

## *Auditory Tuning and Frequency Preferences in Anurans*

### Introduction

Many studies have demonstrated a reasonably good match between auditory tuning and the band or bands of frequencies emphasized in the conspecific advertisement call, which is the main long-range signal in anurans (see Figure 7.1 and below). Such correlations are consistent with the idea, first introduced by Capranica and his colleagues, that the anuran auditory system acts as a matched filter, a concept borrowed from engineering (e.g., Karl 1989). One appeal of this hypothesis is that matched filtering can improve the detection of conspecific signals against a background of broad-band noise, and mixed-species choruses that produce broad-band noise are common in tropical and semitropical environments (e.g., Littlejohn 1977; Drewry and Rand 1983). At a finer level of analysis, the match between auditory tuning and carrier frequency is not so impressive. For example, the average frequency band to which the auditory system is most sensitive often differs by as much as 20% from the average dominant frequency in the advertisement calls of conspecific males in the same population (see Table 7.1 and below). Even small mismatches can potentially mediate directional sexual selection as emphasized by Ryan, Rand, and their colleagues (e.g., Ryan et al. 1990, 1992).

In this chapter we first document correlations between

the spectral content of advertisement calls and maximum auditory sensitivity and discuss some factors that probably contribute to mismatches. We then address the following question: How well do neural estimates of maximum frequency sensitivity correlate with frequency preferences? We raise this issue because most estimates of neural sensitivity are based on audiograms derived from midbrain, multiunit activity or on tuning curves of single neurons, both of which are near-threshold measures. Natural communication and behavioral tests of frequency preferences, however, involve the processing of signals well above threshold, and the auditory system is notoriously nonlinear. Besides saturation, however, such factors as background noise, interactions between differently tuned neural channels, and temperature can affect frequency preferences in unpredictable ways, usually reducing the correlation between auditory tuning and preferences, but sometimes improving it. Finally we examine the assumption that size-dependent mating arises from frequency preferences.

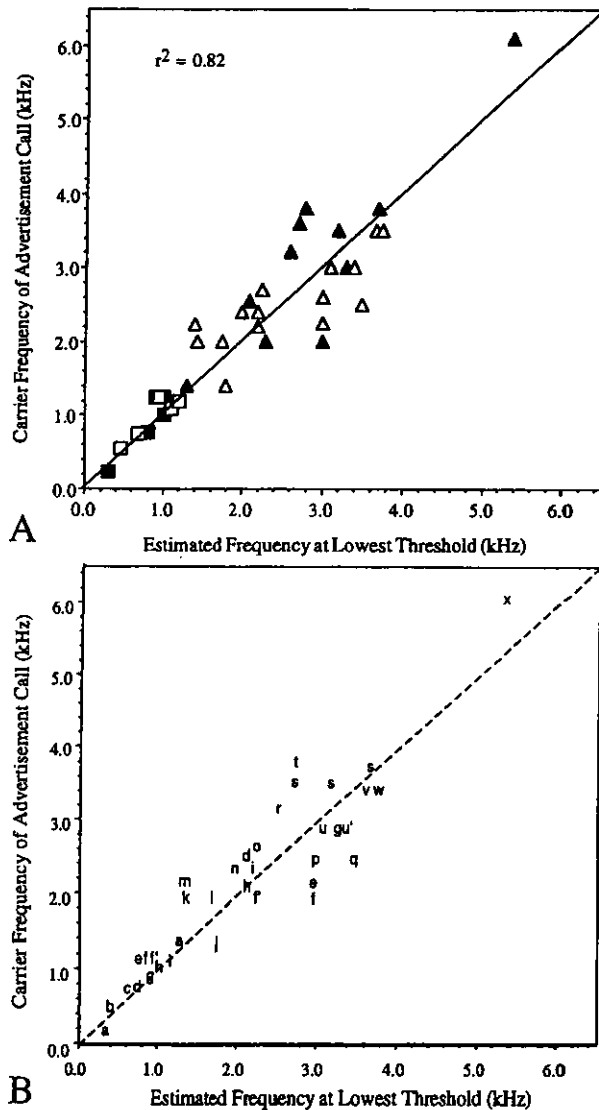
### Evidence for Matched Filtering: Comparative Data

