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Mating Call Recognition in the Green Treefrog (*Hyla cinerea*): Importance of Two Frequency Bands as a Function of Sound Pressure Level

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Summary. 1. Four hundred and ninety-nine females of *Hyla cinerea* made 820 responses in two-choice playback experiments conducted at sound pressure levels (SPL's) between 48 and 85 dB. About one-third of the animals responded at 48 dB, and the percentage increased as a function of SPL.

2. Females did not show a preference for a standard synthetic call with two components (0.9+ 3.0 kHz) of equal relative amplitude over a single-component (0.9 kHz) call at 48 dB SPL, but chose the bimodal stimulus at 54 dB and higher SPL's (Table 1).

3. Females chose the standard call over calls in which the high-frequency peak was attenuated by 12 or 6 dB only when the playback level (overall SPL) of the two sounds was 72 dB or higher (Figs. 1 and 2).

4. Females chose the standard call over one in which the low-frequency peak was attenuated by 12 dB over the entire range of playback levels (48 to 85 dB); when the low-frequency peak was attenuated by 6 dB, females preferred the standard call at low to moderate but not high SPL's (Figs. 3 and 4).

5. The behavioral results are related to acoustic communication in the natural environment and to basic neurophysiological data from the auditory system of *H. cinerea*.

Introduction

Gravid females of *Hyla cinerea* (green treefrog) selectively respond to the conspecific mating call and are also attracted to appropriate 'synthetic' sounds (e.g. Gerhardt 1974a, b; Oldham and Gerhardt 1975). Mating calls of this species have a distinctly bimodal spectrum: one peak, consisting usually of one but sometimes two components, is around 1 kHz; the other, broader peak, consisting of two to five components, is centered around 3 kHz. There is a dip in the relative amplitudes of components between the two peaks. The first behavioral study using synthetic calls established that both spectral peaks are important for call recognition by the female at a moderate sound pressure level (SPL in dB re 20 $\mu N/m^2$) (Gerhardt 1974b). A subsequent study demonstrated that female preferences depended on the playback level: females readily detected a deficiency in the relative amplitude of the low-frequency peak at low and moderate SPL's (65 and 75 dB) but failed to show a clear preference at a high level (85 dB); they detected high-frequency attenuation at moderate and high levels but did not respond preferentially at a low level (Gerhardt 1976).

This paper is a follow-up to the study published in 1976. I have conducted more than 1,100 additional tests of more than 500 animals. Since the playback system and test environment were improved, I was able to use stimuli with more subtle spectral differences and to test animals over a wider range of playback level. The principal goals were (1) to estimate the lowest SPL's at which low- and high-frequency sound energy affects the *selective phonotaxis* of the female; and (2) to assess the effects on selective phonotaxis of 6- and 12-dB attenuations of the relative amplitudes of the two spectral peaks over a SPL range of 48 to 85 dB. This range corresponds to the level just above the behavioral threshold for phonotaxis to the level at about 1 m in front of a typical calling male of *H. cinerea*.

The results relate to sound communication in nature as well as to neurophysiological studies of the green treefrog auditory system. Not only do the spectral properties of the calls of individual males differ somewhat, but also these properties and the overall sound levels change as the signals propagate through

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natural environments. Moffat and Capranica (1974) found that two populations of auditory neurons in the VIIIth nerve of *H. cinerea* are tuned reasonably well to the spectral energy in the mating call of the male. As in the bullfrog, the units tuned to the low-frequency energy (around 1 kHz) probably innervate the amphibian papilla – an inner ear organ unique to amphibians – whereas the high-frequency units probably innervate the basilar papilla (Feng et al. 1975; Capranica 1976). My behavioral results indicate that excitation of the amphibian papilla alone is probably sufficient to elicit phonotaxis. An optimum stimulus, however, excites both inner ear organs, and the behavioral results suggest further that the central auditory system is sensitive to a certain ratio of excitation of the two organs. These behavioral data should become even more important as more quantitative evidence accumulates about the peripheral auditory system and the central nervous system centers upon which the outputs of the two inner ear organs converge.

Materials and Methods

The preferences of gravid female treefrogs were determined by observing their responses in two-choice playback experiments.

