



Parallel female preferences for call duration in a diploid ancestor of an allotetraploid treefrog

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The grey treefrog species complex (*Hyla chrysoscelis* and *H. versicolor*) comprises a single allotetraploid species (*H. versicolor*) that arose multiple times from hybrid matings between an extant diploid species (*H. chrysoscelis*) and at least two other extinct diploid treefrogs. While previous studies have investigated female preferences for call duration in the tetraploid, we know little about these preferences in its putative diploid ancestors. Here, I report results from two-choice phonotaxis experiments investigating call duration preferences in *H. chrysoscelis*. Females preferred an average-length call over shorter-than-average calls (0.5–2.0 standard deviations below average), and they preferred longer-than-average calls over average or shorter-than-average calls when the difference in pulse number was at least 2.0 SD. When the amplitude of the longer alternative was attenuated by 6 dB, females still preferred an average-length call over a shorter-than-average call, but there was no preference for longer-than-average calls over an average call. In the presence of chorus noise, female preferences for both average and longer-than-average calls over shorter alternatives were weakened or reversed. Together, the results from this study reveal patterns of female preferences for call duration that are strikingly similar among two members of a species complex with a novel evolutionary history. In both species, female preferences are directional, nonlinear, and limited by environmental noise. Furthermore, these results also highlight the need for caution in studies of sexual selection when extrapolating from patterns of female preference obtained under ideal laboratory conditions to conclusions about how those preferences are expressed in the real world.

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An important goal in studies of sexual selection is to quantify how variation in male secondary sex traits is related to the patterns of female preferences for those traits (Ryan & Keddy-Hector 1992; Andersson 1994; Jennions & Petrie 1997; Gerhardt & Huber 2002). Quantifying this relationship is one necessary prerequisite for understanding the potential scope and direction of evolution (e.g. stabilizing versus directional), the mechanisms of female choice, and the benefits (if any) that females gain by exercising particular preferences (Ritchie 1996; Castellano & Giacoma 1998; Welch et al. 1998; Gerhardt et al. 2000; Murphy & Gerhardt 2000; Shaw 2000; Shaw & Herlihy 2000; Gerhardt 2005; Bentsen et al. 2006; Rodriguez et al. 2006). Certain aspects of experimental design have important

implications for the conclusions that can be drawn about patterns of female preference. For example, preference functions that are based on many trait values allow better estimations of their shape and make it possible to distinguish between stabilizing and weakly directional selection when preference functions are unimodal (Gerhardt 1991; Gerhardt et al. 2000). Quantitative measures of preference strength (e.g. Wagner 1998) can be especially informative because they can reveal nonlinearities in female preferences even when preferences are strongly directional or open-ended (Gerhardt et al. 1996, 2000; Chenoweth & Blows 2005; Rundle et al. 2005; Wagner & Basolo 2007). Designs that also take into consideration the potential influence of environmental noise can better assess the extent to which such noise could limit or change the expression of female preferences under more natural conditions (Enderler 1992; Wiley 1994, 2006; Jennions & Petrie 1997; Schwartz et al. 2001; Wollerman & Wiley 2002).

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The present experimental study investigated the shapes of female preference functions for acoustic sexual signals, the potential presence of nonlinearities in those functions and the effects of environmental noise on female preferences in a novel vertebrate communication system. The grey treefrog species complex comprises two extant species, Cope's grey treefrog, *Hyla chrysoscelis*, and the eastern grey treefrog, *H. versicolor*. The former is a diploid and the latter is a tetraploid. Earlier phylogenetic analyses based on mitochondrial DNA revealed multiple origins of the tetraploid (Ptacek et al. 1994). More recent analyses based on both mitochondrial and nuclear genes confirm this earlier finding and further indicate that the tetraploid *H. versicolor* comprises one interbreeding species across its geographical range that arose through several independent allopolyploid speciation events resulting from hybrid matings between the extant diploid (*H. chrysoscelis*) and at least two other now extinct diploid species (Holloway et al. 2006). This situation, which appears so far to be unique among vertebrates, presents unparalleled opportunities to compare patterns of sexual selection in closely related species with a novel evolutionary history.

Both grey treefrog species form large choruses in which males produce loud and conspicuous advertisement calls to attract females. Males of both species produce pulsed advertisement calls that differ most notably in pulse rate, and much is already known about the call properties that mediate species recognition in both species (reviewed in Gerhardt 2001). The tetraploid (*H. versicolor*) has also been the subject of numerous studies of acoustically mediated mate choice. One call property that potentially influences patterns of female mate choice in *H. versicolor* is call duration, as determined by pulse number. For example, Klump & Gerhardt (1987) and Gerhardt et al. (1996) showed that females of *H. versicolor* prefer longer calls over shorter calls even when the shorter calls were broadcast at faster call rates and when the longer calls had relatively lower sound amplitudes. This directional preference for longer calls, however, is nonlinear in that females more strongly discriminate against shorter-than-average calls than against average calls, even when the absolute differences in pulse number between shorter and longer alternatives are similar (Gerhardt et al. 2000).

In contrast to the studies cited above, which were conducted under quiet laboratory conditions, additional work suggests that environmental noise could limit the ability of females of *H. versicolor* to exercise preferences for longer calls. In a field study conducted in natural choruses, Sullivan & Hinshaw (1992) failed to find any mating advantage as a function of longer call duration. In a subsequent laboratory study by Schwartz et al. (2001), female preferences for longer calls were reduced when stimuli were broadcast in the presence of background noise from a real chorus (Schwartz et al. 2001). Parallel results were obtained in a study of female choice in small choruses created in an artificial pond (Schwartz et al. 2001). In a field experiment in which females were captured as they responded to speakers broadcasting calls of different duration, fewer females responded to the shortest call, but similar numbers of females responded to speakers

producing average and longer-than-average calls (Schwartz et al. 2001).

