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Mid-frequency suppression in the green treefrog (*Hyla cinerea*): mechanisms and implications for the evolution of acoustic communication

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Abstract Advertisement calls of green treefrogs (*Hyla cinerea*) have two spectral peaks centered at about 1 kHz and 3 kHz. Addition of a component of intermediate frequency (1.8 kHz) to a synthetic call reduced its attractiveness to females relative to an alternative lacking this component. This mid-frequency suppression occurred over a 20-dB range of playback levels. Addition of other intermediate frequencies had weak effects on preferences at some playback levels, in some localities, and at lower-than-normal temperatures. These effects correlate well with the response properties of a population of low-frequency-tuned auditory neurons innervating the amphibian papilla. Males of a closely related species (*H. gratiosa*) produce calls with emphasized frequencies within the range of suppression in *H. cinerea*; however, suppression also occurred in localities well outside the area of geographical overlap with this species. Thus, previous speculation that mid-frequency suppression evolved to enhance species discrimination is probably incorrect. This phenomenon is more likely to reflect a general sensory bias in anurans and other vertebrates, tone-on-tone inhibition. Such negative biases, and other inhibitory mechanisms, almost certainly play an important role in the evolution of communication systems but have received far less attention than positive biases that enhance signal attractiveness.

Keywords Acoustic communication · Mid-frequency suppression · Sensory bias · Tone-on-tone inhibition · Temperature effects · *Hyla cinerea*

Introduction

Numerous studies have shown that signal attractiveness can be enhanced by adding elements that never occur in signals of a given species but are produced by closely related species (review: Ryan 1990). These results have been interpreted as pre-existing sensory biases that can result in immediate positive sexual selection on individuals that produce signals with these new properties (reviews: Ryan 1990; Endler and Basolo 1998). These biases can be peripheral or central in origin (Ryan 1990), and even studies of single species in which longer, louder, or more rapidly repeated signals increase the attractiveness of sexual signals have interpreted such results as fulfilling the predictions of the sensory-bias hypothesis (e.g. Ryan and Keddy-Hector 1992). But sensory discrimination, in general, depends on inhibition as well as excitation (review: Uttal 1973), and adding new elements to signals can, in principle, have the opposite result. These inhibitory effects could thus be interpreted as negative sensory biases that would be expected to constrain and thereby also to shape the evolution of communication signals. Here, we show that adding a single component with frequencies that are not usually emphasized in conspecific advertisement calls and adjusting its amplitude to the same level as “excitatory” spectral components has negative effects on relative signal attractiveness. The widespread occurrence of inhibitory phenomena in the frequency domain, both in the peripheral and central auditory systems (Capranica and Moffat 1983; Manley 1990; Feng and Schellart 1999), indicates that negative sensory biases may have as much influence on the evolution of acoustic signals as positive ones.

As in other anurans, male green treefrogs (*Hyla cinerea*) attract females with advertisement calls (review: Gerhardt and Huber 2002). Females respond by approaching the male and usually initiate sexual contact. The frequency spectrum of the call of *H. cinerea* is bimodal. A low-frequency peak, usually consisting of one

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component, occurs in the range of 0.7–1.2 kHz and has about the same relative amplitude as a high-frequency peak, usually consisting of two to four components, in the range of 2.4–3.6 kHz (Oldham and Gerhardt 1975); components of intermediate frequency have significantly less energy (Fig. 1). A previous study found that the addition of two mid-frequency components (1.5 + 1.8 kHz) to a standard call of 0.9 + 2.7 + 3.0 kHz reduced its attractiveness relative to the standard call (Gerhardt 1974a). Here, we studied this phenomenon, which has been termed mid-frequency suppression (Capranica 1965), in a more quantitative and systematic fashion. First, we determined the range of mid-frequency suppression by varying the frequency of a single intermediate component. Second, we generalized these results over a 20-dB range of stimulus amplitude, thus providing an assessment of the extent to which mid-frequency suppression is likely to affect mate choice at different distances between males and females. We also assessed the strength of suppression by lowering the overall SPL of the preferred call, which lacked a mid-frequency component, relative to a call with the most effective mid-frequency, suppressive component. Third, we assessed the effect of temperature on the frequency range of mid-frequency suppression. These animals call over a wide range of temperature, and previous neurophysiological experiments suggested that temperature effects on mid-frequency suppression are likely (Mudry and Capranica 1987). Fourth, we conducted a geographic survey to explore the characteristics of the suppression in populations throughout the range of distribution. Such a survey is important for establishing whether a sensory bias such as mid-frequency suppression is a general property of a species' communication system and hence an appropriate trait for use in broad-scale comparative studies that include other related taxa. Comparative studies, in turn, have been used to hypothesize about the order of evolutionary change in signals and receivers (Ryan 1990; Endler and Basolo 1998). Alternatively, such a bias might be localized to particular parts of the range, where the negative effects of the presence of such a band could reinforce discrimination against the calls of another species. Indeed, Gerhardt (1974a) hypothesized that mid-frequency suppression in *H. cinerea* might have evolved or been modified to enhance discrimination against the calls of a congener, *H. gratiosa*.