Experimental Stimuli, Playback System, and Test Environments. Synthetic calls were generated by a custom-built synthesizer (Gerhardt 1974b). Most of the synthetic calls used in this study consisted of just two phase-locked components: 0.9 and 3.0 kHz. Most of the experiments done in 1976 employed sounds with three phase-locked components: 0.9, 2.7 and 3.0 kHz. In one experimental series (1977–1979) a synthetic call consisting of just 0.9 kHz was used. The frequency-responses of the two Nagra DH speakers that were used in 1975–1977 were taken into account during call synthesis, and the relative amplitudes of the various components adjusted accordingly. This became unnecessary in late 1977–1979 when Analog-Digital-Systems 200 speakers and a stereo equalizer (Crown EQ synergistic) were incorporated into the playback system.

Synthetic calls lasted 160 ms and repeated every 0.8 s. They were recorded and played back with a Nagra IV-S or ReVox A77 stereophonic recorder. There was one kind of synthetic call per channel on tape programs that lasted 7 to 12 min. The two signals on each channel alternated (every 0.4 s) and their amplitude-time envelopes were the same as those used in previous studies (Gerhardt 1974b, 1978).

Fidelity of sound reproduction was checked at the release point of the frogs midway between the speakers. In 1975 this was done by recording the signals with a Nagra IV recorder and Sennheiser 815 microphone and then analyzing the recordings in the laboratory with a wave analyzer (General Radio (GR) 1900A) and oscilloscope (Tektronix D13). The results of these analyses indicated that the accuracy of the tape equalization during synthesis was about ±3 dB. In 1976 and subsequent years checks were made at the release point by means of a Brüel and Kjaer (B&K) 1621 3%–1/3 octave tunable filter and a B&K 2209 or GR 1933 sound level meter. This equipment was also used to equalize the overall SPL's of the sounds (±1 dB) and the relative amplitudes of particular frequency components (±1 dB of the desired amplitudes). A Tektronix 214 storage oscilloscope was used to spot check for sound reflections at the release point beginning in 1976.

In 1975 all experiments were done outdoors on a level cement driveway. In 1976 fewer than one-half of the experiments were conducted outdoors on a level asphalt golf cart path. The remainder of the experiments were done in a large exhibit hall in the Savannah Science Museum or in a room at the Oatland Island Educational Center, Savannah, Georgia. Sound reflections were reduced by means of anechoic wedges of acoustic foam (Soundcoat Inc.), and the distance between the speakers was reduced to 2 m (from 4 m). This change improved the signal-to-noise ratio at the release point and reduced the magnitude of harmonic distortion at high SPL's, even though the level of such distortion was acceptable (1% or less) when the speakers were 4 m apart. Another advantage of the indoor experimentation was the absence of extraneous light sources, i.e. moonlight, distant floodlights, etc. These lights might bias a female's initial orientation, especially when the difference between the two sounds is slight. Ambient temperature during the experiments ranged from 23 to 29 °C but was usually 25±2 °C.

Experimental Procedure. Two female treefrogs were collected in Bryan County, Georgia near Richmond Hill. All others were from Chatham County, within 30 km of Savannah, Georgia. The majority of these were from Wilmington Island. Most females were collected in amplexus late on one night, refrigerated to inhibit oviposition, and acclimated (15 min or longer) to the room temperature prior to testing on the following night. A few females were tested on the night of capture, and a few others were tested as many as four nights later. These different treatments did not seem to affect the choices of the animals, but holding an animal for more than one day decreased the probability that it would respond at all.

Each female was placed individually midway between the two speakers, where she was restrained in a small hardware cloth cage until she recovered from handling (usually less than 30 s). The top of the cage was then removed at a time when the female did not face either speaker, and the playback of sounds begun. A response was tabulated when a female approached within 30 cm of a speaker; usually she touched it. Many animals were tested in more than one two-choice experiment. Several minutes to about an hour usually elapsed between experiments using the same stimuli at different SPL's. There was, however, little evidence to suggest that a female's choice in one experiment influenced her choice in a subsequent experiment, even when the tests followed one another closely in time (see Appendix). In any event the order of presentation of experiments was varied so that there was usually an adequate number of 'first responses', upon which a confident decision could be made about the existence or absence of a preference. I emphasize that only one response per female in a given test at a given SPL was tabulated; the term 'first response' merely refers to the situation in which a female had no prior exposure to either of the alternatives in some previous test. Stimuli were exchanged between speakers often so that any undetected position biases (playback system) or directional biases of the females were neutralized.