Together, these previous studies of the allotetraploid grey treefrog (*H. versicolor*) reveal female preferences for call duration that are directional, nonlinear, and reduced in the presence of environmental noise. How might these patterns compare to those in the putative diploid ancestors? Here, I extend earlier work in the grey treefrog species complex by investigating patterns of female preference for call duration in the diploid *H. chrysoscelis*. My aims in this study were to (1) characterize natural variation in pulse number, (2) assess the shape of female preference functions for pulse number, (3) estimate preference strengths for several trait values across the range of variation and (4) evaluate preference functions under the simulated conditions of a noisy breeding chorus.

METHODS

Recordings and Analyses of Male Calls

Recordings of males of *H. chrysoscelis* were made between 8 May and 14 June 2006 from ponds and marshes at three field sites in east-central Minnesota (Carver Park Reserve, Carver Co., MN, U.S.A.; Carlos Avery Wildlife Management Area, Anoka Co., MN, U.S.A.; Tamarack Nature Center, Ramsey Co., MN, U.S.A.). Additional details regarding these field sites are provided elsewhere (Bee 2007, 2008; Bee & Swanson 2007; Swanson et al. 2007). I recorded 20 advertisement calls from each of 16 male *H. chrysoscelis* using a Marantz PMD670 solid-state recorder and Sennheiser ME66 directional microphone. Recordings were made between 2130 and 0000 hours during the peak of calling activity in moderately dense choruses. Most recorded males had at least one calling neighbour within 1 m. The microphone was mounted on a Sennheiser shock-mount pistol grip (MZS20-1) and housed inside a Sennheiser blimp windscreen (MZW60-1) held 50–100 cm from the calling male. In this study, males called from the surface of the water, so at the completion of each recording, I determined the water temperature at the male's calling site (range 15–26 °C). For 10 of the recorded males, I also measured body mass (to 0.1 g) using a Pesola spring scale and snout-to-vent length (SVL; to 0.1 mm) using dial callipers.

I used the automatic recognition tool of Avisoft-SASLab Pro (Avisoft Bioacoustics, Berlin) to count the number of pulses in the 20 calls recorded from each male (320 calls total). From these data, I determined (1) the mean (and SD) number of pulses/call for each individual male, (2) the within-individual (i.e. within-recording-session) coefficient of variation ($CV_w = 100\% \times SD_w / \text{mean}_w$) for each male, (3) the among-male CV based on the mean and SD of the 16 individual means ($CV_a = 100\% \times SD_a / \text{mean}_a$) and (4) the standard deviation of the number of pulses/call around the population mean determined over all 320 calls. I used model II analysis of variance (ANOVA) and a significance criterion of $\alpha = 0.05$ to compare the magnitude of among-individual and within-individual variability in pulse number.

Female Phonotaxis Tests

General testing procedures

Nightly collections of gravid females were made between 25 May and 15 June 2007 from the same field sites at which male calls were recorded. Full procedural details regarding the collection and handling of amplexant male–female pairs are provided elsewhere (Bee 2007, 2008; Bee & Swanson 2007; Swanson et al. 2007). Briefly, collected females were maintained at 2 °C to delay egg deposition prior to testing. On the day of testing, females were transferred to a 20 °C incubator and held there at least 1 h until their body temperatures had reached 20° ($\pm 1^\circ$) C. Females and their mates were returned to their original location of capture within 1–3 days of collection.

I tested females in two custom-built, hemianechoic sound chambers (Industrial Acoustics Company, IAC, Bronx, NY, U.S.A.; inside dimensions, L \times W \times H: 300 \times 280 \times 216 cm and 220 \times 280 \times 216 cm). The floor of each chamber was covered with dark grey carpet, and the inside walls and ceiling were treated with IAC's Planarchoic panel system (dark grey) to reduce reverberations. The baseline noise levels in both chambers were the same as those reported in Bee (2008). The temperature inside each chamber was maintained at 20° ($\pm 2^\circ$) C, and the frequency response of the playback system was flat (± 3 dB) between 500 and 4000 Hz. I used either a Brüel & Kjær Type 2250 or a Larson–Davis System 834 sound level meter to calibrate the playback levels of acoustic stimuli by placing the microphone of the meter at the approximate position of a female's head at the location from which females were released. Acoustic stimuli were calibrated at least twice daily.

Testing procedures and equipment are fully described elsewhere (Bee 2007, 2008). Briefly, I tested females under infrared (IR) illumination and observed behavioural responses from outside the sound chambers using an overhead IR-sensitive video camera. Digital acoustic signals (44.1 kHz, 16-bit) were broadcast through A/D/S L210 speakers using computers running Adobe Audition version 1.5 (Adobe Systems Inc, San Jose, CA, U.S.A.) interfaced with an M-Audio FireWire 410 multichannel soundcard. Output from the soundcard was amplified using Sonamp 1230 (Sonance) or HTD 1235 (Home Theater Direct) multichannel amplifiers.

I conducted phonotaxis tests in circular test arenas (2 m diameter) as described in Bee (2007, 2008). Two playback speakers were positioned on the floor of each chamber (2 m and 180° apart) just outside the acoustically transparent wall of the test arena and aimed towards the centre of the arena. At the beginning of a test, the female was placed in an acoustically transparent release cage positioned at the centre of the arena floor. After a 1 min acclimation period, I started broadcasts of the alternating signals, which were broadcast as a repeating loop during the entire duration of a test. Females were released after four presentations of both signals and given up to 5 min to make a choice, which consisted of touching the wall of the arena inside the 15° arc directly in front of one of the two speakers. I periodically switched the outputs from the amplifier going to each speaker or switched the

positions of the two speakers to control for directional response biases in each chamber. No such bias was detected. Playback levels were recalibrated after switching the relative positions of the two stimuli.