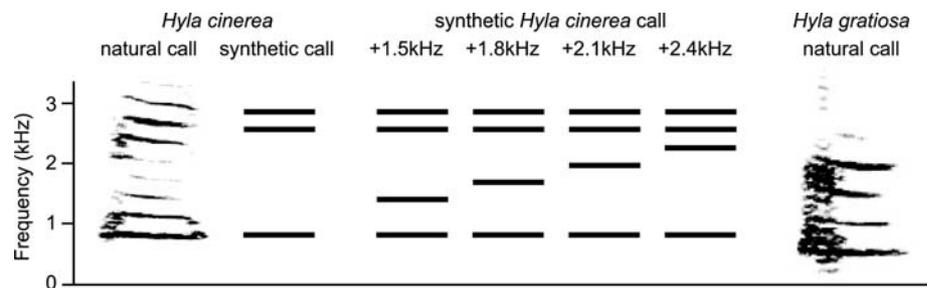
Our study represents the most comprehensive behavioral study of mid-frequency suppression published to date. Even studies that have focused on positive sensory biases have usually tested animals from one or a few localities and have not generalized the results over the range of signal intensities likely to be experienced by receivers. Because selective phonotaxis to advertisement calls is the sole basis for mate choice in green treefrogs and other anurans, mid-frequency suppression is probably a potent source of selection on the spectral structure of advertisement calls.

Materials and methods

We tested the suppressive effect of adding single, mid-frequency components on selective phonotaxis by female green treefrogs with two-speaker playbacks of synthetic advertisement calls. In most experiments, the spectra of these calls consisted of phase-locked sinusoids of equal relative amplitude (see Fig. 1). The low frequency peak of the natural call was represented by a single component of 0.9 kHz, the broader high frequency peak by two components: 2.7 and 3.0 kHz. The duration and envelope shape of the synthetic calls were similar to those of a typical advertisement call, except for the lack of a pulsatile beginning (oscillograms in Gerhardt 1974a). The standard three-component synthetic call (0.9 + 2.7 + 3.0 kHz) was as attractive to females as a typical natural pre-recorded advertisement call in two-speaker playback tests (Gerhardt 1974a).

In our experiments, the standard signal was broadcast through one loudspeaker, and an alternative stimulus with one additional, mid-frequency component, through another loudspeaker. The frequency of the added component was always a multiple of 300 Hz (in the range of 1.2–2.4 kHz) so that the waveform periodicity in all stimuli was 300/s, which is a value typical of natural calls and which is preferred by females of this species (Gerhardt 1978). The timing relationship of the alternative stimuli was fixed so that there were equal periods of silence between alternate presentations, i.e., neither stimulus led or lagged the other in time. The sound pressure levels of the alternative stimuli (SPL in decibels re: 20 μ Pa; "fast" RMS meter setting) were equalized at the female release point, which was midway between the loudspeakers, using either a Brüel and Kjaer

Fig. 1 Sonograms of natural calls of *Hyla cinerea* (left) and *Hyla gratiosa* (right). Diagrams between these two sonograms show the frequency components of synthetic calls used in choice trials. Note the lack of a prominent mid-frequency component in the *H. cinerea* natural call



2209 or Lutron SL-4001 sound level meter. Because the overall SPL of the two sounds was equalized in most tests, the absolute values of the “excitatory” components in the standard three-component call were about 1.25 dB greater than those in the four-component calls. However, we are confident that this difference in absolute values of excitatory components was not the reason for the reduction in the attractiveness of sounds with mid-frequency components of particular frequencies. First, in tests of eight females, the overall level of the stimulus with an added 1.8 kHz mid-frequency component was adjusted to be 2 dB higher (87 dB SPL) than that of the standard, three-component call (85 dB SPL); all eight females chose the standard call. Second, in all the tests of two- versus three-component calls that were used to assess temperature effects (see below), we adjusted the two-component standard call to 75 dB and the three-component alternatives to 77 dB in order to equalize the absolute values of the “excitatory” frequencies (0.6 and 3.0 kHz) in the alternatives at the release point. The values were confirmed at the release point using a 3% tunable filter set (Brüel and Kjaer Model 1621) in conjunction with the Brüel and Kjaer sound level meter. As shown below (Results), the addition of some mid-frequency components had a suppressive effect under these conditions.

