimental Protocol

At the start of each trial, we placed the female in the acoustically transparent release box in the centre of the arena. After four repetitions of the stimulus, we removed the lid of the release box and measured the time until the female touched the wall of the arena, directly in front of the speaker. We recorded a score of No Response if the female remained in the release box for 3 min, left the release box but did not reach the speaker within 5 min, or arrived at the wall of the arena in the semicircle opposite the speaker.

Each female began an experimental session with two control trials in which she was presented with the model of the conspecific call (control stimulus, see above). The first of these control trials was intended to acclimate the female to the arena; the data from this trial were discarded. Her time to respond to the second of the control trials was used as a control time in the data analysis. Following the initial two control trials, we presented each female with two different test trials, another control trial, two different test trials, a control trial, and so forth, until the female's response to the control trial began to weaken, as evidenced by an increasing response time. At this point we removed the female from the experiment and discarded her previous two test scores. In order to abolish possible memory effects between trials, we returned each female to the incubator for a minimum of 5 min between trials. We presented the test trials in random order, which differed among females, until each test stimulus had been presented to 10 different females.

Calculation of the Phonotaxis Score

We calculated a phonotaxis score for each trial completed by each female. The score is the relative time required during each trial to reach the loudspeaker. It was calculated as the ratio of her response time during the control trials to her response time during the test trial ($t_{control}/t_{test}$). The control time was the average of the control trials immediately before and after the test trial of interest. A phonotaxis score of 1, therefore, indicates that the female approached the test stimulus with the same response time as the average of the two surrounding control stimuli; a score of less than 1 indicates that she approached the test stimulus more slowly than she approached the control. A phonotaxis score of zero was assigned to all trials in which the female received a No Response (see above). Note that the phonotaxis score can exceed 1 if the female approached the test stimulus more rapidly than the control stimulus.

Response times of individual females to the control did not vary systematically during the course of an experimental session. To check for this, we plotted the phonotactic score for all control trials by dividing each control trial by the mean of control trials of that female (Fig. 2). Although there was considerable variation within the controls of individual females, there were no tendencies for response times to change over the course of any of the experimental series. Even if gradual changes of response times were to occur (e.g. due to fatigue), our analysis
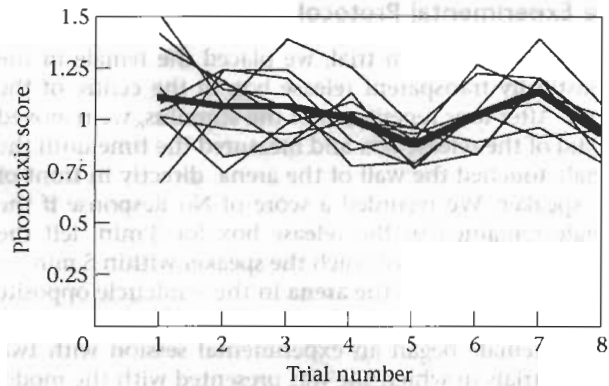
Figure 2. Phonotaxis scores for the control trials of each female of *H. versicolor* used in the experiment varying pulse number. Scores were calculated by dividing each control trial by the mean control trial of that female. The bold line represents the mean of all females. There were no trends in the response times to the controls for any of the females.

would be unaffected, since we used the control trials surrounding each test trial for normalization.

The mean response time to the conspecific control was 53.7 ± 13.8 s (grand mean $\pm$ SD for $N=73$ females, 5–13 trials/female) for *H. versicolor* and 56.8 ± 11.8 s ($N=20$, 5–15 trials/female) for *H. chrysoscelis*.

RESULTS

Because the tests measured response strength to a single stimulus rather than a preference between different stimuli, we prefer to use the term 'response function' to describe the data from single-stimulus tests, and reserve the term 'preference function' for data obtained in situations in which females were allowed to choose among two or more stimuli. Of course the difference in response strength can be used to predict preferences (see Discussion).