Experimental design

I conducted 20 two-choice discrimination experiments (Gerhardt 1995; Ryan & Rand 2001) in which females were given a choice between two synthetic calls that alternated between the two speakers. I used a custom-made program (courtesy of J. J. Schwartz) to synthesize a single pulse that had temporal and spectral properties based on temperature-corrected (20 °C) average values from my sample of recordings from local Minnesota populations (Fig. 1a; also see Bee 2008). I used this synthetic pulse to create synthetic calls that differed in pulse number (see Fig. 1). Within each call, the pulse duty cycle was 50%

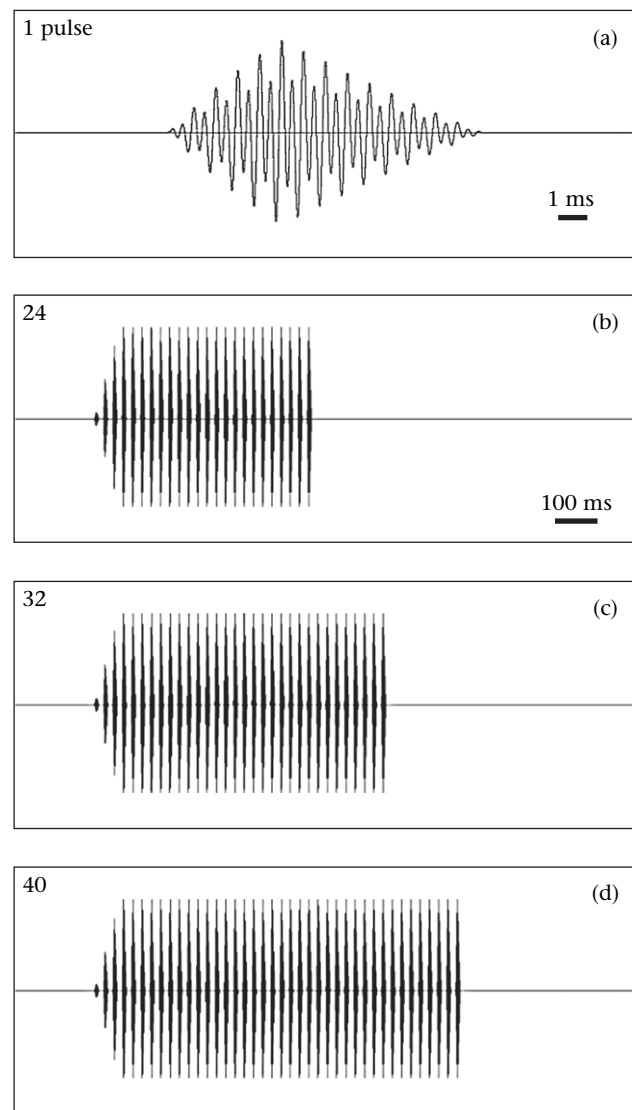


Figure 1. Oscillograms showing (a) a single synthetic *H. chrysoscelis* pulse and (b–d) examples of synthetic calls with (b) 24 pulses, (c) 32 pulses (i.e. the average call) and (d) 40 pulses. The scale bar in (b) also applies to panels (c) and (d).

and the pulse rate was 45.5 pulses/s, which is close to the average pulse rate in local populations at 20 °C (M. A. Bee, unpublished data). The two alternative stimuli in a given choice test were presented so that there were equal intervals of silence preceding and following each call; therefore, no alternative was a leading or lagging call. Each call repeated with a period of 5 s, which is within the range of temperature-corrected (20 °C) call periods recorded in local populations (M. A. Bee, unpublished data). Unless indicated otherwise, the sound pressure level (SPL re. 20 µPa) of each stimulus was 85 dB (LCF) at the female release site (1 m). This SPL falls in the range of call amplitudes recorded in grey treefrogs at a distance of 1 m (Gerhardt 1975).

In a series of 10 discrimination tests, I paired a 32-pulse call (Fig. 1c), which is close to the population average (see below), against alternatives that differed from the average pulse number by 0, ± 0.5 , ± 1.0 , ± 1.5 , ± 2.0 and ± 5 SD based on the distribution of average pulse number in the calls of the 16 recorded males. In my sample of recordings, the SD of the distribution of male averages was approximately four pulses (see below). Hence, in these 10 tests, females heard the 32-pulse call paired against an alternative with 24, 26, 28, 30, 32, 34, 36, 38, 40 and 52 pulses (Fig. 1b–d). In one additional test, I paired the shortest (24-pulse) call against the 40-pulse alternative, which corresponds approximately to calls from the two ends of the distribution of average pulse number (see below) and is equivalent to a difference of about 4 SD.

To derive estimates of preference strength for longer calls, I repeated three of the phonotaxis tests described above (24 versus 32, 32 versus 40, and 32 versus 52) after attenuating the amplitude of the longer alternative by 6 dB. Hence, the amplitude of the shorter alternative was 85 dB SPL (at 1 m) and that of the longer alternative was 79 dB SPL (at 1 m). This manipulation measures preference strength as the willingness of a female to approach a longer call that would be perceived as originating at twice the distance of the shorter call at the female's release site according to the inverse square law relating sound attenuation to distance (Gerhardt et al. 1996, 2000).

In a final series of six tests, I broadcast the two alternatives in the presence of artificial 'chorus-shaped' noise similar to that used in recent studies of auditory masking in grey treefrogs (Bee 2007, 2008; Bee & Swanson 2007). In four of these six tests, I gave females a choice between the 32-pulse (average) call and an alternative stimulus that had 24 (-2.0 SD), 28 (-1.0 SD), 36 ($+1.0$ SD), or 40 ($+2.0$ SD) pulses. In two additional tests, I paired the 32-pulse call against the 24-pulse call (-2.0 SD) and the 40-pulse call ($+2.0$ SD) after attenuating the longer call alternative by 6 dB, so that it was broadcast at 79 dB SPL (at 1 m).