The experiments generalizing suppressive effects over a 20-dB range of stimulus amplitudes (65, 75, and 85 dB SPL) were carried out in 1975, 1977, 1979, 1981 and 1982. A few tests of the strength of the preference (in terms of overall SPL differences favoring the standard, preferred call) were conducted in 1982. The females ($n = 111$) for these experiments were collected from two populations near Savannah, Georgia. The calls for these experiments were generated with a custom designed analog synthesizer described in Gerhardt (1974a). These stimuli were broadcast from Analog-Digital-Systems 200 loudspeakers (separated by 2 m) after being amplified by Nagra DH speaker-amplifiers. The frequency-response of the system was flat (± 3 dB) at the release point of the females; further, the specific frequency components were adjusted with a Crown EQ equalizer so that their relative amplitudes were within about ± 1 dB. Experiments were conducted in a semi-anechoic environment treated with sound-absorbing wedges (Soundcoat, Inc.).

A previous study had shown that frequency preferences in the low-frequency range reversibly shift to lower values (0.5–0.6 kHz from 0.9 kHz) when the animals are cooled to 16–20°C from their normal breeding temperature range of 24–28°C (Gerhardt and Mudry 1980). We assessed temperature effects on mid-frequency suppression using the same setup described in the previous paragraph during 1979. Females ($n = 21$) were acclimated to a test temperature range of about 18–20°C for at least 30 min prior to testing, and we measured their cloacal temperature after every response. The standard call had two equal-amplitude components of 0.6 and 3.0 kHz, and alternatives had an additional component

of the same relative amplitude and intermediate frequency (0.6+0.9+3.0 kHz, 0.6+1.2+3.0 kHz or 0.6+1.8+3.0 kHz).

The experiments that tested for suppressive effects over the range of distribution of *H. cinerea* were conducted in 1999–2001. Females ($n = 69$) were collected from six different populations in the eastern and central USA (see Fig. 2). In these experiments, we used 16-bit digital signals, which were created with custom-designed software and broadcast at 44 kHz sampling rate using commercial D/A hardware and interface software (CoolEdit96, Syntrillium Software Corp). Signals were amplified and broadcast through Radio Shack Optimus XTS 40 speakers situated just outside a portable playback arena located outdoors. The arena was rectangular and had opaque but acoustically transparent sides. The frequency-response of the speakers and arena was taken into account during sound synthesis so that the relative amplitudes of all components were within ± 2 dB. The SPL of the standard call and alternatives were always equalized at 85 dB SPL.

During an experiment, a female was placed singly in an acoustically transparent cage at the release point. After several repetitions of the alternative stimuli, the female was released and a choice was scored when a female moved to within 10 cm of one of the speakers, which continued to playback synthetic calls until after a response occurred. Only one response per female per test was tabulated, and if a female was tested in more than one test, there was a time-out period of at least 5 min (see Gerhardt 1981a for an analysis showing a lack of carry-over effects from one test to another in *H. cinerea*). Stimuli were switched between speakers periodically to minimize any side biases, none of which were detected. We report the proportions of females choosing the alternative to the standard call and show the 95%

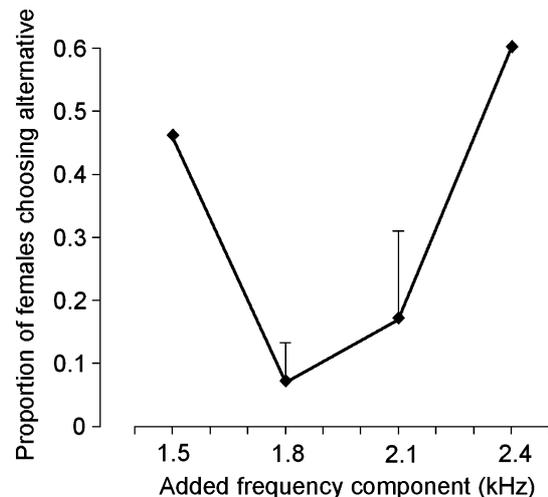


Fig. 2 Addition of a mid-frequency component of 1.8 or 2.1 kHz resulted in behavioral suppression. Shown is the combined data set from all tests performed at 85 dB SPL. Error bars are upper 95% credible limits. For details on each of the populations tested see Table 1

