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Effects of heterospecific call overlap on the phonotactic behaviour of grey treefrogs

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We investigated the effects of overlap between the advertisement calls of two closely related and often-sympatric species of grey treefrogs on female phonotactic behaviour and signal selectivity. The tetraploid *H. versicolor* and the diploid *H. chrysoscelis* have advertisement calls consisting of trains of stereotyped pulses that are similar in their spectral composition. All females of *H. versicolor* from a population sympatric with *H. chrysoscelis* approached a speaker broadcasting synthetic advertisement calls modelled after conspecific calls when the calls of the two species alternated from speakers with a 90° angular separation with respect to the release point. Surprisingly, the same females almost exclusively approached the source of synthetic calls modelled after the heterospecific advertisement calls when these calls completely overlapped the conspecific stimuli. Female *H. chrysoscelis* from the same sympatric population approached the source of synthetic conspecific calls even when these calls were overlapped by the heterospecific stimuli, indicating that females in these closely related species may differ in their susceptibility to the negative consequences of call overlap. When female *H. versicolor* were presented with conspecific advertisement calls that were completely overlapped by those of *H. chrysoscelis*, many females failed to approach either signal source. Response times of females that did respond were also longer, and the direction of phonotaxis was shifted in the direction of the heterospecific signal source. These negative influences of acoustic interference were reduced or eliminated as the degree of overlap between the calls of the two species was reduced. The results demonstrate that the effects of signal overlap on female phonotactic behaviour may be complex.

Communication is important in the social lives of many organisms. The efficiency of interactions between signallers and receivers, however, will be constrained by an array of intrinsic physical and biotic aspects of the communication channel (Wiley 1994; Bradbury & Vehrencamp 1998). Biotic noise arising from the signals of other individuals may be a particularly severe problem for socially signalling organisms. For most frogs, the successful coordination of reproductive behaviour depends upon the ability of females to assess and respond to the acoustic advertisement signals of males. Although female frogs can recognize and accurately locate sources of conspecific advertisement calls in simple acoustic environments (reviewed in Gerhardt & Huber 2002), the inherent acoustic complexity of anuran choruses may significantly reduce the ability of females to accomplish these tasks in natural settings (Wollerman 1999; Schwartz et al. 2001; Wollerman & Wiley 2002). A major problem of communicating in a frog chorus arises when the signals of neighbouring males temporally overlap, which occurs as an incidental by-product of the high call rates of signalling males at high densities or, in some cases, because signallers actively overlap their signals (Schwartz 2001). Female frogs of a number of species discriminate against overlapping conspecific advertisement signals in favour of calls without such interference (e.g. Schwartz 1987; Bosch & Márquez 2000), and the potential for lost mating opportunities arising from call interference is probably an important factor shaping male signalling behaviour, including the distinctive call-timing interactions found in many species (Schwartz 2001).

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While many studies of acoustic interference have examined the consequences of overlapping signals arising from males of the same species (e.g. Bosch & Márquez 2000), choruses consisting of multiple species with similar breeding ecologies, phenologies and signals are common features of anuran breeding assemblages. In such environments, acoustic interference between the signals of heterospecific neighbours is potentially a serious problem for intraspecific communication. The costs associated with heterospecific signal interference have probably contributed to the increased differentiation in the carrier frequencies of the calls of some sympatric species and to temporal or spatial separation of breeding activity in others; these costs have probably also influenced the evolution of the interspecific signal-timing behaviours that reduce such interference (reviews in Gerhardt & Schwartz 1995; Gerhardt & Huber 2002). Given the potential importance of cross-species signal overlap in the evolution of anuran communication, it is surprising that its significance for intersexual communication has been investigated in only a few species (e.g. Schwartz & Wells 1984).

Our current understanding of the significance of call overlap for anuran communication is limited in large part by experimental designs used in previous studies. One major difficulty of previous studies is that they have almost exclusively used an experimental paradigm in which female frogs must choose between overlapping and nonoverlapping signals (e.g. Schwartz 1987; Bosch & Márquez 2000). Although estimating the extent to which females of a particular species discriminate against overlapping calls, such experiments do not provide information about how signal overlap influences female phonotactic behaviour per se. Phonotactic responses that may be affected by signal overlap include signal detection and recognition, the speed of the phonotactic approach, and the accuracy of sound-source localization. Evaluating the manner in which advertisement call overlap affects such behavioural responses is important for two main reasons. First, females may incur fitness costs, such as an increased assessment time, risk of predation or risk of mating with a male of inferior quality (Gerhardt et al. 1994; Grafe 1997). Such fitness costs may act as selective forces with the potential to shape the evolution of sensory systems and mating behaviour of receivers. Second, such studies may provide insights and generate hypotheses about sensory mechanisms as well as sensory constraints arising from the complex acoustic environments in which many organisms must communicate.

Another limitation of previous studies of acoustic interference is that few have systematically examined how the degree of overlap affects receiver responses (but see Schwartz 1987; Schwartz & Rand 1991). The extent of signal overlap varies, depending on such factors as the density of males in a chorus and the extent to which signallers are able to adjust the timing of their calls relative to those of neighbours (Schwartz et al. 2002). Most studies have only examined the consequences of completely overlapping calls, and hence may overestimate the negative consequences of signal overlap. We predict that, in general, reducing the extent of overlap should reduce or eliminate the negative effects of signal overlap on signal recognition and localization.

The grey treefrog complex represents a particularly good system for examining the significance of cross-species interference for female phonotactic behaviour. The complex comprises two strikingly similar species, *Hyla chrysoscelis* and *H. versicolor*, which breed syntopically in many parts of their range. Cross-species signal interference may be particularly significant for grey treefrog communication because of the overall similarity in the spectral and gross-temporal structure of their advertisement calls, which function primarily in mate attraction (Fig. 1). The similarity in call structure arises from the close phylogenetic relatedness of these two taxa: *Hyla versicolor* is a polyploid treefrog that arose from the diploid *H. chrysoscelis* and similar extinct species (Ptacek et al. 1994; Holloway et al. 2006). Female grey treefrogs rely on the pulse structure of the male's advertisement call for signal recognition, and such selectivity mediates species identification in sympatric choruses (Gerhardt 2001).