I created the artificial chorus noise using the Adobe Audition 1.5 software. I used 10th-order Butterworth filters to shape two white noises so that one had a bandwidth of 600 Hz and a centre frequency of 1.3 kHz, and the other had bandwidth of 1200 Hz and a centre frequency of 2.6 kHz. I then digitally added these two noises together so that the spectrum of the resulting sound had the approximate shape of a grey treefrog

chorus (Swanson et al. 2007). The chorus-shaped noise was broadcast from an overhead speaker (Kenwood KFC-1680ie) that was suspended from the chamber ceiling 190 cm directly above the female release site. The use of an overhead speaker from this height creates a uniform (± 3 dB) noise level across the entire floor of the circular test arena. The amplitude of the noise was calibrated to be 75 dB SPL at the female release site. This SPL falls in the range of SPLs that can be recorded in natural choruses (Swanson et al. 2007) and is similar to the level of 77 dB SPL for chorus noise used in a previous study of call duration preferences in the other grey treefrog (Schwartz et al. 2001). Thus, for stimuli broadcast at 85 dB SPL, the signal-to-noise ratio (SNR) was $+10$ dB; for stimuli attenuated by 6 dB, the SNR was $+4$ dB. Previous work on auditory masking in grey treefrogs suggests that signals presented at SNRs of both $+4$ dB and $+10$ dB are audible to females in the presence of chorus noise (Bee 2007, 2008; Bee & Swanson 2007). During tests with chorus-shaped noise, the noise started 30 s before the onset of the alternating signals and was broadcast continuously during the duration of a test.

Data analysis

A total of 147 females were collected and tested in 420 individual phonotaxis tests. Most females were used as subjects in more than one test (1 test: $N = 40$; 2 tests: $N = 41$; 3 tests: $N = 18$; 4 tests: $N = 19$; 5 tests: $N = 11$; 6 tests: $N = 15$; 7 tests: $N = 1$; 8 tests: $N = 2$). Results from 15 tests were excluded from analyses because the female did not make a choice within 5 min. With the single exception of the test of a 32-pulse call versus a 32-pulse call ($N = 25$), all tests had a sample size of $N = 20$ females. I used separate two-tailed binomial tests to test the null hypothesis that 50% of females would approach each stimulus against the alternative hypothesis that greater than 50% of females would choose one of the two alternative stimuli.

RESULTS

Patterns of Variability in Pulse Number

The average number of pulses in a male's calls (Fig. 2a) ranged from 23.8 to 41.0 pulses, with a grand mean (\pm SD) of 32.8 (± 4.0) pulses/call. In the total sample of 320 calls, pulse number ranged from 20 pulses to 60 pulses (Fig. 2b), and the SD of the entire sample was 5.3 pulses/call. Within males, the SD of the mean number of pulses per call ranged from 1.4 to 7.7 pulses/call (mean SD = 3.2 pulses/call, $N = 16$), and the within-individual CVs ranged from 4.1% to 18.8% (mean $CV_w = 9.6\%$, $N = 16$). The among-individual CV (CV_a) was 12.2%. Pulse number varied significantly more among males than it did within males (ANOVA: $F_{15,304} = 24.0$, $P < 0.001$). Generally similar patterns of variability have been reported for pulse number (or signal duration) in other frogs and insects with pulsed signals (reviewed in Gerhardt & Huber 2002). Neither temperature nor body size were significantly correlated with the average number of pulses in

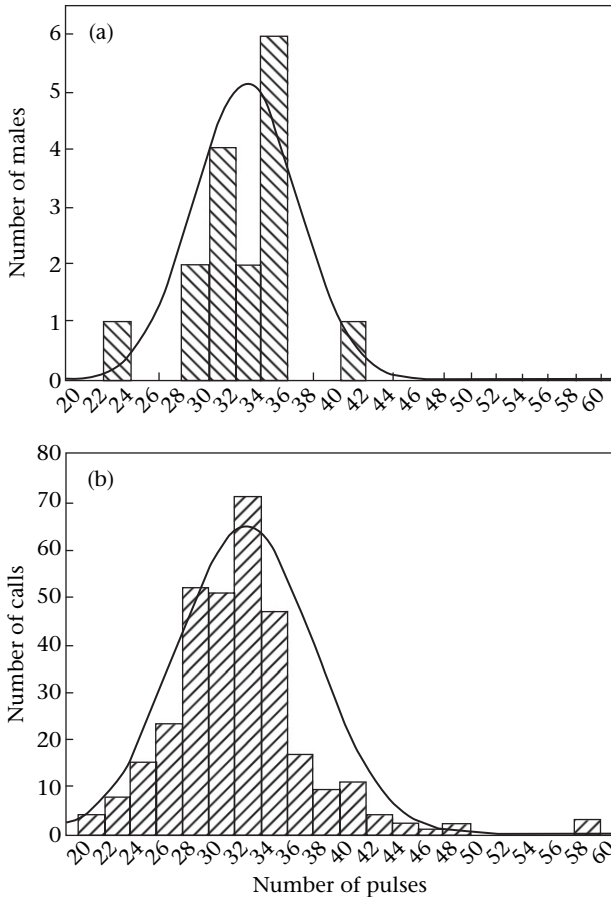


Figure 2. Histograms showing the distribution of pulse number in the calls of *H. chrysocelis* in Minnesota. (a) Distribution of individual means from 16 males. (b) Distribution of 320 calls recorded from 16 males (20 calls/male). In both (a) and (b) a normal curve is fitted to each distribution. Values along the X axis depict inclusive upper bounds.

a male's calls (Pearson correlation: temperature: $r_{14} = -0.23, P = 0.380$; SVL: $r_8 = -0.17, P = 0.640$; body mass: $r_8 = -0.42, P = 0.221$).