In Figure 7.1 we plot estimates of average carrier frequency against estimates of auditory tuning, as estimated from

audiograms (evoked potentials or multiunit recordings from the midbrain) or distributions of characteristic frequency (frequency at lowest threshold) of neurons recorded in the auditory nerve. As shown below, these two kinds of measurements yield similar estimates in the few species for which both kinds of data are available. We refer to these minimum thresholds as "best excitatory frequencies" (BEF) in the rest of this chapter. In those species whose calls have a bimodal spectrum, the two dominant frequencies and the two corresponding threshold-minima are plotted. We assume that sensitivity to relatively low frequencies is mediated by the amphibian papilla, one of the two inner ear organs of anurans, whereas sensitivity to relatively high frequencies is mediated by the basilar papilla, the other inner ear organ (review: Zakon and Wilczynski 1988). Neurons receiving inputs from both papillae contribute some-

what to the responses of some neurons recorded in the midbrain (Fuzessery 1988), but contamination is unlikely to distort midbrain audiograms (based on stimulation with a single frequency at a time) in the regions to which the two organs are maximally sensitive because these regions are usually separated by one to two octaves.

For many species, the data are based on threshold estimates from a sample of both males and females; in other species, which we identify in Figure 7.1, separate estimates are available for each sex. In three species (*Eleutherodactylus coqui*, *Pseudacris crucifer*, and *Acris crepitans*), good evidence exists for sexual differences in tuning, probably influenced by size differences (Narins and Capranica 1976; Wilczynski et al. 1984; Keddy-Hector et al. 1992, respectively; additional discussion below). Sample sizes, and the extent to which estimates for thresholds in individuals were based on data



**Figure 7.1.** (A) Scatter diagram of spectra peaks in the advertisement calls of 24 species of anurans (plus three populations of one species) against estimates of minimum threshold (BEF). Solid symbols show data from studies in which closed-system stimulation was used, and open symbols show data from studies in which free-field stimulation was used. Squares indicate low-frequency sensitivity attributed to the amphibian papilla, and triangles indicate high-frequency sensitivity attributed to the basilar papilla. The line shows where all points would lie if the correlation were perfect. (B) Key to the data points plotted in A; SU = single-unit data (auditory nerve); MU = multi-unit spike data (torus semicircularis); EP = evoked potentials (torus semicircularis). a - *Rana catesbeiana*, SU, Frishkopf et al. 1968; b - *Bombina variegata*, MU, Mohnke and Schneider 1979; c - *Limnodystes dorsalis*, EP, Loftus-Hills and Johnstone 1970; d - *Physalaemus pustulosus*, MU, Ryan et al. 1990; e - *Hyla arborea*, MU, Hubl and Schneider 1979; f - Male *Eleutherodactylus coqui*, SU, Narins and Capranica 1980; f' - Female *Eleutherodactylus coqui*, SU, Narins and Capranica 1980; g - *Hyla cinerea*, SU, Capranica and Moffat 1983; h - *Hyla versicolor*, MU, Schwartz and Gerhardt, unpublished; i - *Hyla chrysoscelis*, EP, Hillery 1984; j - *Alytes obstetricans*, MU, Mohnke and Schneider 1979; k - *Limnodystes tasmaniensis*, EP, Loftus-Hills and Johnstone 1970; l - *Litoria verreauxii*, EP, Loftus-Hills and Johnstone 1970; m - *Rana ridibunda*, MU, Hubl and Schneider 1979; n - *Atelopus chiriquiensis*, MU, Jaslow and Lombard 1996; o - *Litoria ewingii*, EP, Loftus-Hills and Johnstone 1970; p - *Hyla regilla*, MU, Jaslow and Lombard 1996; q - *Hyla savignyi*, MU, Hubl and Schneider 1979; r - *Hyla ebraccata*, MU, Wilczynski et al. 1993; s - *Acris crepitans*, SU + MU, Ryan et al. 1992; t - *Hyla phlebodes*, MU, Wilczynski et al. 1993; u - Male *Pseudacris crucifer*, MU, Diekamp and Gerhardt 1992; u' - Female *Pseudacris crucifer*, MU, Schwartz and Gerhardt 1998; v - *Ranidella parinsignifera*, EP, Loftus-Hills and Johnstone 1970; w - *Ranidella signifera*, EP, Loftus-Hills and Johnstone 1970; x - *Hyla microcephala*, MU, Wilczynski et al. 1993.