Hyla versicolor has been the subject of several studies examining the consequences of intraspecific signal interference for signal selection (Schwartz 1987; Schwartz & Gerhardt 1995; Schwartz & Marshall, in press). Comparative data from *H. chrysoscelis* is of particular interest because of the differences in the acoustic criteria used by

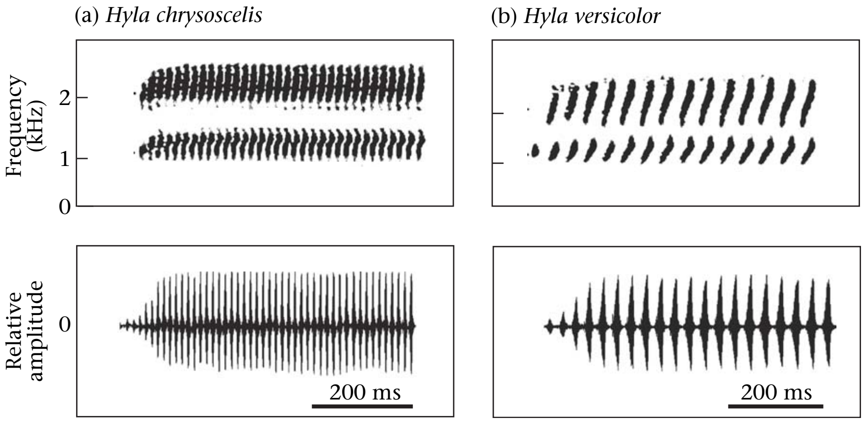

Figure 1. Spectrograms (top) and oscillograms (bottom) of natural advertisement calls of (a) *Hyla chrysoscelis* and (b) *H. versicolor*.

females of these two species (Gerhardt 2001, 2005; Schul & Bush 2002).

In this study, we examined the consequences of overlap between the advertisement calls of the two species of grey treefrogs for female signal selection and phonotactic behaviour. In the first experiment, we determined whether females of *H. versicolor* and *H. chrysoscelis* from a sympatric population in central Missouri, U.S.A., display phonotactic responses towards conspecific advertisement calls that are overlapped by the advertisement calls of the other species. In the second experiment, we examined the effects of heterospecific interference on several aspects of the phonotactic behaviour of female *H. versicolor*, including response times and the phonotactic accuracy (directionality) of responses under varying degrees of interspecific temporal overlap.

METHODS

In central and south-central Missouri, male grey treefrogs form breeding choruses around semipermanent ponds from late April through to July. While there is a report of character displacement in calling perch height in sympatric populations in Missouri (Ptacek 1992), males of the two species overlap broadly in choice of perch sites in populations where the two species co-occur. There is no evidence that males of either species preferentially associate with conspecifics within choruses, and mixed-species groups of males are commonly observed with intermale distances between heterospecific neighbours of 1 m or less (V. T. Marshall, unpublished data). Previous work has demonstrated that female *H. versicolor* discriminate against overlapping conspecific calls in favour of advertisement calls free from interference, and such discrimination has been suggested to be a consequence of degradation of the species-typical call structure within overlapping calls (Schwartz 1987; Schwartz & Gerhardt 1995). Additionally, males of both species adjust the timing of their calls to avoid overlap with the calls of neighbours, and such timing interactions occur with males of the other species in sympatric populations (V. T. Marshall & H. C. Gerhardt, unpublished data).

In the first experiment, we tested females of *H. versicolor* and *H. chrysoscelis* collected in amplexus from a sympatric population in Crawford County, Missouri in June of 2000. For the second experiment, we collected gravid female *H. versicolor* from a pond at the Thomas Baskett Wildlife Area in Boone County, Missouri in May and June of 2002 that did not contain *H. chrysoscelis*, although populations of *H. chrysoscelis* occurred within 20 km. Signal selectivity and phonotactic behaviour of females from these populations and from those found in sympatry with *H. chrysoscelis* do not differ (Gerhardt 2005). Females from the amplexed pairs were separated from the male, placed in containers within an ice-filled cooler to retard oviposition, and returned to the University of Missouri for testing. Some females were kept in coolers for up to 3 days before testing, and all females were returned to their point of collection subsequent to testing. The handling and use of study animals was approved by the University of Missouri IACUC (Protocol No. 1910).

Stimuli and General Methods

For experiment 1, we used computer-generated synthetic advertisement calls of the two species. The stimulus calls were created with a custom signal synthesis program (written by J. J. Schwartz) at a sampling rate of 22.05 kHz and 16-bit resolution. The spectra of the calls consisted of two phase-locked, constant-frequency bands of 1.1 kHz and 2.2 kHz, with the amplitude of the 2.2-kHz frequency band set to be 6 dB greater than that of the 1.1-kHz band. The pulses in the alternative calls had the pulse structure of the advertisement calls of *H. chrysoscelis* and *H. versicolor*, respectively. The pulses in the *H. chrysoscelis* and *H. versicolor* calls were 10 and 25 ms in duration, respectively, and the interpulse intervals were equal to the pulse durations for each stimulus, maintaining a 50% pulse duty-cycle. The pulse duration and interval values were set to the mean values of central Missouri males of each species calling at 20 °C. Both stimulus durations were approximately 870 ms. Synthetic stimuli with these parameters have been found to be as effective as natural calls in eliciting normal phonotactic responses from female grey treefrogs of both species (Gerhardt 2001, 2005).