Bayesian credible intervals. These intervals are numerically the same as confidence intervals (Burstein 1971) because we assumed a uniform prior probability function; however, unlike confidence intervals their validity does not depend on a stopping rule, such as a predetermined sample size. Credible intervals are also interpreted in a more straightforward fashion than confidence intervals (Gerhardt 1992). We consider a preference to be statistically significant if the upper 95%-credible limit on the proportion of females choosing the alternative stimulus was < 0.40 ; results meeting this criterion would also be significant at the 0.05-level in a two-tailed binomial test if sample sizes had been predetermined.

Results

Mid-frequency suppression resulting from the addition of a single frequency component (1.8 kHz) occurred over a wide range of playback levels in tests of females from eastern Georgia (USA): fewer than 10% of the females tested at each level chose the alternative that had this component (Fig. 3; Table 2). Other mid-frequency components had relatively weaker effects at some playback levels. About 30% of females also chose the alternative of 2.1 kHz at 75 dB SPL, and this proportion dropped to a significant level (about 0.17, 95% upper credible limit of 0.31) at 85 dB SPL, perhaps suggesting a trend for an upward shift in the inhibitory range with increasing playback levels.

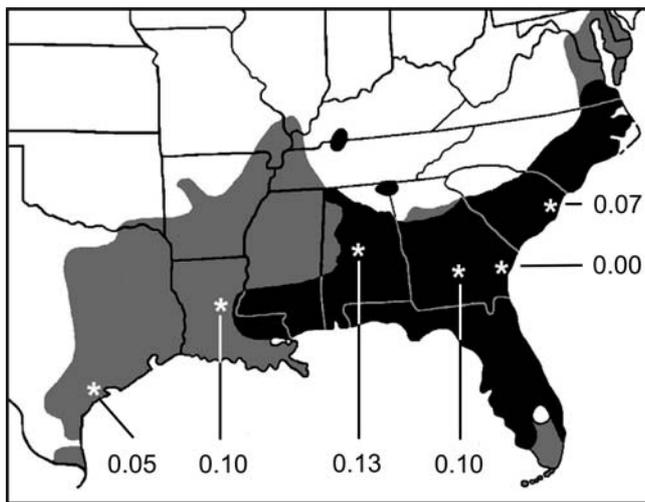


Fig. 3 The negative bias against added mid-frequency components is present across the geographic range of *H. cinerea*. The gray shaded area denotes allopatric occurrence of *H. cinerea*, the black shaded area the sympatric range where both *H. cinerea* and *H. gratioiosa* occur. Symbols indicate the specific test localities, and the proportions of females from each location that chose the alternative call with a mid-frequency component of 1.8 kHz are indicated (95%-credible limits are listed in Table 2). Females were collected from ponds near Sinton (Texas), Forest Hill (Louisiana), Marion (Alabama), Tifton and Savannah (Georgia) and Georgetown (South Carolina)

Only 30% of the females tested at 65 and 75 dB SPL chose the alternative with a component of 1.5 kHz, but upper credible limits exceeded our significance criterion. The strength of suppression does not appear to be particularly strong in the face of intensity differences between the two alternatives. In tests in which the overall SPL of the standard call was reduced by 6 dB relative to that of the alternative, five females chose the alternative with a mid-frequency component of 1.8 kHz and one chose the standard call. Notice, too, that one or even a few females chose the call with the 1.8 kHz component in most tests (see Table 1).

Most females tested at lower-than-normal temperatures (body temperature range: 16.8–22°C) chose the standard call of 0.6+3.0 kHz to alternatives to which mid-frequency components of 1.2 kHz and 1.8 kHz were added. Nine of 11 females chose the standard call in the first of these tests (0.82, 95% lower credible interval of 0.53), and thirteen of 15 did so in the second test (0.87, 95% lower credible interval of 0.64). Females did not prefer the standard call to an alternative of 0.6+0.9+3.0 kHz; three females chose the standard call and three chose the alternative. At normal breeding temperatures (24–28°C), the mid-frequency component of 1.2 kHz was within the excitatory range and did not have suppressive effects (Tables 1 and 2; Fig. 2). Thus, the frequency range of mid-frequency suppression was much broader because the mid-frequency component of 1.8 kHz was still effective.