Results

Data from a previously published note (Gerhardt 1976) are included and clearly labeled. Four hundred and ninety-nine (78%) of the 642 females responded in at least one two-choice test. There were 820 (71%) responses in the 1163 tests conducted. Not sur-

Table 1. Responses of *Hyla cinerea* females given a choice between a synthetic call of 0.9 kHz and one of 0.9+3.0 kHz. The two components of the bimodal stimulus had the same relative amplitude. The absolute levels of the 0.9 kHz component of the bimodal stimulus was equalized to the same sound pressure level (SPL) as the 0.9 kHz stimulus. *N.S.* not significant. *N.R.* number of females that did not respond

Sound pressure level of 0.9 kHz component and 0.9 kHz call	Number of females choosing 0.9+3.0 kHz call	Number of females choosing 0.9 kHz call	Probability (two-tailed binomial)	N.R.
48 dB	16	10	N.S.	43
54 dB	21	5	0.002	32
60 dB	16	1	0.001	14
66–72 dB	7	0	<0.015	2

prisingly, there was a direct relationship between the percentage of responding animals and the SPL at which they were tested (see Figs. 1–5). Detailed tables of the results to be summarized in this paper are available from the author.

A Single-Component vs a Biomodal Synthetic Call. Given a choice between a synthetic call consisting solely of 0.9 kHz and one consisting of 0.9+3.0 kHz components of equal relative amplitude (standard call), a sample of 26 females failed to show a preference at a level of 48 dB (Table 1). This occurred even though the 0.9 kHz components of the two sounds were equalized so that the overall SPL of the standard (bimodal) stimulus was 3 dB greater than that of the single-component call. Since only 36% of the females tested at 48 dB responded to any acoustic stimulus, I consider this SPL to be a reasonable estimate of the 'behavioral threshold' for phonotaxis in this species. (Four of six females tested at 42 dB also responded, but this was a highly selected group of females that had responded with unusual rapidity at 48 dB). When, however, the SPL of the playback was increased to 54 dB, females preferred the standard bimodal call, and at 60 dB and higher the preferences were even clearer (Table 1).

Effects of Attenuating the High-Frequency Peak. Females given a choice between the standard call and one in which the high-frequency peak was attenuated by about 12 dB (Upper peak (UP) −12 dB) chose the standard call at moderate to high SPL's but did not show a preference at low levels (Fig. 1). Similar results were obtained when the high-frequency attenuation was 6 dB (Fig. 2). In two experiments the 0.9 kHz components of the standard call and of the UP −6 dB call were equalized rather than the overall SPL's of the two sounds. Females chose the standard call at 66 dB but did not show a preference at 54 dB, despite the fact that the overall level of the standard call was 2 dB greater than that of the alternative.

In 1976 both the standard call and alternatives had high-frequency peaks consisting of two components (2.7 and 3.0 kHz). Results of experiments in which the relative amplitudes of these components were attenuated relative to the 0.9 kHz low-frequency peak are presented in Table 2. When the UP was −10 dB, the pattern of responses was similar to that described in the previous paragraph; females did not show a preference at 60 dB, but chose the standard call at 75 and 85 dB. When the UP of an alternative was −15 dB, most females chose the standard call at all playback levels, including 60 dB. These results are important because the standard call used is as attractive to females as a typical natural mating call (Gerhardt 1974b and unpublished); the standard call used in all other experiments (0.9+3.0 kHz) is not as attractive (Gerhardt 1978). Because the beating (300/s) of the two high-frequency components (2.7 and 3.0 kHz) is itself a pertinent (fine-temporal) property, the interpretation of experiments in which these components are attenuated is not straight forward (Gerhardt 1976, 1978). Preferences could be based on the deficiency of high-frequency energy per se, on the degradation of the fine-temporal information (beat amplitude) or on a combination of these two factors.

Effects of Attenuating the Low-Frequency Peak. When a standard call was tested against an alternative in which the low-frequency peak was attenuated by 12 dB relative to the high-frequency peak (Lower peak (LP) −12 dB), females preferred the standard call over the entire range of playback levels: 48 to 85 dB (Fig. 3). Some of the experiments conducted at 60 dB and at 85 dB (in 1976) used a three-component standard (0.9+2.7+3.0 kHz) and alternatives; the other experiments used the two-component standard depicted in Fig. 3. Females did not show a preference for the standard call at 72 and 84 dB when the low-frequency peak of the alternative was attenuated by only 6 dB (LP −6 dB) (Fig. 4). Even at SPL's where statistically significant preferences were established, substantial numbers of females chose the LP −6 dB stimulus. At 54 dB females chose the standard call when the 3.0 kHz components of the standard and alternative were equalized (Fig. 4); three females chose the standard and two chose the LP −6 dB call when the overall SPL's of the two sounds were 54 dB.