For the second experiment, we used digitized natural advertisement calls of *H. versicolor* and *H. chrysoscelis* that had been recorded using a Sony TCD-D100 DAT recorder and Sennheiser ME-66 shotgun microphone on the same night in a syntopic population in Phelps County, Missouri in May of 2001. The recordings were digitized on an Apple iMac computer using the program Sound Edit 16 v.2 (Macromedia, San Francisco, California, U.S.A.) at a sampling rate of 22.05 kHz and 16-bit resolution. The recordings were bandpass-filtered (500 to 5000 Hz pass) to remove noise outside of the frequency range of the species' calls. Three advertisement calls from each of four males of each species (body temperatures of males: 22 ± 1.0 °C) were used for the stimuli. These calls were recorded where the background noise level was low, and they did not overlap with the calls of nearby neighbours. Some of these calls were edited to equalize the duration (~800 ms) of alternative stimuli by removing pulses from the end of the longer call. The resulting calls had 18 pulses (*H. versicolor* calls) or between 42 and 45 pulses (*H. chrysoscelis* calls). Using Cool Edit PRO v 1.2 (Syntrillium, Phoenix, Arizona, U.S.A.), we pasted the three calls from a male in sequence into a sound file with a call period of 4 s. For each timing relationship (see below), four stereo files, each using calls from a different pair of males of the two species, were then created; the calls of a *H. versicolor* and a *H. chrysoscelis* were on separate channels. Varying the amount of overlap between the calls was accomplished by delaying the start time of the *H. versicolor* calls by a fixed time period relative to the start of the *H. chrysoscelis* calls. In tests with a particular timing relationship between the calls, each female was presented with a playback from one of the four stereo files (exemplars).

Playbacks were performed in a temperature-controlled acoustic chamber lined with wedges of echo-attenuating acoustic foam (a description of the chamber set-up is provided in Gerhardt 1994). The digital signals were output using Cool Edit Pro from the PCM-CIA card slot of a Dell Inspiron 3500 laptop computer into a digital-to-analogue signal converter (WAMI Box, EGO Systems, Cupertino, California, U.S.A.). The output of the converter was amplified by a pair of Nagra DSM amplifiers, which drove two ADS 200-C speakers. Stimulus amplitudes were adjusted using built-in potentiometers in the amplifiers, and were checked using a Larson-Davis Precision Integrating Sound Level Meter (Model 800B; C-weighting, fast response).

Experiment 1: Cross-species Overlap and Signal Recognition by *H. versicolor* and *H. chrysoscelis*

We examined whether overlap from the calls of the other species would affect the ability of females to detect and recognize a source of conspecific advertisement calls. We presented female *H. versicolor* ($N = 11$) and female *H. chrysoscelis* ($N = 17$) collected from a sympatric population at Reiss Biological Station in Crawford County, Missouri with two different timing relationships between the synthetic advertisement calls of the two species: in an alternating fashion (test 1A) and in a completely overlapping fashion (test 1B). The speakers broadcasting the alternative stimuli had a 90° angular separation relative to the female release site (interspeaker distance: 1 m). The order in which females were presented with the two tests was switched from female to female. Females were placed in a small, acoustically transparent cage at the release site. After a female was exposed to four repetitions of the stimulus, the top of the release cage was removed remotely, and the female was monitored under infrared illumination via an IR sensitive television camera on a video monitor outside of the chamber. We recorded a choice of a stimulus if a female approached within 10 cm of one of the speakers during the 10-min test period. Females failing to reach the speakers during the test period were recorded as a ‘no-choice’. The *H. versicolor* and *H. chrysoscelis* calls were presented at 86 and 83 dB SPL (sound pressure level in dB; re: 20 μPa; C-weighted; fast RMS meter setting), respectively, at the release point. The 3-dB difference reflects the average lower amplitude of *H. chrysoscelis* advertisement calls relative to those of *H. versicolor* as measured at the same distance (Gerhardt 1975). Tests were performed at 20 °C.

Experiment 2: Effects of Degree of Overlap on the Phonotactic Behaviour of Female *H. versicolor*

We examined how the extent of cross-species signal overlap influenced the propensity of females of *H. versicolor* to respond to either of the alternative signals, the speed of responses, and the accuracy of signal localization. We placed a portable circular arena (1.5-m diameter) surrounded by acoustically transparent hardware cloth (height of 25 cm) in the anechoic chamber. The speakers that broadcast the stimuli were positioned 10 cm from the edge of the arena behind the hardware cloth and oriented towards the female release site in its centre. We marked the wall of the arena at 10-degree intervals along its circumference so that we could record where females initially contacted the arena wall relative to the speakers during a trial. The two speakers were placed on the edge of the arena with a 90° angular separation relative to the female release site (interspeaker distance was 1.15 m; Fig. 2a). For analysis and data presentation, the location of the *H. versicolor* speaker is referenced at 0° and the *H. chrysoscelis* speaker at 90°, although we moved the location of the speakers around the arena between tests to control for the possibility of nonacoustic biases in the direction of female phonotaxis. Stimulus amplitudes were set to 86 dB SPL for the *H. versicolor* call and 83 dB SPL for the *H. chrysoscelis* call (fast RMS) at the release point. These tests were performed at 22 °C, reflecting the average temperatures of the eight males whose calls were used to create the playback stimuli.

We varied the amount of overlap between the *H. versicolor* and *H. chrysoscelis* calls in different tests. The degrees of overlap between the calls that females received were complete overlap (100% of call duration; Fig. 2b), three degrees of partial overlap (83%, 67% and 33% of call duration) and no overlap (0%). In the no-overlap condition, the calls of the two species were timed to alternate with each other. In addition to the tests just described, we also performed tests in which each female was presented with just *H. versicolor* calls broadcast from one of the speakers. These tests provided baseline data for the probability, speed and accuracy of phonotaxis to conspecific calls in the absence of call overlap. Moreover, because these single-speaker tests were conducted immediately before or after a test with overlapping or alternating stimuli, they served as a check on the female’s phonotactic motivation. In another control test, we presented 12 females with heterospecific (*H. chrysoscelis*) advertisement calls from one of the speakers, thus providing the same baseline data for female phonotactic behaviour in response to the heterospecific stimulus in the absence of call overlap.