In choice-tests at 85 dB SPL, the addition of the mid-frequency component of 1.8 or 2.1 kHz resulted in a reduction in the relative attractiveness of a synthetic call in populations throughout the range of distribution of green treefrogs (Table 1; Fig. 2).

Fewer than 15% (maximum 95% upper credible interval < 0.40) of the females in any population chose the alternative with a mid-frequency component of 1.8 kHz over the standard call. In Louisiana, females also discriminated against an alternative with a mid-frequency component of 2.1 kHz (95% upper credible limit: 0.39); there was also a non-significant trend in the same direction in Alabama and Tifton, GA (95% upper credible limits: 0.44 and 0.51, respectively). Across the range of distribution, addition of a mid-frequency component of 1.5 or 2.4 kHz had no significant effect on call attractiveness.

Discussion

Our results robustly demonstrate a consistent, geographically widespread sensory bias in the communication system of the green treefrog. The negative effect of adding a mid-frequency component on signal effectiveness could be interpreted as stronger than in the only other behavioral example of mid-frequency suppression that has been discussed in the context of communication in anurans. That is, in the green treefrog, the mid-frequency component effectively reduced signal

Table 1 Effective frequency components and geographic distribution of mid-frequency suppression in *Hyla cinerea*. Playback level in all experiments was 85 dB SPL. Significant results are indicated by asterisks

Added Frequency (kHz)	Study site	Females choosing alternative with mid-frequency component			<i>n</i>
		Proportion	95% lower credible intervals	95% upper credible intervals	
1.5	Louisiana	0.50		0.778	10
	Alabama	0.40		0.696	10
	Georgia (Tifton)	0.70	0.393		10
1.8*	Georgia (Savannah)	0.35		0.558	20
	Texas	0.05		0.216*	20
	Louisiana	0.10		0.394*	10
	Alabama	0.13		0.363*	15
	Georgia (Tifton)	0.10		0.394*	10
	Georgia (Savannah)	0.00		0.162*	17
	South Carolina	0.07		0.297*	14
	Louisiana	0.10		0.394*	10
2.1*	Alabama	0.20		0.439	15
	Georgia (Tifton)	0.20		0.507	10
2.4	Louisiana	0.40		0.696	10
	Georgia (Tifton)	0.80	0.493		10

Table 2 Discrimination by female green treefrogs against synthetic calls with added mid-frequency components as a function of playback level. Significant discrimination against an alternative with an added component is indicated by asterisks. Females from eastern Georgia (Savannah) were tested at 65, 75, and 85 dB SPL; females from other study sites (see Fig. 3 and Table 1) were only tested at 85 dB SPL, thus the difference in sample size

Playback level (dB SPL)	Added frequency component (kHz)	Females choosing alternative with mid-frequency component			<i>n</i>
		Proportion	95% lower credible intervals	95% upper credible intervals	
65	1.5	0.31		0.548	16
	1.8*	0.07		0.250*	17
75	1.2	0.50		0.778	10
	1.5	0.33		0.536	21
	1.8*	0.06		0.238*	18
	2.1	0.31		0.548	16
85	1.5	0.46		0.585	50
	1.8*	0.07		0.133*	86
	2.1*	0.17		0.311*	35
	2.4	0.60	0.394		20

attractiveness when its relative amplitude was the same as that of the “excitatory” components that are normally emphasized in the advertisement call. In by contrast, in the bullfrog (*Rana catesbeiana*) the amplitude of a suppressive frequency component had to be 10 dB greater than an “excitatory” low-frequency component to reduce evoked calling (Capranica 1965). Evoked calling, however, is a “no-choice” (single speaker) design, and the forced-choice testing paradigm used with green treefrogs is undoubtedly more sensitive. In favor of this interpretation, we point out that females responded to a stimulus consisting solely of pairs of mid-frequency components (1.8 + 2.1 kHz) in no-choice tests (Klump et al. 2004) designed to assess the frequency-dependence of sound localization. Nevertheless, females in nature have the chance to choose among several nearby calling males (Gerhardt and Klump 1988) even in dense choruses, and our results indicate that a male producing calls with strong mid-frequency components around 1.8 kHz, would be less likely to attract a mate than a male with the usual spectral structure.