**Table 7.1** Relatively large mismatches between auditory tuning and average carrier frequency (CF)

Species	Stimulation	Auditory Organ	BEF	
			< av. CF (%)	> av. CF (%)
<i>Acris crepitans</i> (Austin) <sup>a</sup>	CS	BP	25	—
<i>Alytes obstetricans</i> <sup>b</sup>	FF	BP	—	30
<i>Eleutherodactylus coqui</i> (male) <sup>c</sup>	CS	BP	—	50
<i>Hyla arborea</i> <sup>d</sup>	FF	AP	24	—
<i>Hyla arborea</i> <sup>d</sup>	FF	BP	—	33
<i>Hyla phlebodes</i> <sup>c</sup>	CS	BP	27	—
<i>Hyla savignyi</i> <sup>d</sup>	FF	BP	—	40
<i>Limnodynastes tasmaniensis</i> <sup>d</sup>	FF	BP	28	—
<i>Rana ridibunda</i> <sup>d</sup>	FF	BP	38	—

Sources: Data from <sup>a</sup>Ryan et al. 1992; <sup>b</sup>Mohneke and Schneider 1979; <sup>c</sup>Narins and Capranica 1980; <sup>d</sup>Hubl and Schneider 1979; <sup>e</sup>Wilczynski et al. 1993; <sup>f</sup>Loftus-Hills and Johnstone 1970.

BEF = best excitatory frequencies; CS = closed stimulation system; FF = free-field stimulation; BP = basilar papilla; AP = amphibian papilla.

from multiple recording sites, differ considerably among these studies, as does the way in which threshold estimates are presented. We usually used mean or median values of BEF presented in the text or tables, but sometimes we had to estimate these values from audiograms. As discussed below, tuning is often so shallow that deciding on a minimum is somewhat arbitrary; this procedure thus masks the fact that the range of near-maximum sensitivity usually covers a considerable range of frequency. Similarly by taking mean values for carrier frequencies or estimating the mean as the midpoint in the range of variation (in studies where mean values were not reported), the analysis ignores that the calls of many of the species considered have a broad-band spectrum, with substantial acoustic energy present over two or more octaves. The extent of some of the mismatches we report are likely to be reduced as better estimates of mean carrier frequency become available.

Some studies used free-field acoustic stimulation, whereas others used a closed-stimulation system sealed onto one of the tympanic membranes (see symbols in Figure 7.1). The latter arrangement might be expected to exaggerate low-frequency sensitivity in comparison with free-field stimulation (Pinder and Palmer 1983). Free-field stimulation adds another complication, however, because the anuran system acts as a pressure-difference system in which there are multiple inputs of sound, including internal pathways, to the tympanic membrane and probably directly to the inner ear organs (Hetherington 1992). Because the frequency-responses of the input surfaces and pathways are likely to be influenced by the sound direction, some variation among studies would be expected because of different placements of speakers relative to the orientation of

the animal. Finally only two studies formally investigated the effect of temperature on auditory tuning as estimated by midbrain audiograms (Hubl and Schneider 1979; Mohneke and Schneider 1979). As discussed in a later section, if the temperature is significantly different from that experienced by the frogs during breeding periods, then relatively large mismatches between estimates of frequency sensitivity and emphasized frequencies in advertisement calls are possible (see also Narins, this volume). In the studies just cited, however, the main effect of increasing temperature was usually a decrease in threshold rather than a major shift in the BEF of the audiogram. In choosing an estimate of BEF from these studies for Figure 7.1, we used either values that were common to two or three of the temperatures used by the authors or the temperature at which most breeding occurs.