For each trial with a female, we recorded which stimulus the female approached, the time to make a choice of a stimulus, and where, relative to the speakers broadcasting the stimuli, the female initially contacted the arena wall (±10°). Our choice criterion was the movement of the female into an area within a 10-cm radius that was marked around the arena wall in front of a speaker (Fig. 2a). Females were given 10 min to make a choice. Females that climbed the wall and exited the arena away from the speakers or failed to reach the choice area within 10 min were recorded as no-choice.

To ensure that there were no nonacoustic biases within the test apparatus that may have influenced the directionality of female responses, we observed the behaviour of 12 females in the absence of any playback. Three of the 12 females tested in the arena with no stimulus failed to

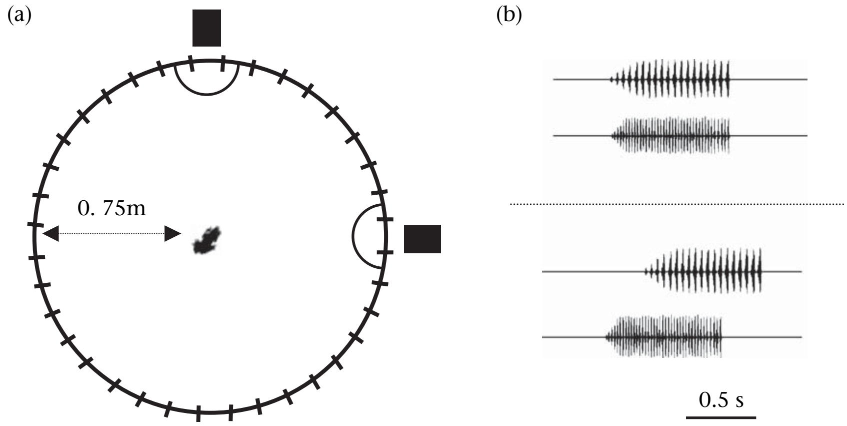

Figure 2. (a) Diagram of the playback arena used in experiment 2. The 10-cm choice regions are in front of each speaker. (b) Oscillograms show two of the timing relationships between the *H. versicolor* and *H. chrysoscelis* calls. Top trace: complete overlap between the two calls. Bottom trace: 67% overlap between the two calls.

reach the wall of the arena during the 10-min test period, and the distribution of female contacts with the wall was random for the remaining females (Rayleigh test: $r = 0.25$, $N = 9$, $P = 0.58$).

We used 29 female *H. versicolor* in the six tests (five tests with overlapping or alternating *H. versicolor* and *H. chrysoscelis* calls plus the test with only *H. chrysoscelis* calls), and each trial with a female was paired with a control test with the conspecific calls. While many females were used in more than one test (mean number of test and control trials per female: 3.5 ± 2.4; range 1−6), no female was used in a single test more than once.

RESULTS

Experiment 1: Cross-species Overlap and Signal Recognition by *H. versicolor* and *H. chrysoscelis*

The choices made by females in experiment 1 are shown in Fig. 3. Each of the 11 female *H. versicolor* from sympatry approached the speaker broadcasting the conspecific advertisement calls when the *H. versicolor* and *H. chrysoscelis* calls alternated with each other in test 1A (two-tailed binomial test: $P = 0.001$). Similarly, all 17 *H. chrysoscelis* females from the same population exclusively approached the conspecific call source in the same test (two-tailed binomial test: $P < 0.0001$). However, when the calls of the two species were timed to completely overlap in test 1B, nine of the female *H. versicolor* approached the speaker broadcasting the *H. chrysoscelis* calls and one approached the speaker playing the *H. versicolor* calls (two-tailed binomial test: $P = 0.02$). One female failed to approach either speaker during the 10-min test period. In contrast to the responses of *H. versicolor*, all 17 female *H. chrysoscelis* approached the speaker broadcasting the *H. chrysoscelis* calls when the two species' calls overlapped (two-tailed binomial test: $P < 0.0001$). When the calls overlapped, the proportions of females choosing the conspecific and heterospecific calls were different for the two species ($G_2 = 9.62$, $P < 0.01$).

Experiment 2: Effects of Degree of Overlap on the Phonotactic Behaviour of Female *H. versicolor*

Responses of H. versicolor *females to conspecific calls*

When presented with just conspecific advertisement calls, female *H. versicolor* demonstrated strong positive responses towards the stimulus source. In each of the 100 tests with the conspecific calls, the female met the choice criterion. Phonotactic movements were vigorous during these trials, and most females reached the choice area rapidly (mean response time: 28 ± 13 s, $N = 100$). After reaching the arena wall in front of the speaker, females continued moving in the vicinity of the source of the conspecific calls and often climbed over the hardware cloth and jumped on top of the speaker. Females typically made their initial contact with the arena wall close to

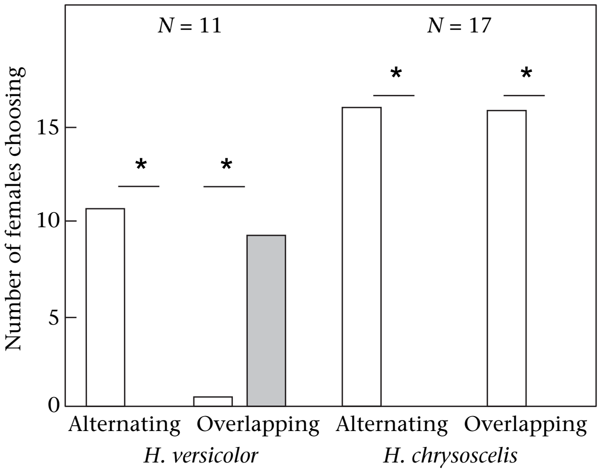

Figure 3. Choices made by *H. versicolor* and *H. chrysoscelis* females in experiment 1. Bars show the number of females choosing the speaker broadcasting the conspecific advertisement calls (□) and the speaker broadcasting the heterospecific advertisement calls (▨). Numbers above each category show the number of females tested. *$P < 0.001$, two-tailed binomial test.

the speaker (mean vector angle of contact with wall = 358°, circular deviation = 7°; location of speaker broadcasting *H. versicolor* calls referenced at 0°). The direction of phonotaxis was significantly different from random and oriented towards the location of the conspecific calls (*V* test: $r = 0.99$, $N = 100$, $P > 0.001$).