Two other studies of anurans have found that adding a second component of lower frequency to a synthetic

call with a high-frequency component reduced its relative attractiveness (Witte et al. 2001; Bosch and Boyero 2003). In these studies, however, the frequency of the added component matched the estimated frequency sensitivity of the amphibian papilla and was therefore expected to enhance the signal’s attractiveness relative to single, high-frequency components, even though conspecific calls in both species have only a single high-frequency band that matches the tuning of the basilar papilla. These low-frequency signals did not attract females in no-choice situations, but the preferences cannot unequivocally be interpreted as inhibition because in both the studies the overall levels of the alternative sounds were equalized. Instead, females could have based their choices on the absolute difference of 3 dB in the high-frequency range, which favored the single-component alternatives.

Correlations with neurophysiological studies

Paralleling the behavioral results in *R. catesbeiana*, an inhibitory tone with an amplitude that was 10 dB

greater than an excitatory tone effectively inhibited the responses of auditory nerve fibers to an excitatory tone at the neuron's characteristic frequency (Frishkopf et al. 1968). Such peripheral tone-on-tone inhibition is well known in vertebrate auditory systems (review: Manley 1990; Feng and Schellart 1999). In *H. cinerea*, the response of low-frequency tuned auditory nerve fibers was also suppressed reliably when the amplitude of the second, higher-frequency tone was, on average, about 11 dB greater than the excitatory tone; in some neurons suppression occurred when the difference was of the order of 6 dB (Ehret et al. 1983). We therefore suggest that mid-frequency suppression in *H. cinerea* is also likely to be mediated mainly by peripheral tone-on-tone inhibition, although we cannot rule out the possibility that central inhibition (Fuzessery 1988; Mudry and Capranica 1987) may contribute to the greater effect of mid-frequency suppression (at 0 dB relative amplitude) in behavioral tests.

Further support for the hypothesis that behavioral suppression is mainly caused by peripheral mechanisms is the correlation between the results of neurophysiological and behavioral studies of temperature effects. Mudry and Capranica (1987) reported non-linear facilitated responses in the auditory thalamic area of *H. cinerea*: combinations of two tones with the appropriate frequencies elicited far greater responses than either tone in isolation. Furthermore, these facilitated responses could be suppressed by the addition of a third tone of intermediate frequency. These results were consistent with behavioral studies (Gerhardt 1974a) in that synthetic calls with two frequency bands were more attractive than calls with either band alone. A major discrepancy between the two studies was that the low-frequency "excitatory" band and the mid-frequency suppressive band were both far lower in frequency in the neurophysiological studies (0.5 and 1.2 kHz, respectively) than in the behavioral one (0.9 and 1.8–2.1 kHz, respectively). Accordingly, Gerhardt and Mudry (1980) tested and found temperature effects on female green treefrogs; females tested at lower-than-normal temperatures reversibly changed their preference for calls with a low-frequency peak of 0.9 kHz to calls with low-frequency peaks of 0.5 and 0.6 kHz. These behavioral results, in turn, prompted additional neurophysiological experiments that showed parallel temperature-dependent changes in optimal low-frequency component for facilitated responses in the thalamus, which were included in the paper reporting auditory responses in the thalamus (Mudry and Capranica 1987).

In the present study we have shown that at below-normal temperatures, the range of behavioral suppression expands to a lower range of frequencies that includes the "optimum" suppressive frequency of 1.2 kHz found by Mudry and Capranica (1987). Support for the hypothesis that this shift originates in the periphery, rather than in the central auditory system as suggested by Mudry and Capranica (1987), comes from studies of temperature effects on the tuning and