In the first experimental series, we measured the female response function based on pulse rate (Fig. 3a). In *H. versicolor*, high scores occurred only at 20 Hz (i.e. the mean pulse rate of the conspecific call), while higher and lower rates resulted in lower response values. For higher pulse rates (50 and 100 Hz) phonotactic scores were close to zero (i.e. females did not reach the loudspeaker), whereas for lower rates (down to 5 Hz) the scores remained well above zero. In *H. chrysoscelis*, we measured strongest responses not only at the mean conspecific pulse rate of 50 Hz, but also 10 Hz above this value (Fig. 3a). For higher and lower pulse rates, the roll-off was fairly symmetrical, reaching zero at both 120 and 25 Hz. In both species, the phonotactic scores for the hetero-specific pulse rate (*H. versicolor* responding to 50 Hz, *H. chrysoscelis* to 20 Hz) was close to zero. Thus, the calls of heterospecific males were not at all attractive and the females did not approach them.

Comparison with previously published data from choice experiments revealed general agreement, but also subtle differences (Fig. 3b). The choice experiments indicated asymmetrical preference functions for both species,
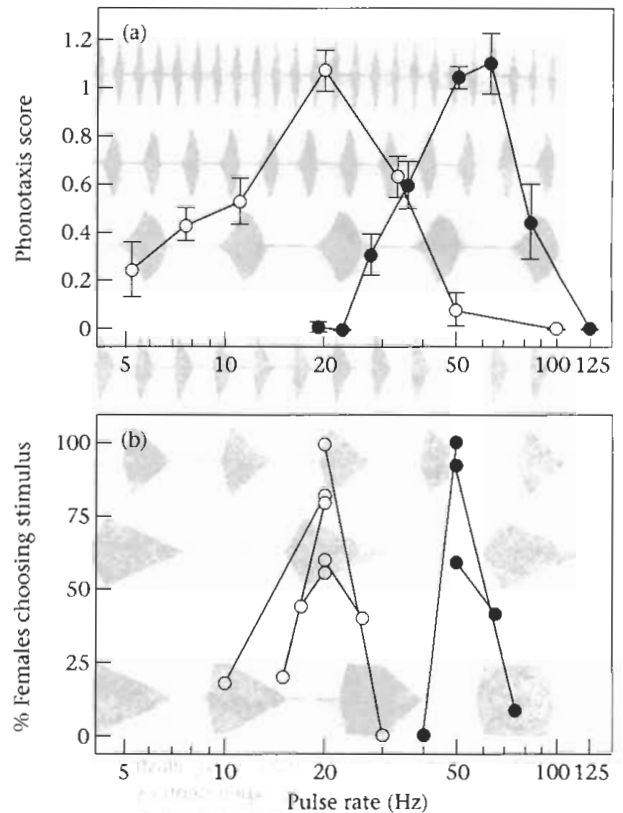

Figure 3. (a) Female responses to song models varying in pulse rate in the single-stimulus experiment. Each point represents the mean ($\pm$SE) phonotaxis score of 10 females. The pulse rates of the standard calls used in the control trials were 20 Hz (*H. versicolor*: ○) and 50 Hz (*H. chrysoscelis*: ●). (b) Percentage of females choosing each stimulus in two-choice experiments (from Gerhardt & Doherty 1988; H. C. Gerhardt, unpublished data). The points connected by each line represent the two stimuli presented in each choice presentation.

with steeper roll-offs as the pulse rate approached the heterospecific calls. Our data for *H. versicolor* is in good agreement with the choice experiment results. For *H. chrysoscelis*, however, our single-speaker results indicate a more symmetrical function than was found in the choice experiments (see Discussion).

Choice experiments suggest that the pulse rise time is an important cue for female selectivity in *H. versicolor*, and that this property can override the preference for pulse rates when the difference in pulse rate is relatively small (see Introduction). The influence of pulse rise time on female responses in our single-stimulus situation is shown in Fig. 4. Phonotactic scores were high (>0.8) for rise times longer than 9 ms, but for rise times shorter than 9 ms response magnitude dropped sharply. Even for the shortest rise time tested (3.2 ms), however, the mean score was above 0.4, indicating that this stimulus was still reasonably attractive.