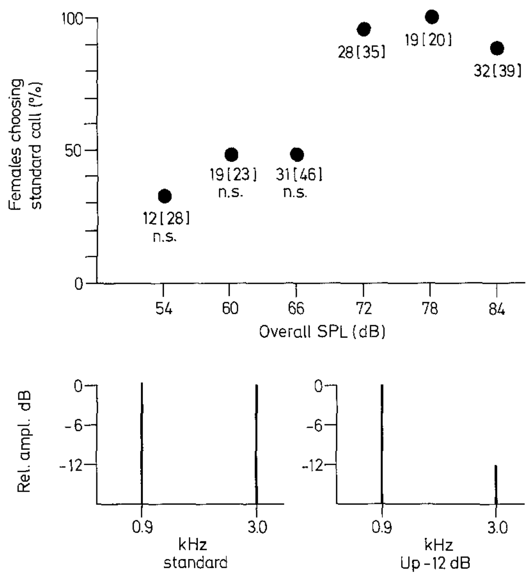

Fig. 1. Responses of females given a choice between the standard call and a call in which the high-frequency component was attenuated by 12 dB[1]. The number of responding animals is indicated under each point and the total number tested is indicated in parentheses. Unless otherwise indicated (*n.s.* not significant), the probability (two-tailed binomial) that the animals preferred the standard call was 0.001 or less. Data from Gerhardt (1976) are included in the results at 65 dB (12 animals), 70 dB (5 animals), and 84 dB (9 animals)

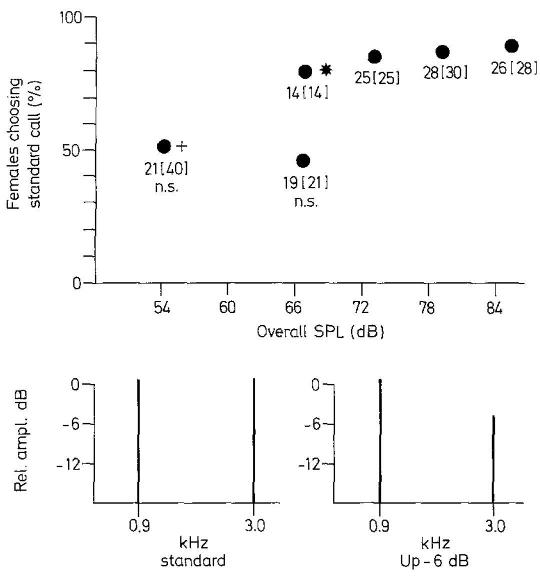

Fig. 2. Responses of females given a choice between the standard call and a call in which the high-frequency component was attenuated by 6 dB. Sample sizes are indicated as in Fig. 1. Unless otherwise indicated (*n.s.* not significant), the probability (two-tailed binomial) that the animals preferred the standard call was 0.001 or less, except at 66 dB, where the probability was 0.057. *0.9 kHz components of the standard and UP −6 dB were equalized at 66 dB. +0.9 kHz components of the standard and UP −6 dB were equalized at 54 dB

1 Analysis of a recording of the stimulus playback under test conditions revealed that the 3.0 kHz component was only − 9 dB relative to the 0.9 kHz component in the experiments done in 1975 (Gerhardt 1976)