Population-level Preference Functions for Pulse Number

In the test that paired the 32-pulse call against itself, 13 females approached one speaker, and 12 approached the other ($P = 1.0$). For 20 of these females, I noted whether or not the speaker they chose was also the speaker that broadcast the signal initiating the continuous loop prior to the female's release. Nine females approached the speaker that initiated the loop, and 11 females approached the speaker that broadcast the second signal to be heard in the initial loop ($P = 0.824$). Thus, in my experimental set-up, females showed no significant preference between two alternatives with equal pulse numbers and amplitudes, and no preference based on whether the signal was the first or the second signal presented at the start of the continuous loop of alternating signals.

The population preference function based on equal amplitude calls is depicted in Fig. 3a in comparison to

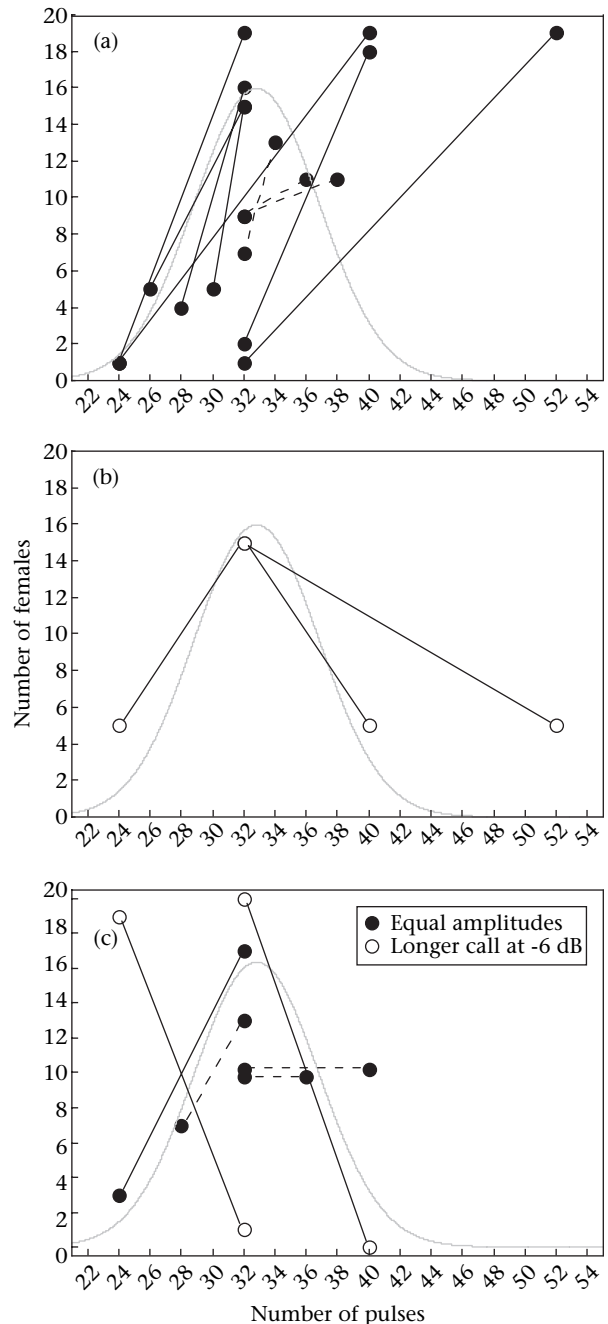


Figure 3. Female preference functions for call duration showing the number of females that chose each alternative as a function of pulse number in two-choice discrimination tests. In each graph, the normal curve depicts the curve fitted to the distribution of average pulse number and is redrawn from Fig. 2a. Lines join the two alternatives in each test; solid lines indicate a significant difference (two-tailed binomial: $P < 0.05$) and dashed lines indicate no significant difference (two-tailed binomial: $P > 0.05$). Closed symbols depict results from equal-amplitude tests; open symbols depict results from tests with the longer alternative attenuated by 6 dB. (a) Results when both alternatives were broadcast at 85 dB SPL with no noise. (b) Results when the amplitude of the longer call was attenuated by 6 dB (79 dB SPL) relative to the shorter call (85 dB SPL). (c) Results when signals were broadcast in the presence of a chorus-shaped noise.

the distribution of the average number of pulses per call. Females chose the average 32-pulse call more often than expected by chance when it was paired against an equal-amplitude alternative with fewer pulses (i.e. shorter calls with 24, 26, 28 or 30 pulses; Fig. 3a). Females also chose the 40-pulse and 52-pulse alternatives when these were paired against the equal-amplitude 32-pulse call (Fig. 3a). When the number of pulses in the longer call alternative was 34, 36 or 38 pulses, females failed to choose the longer call in significantly greater proportions than they chose the average 32-pulse call (Fig. 3a). When a longer-than-average call with 40 pulses was paired against a shorter-than-average call with 24 pulses, significantly more females chose the longer call (Fig. 3a).

Preference Strength Based on Unequal Amplitudes

When the average 32-pulse call was attenuated by 6 dB and paired against the 24-pulse call (-2 SD), significantly more females than expected by chance still chose the average call (Fig. 3b). In contrast, when longer-than-average calls with 40 pulses ($+2$ SD) or 52 pulses ($+5$ SD) were attenuated by 6 dB and paired against the average call with 32 pulses, females chose the average (shorter) call (Fig. 3b). Hence, attenuating the longer-than-average signals and pairing these alternatives against an average call actually reversed female choices compared to the equal-amplitude conditions (cf. Fig. 3a and 3b).