On the one hand, despite all of the experimental variation and sources of error, the correlation between BEF and call frequency in this sample of species (and populations) is remarkably good, with a coefficient of determination of 0.82. At this level of analysis, the matched filter hypothesis appears to be well supported, and we might expect auditory tuning to conspecific signals in many species to provide some measure of improved detection in mixed species choruses as well as mediating weak stabilizing selection on carrier frequency (see below). On the other hand, discrepancies between average carrier frequency and BEF of 15% or more were found for half of the 36 estimates in Figure 7.1; Table 7.1 provides details for mismatches of about 25% or more. Two-thirds (12 of 19) of the (15%) mismatches involved tuning to frequencies lower than the estimated average carrier frequency, but there was no trend for this kind of mismatch

to be correlated with the type of stimulation: 6 of the estimates were obtained from closed-system stimulation and the other 6 from free-field stimulation. Substantial mismatches (in either direction) involved 4 of 10 estimates of low-frequency (amphibian papilla) tuning and 14 of 26 estimates of high-frequency (basilar papilla) tuning.

As previously suggested, however, many of these estimates could probably be improved by one or more of the following actions: increasing the sample size of animals, recording from multiple sites within each animal, and adjusting the temperature of preparations to the middle of the normal range of breeding temperatures. It will also be important to conduct systematic studies of the effects of the mode of stimulation and the direction of free-field stimulation using the same species, or better yet, the same animals. Separate analyses for the two sexes to check for sexual differences in tuning should also be conducted whenever significant sexual size dimorphism occurs. Size-dependent tuning and frequency preferences have been widely documented within females of the same species (Wilczynski et al. 1984; Keddy-Hector et al. 1992; Ryan et al. 1992; Jennions et al. 1995; Márquez and Bosch 1997). No sexual difference in tuning was reported in *Physalaemus pustulosus* despite the pronounced sexual size dimorphism in this species, but the sample of audiograms was only five, and the number of animals of each sex was unspecified (Ryan et al. 1990).

## Estimates of Tuning and Frequency Preferences

### Túngara Frogs

Several studies of anurans have correlated estimates of auditory tuning with the frequency preferences of females from the same populations (Gerhardt 1974, 1987; Ryan et al. 1990, 1993; Diekamp and Gerhardt 1992; Schwartz and Gerhardt 1998). In the túngara frog, *P. pustulosus*, audiograms based on averaging multiunit thresholds from five individuals suggested that these frogs would be more sensitive to chucks of lower-than-average frequency (about 2.13 kHz) than to chucks of average (2.55 kHz) and higher-than-average frequencies (Ryan et al. 1990). In behavioral tests, 63% of the females chose a synthetic call with a chuck-frequency that was lower than average (2.1 kHz) to an alternative with a chuck-frequency that was higher than average (3.0 kHz) (Wilczynski et al. 1995). This trend is consistent with the neural data even though it is not statistically significant.

### Cricket Frogs

Estimates of auditory tuning in three populations of Blanchard's cricket frog (*Acris crepitans blanchardii*) were based on estimates of the characteristic frequency of primary auditory neurons and multiunit recordings (Ryan et al. 1992).