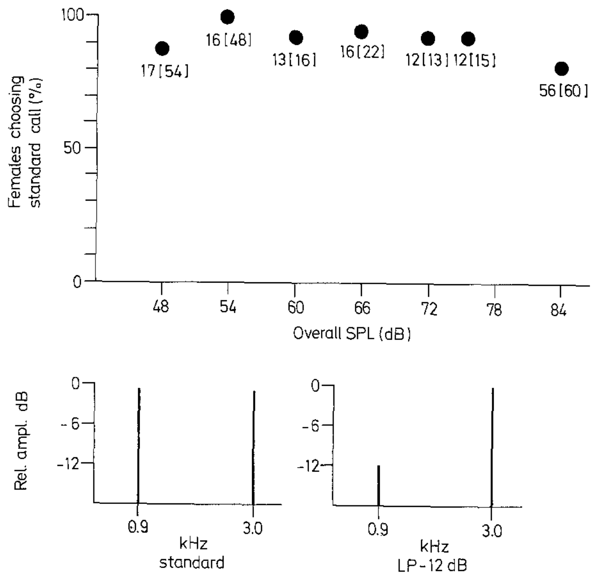

Fig. 3. Responses of females given a choice between the standard call and a call in which the low-frequency peak was attenuated by 12 dB. Sample sizes are indicated as in Fig. 1. The probability that the animals preferred the standard call was 0.006 or less at every SPL. In 10 tests at 60 dB and 9 tests at 85 dB, the standard and the alternative stimulus had a high-frequency peak consisting of 2.7 and 3.0 kHz. Data from Gerhardt (1976) are included in the results at 66 dB (14 animals), 72 dB (3 animals), 75 dB (all data), and 84 dB (23 animals). The only discrepancy between the results of the 1976 study and this study was at 85 dB. The failure of the animals to show a clear preference for the standard call in the earlier study was probably attributable to extraneous lights in the outdoor test environment

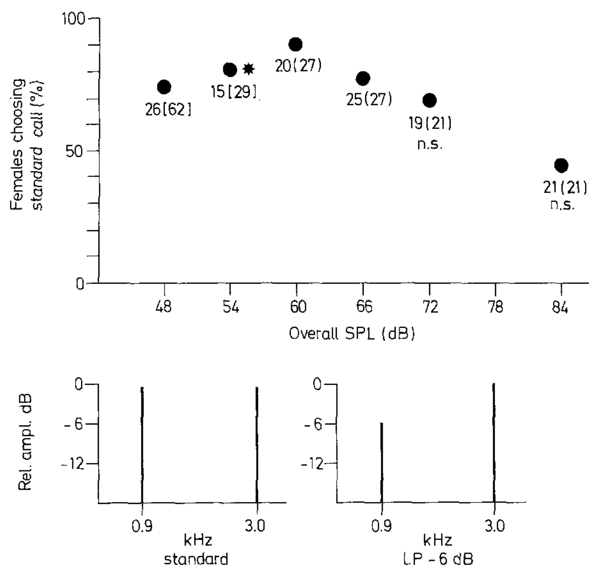

Fig. 4. Responses of females given a choice between the standard call and a call in which the low-frequency peak was attenuated by 6 dB. Sample sizes are indicated as in Fig. 1. Unless otherwise indicated (*n.s.* not significant), the probability (two-tailed binomial) that the animals preferred the standard call was 0.035 or less. The 3.0 kHz components rather than the overall SPL's were equalized in experiments at 54 dB; see for results when the overall levels were the same

Table 2. Responses of females given a choice between a standard synthetic call of three components (0.9+2.7+3.0 kHz) and synthetic calls in which the high-frequency components were attenuated relative to 0.9 kHz. Number of first responses given in parentheses. *NR* no response; *NS* not significant; *P* probability (two-tailed binomial). All experiments were done in 1976

SPL	Number of females choosing standard call	Number of females choosing UP-10 dB	*P*	NR
60 dB	4 (4)	8 (8)	NS	4
75 dB	18 (10)	6 (3)	0.022	3
85 dB	21 (10)	3 (0)	<0.001	1

SPL	Number of females choosing standard call	Number of females choosing UP-15 dB	*P*	NR
60 dB	20 (19)	4 (4)	0.001	5
65 dB	8 (5)	1 (1)	0.038	4
75 dB	9 (5)	0	0.004	2
85 dB	10 (10)	3 (1)	NS	1