Preference Functions in Noise

In the presence of a chorus-shaped noise, females chose the 32-pulse (average) call significantly more often than an alternative with just 24 pulses (-2.0 SD), but not when the shorter alternative had 28 pulses (-1.0 SD) (Fig. 3c). When the 32-pulse call was paired against the longer-than-average 36-pulse ($+1.0$ SD) and 40-pulse ($+2.0$ SD) alternatives, females showed no significant preferences (Fig. 3c). These results differ somewhat from those obtained without the chorus noise; under quiet conditions, females chose the 32-pulse call over the 28-pulse call, and the 40-pulse call over the 32-pulse call (cf. Fig. 3a and 3c). When the longer alternative was attenuated by 6 dB (i.e. 24 pulses versus 32 pulses (-6 dB) or 32 pulses versus 40 pulses (-6 dB)) in the presence of chorus noise, females chose the louder (and also shorter) call significantly more often than expected by chance (Fig. 3c, open symbols). These results also differ from those obtained in parallel tests conducted in quiet conditions, in which females chose the 32-pulse call broadcast at 79 dB SPL over the 24-pulse call at 85 dB SPL (cf. open symbols in Fig. 3b and 3c).

DISCUSSION

Acoustic communication plays important roles in sexual selection in anuran amphibians (reviewed in Ryan 2001; Gerhardt & Huber 2002; Gerhardt & Bee 2006; Wells &

Schwartz 2006). Among anurans, and indeed among all animals, the grey treefrog species complex represents a particularly interesting case study for investigating patterns of female preferences among closely related species with an unusual evolutionary history (Holloway et al. 2006). Of the three putative diploid ancestors of the allotetraploid *H. versicolor*, the only extant species is Cope's grey treefrog (*H. chrysoscelis*). Results from this study of female preferences for call duration in *H. chrysoscelis* are strikingly similar to those reported in earlier studies of *H. versicolor*.

Directional and Nonlinear Preferences for Call Duration

Directional female preferences for more elaborate or exaggerated traits may evolve because such traits provide greater stimulation of the female's sensory system (e.g. Ryan & Keddy-Hector 1992) or because they are more easily detected against a background of environmental noise (e.g. Endler 1992). Results from the present study of the diploid and those of earlier studies of the tetraploid (Klump & Gerhardt 1987; Gerhardt 1991; Gerhardt et al. 1996, 2000) indicate that female preferences for call duration in both species are highly directional in favour of calls with higher pulse numbers, at least in quiet laboratory conditions. On the surface, this result is consistent with expectations regarding female preferences for elaborate or exaggerated male traits. As in the tetraploid species, however, female preferences for longer calls in the diploid were nonlinear. While females in the present study consistently preferred an average-length call over shorter-than-average calls (-2.0 , -1.5 , -1.0 and -0.5 SD), preferences for longer-than-average calls over an average-length call were only found when the longer call was at least 2 SD longer than the average call length. Hence, females more strongly discriminated against shorter-than-average calls than against average-length calls when the differences in pulse number between the shorter and longer alternatives were equivalent (i.e. ± 0.5 , ± 1.0 and ± 1.5 SD). This non-linearity was corroborated in the unequal-amplitude tests. Females still preferred an average-length call attenuated by 6 dB when it was paired against a shorter-than-average call, but attenuating a longer-than-average call was sufficient to reverse female preferences for this signal over a call of average length.

The nonlinear preference functions depicted in Fig. 3 represent a departure from Weber's law, which would predict similar preferences when the two alternatives in each test differ by the same percentage. In the equal-amplitude tests, for example, when the difference in pulse number was about 6–7% (expressed as a percentage of the number of pulses in the shorter alternative), 75% of females chose the longer alternative when it was paired against a shorter-than-average call, but only 65% did so when the longer alternative was paired against an average call (Comparison 1, Table 1). When the difference in pulse number between the two alternatives was about 13–14%, only 55% of females chose the longer call when it was paired against an average call, but 80% chose an average call paired against a shorter call (Comparison 2, Table 1). Only

Table 1. Comparisons among test results based on percentage differences in pulse number (as a percentage of shorter call alternative)

Test comparison	Shorter alternative		Longer alternative	Tested % difference	Tested SD difference	% Choosing longer call	Two-tailed binomial <i>P</i>
1	30	vs	32	7	-0.5	75	0.0414
	32	vs	34	6	+0.5	65	0.2632
2	28	vs	32	14	-1.0	80	0.0118
	32	vs	36	13	+1.0	55	0.8238
3	26	vs	32	23	-1.5	75	0.0414
	32	vs	40	25	+2.0	90	0.0004
4	24	vs	32 (-6 dB)	33	-2.0	75	0.0414
	32	vs	52 (-6 dB)	63	+5.0	25	0.0414

when the difference in pulse number was at least 23–25% did females choose a longer-than-average call over an average call in proportions that exceeded those that chose an average call over a shorter-than-average call (Comparison 3, Table 1). This departure from Weber's law was particularly striking in the unequal-amplitude tests. For example, when the difference in pulse number was 33%, 75% of females still chose the average call (32 pulses) over the shorter-than average call (24 pulses) when the former was attenuated by 6 dB (Comparison 4, Table 1). When the difference in pulse number was even larger (63%), however, females chose the average call (32 pulses) over a longer alternative (52 pulses) that was attenuated by 6 dB (Comparison 4, Table 1). Although statistical analyses of these comparisons were not conducted because of nonindependence from partial overlap among subjects in different tests, these qualitative comparisons are consistent with the conclusion that female preferences for call duration in *H. chrysoscelis* do not follow Weber's law. Similar results have been reported for *H. versicolor* (Gerhardt et al. 2000).