Phonotactic responses were weak or absent when female *H. versicolor* were presented the *H. chrysoscelis* calls alone. Of the 12 females that were tested, only nine reached the wall of the arena during the 10-min test period. The directions of female contact with the wall were not significantly different from random (Rayleigh test: $r = 0.29$, $N = 9$, $P = 0.48$), and only one female entered the choice area in front of the speaker during the test period. This female failed to repeat the choice when tested with the *H. chrysoscelis* calls a second time.

Responses to overlapping and alternating H. versicolor *calls*

When the calls overlapped by 100% and 83%, 68% and 88% of the females met the choice criterion, respectively. With less overlap and when the calls alternated, all females met the choice criterion. The choice times of responding females varied with the degree of overlap between the calls (repeated measures ANOVA: $F_{4, 16} = 4.35$, $P < 0.05$). Choice times were positively associated with the degree of overlap ($r^2 = 0.87$, $F = 29.57$, $P = 0.012$). In all tests in which the calls of the two species overlapped, the response times were significantly longer than in the corresponding control tests (*t* test for dependent samples: $P < 0.01$; Fig. 4). However, when the calls of the two species alternated (0% overlap), the response times of females did not differ significantly from their response times in the control trials (*t* test for dependent samples: $P = 0.11$).

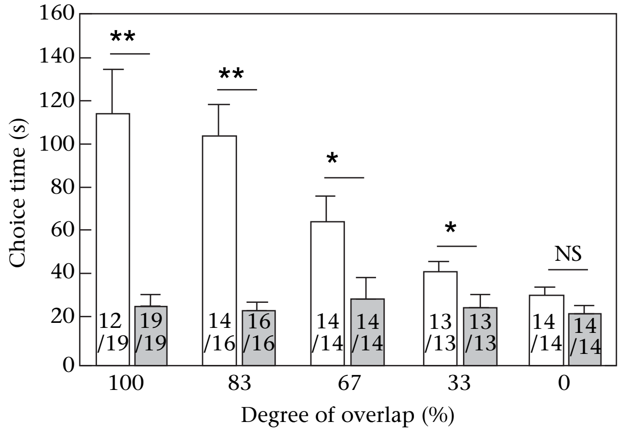

Figure 4. Response times (mean + SE) of *H. versicolor* females given choices of stimuli with varying degrees of overlap between the advertisement calls of *H. versicolor* and *H. chrysoscelis* (□) and their response times in the corresponding control tests in which females were presented with only *H. versicolor* advertisement calls (▨). Numbers in bars show the number of females making choices during the tests relative to the number of females tested. Asterisks show results of comparison between response times of females during controls and with the test stimuli. *$P < 0.01$; **$P < 0.0001$, two-tailed *t* test for repeated samples.

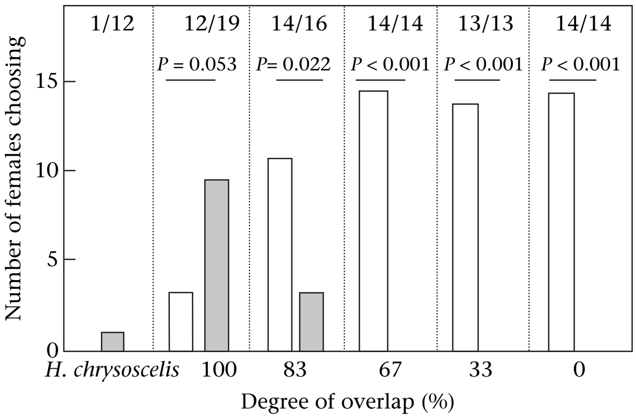

Figure 5. Choices made by *H. versicolor* females in the various tests. Bars show the number of females choosing the speaker broadcasting the *H. versicolor* advertisement bars (□) and the speaker broadcasting the *H. chrysoscelis* calls (▨). Numbers above each category show the number of females that made choices during the test out of the total number tested. *P* values are for the result of two-tailed binomial tests.

The choices made by the females in the various tests in the second experiment are summarized in Fig. 5. In the tests in which the advertisement calls of *H. versicolor* were overlapped by 67% or less of their total duration by the *H. chrysoscelis* calls, all females approached the speaker broadcasting the *H. versicolor* calls. When the calls of the two species overlapped completely, nine of the 12 females (75%) that met the choice criterion chose the speaker broadcasting the *H. chrysoscelis* calls, and three of the 14 responding females (21%) chose the heterospecific call when there was 83% overlap.

In all of the tests in which *H. versicolor* advertisement calls were broadcast, the direction of female phonotaxis was significantly different from random (Table 1). In most of these tests, the females made contact with the wall of the arena near the location of the speaker broadcasting the *H. versicolor* calls (referenced at 0°), and the direction of phonotaxis was strongly oriented towards the location of the conspecific calls (Fig. 6a). However, when the degree of overlap was 100%, the mean direction of phonotaxis was shifted away from the location of the speaker broadcasting the *H. versicolor* call and towards the speaker broadcasting the *H. chrysoscelis* calls (mean vector angle: 66.8°, circular deviation = 40.9°; Rayleigh test: $r = 0.78$, $N = 17$, $P < 0.001$; *H. chrysoscelis* stimulus referenced at 90°; Fig. 6b).