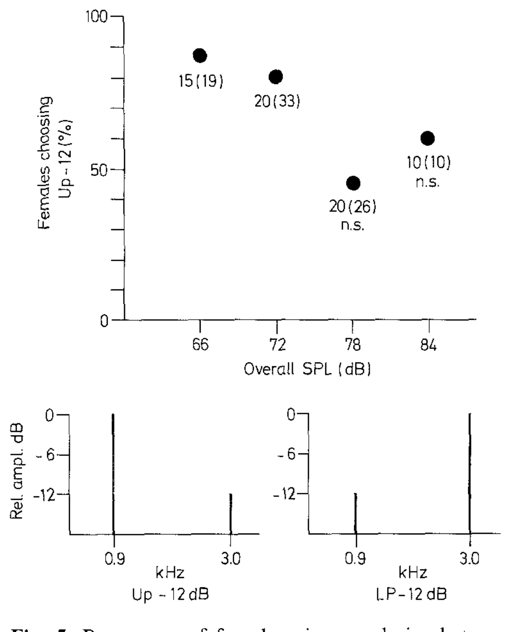

Fig. 5. Responses of females given a choice between a call with an attenuated (12 dB) high-frequency peak and a call with an attenuated (12 dB) low-frequency peak. Sample sizes are indicated as in Fig. 1. Unless otherwise indicated (*n.s.* not significant), the probability (two-tailed binomial) that the animals preferred the call with attenuated high-frequency peak (UP-12 dB) was 0.012 or less

High-Frequency Attenuation vs Low-Frequency Attenuation. The results presented above indicate that low-frequency attenuation of 12 dB is behaviorally significant over a wide range of SPL; high-frequency attenuation is effective only at moderate to high SPL. This was tested in a direct fashion by offering females a choice between LP −12 dB and UP −12 dB. Females preferred the call with a deficiency of high-frequency energy at low levels, but failed to show preferences at higher SPL's (Fig. 5).

Discussion

One of the most striking and stereotyped characteristics of the green treefrog's mating call is its bimodal spectrum; both spectral peaks are pertinent for selective phonotaxis and their relative importance depends on SPL.

Significance of High-Frequency Energy: Single-Component vs a Bimodal Signal. A previous study showed that a single-component synthetic call of 0.9 kHz attracted females at a fairly low SPL (about 55 dB) whereas synthetic calls consisting of one (3.0 kHz) or two (2.7+3.0 kHz) high-frequency components were much less effective, having 'behavioral thresholds' around 90 and 72 dB respectively (Gerhardt 1976). Subsequently, two-choice experiments were conducted indoors where the noise level was lower, and about one-third of the females were attracted to bimodal (0.9+3.0 kHz) and single-component (0.9 kHz) sounds at a SPL of 48 dB. Furthermore, females failed to show a preference between these two sounds at 48 dB, indicating that the high-frequency energy was undetected or irrelevant for selective phonotaxis.

The fact that high-frequency energy *in combination with low-frequency energy* becomes important for *selective phonotaxis* at SPL's of about 54 dB and higher is one of the most important results of this study (Table 1). This means that the ineffectiveness of high-frequency energy alone ('thresholds' of 72 to 90 dB) is almost certainly not attributable to the insensitivity of the animals to high-frequency sounds. I suggest that the high threshold is related to the fact that female green treefrogs can localize high-frequency sounds (3 kHz) only with extreme difficulty (Rheinlaender, Gerhardt and Walkowiak, in preparation).

Effects of Varying the Relative Amplitudes of the Components of Bimodal Signals. As the SPL's of synthetic stimuli are increased, females of *H. cinerea* apparently become sensitive not only to the presence or absence of high-frequency energy but also to its amplitude relative to the low-frequency peak or to absolute amplitude differences. When the overall SPL's of the standard call and calls with peaks of unequal relative amplitude are equalized, there are differences in the absolute levels of *both* spectral peaks. If, for example, the standard call and UP −12 dB are equalized at

66 dB, then the absolute levels of the 0.9 and 3.0 kHz components of the standard call are both 63 dB; the absolute levels of these same components in the UP −12 dB stimulus are 66 and 54 dB respectively. If the overall level of UP −6 dB is adjusted to 66 dB, its 0.9 kHz component has an absolute level of 65 dB and its 3.0 kHz component, 59 dB. At 66 and 54 dB females did not show a preference for the standard call over UP −12 dB nor UP −6 dB (Figs. 1 and 2) when the overall levels were equalized. The greater absolute energy at 0.9 kHz could have been a factor, and this idea was supported by the results of an experiment in which the 0.9 kHz component in the standard call was adjusted with the tunable filter to the same level as the 0.9 kHz component in the UP −6 dB stimulus (Fig. 2). However, when the same equalization procedure was done at 54 dB, the animals failed to show a preference (Fig. 2). Neither the discrepancy in overall levels (the standard call was 2 dB greater) nor the differences in absolute and relative amplitudes (both 6 dB) were sufficient to elicit selective phonotaxis.