Implications for Mate Choice in a Noisy World

To receive any benefits from preferentially mating with males having particular signal traits, females must be able to discriminate among different signal variants under the noisy conditions that occur in nature (Endler 1992; Wiley 1994, 2006; Brumm & Slabbekoom 2005). Calling is energetically expensive in grey treefrogs, and longer calls may be more expensive to produce than shorter calls (Taigen & Wells 1985; Wells & Taigen 1986; Wells et al. 1995). Quantitative breeding studies indicate that females of *H. versicolor* that preferentially mate with males that produce long calls could receive indirect benefits in the form of increased offspring fitness (Welch et al. 1998; Doty & Welch 2001; Welch 2003). Hence, pulse number is considered to be an honest indicator of a male's genetic quality or physiological condition. But how robust are female preferences for longer calls under the noisy conditions of a breeding chorus?

Previous results reported by Schwartz et al. (2001) indicate that preferences for longer calls are reduced in *H. versicolor* when signals are perceived in noisy environments. These findings may provide the explanation for why Sullivan & Hinshaw (1992) failed to find a relationship between call duration and mating success in their field study of the tetraploid. In the present study, females of

the diploid species continued to discriminate against the shortest call (24 pulses; -2.0 SD) in favour of an average 32-pulse call broadcast at equal amplitudes in the presence of background chorus noise. But females preferred neither the average 32-pulse call over the 28-pulse call (-1.0 SD) nor the 40-pulse call (+2.0 SD) over the average 32-pulse call in the presence of chorus noise, although both of these preferences were significant in favour of the longer call under quiet conditions. These results are consistent both with general predictions from signal detection theory, which hold that noise should make discriminating among signal variants more difficult (Wiley 1994, 2006), and with results from previous studies of masked signal discrimination in animal communication (e.g. Wollerman & Wiley 2002; Lohr et al. 2003; Bee 2008). Results from the present study further suggest the possibility that female preferences for longer calls might actually be reversed in the presence of chorus noise. Under quiet conditions, females preferred the average-length call (32 pulses) when it was attenuated by 6 dB and paired against the 24-pulse call (-2.0 SD). When this same test was repeated in the presence of chorus noise, females instead preferred the shorter (24-pulse) call. Thus, under some conditions, females may prefer even the shortest calls if males that produce these signals are perceived as being closer than a more distant male that produces average calls. Together, results from this study and previous studies of *H. versicolor* (Gerhardt et al. 2000; Schwartz et al. 2001) suggest that directional selection operating on call duration might be very weak in nature and may differentially select against males that produce signals with trait values far below the population average as opposed to selecting for males with above-average signals.

Conclusions

This study has demonstrated a pattern of female preferences for call duration in a putative diploid ancestor (*H. chrysoscelis*) that closely parallels that described previously for its allotetraploid descendent (*H. versicolor*). These parallel patterns extend to (1) the shape of a directional preference function determined in quiet conditions, (2) nonlinearities in the preference function and (3) the ability of environmental noise to limit the expression of female preferences. At present, it is unclear whether these parallel patterns of preference are the result of convergence between *H. chrysoscelis* and *H. versicolor* or the evolutionary

conservation of ancestral preferences in the tetraploid. One of these two alternative hypotheses might be rejected by using a phylogenetic approach to compare female preferences in the recognized separate lineages of the diploid and the multiple lineages of the tetraploid that arose independently (Ptacek et al. 1994; Holloway et al. 2006).

Studies of sexual selection in grey treefrogs further highlight the importance of studying mate choice preferences under more natural conditions that exploit or simulate the noisy conditions in which mate choice operates in the real world. In quiet conditions, female preferences for call duration conform to the widely held view that females generally prefer more extravagant or elaborate male traits (Ryan & Keddy-Hector 1992; Andersson 1994). As shown here, however, nonlinearities and environmental noise may, in some situations, interact to weaken or reverse a directional preference obtained under quiet laboratory conditions. Nonlinear preferences and the breakdown of signal discrimination in noisy conditions probably contribute to the maintenance of heritable genetic variation for call duration in natural populations. Clearly, extrapolating from preference functions determined under unrealistic conditions with no environmental noise to the operation of sexual selection in nature should be done with caution.

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References

- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Bee, M. A. 2007. Sound source segregation in grey treefrogs: spatial release from masking by the sound of a chorus. *Animal Behaviour*, **74**, 549–558.
- Bee, M. A. 2008. Finding a mate at a cocktail party: spatial release from masking improves acoustic mate recognition in grey treefrogs. *Animal Behaviour*, **75**, 1781–1791.
- Bee, M. A. & Swanson, E. M. 2007. Auditory masking of anuran advertisement calls by road traffic noise. *Animal Behaviour*, **74**, 1765–1776.
- Bentsen, C. L., Hunt, J., Jennions, M. D. & Brooks, R. 2006. Complex multivariate sexual selection on male acoustic signaling in a wild population of *Teleogryllus commodus*. *American Naturalist*, **167**, E102–E116.
- Brumm, H. & Slabbekoorn, H. 2005. Acoustic communication in noise. *Advances in the Study of Behavior*, **35**, 151–209.
- Castellano, S. & Giacoma, C. 1998. Stabilizing and directional female choice for male calls in the European green toad. *Animal Behaviour*, **56**, 275–287.
- Chenoweth, S. F. & Blows, M. W. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *American Naturalist*, **165**, 281–289.
- Doty, G. V. & Welch, A. M. 2001. Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (*Hyla versicolor*). *Behavioral Ecology and Sociobiology*, **49**, 150–156.
- Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist*, **139**, S125–S153.
- Gerhardt, H. C. 1975. Sound pressure levels and radiation patterns of vocalizations of some North American frogs and toads. *Journal of Comparative Physiology*, **102**, 1–12.
- Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour*, **42**, 615–635.
- Gerhardt, H. C. 1995. Phonotaxis in female frogs and toads: execution and design of experiments. In: *Methods in Comparative Psychoacoustics* (Ed. by G. M. Klump, R. J. Dooling, R. R. Fay & W. C. Stebbins), pp. 209–220. Basel: Birkhäuser Verlag.
- Gerhardt, H. C. 2001. Acoustic communication in two groups of closely related treefrogs. *Advances in the Study of Behavior*, **30**, 99–167.
- Gerhardt, H. C. 2005. Advertisement-call preferences in diploid–tetraploid treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*): implications for mate choice and the evolution of communication systems. *Evolution*, **59**, 395–408.
- Gerhardt, H. C. & Bee, M. A. 2006. Recognition and localization of acoustic signals. In: *Hearing and Sound Communication in Amphibians* (Ed. by P. M. Narins, A. S. Feng, R. R. Fay & A. N. Popper), pp. 113–146. New York: Springer.
- Gerhardt, H. C. & Huber, F. 2002. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: Chicago University Press.
- Gerhardt, H. C., Dyson, M. L. & Tanner, S. D. 1996. Dynamic properties of the advertisement calls of gray tree frogs: patterns of variability and female choice. *Behavioral Ecology*, **7**, 7–18.
- Gerhardt, H. C., Tanner, S. D., Corrigan, C. M. & Walton, H. C. 2000. Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behavioral Ecology*, **11**, 663–669.
- Holloway, A. K., Cannatella, D. C., Gerhardt, H. C. & Hillis, D. M. 2006. Polyploids with different origins and ancestors form a single sexual polyploid species. *American Naturalist*, **167**, E88–E101.
- Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society*, **72**, 283–327.
- Klump, G. M. & Gerhardt, H. C. 1987. Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. *Nature*, **326**, 286–288.
- Lohr, B., Wright, T. F. & Dooling, R. J. 2003. Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. *Animal Behaviour*, **65**, 763–777.
- Murphy, C. G. & Gerhardt, H. C. 2000. Mating preference functions of individual female barking treefrogs, *Hyla gratiosa*, for two properties of male advertisement calls. *Evolution*, **54**, 660–669.