tone-on-tone suppression of auditory nerve fibers in other species of anurans. First, Stiebler and Narins (1990) showed that the characteristic frequency (tuning) of low- and mid-frequency fibers in *Eleutherodactylus coqui* and *Hyla regilla* innervating the amphibian papilla was highly temperature dependent. The magnitude of these changes alone is sufficient to explain the changes in low-frequency preferences in *H. cinerea* even if central processing were unaffected. In contrast the tuning of high-frequency fibers innervating the basilar papilla was temperature-independent, paralleling the lack of a frequency-preference change with respect to the high-frequency peak in *H. cinerea* (Gerhardt and Mudry 1980). Second, Benedix et al. (1994) showed that the suppressive range above the characteristic frequency of mid- and low-frequency tuned fibers innervating the amphibian papilla of the leopard frog (*Rana pipiens*) decreased when the body temperature of the frog was increased. Obviously, this also means that the range of suppressive frequencies would be expected to expand when temperature decreased as it did in the behavioral experiments with *H. cinerea* (this study). Recent research suggests that temperature-dependence in the amphibian papilla is caused by effects on the electrical properties of hair cells that determine their resonant frequency (review: Narins 2001). A remaining puzzle is that the tuning of the non-inhibitable mid-frequency-tuned fibers better matches the frequency range of the low-frequency peak in advertisement calls than does the tuning of the low-frequency tuned inhibitable fibers (Capranica and Moffat 1983; Klump et al. 2004). Our behavioral results suggest that these inhibitable neurons must nevertheless be playing some role in selective phonotaxis, perhaps by contributing to sound localization (Klump et al. 2004).

Implications for mate choice and evolution of acoustic communication

What are the consequences of mid-frequency suppression for the communication system of green treefrogs? First, this negative sensory bias is likely to be a potent factor in maintaining the distinctly bimodal call spectrum in this species. We are unaware of any population of green treefrogs in which male advertisement calls have strongly emphasized frequency components that fall within the effective range of mid-frequency suppression. The energy in both spectral peaks is always substantially greater (> 6 dB) than that in the mid-frequency range (see also Oldham and Gerhardt 1975). Other anuran species have broad-band signals, such as the advertisement calls of a closely related species (*Hyla andersonii*; Gerhardt 1974b), without significant attenuation of intermediate frequencies. It will be important to conduct both behavioral and neurophysiological studies of mid-frequency suppression in such species. Second, as suggested by Gerhardt (1974a), mid-frequency suppression may contribute to discrimination by females of *H. cinerea* against heterospecific signals, such as those of

the barking treefrog (*H. gratiosa*), whose calls strongly emphasize frequency components around 1.5–2.0 kHz. The uniformity of the behavioral suppression in *H. cinerea* in both allopatry and sympatry suggests, however, that any contribution to such discrimination is a fortuitous consequence of a general sensory bias rather than an adaptation resulting from selection against mismatching. Barking treefrogs are larger than green treefrogs and their frequency preferences and auditory tuning (Capranica and Moffat 1983) are also lower; hence frequencies in the range of 1.5–2.0 kHz are attractive to females (Gerhardt 1981b). Playback experiments at one level (65 dB) did not reveal mid-frequency suppression in *H. gratiosa* (0.5 + 1.5 + 2.0 kHz was not preferred to 0.5 + 1.0 + 1.5 + 2.0 kHz) even though, as in *H. cinerea*, the spectrum of the advertisement calls is bimodal (see Fig. 1). It will be important, however, to conduct additional experiments at higher playback levels before concluding that mid-frequency suppression does not exist in this species.

Alternatively, mid-frequency suppression could have been selected for in sympatry and been maintained when ranges of distribution changed, i.e., expansion of the range of *H. cinerea*, contraction of the range of *H. gratiosa*, or both. Arguing against this alternative is the existence of a strong pattern of reproductive character displacement in the selectivity of female green treefrogs for synthetic calls with the conspecific spectrum in tests against calls with a spectrum typical of *H. gratiosa* (Höbel and Gerhardt 2003). Evidently, mid-frequency suppression contributes relatively little, if at all, to the mechanisms responsible for this geographic difference in spectral selectivity. Indeed, the reversal of the preference when the SPL of the standard call was reduced by 6 dB (see Results) occurred in tests of females from a sympatric locality.

Regardless of the evolutionary origins or the specific mechanisms of mid-frequency suppression in green treefrogs, the important message of this study is that negative as well as positive sensory biases can shape signal evolution, mate choice and the evolution of communication systems. These consequences have not been widely appreciated by either evolutionary biologists or physiologists despite the widespread occurrence of inhibitory phenomena, both at the periphery and in the central nervous system (Capranica and Moffat 1983; Manley 1990; Feng and Schellart 1999). In fact, we are unaware of any example in which such effects have been discussed in this context. The next step will be to conduct comparative studies of negative biases. These studies have the potential to estimate broader-scale patterns of evolution that may provide evidence about the evolutionary origins of such biases in treefrogs and other anurans (e.g., Ryan and Rand 1993).

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