DISCUSSION

Grey treefrogs produce intense advertisement calls at high rates within breeding aggregations. Females entering into choruses to choose a mate are usually faced with evaluating and responding to signals that are temporally overlapping (Schwartz et al. 2002). In sympatric choruses of *H. chrysoscelis* and *H. versicolor*, overlapping signals may

Table 1. Direction of female phonotaxis as measured by the area of the wall that females contacted relative to the speakers broadcasting the *H. versicolor* calls (0°) and *H. chrysoscelis* calls (90°)

Degree of overlap (%)	Number tested	Number reaching wall	Mean vector angle (degree)	Circular variance	95% CI of mean	*r*	*P**
100	19	17	66.8	40.9	44.8–88.7	0.78	<0.001
87	16	14	22.0	43.4	358.2–45.8	0.75	<0.001
67	14	13	352.1	6.7	336.2–8.1	0.99	<0.001
33	13	13	353.8	7.4	337.2–10.5	0.99	<0.001
0	14	14	350.0	19.8	339.5–0.51	0.95	<0.001

*Rayleigh tests for nonrandomness in phonotactic direction.

arise from members of the same species or from heterospecific neighbours. As our experiments demonstrate, overlap of conspecific advertisement calls by those of the other species can have negative consequences for female phonotactic behaviour. The results of the first experiment also show that the two species may differ in their susceptibility to the negative effects of acoustic interference. Female *H. versicolor* made more errors in signal choices than did female *H. chrysoscelis* when conspecific calls were overlapped by those of the other species. Indeed, cross-species overlap did not appear to significantly affect the ability of female *H. chrysoscelis* to respond to conspecific signals.

Although males of the two species produce advertisement calls that typically differ in intensity, this difference is unlikely to account for the asymmetry in the effects of acoustic signal overlap, because the SPL of the *H. versicolor* stimuli was actually 3 dB greater than that of the *H. chrysoscelis* calls in both experiments. Thus, even though the signals of *H. versicolor* were favoured in intensity, female *H. versicolor* were still more likely to make errors in response to call overlap than were female *H. chrysoscelis*. This result, however, does not imply that heterospecific interference has no negative consequences for *H. chrysoscelis* communication. Female *H. chrysoscelis* do discriminate against conspecific calls that are overlapped by those of *H. versicolor* in favour of those that are free from overlap (V. T. Marshall, unpublished data).

In the second experiment, we found that when the calls of the two species completely overlapped, there were several consequences for the phonotactic behaviour of *H. versicolor*. First, a smaller proportion of females met the choice criterion for either stimulus compared to the trials with just the conspecific calls (68% versus 100%, respectively). Second, for the females that did meet the choice criterion, their response times to the overlapping calls were significantly longer than their response times to just the conspecific calls. Longer response times to the overlapping stimulus presentations were also observed in the several tests in which there was partial overlap. Finally, there were significant numbers of errors in signal identification when calls overlapped completely, with the average direction of phonotaxis not only shifted towards the source of the heterospecific advertisement calls, but also with over twice as many females meeting the choice criterion for the heterospecific call as for the conspecific call. The latter effect was consistent with the approaches of females of *H. versicolor* to the source of heterospecific calls observed in the first experiment when there was complete overlap.

One concern is that, by limiting the stimuli in the second experiment to the calls of four males of each species, we may have failed to capture natural variation in call structure, which could influence the responses to interfering calls (Kroodsma 1989). Because male grey

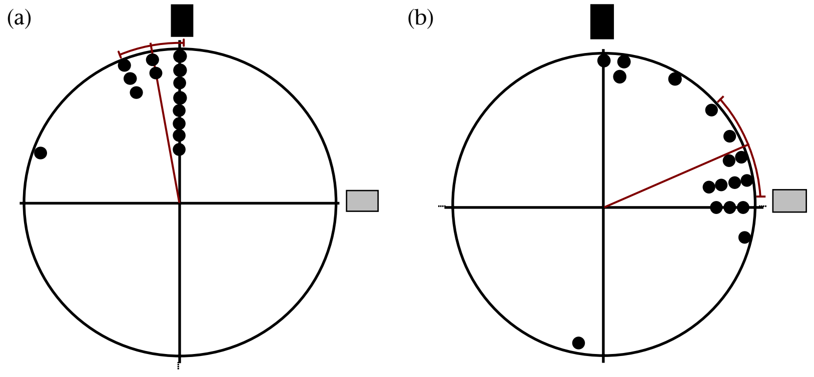

Figure 6. Direction of phonotaxis in the tests where female *H. versicolor* were presented with advertisement calls of conspecifics and advertisement calls of *H. chrysoscelis* that were timed to alternate (a) and overlap completely (b) in experiment 1. The position of the speakers broadcasting the *H. versicolor* and *H. chrysoscelis* advertisement calls are shown by the black and grey rectangles, respectively. A point reflects where an individual female contacted the wall of the arena. The mean vector angle of phonotaxis and its 95% confidence interval are shown by the line and brackets.

treefrogs call in dense choruses, we were ultimately limited in the number of exemplars for the second experiment by the availability of sufficiently low-noise and nonoverlapped call examples. Similarly, our use of a single set of synthetic calls for the first experiment may have also failed to capture relevant variation in signal parameters. The similar patterns of responses by female *H. versicolor* in the two experiments nevertheless indicate that the effects of cross-species interference were robust to the differences in the type of stimuli used (synthetic versus natural advertisement calls) as well as to whether the females were from sympatry or near-sympatry. We did not test females with a range of synthetic call models or large numbers of natural call exemplars, so we recognize that our results may not be generalized to all possible cases of heterospecific overlap in nature.

Another concern is that, because some females were tested with the same call exemplars in each test in the second experiment, the responses were not completely independent of each other. Pulse shape, pulse duration and pulse rate, as well as frequency features of calls, are static call properties and typically vary little within and between males of each species within a chorus (Gerhardt 2001). In light of the small variation in call structure among males within a chorus, as well as the consistency of the responses of females in the two experiments, we feel confident that differences between the exemplars were unlikely to significantly influence the behaviour of females. Nevertheless, we cannot rule out detecting such effects if we tested larger numbers of females with the calls of different males.