- Ptacek, M. B., Gerhardt, H. C. & Sage, R. D.** 1994. Speciation by polyploidy in treefrogs: multiple origins of the tetraploid, *Hyla versicolor*. *Evolution*, **48**, 898–908.
- Ritchie, M. G.** 1996. The shape of female mating preferences. *Proceedings of the National Academy of Sciences, U.S.A.*, **93**, 14628–14631.
- Rodriguez, R. L., Ramaswamy, K. & Cocroft, R. B.** 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proceedings of the Royal Society of London, Series B*, **273**, 2585–2593.
- Rundle, H. D., Chenoweth, S. F., Doughty, P. & Blows, M. W.** 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology*, **3**, 1988–1995.
- Ryan, M. J.** 2001. *Anuran Communication*. Washington, DC: Smithsonian Institution Press.
- Ryan, M. J. & Keddy-Hector, A.** 1992. Directional patterns of female mate choice and the role of sensory biases. *American Naturalist*, **139**, S4–S35.
- Ryan, M. J. & Rand, A. S.** 2001. Feature weighting in signal recognition and discrimination by túngara frogs. In: *Anuran Communication* (Ed. by M. J. Ryan), pp. 86–101. Washington, DC: Smithsonian Institution Press.
- Schwartz, J. J., Buchanan, B. W. & Gerhardt, H. C.** 2001. Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behavioral Ecology and Sociobiology*, **49**, 443–455.
- Shaw, K. L.** 2000. Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. *Evolution*, **54**, 1303–1312.
- Shaw, K. L. & Herlihy, D. P.** 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proceedings of the Royal Society of London, Series B*, **267**, 577–584.
- Sullivan, B. K. & Hinshaw, S. H.** 1992. Female choice and selection on male calling behavior in the gray treefrog *Hyla versicolor*. *Animal Behaviour*, **44**, 733–744.
- Swanson, E. M., Tekmen, S. M. & Bee, M. A.** 2007. Do female anurans exploit inadvertent social information to locate breeding aggregations? *Canadian Journal of Zoology*, **85**, 921–932.
- Taigen, T. L. & Wells, K. D.** 1985. Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *Journal of Comparative Physiology B*, **155**, 163–170.
- Wagner, W. E.** 1998. Measuring female mating preferences. *Animal Behaviour*, **55**, 1029–1042.
- Wagner, W. E. & Basolo, A. L.** 2007. The relative importance of different direct benefits in the mate choices of a field cricket. *Evolution*, **61**, 617–622.
- Welch, A. M.** 2003. Genetic benefits of a female mating preference in gray treefrogs are context-dependent. *Evolution*, **57**, 883–893.
- Welch, A. M., Semlitsch, R. D. & Gerhardt, H. C.** 1998. Call duration as an indicator of genetic quality in male gray treefrogs. *Science*, **280**, 1928–1930.
- Wells, K. D. & Schwartz, J. J.** 2006. The behavioral ecology of anuran communication. In: *Hearing and Sound Communication in Amphibians* (Ed. by P. M. Narins, A. S. Feng, R. R. Fay & A. N. Popper), pp. 44–86. New York: Springer.
- Wells, K. D. & Taigen, T. L.** 1986. The effect of social interactions on calling energetics in the gray treefrog (*Hyla versicolor*). *Behavioral Ecology and Sociobiology*, **19**, 9–18.
- Wells, K. D., Taigen, T. L., Rusch, S. W. & Robb, C. C.** 1995. Seasonal and nightly variation in glycogen reserves of calling gray treefrogs (*Hyla versicolor*). *Herpetologica*, **51**, 359–368.
- Wiley, R. H.** 1994. Errors, exaggeration, and deception in animal communication. In: *Behavioural Mechanisms in Evolutionary Ecology* (Ed. by L. A. Real), pp. 157–189. Chicago: Chicago University Press.
- Wiley, R. H.** 2006. Signal detection and animal communication. *Advances in the Study of Behavior*, **36**, 217–247.
- Wollerman, L. & Wiley, R. H.** 2002. Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Animal Behaviour*, **63**, 15–22.