A third factor that is known to influence call attractiveness in *H. versicolor* in choice situations is call duration (see Introduction). In our single-speaker tests, phonotactic scores increased logarithmically with increasing call duration (Fig. 5). This trend occurred over the complete
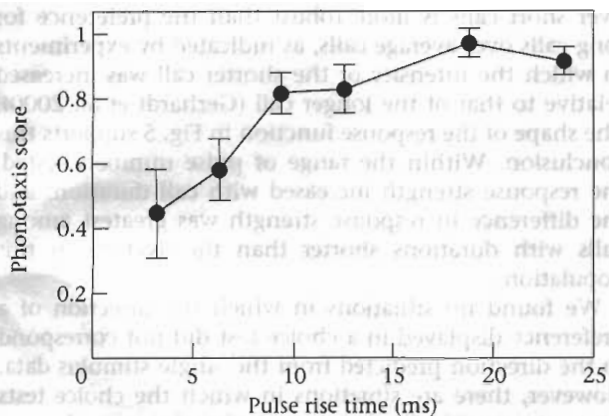
Figure 4. Phonotaxis scores of *H. versicolor* females to song models varying in pulse rise time. Each point represents the mean (±SE) of 10 females. The rise time of the standard call used in the control trials was 18 ms.
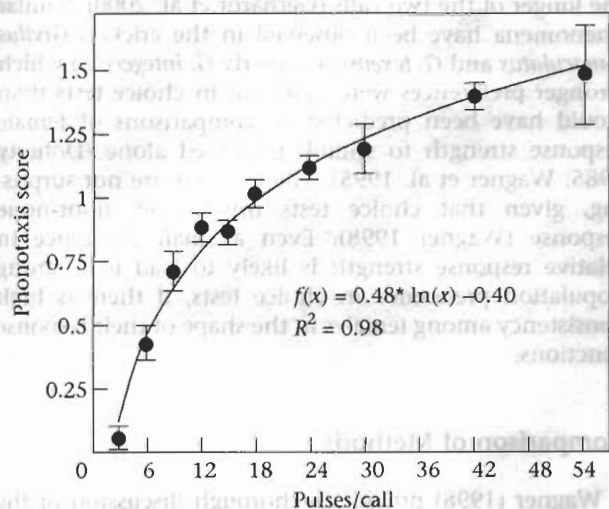

$$f(x) = 0.48 \ast \ln(x) - 0.40$$
$$R^2 = 0.98$$

Figure 5. Phonotaxis scores of *H. versicolor* females to song models varying in pulse number. Each point represents the mean (±SE) of 10 females. A logarithmic curve is fitted to the data. The standard song model used in the control trials had 18 pulses/call.

range tested from call durations of 125 ms (three pulses/call) to 2675 ms (54 pulses/call), which is three times the duration of the control stimulus (18 pulses/call). The logarithmic form of the response function means that each doubling of call duration resulted in a roughly equal increase in response strength, even well beyond the mean natural call length.

DISCUSSION

Following Wagner's (1998) recommendations regarding experimental designs for measuring female mating preferences, we used a single-stimulus design to construct quantitative response functions in female treefrogs. Our response functions are based on the relative response time of phonotaxis to signals varying in pulse rate, pulse rise time and pulse number. Comparisons of our single-stimulus results with earlier data based on

choice experiments reveal strong similarities and some important differences.

Signal Recognition as a Graded Response

Our approach serves to emphasize that phonotaxis towards an acoustic stimulus reflects a graded response that varies over a range of stimulus variations, rather than being governed by a simple categorization of 'recognized' versus 'not recognized'. Within an individual female, the rate at which she approaches the speaker reflects the strength of her attraction to the signal. Response strength varies on a continuous scale ranging from no attraction, in which the female does not approach the speaker, through graded responses in which the speed of approach gradually increases as the signal parameters approach those of the conspecific call. Variation of some call parameters (e.g. pulse number/call) may even identify 'supernormal' stimuli that are more attractive and elicit more rapid phonotaxis than the conspecific call. The resulting response functions thus provide a quantitative measure of the strength of female attraction to continuous variation of acoustically relevant call properties. The dichotomous scoring of responses into either 'recognized' or 'not recognized', as has been used in some single-stimulus experiments with anurans (e.g. Ryan & Rand 1993), implies that females reach a threshold point at which signal recognition occurs and phonotaxis behaviour is consequently released. We assert, however, that signal recognition occurs along a continuous scale, and that categorization of responses into 'recognized' versus 'not recognized' is a simplification of the process that requires some arbitrary cutoff criterion.