At 72 dB and higher levels females chose the standard call despite the fact that calls with attenuated high-frequency peaks had more energy at 0.9 kHz. This suggests that preferences must be based on the relative amplitude differences (0 dB in the standard vs 6 dB or 12 dB in the alternatives) or on the smaller absolute difference in energy at 3.0 kHz (i.e. 9 dB: standard vs UP −12 dB; and 4 dB: standard vs UP −6 dB).

Acoustic Communication in Natural Environments. Can the pattern of preferences based on spectral differences at various SPL's be related to the acoustic behavior of the green treefrog in nature? Males of *H. cinerea* often form large breeding aggregations, where dozens to hundreds of individuals call from the edges of ponds or swamps and from emergent vegetation (lily pads, cattails, bushes, logs, algal mats, etc.). When a gravid female is ready to lay eggs, she enters the breeding site, locates a calling male and usually initiates sexual contact. If she approaches the chorus from a distance, the low-frequency components of the male's call or even the nearly continuous low-frequency energy produced by the chorus is sufficient to attract and guide her (Ehret and Gerhardt 1980; this study). As she gets closer the high-frequency components in the mating call begin to affect the selectivity of her phonotaxis (though not its accuracy: see Rheinlaender et al. 1979). At still closer distances where the SPL's of the calls are even greater, the female becomes sensitive to rather small deficiencies in the relative or absolute amplitudes of the high-frequency components.

Male treefrogs are usually near the shoreline and females usually approach from land. Individuals of either sex may be at ground level or elevated (up to 1.5 m). If both are at ground level and the sound propagates over a porous substrate (with or without vegetation), severe excess attenuation (drop in sound level in addition to the 6 dB per doubling of distance expected from spherical spreading) of the high-frequency components of the male's call will occur because of the ground impedance, which strongly affects sounds propagating at near-grazing angles of incidence (Embleton et al. 1976). If, however, either the male or female is elevated (but not both), then excess attenuation of the high-frequency components is insignificant over relatively short distances. For example, when both a source and receiver are on a grass-covered substrate, a 900 Hz sound will have a SPL more than 15 dB greater than that of a 3,000 Hz sound after propagating 50 ft. If the receiver height is increased to 4 ft, there is very little difference in the SPL's of these two frequencies (see Fig. 5 in Embleton et al. 1976). Acoustic analyses of the calls of *H. cinerea* over grassy substrates yield similar results. Relative amplitude differences of behavioral significance can occur over distances of 5 to 7 m. Furthermore the relationship between source and receiver height is reciprocal. That is, elevating the source when the receiver is at ground level results in a dramatic recovery of the high-frequency components.

The relative amplitudes of high-frequency components are not severely affected when both source and receiver are near the surface of water and sound propagates across this medium, with its much higher impedance (Embleton et al. 1976). The situation is very complicated when both source and receiver are elevated. There are then two sound paths, direct and reflected, and interference between the two waves is a function of frequency (wavelength and thus distance) and the impedance of the substrate, which may cause phase changes in the reflected wave (Embleton et al. 1976). Thus the relative amplitudes of the spectral components in the green treefrog's call should change in a non-monotonic fashion with distance.

Because of the variability of calling positions (elevated or at ground level) and approaches of females (elevated or at ground level), the source/receiver geometry and environmental differences (substrate) will give rise to spectral differences in mating calls that are more than sufficient to affect the choices of female green treefrogs. Rather than providing the female with stable, reliable information about the male and his calling site, however, these complex environmental effects on spectral structure may actually obscure differences among conspecific males. It will be necessary to analyze actual patterns of female movements and assessment of males prior to mating in order to learn if spectral properties play a significant role in female mate choice.

Neurobiological Implications. Neurophysiological and anatomical studies of the peripheral auditory system of *H. cinerea* and other anurans indicate the low- and high-frequency components of the mating call are processed primarily by two different inner ear organs, the amphibian and basilar papillae respectively (see Capranica 1976 for a general review). Moffat and Capranica (1974, personal communication) have also found that many of the non-inhibitable auditory nerve fibers that probably innervate the amphibian papilla have absolute thresholds that are lower than those of the most sensitive nerve fibers that innervate the basilar papilla (about 20–30 dB SPL at 600–1,200 Hz vs about 55–60 dB at 3 to 3.6 kHz). Since females are attracted to 0.9 kHz signals at 42 to 48 dB SPL, excitation of the amphibian papilla alone is almost certainly sufficient to elicit phonotaxis. I suggest that excitation of the basilar papilla is important for selective phonotaxis: females select 0.9+3.0 kHz over 0.9 kHz alone at about 54 dB, which corresponds to the absolute thresholds of the most sensitive high-frequency neurons. (The fact that females chose the standard call over LP −6 dB and LP −12 dB at 48 dB indicates that the failure of the females to show a preference between the standard call and 0.9 kHz alone is almost certainly not attributable to the low playback level per se.)