We expected that overlap between the calls of the *H. versicolor* and *H. chrysoscelis* would have negative consequences for phonotactic behaviour of female grey treefrogs because of the similarity in the structure of the two species' calls, particularly in the spectral domain. Although the auditory systems of most frogs are generally tuned to the frequencies emphasized within the advertisement calls of conspecifics (reviewed in Gerhardt & Schwartz 2001), the broad overlap in call frequencies of *H. versicolor* and *H. chrysoscelis* may preclude the use of matched spectral filtering as a strategy to reduce the negative consequences of cross-species interference. Next, we consider some possible explanations for why the overlap of conspecific advertisement calls by those of *H. chrysoscelis* influenced the behaviour of female *H. versicolor* in the manner observed.

Auditory masking, an increase in the threshold for detection of a target signal in the presence of a competing signal (Moore 1997), is potentially a problem when spectrally similar signals overlap in time. Masking of conspecific signals by overlapping heterospecific songs may account for the abolition of phonotactic responses to conspecific songs in some chorusing insects (Pollack 1986; Greenfield 1993). Auditory masking is also likely to be a common problem for female frogs choosing mates within dense and noisy choruses (Gerhardt & Huber 2002). In support of this, several studies have shown that simultaneous broadcasts of male advertisement signals and a sufficiently intense noise stimulus (such as broadband or natural chorus noise) can significantly weaken or abolish phonotactic responses of female anurans (e.g. Gerhardt & Klump 1988; Schwartz & Gerhardt 1989; Wollerman 1999; Wollerman & Wiley 2002).

In the case of overlap between the advertisement calls of *H. versicolor* and *H. chrysoscelis*, auditory masking could result for females of *H. versicolor* if the rapidly produced pulses of the *H. chrysoscelis* advertisement calls render the pulses of overlapped *H. versicolor* calls undetectable. For females of *H. chrysoscelis*, the longer pulses of the *H. versicolor* calls may mask the shorter pulses in conspecific signals. In this study, however, we failed to detect behavioural correlates of auditory masking in either experiment. All females of *H. chrysoscelis* in the first experiment and most females of *H. versicolor* in both experiments approached one of the speakers broadcasting conspecific or heterospecific advertisement calls or synthetic equivalents, even when the calls completely overlapped.

Another potential consequence of overlap between the calls of the two species is that it may have degraded the call's species-typical structure, rendering them less attractive to females. Degradation of call structure has been proposed as a negative consequence of signal overlap for species in which the temporal properties of advertisement calls are important for signal recognition (Schwartz 1987; Schwartz & Gerhardt 1995). Given the distinct differences in the temporal structure of the advertisement calls of the two species of grey treefrogs, our a priori expectation was that cross-species overlap would have negative influences on female phonotactic behaviour primarily through interference with their ability to extract the conspecific temporal structure from the composite signal. Bush et al. (2001) used single-stimulus experiments with both species of grey treefrogs to show that the speed of the phonotactic response of females to synthetic advertisement signals shows a graded decrease as the pulse structure of advertisement calls increasingly diverges from species-typical values. The significantly longer phonotaxis times of female *H. versicolor* when the calls of the two species overlapped either completely or partially in the second experiment is consistent with a decrease in the attractiveness of the calls relative to conspecific advertisement calls played back in isolation.

Temporal degradation of conspecific call structure cannot, however, explain the localization behaviour of female *H. versicolor* in response to completely overlapping calls of the two species. In both experiments, significant proportions of female *H. versicolor* approached the speaker broadcasting the heterospecific calls under this condition. It is unlikely that the overlap simply reduced female selectivity for the species-typical temporal structure of calls, thereby leading to a lack of discrimination between the calls of conspecifics and heterospecifics. First, recall that females were not reliably attracted to the calls of *H. chrysoscelis* that were presented alone in experiment 2. Bush et al. (2001) also showed that females of *H. versicolor* do not reliably respond to synthetic models with properties typical of *H. chrysoscelis*. Second, and most significantly, female *H. versicolor* presented with completely overlapping calls in the first experiment did not simply fail to prefer conspecific calls. Instead, they showed a clear bias for the speaker broadcasting the heterospecific calls. Similarly, in the second experiment, the mean direction of phonotaxis was shifted away from the speaker broadcasting the conspecific calls towards the speaker broadcasting the *H. chrysoscelis* advertisement calls (rather than showing a bimodal distribution) when the calls of the two species completely overlapped. One interpretation of these results is that the composite signal resulting from overlap was recognized as a somewhat attractive signal whose location was interpreted as emanating from the speaker playing back the calls of *H. chrysoscelis*.

In the case of overlap between the calls of *H. versicolor* and *H. chrysoscelis*, we hypothesize that the timing relationship among the pulses of the overlapping calls may play a role in the phonotactic bias of females of *H. versicolor* towards the heterospecific signals, which otherwise would be ineffective in eliciting responses on their own. The relative time of arrival of alternative conspecific calls or songs can influence the signal selectivity of females in a variety of acoustic organisms (reviews in Greenfield et al. 1997; Schwartz 2001; Gerhardt & Huber 2002). In most such species, females have strong preferences for signals in a temporally leading position, although preferences for following signals under some conditions have also been found (Grafe 1999). While these preferences may arise as a consequence of directional masking (Römer et al. 2002), relative signal timing has been shown to influence the perceived location of sounds in humans, as well as other mammals and birds (Zurek 1987; Dent & Dooling 2004). Specifically, over very short delays, leading sounds may be weighed more heavily in the perceived location of a sound than those in a following position; a psychoacoustic phenomenon termed the precedence effect. Could such an effect on signal localization account for the bias in phonotactic direction towards heterospecific signals by female *H. versicolor* and the lack of such a bias in female *H. chrysoscelis*?