Comparison of Single-speaker and Choice Test Data

The response functions based on pulse rate indicate that response strength peaks at the population mean (at 20 °C) for *H. versicolor*, and at 5–10 Hz above the population mean for *H. chrysoscelis*. These results are broadly consistent with those of choice tests, which indicate stabilizing preferences based on pulse rate in both species. Although the asymmetry of the preference functions based on choice experiments persists in the single-speaker data for *H. versicolor*, we found a more symmetrical response function for *H. chrysoscelis* in the single-speaker data than was reported earlier in choice tests. The results of the choice experiments suggested that the preference function was skewed, such that females discriminated more strongly against lower pulse rates than against higher pulse rates; a pulse rate difference of 10 Hz below the mean (40 versus 50 Hz) was enough to generate a significant preference, whereas a pulse rate difference of 25 Hz above the mean (50 versus 75 Hz) was required to obtain a similar level of preference for the conspecific pulse rate (Fig. 3b). The results of the single-speaker tests reveal that the response function itself is not strongly skewed, but rather that the peak of the function is shifted to a pulse rate 5–10 Hz higher than the mean population

value (Fig. 3a). Such a shift in the peak of a response function is unlikely to be detected using choice tests, because for characters exhibiting stabilizing preference functions, one of the two signals presented in each test usually corresponds to the population mean. As a result, preference functions derived from choice tests may be misleading if the population mean for that signal character does not coincide with the strongest female response.

The asymmetry in the preference function derived from choice experiments may still be ecologically relevant: selection against males calling at a pulse rate lower than the population mean is stronger than selection against males calling at a pulse rate higher than the population mean. The data from the single-stimulus experiments indicate that this difference is not due to an asymmetry in the response functions of the females, however, but rather to a shift in the central frequency of a symmetrical band-pass filter towards a higher pulse rate than the population mean. In this sense, the asymmetry in the preference function may be biologically meaningful, even though the underlying mechanism of pattern recognition appears to rely on a symmetrical function.

We emphasize that the response functions in Fig. 3 are not intended to suggest that the underlying neuronal mechanism for the pulse rate preferences is pulse rate recognition in either species. To make this claim additional data showing, for example, that variation in pulse duration is irrelevant, would be required.

The response functions based on pulse rise time indicate that rise times of 9 ms or longer elicited a strong response from female *H. versicolor*. The drop in response strength as rise time fell below 9 ms lends support to earlier conclusions that differences in rise time are used by *H. versicolor* for call discrimination. Using choice tests, Gerhardt & Schul (1999) showed that differences in rise time of as little 5 ms result in significant preferences for the stimuli with the longer rise time, and that the strength of this preference is greater between short (7.5 ms) and intermediate (12.5 ms) rise times than between intermediate and long (17.5 ms) rise times. Both of these conclusions from the choice tests would be predicted by the shape of the response functions derived here from single-stimulus tests. Nevertheless, even the shortest rise times tested in our single-stimulus presentations elicited a phonotaxis score above 0.4. Thus although this rise time is even shorter than that of the heterospecific call, females often responded reliably to the stimulus. The implications of this result are discussed below.

The results of the pulse number experiment indicate that response strength in *H. versicolor* increased logarithmically as the duration of the stimuli increased. Female preferences for long calls have been demonstrated repeatedly with choice tests in this species, and there is evidence that the offspring of females mated with long-calling males receive fitness benefits (Welch et al. 1998). Recent studies have indicated that the strength of the preference for the longer of two calls depends upon the call durations presented: the preference for average calls

over short calls is more robust than the preference for long calls over average calls, as indicated by experiments in which the intensity of the shorter call was increased relative to that of the longer call (Gerhardt et al. 2000). The shape of the response function in Fig. 5 supports this conclusion. Within the range of pulse numbers tested, the response strength increased with call duration, and the difference in response strength was greatest among calls with durations shorter than the average in this population.