The detection of differences in the relative and/or absolute amplitude of the high-frequency peak must be a more difficult task than merely detecting the presence or absence of high-frequency energy. Females showed preferences for the standard call over UP −12 dB and UP −6 dB only when the overall playback level was 72 dB or greater. I suggest therefore that detection of high-frequency attenuation depends on more than the excitation of just the most sensitive high-frequency units. Moreover, I conclude that mating call recognition in *H. cinerea* must depend not merely on the simultaneous excitation of the two auditory organs; rather, an optimum call probably elicits an optimum ratio (or range of ratios) of excitation. Obviously both the standard call and alternatives with attenuated peaks must stimulate both organs at moderate to high SPL's and yet the females show consistent preferences in many experiments. The data presented in this paper will become even more important when it is possible to estimate the relative excitation of the two organs in response to a given acoustic stimulus.

Mudry (1978) has discovered what might be a central nervous system correlate of the basic behavioral results presented here. Recording evoked potentials from the auditory area of *H. cinerea,* she found that a combination of a low-frequency tone and a high-frequency tone elicited responses that were significantly greater than the sum of the responses to the individual tones presented in isolation. This non-linear facilitation was first evident at about 60 to 65 dB, and it probably reflects a convergence of information from the two auditory organs. If this area is involved in the processing of biologically-significant sounds – and the available evidence supports this idea (see Gerhardt and Mudry 1980) – then the non-linear facilitation is best correlated with selective phonotaxis and not merely phonotaxis per se. The estimated threshold for the non-linear facilitation, as the thresholds of the most sensitive high-frequency fibers in the periphery, is far above the behavioral threshold for a 0.9 kHz acoustic stimulus. Once again it may be possible to relate the magnitude of the non-linear response in the thalamus (evoked potentials or single units) to the ratio of excitation of the two inner ear organs on the one hand and to selective phonotaxis on the other.

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Appendix

Evidence that the Choices of Female Hyla cinerea Are Not Biased by Their Choices in a Previous Experiment. A female chooses one of two sounds in a first test; she finds a loudspeaker instead of a male and therefore does not achieve a mating. Possibly she could be expected to choose a different stimulus on her next trial. Alternatively, a response to a given stimulus in one trial could sensitize the female so that she would tend to choose the same stimulus in the next test. In Table 3 I have tabulated the choices that females made according to whether the choice was the same or different than the choice made in a previous test. The data are further analyzed in terms of the preference, if any, expected on the basis of *independent* samples of 'first responses', i.e. choices that females made in the first tests in which they were exposed to a sound that was also presented in a second test. The first row of the table shows that females not expected to prefer either of the two sounds tended to choose the stimulus that they did not choose in the previous test (the 'different' stimulus); however, the difference is not statistically significant. The next row analyzes the choices of animals that did *not* make the expected (based on the independent sample) choice. One obvious hypothesis is that their previous choice and/or exposure biased them. However, just as many females chose the 'same' stimulus contrary to expectations (independent sample) as chose the 'different' stimulus contrary to expectations. The third row analyzes the choices of females which chose the 'standard' call as expected (based on independent

Table 3. Choices of females that previously responded to one of the same alternatives[a]

	Number of choices that were the same as in the previous experiment	Number of choices that were different than in the previous experiment
Neutral (animals not expected to show a preference)[b]	24	32
Animals which chose the stimulus which an independent sample did *not* prefer[b]	12	12
Animals which chose the standard stimulus as expected[b]	101	42

[a] LP −12 dB versus UP −12 dB experiment is excluded from these tabulations

[b] Based on independent samples of 'first responses'

samples). More females chose the 'same' stimulus because the 'standard' call was likely to have been chosen in the previous test. Recall that the standard call was an alternative in every experiment (except LP-12 vs UP-12 dB) and that in the majority of experiments it was preferred over alternatives having an attenuated component.

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