In a post hoc analysis, we quantified the proportion of pulses in the calls of each species that could be defined as following relative to the pulses in the alternative call in the completely overlapping stimuli. We defined a pulse as following if it had a pulse of the other species beginning before it that overlapped or abutted it temporally. We used this criterion because such timing relationships among calls usually have the strongest effects on the signal-timing preferences of female frogs (reviewed in Gerhardt & Huber 2002). In experiment 1, 56% of the *H. versicolor* pulses were defined as following relative to *H. chrysoscelis* pulses; in experiment 2, a mean of 61% were in a following position. By contrast, 38% and 39%, respectively, of the *H. chrysoscelis* pulses were in a following position relative to *H. versicolor* pulses in the two experiments. The extent to which these differences in the proportion of pulses that are in a temporally following position can explain the species difference in phonotactic errors is an open question. However, playback experiments using overlapping *H. versicolor* advertisement calls in which the phases of pulses in overlapping calls were constant (leading versus following) showed that calls with leading pulses were more attractive to female *H. versicolor* (V. T. Marshall & H. C. Gerhardt, unpublished data), supporting the idea that the temporal relationship among pulses in overlapping calls can influence receiver responses.

Although closely related species of frogs often differ in the temporal structure of their advertisement calls, it is largely unknown how such differences influence the susceptibility of a species to the negative effects of acoustic signal overlap from members of their own or other species. For example, in the overlapping synthetic calls used in these experiments, 63% of the *H. chrysoscelis* pulses and 62% of the interpulse intervals were overlapped by *H. versicolor* pulses in the first experiment. Similar proportions of pulse (mean: 55%) and interval (mean: 54%) overlap occurred with the completely overlapping natural stimuli used in experiment 2 (Fig. 7). In contrast, 100% of the pulses and interpulse intervals of the *H. versicolor* calls were overlapped by *H. chrysoscelis* pulses in these experiments. Differences in susceptibility may also arise from differences in the signal recognition criteria used by different species. For females of *H. chrysoscelis*, the pulse rate of the call is the temporal feature used for signal recognition, whereas females of *H. versicolor* are sensitive to the combination of pulse duration and interpulse interval duration, as well as the rise time and shape of the pulse (Gerhardt 2001; Schul & Bush 2002). Perhaps

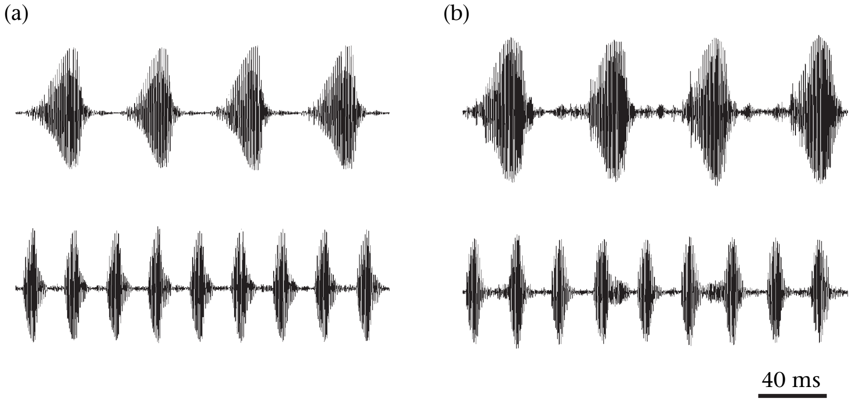

Figure 7. Oscillograms showing fine-scale timing of pulses when the advertisement calls of *H. versicolor* (top) and *H. chrysoscelis* (bottom) overlap. (a) Timing of pulses in one of the stimulus exemplars used in experiment 1. (b) Timing in naturally overlapping calls recorded from a pair of males (intermale distance = 0.75 m) in a sympatric pond in Phelps County, Missouri, U.S.A.

H. chrysoscelis females are less susceptible than *H. versicolor* to masking or degradation of the relevant call structure during signal overlap because they can extract sufficient pulse rate information from the nonoverlapped pulses and interpulse intervals within the gaps separating individual *H. versicolor* pulses. The reliance of females of *H. versicolor* on the combination of pulse shape, pulse duration and interpulse interval duration may make them more susceptible to disruption of signal recognition mechanisms by acoustic interference; disruption of any of these features may decrease the attractiveness of conspecific signals to females. Future studies comparing the effects of call interference on closely related species should address the degree to which differences in signal structure and signal recognition mechanisms may interact to influence susceptibility to acoustic interference.

Implications for Female Fitness

Factors that decrease the efficiency with which breeding individuals are able to respond to the signals of potential partners may increase the costs of mate choice. Female grey treefrogs are probably subject to two main kinds of costs when searching for mates within choruses. First, predation risks may be significant for females as they move through a chorus (Grafe 1997). Female grey treefrogs face predation from bullfrogs (*Rana catesbeiana*), green frogs (*R. clamitans*) and semiaquatic snakes of the genera *Nerodia* and *Thamnophis* within breeding ponds (Schwartz et al. 2000; personal observation). Second, females risk mating with a nonpreferred mate or, more significantly, a heterospecific male. Male frogs indiscriminately clasp any other frog that accidentally contacts them or moves nearby. Because of the broad overlap in the calling sites of the two species of grey treefrogs in sympatry, mismating is a particularly significant problem for these frogs, and heterospecific pairings may account for nearly 6% of mating in some populations (Gerhardt et al. 1994). Hybrids between the two species have decreased viability and are infertile, and such mating mistakes therefore carry a significant fitness cost for females. Reductions in signal attractiveness or difficulty in localizing signals resulting from partial or irregular call overlap may increase the chances of encountering a male of the wrong species or a predator if they increase the time that females spend searching for mates in a chorus. Recall that in all tests in which the stimulus calls overlapped in experiment 2, females took significantly longer to approach the stimulus calls than they did the nonoverlapped control calls. Such search inefficiency is likely to be amplified in choruses in which there will be numerous interfering sources and high levels of background noise.

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