We found no situations in which the direction of a preference displayed in a choice test did not correspond to the direction predicted from the single stimulus data. However, there are situations in which the choice tests appear to amplify the difference in female responsiveness to two stimuli. For example, although the phonotaxis scores of females approaching calls of 18 and 24 pulses differed by only 0.1 (at 1.01 and 1.11, respectively), choice tests revealed a highly significant preference for the longer of the two calls (Gerhardt et al. 2000). Similar phenomena have been observed in the crickets *Gryllus bimaculatus* and *G. texensis* (formerly *G. integer*), in which stronger preferences were indicated in choice tests than would have been predicted by comparisons of female response strength to stimuli presented alone (Doherty 1985; Wagner et al. 1995). These results are not surprising, given that choice tests involve an all-or-none response (Wagner 1998). Even a small difference in relative response strength is likely to lead to a strong population preference in choice tests, if there is high consistency among females in the shape of their response functions.

Comparison of Methods

Wagner (1998) provides a thorough discussion of the relative advantages and disadvantages of common experimental designs for measuring the strength of attraction to stimuli. If a choice design is used, the shape of the preference function can only be determined if multiple stimulus pairs are presented, and if the stimuli cover a broad range of signal values; tests involving only the extremes of the distribution reveal little about the shape of the preference function, or the relative strength of attraction for signals with intermediate characteristics (Gerhardt 1991; Wagner 1998). An additional caveat is illustrated by the misleading skew in the pulse rate preference function as described above. Single-speaker presentations are less likely to misrepresent the shape of the response function, as no prior assumptions are made regarding the location of its peak. Alternatively, this problem with choice tests can be avoided by using a sliding window design with no fixed point of comparison (Shaw 2000; Shaw & Herlihy 2000). Another approach that may overcome this problem is cubic spline analysis to compute the shape of a preference function derived from choice tests (e.g. Ritchie 1999).

A more important advantage of using a single-stimulus test to construct response functions is that it enables predictions about the behaviour of females confronted by

heterospecific calls in the absence of localizable conspecific calls. A dense chorus of males probably prevents a female from attending to the calls of more than the few nearest males ('selective attention', Gerhardt & Klump 1988; Pollack 1988). Although choice tests clearly indicate that both *H. versicolor* and *H. chrysoscelis* prefer the calls of conspecific males, these results provide little information regarding the outcome of a situation in which all of the nearby males are heterospecific, a situation that is likely to arise frequently in the mixed-species choruses of sympatric populations. Single-stimulus tests are a good predictor of whether such a female is likely to mate with a heterospecific male, or to wait (or search) for a conspecific male instead. Our results suggest that both *H. versicolor* and *H. chrysoscelis* are unlikely to mate with a heterospecific in the absence of a conspecific male; the phonotaxis scores in response to the call model with the heterospecific pulse rate were only 0.08 and 0.01 for *H. versicolor* and *H. chrysoscelis*, respectively. Thus, the difference in pulse rate alone is sufficient to ensure that females would rarely approach a heterospecific male even if conspecific males were rare or absent. In contrast, the pulse rise time does not constitute such a barrier for *H. versicolor*. Although females display a strong preference for the conspecific rise time in choice experiments, females in our single-stimulus tests responded with a phonotaxis score of 0.4 to calls with rise times even shorter than that of the heterospecific call. These results suggest that pulse rate alone, but not pulse rise time, is a sufficient cue to avoid phonotaxis to heterospecific males.

Our conclusions regarding species isolation contradict the suggestion by Doherty (1985) that choice tests are more relevant than single-stimulus tests for studying species recognition in sympatric populations. We argue that the likelihood of heterospecific matings should not be assumed based on a strong preference in choice experiments. In a bushcricket species complex (Tettigoniidae), for example, both *Tettigonia viridissima* and *T. cantans* prefer the conspecific song to the heterospecific song in choice situations. However, when presented with each signal separately, *T. cantans* (but not *T. viridissima*) approaches the heterospecific song with a phonotaxis score of ca. 0.5, and is probably in greater danger of heterospecific matings than are females of *T. viridissima*, which do not respond at all to the heterospecific calls when presented alone (Schul et al. 1998). The quantitative analysis of single-speaker experiments yields data of ecological relevance that cannot always be derived from choice experiments.

We are not suggesting that choice experiments have limited value, but rather that the data derived from choice and no-choice experiments are complimentary and have different strengths: choice experiments are a sensitive tool for exploring the ability to discriminate signals, whereas no-choice experiments are more useful for identifying and characterizing the features of a signal that release phonotaxis or other courtship behaviours. In this sense, single-stimulus presentations may provide insights into the underlying mechanisms of both pattern recognition and female preferences.

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