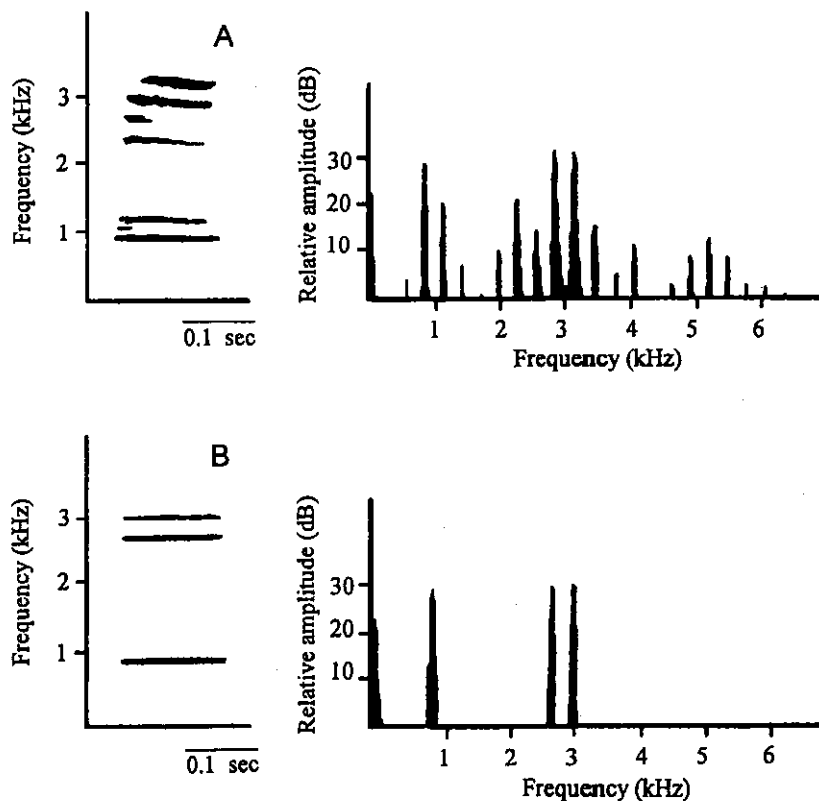
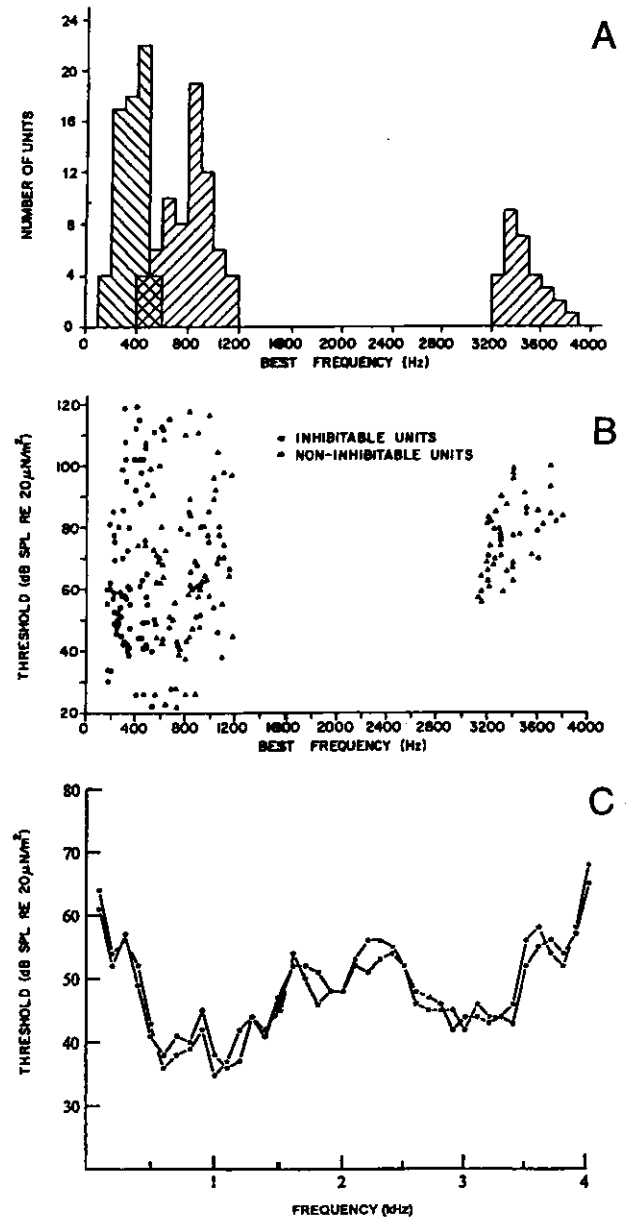


Figure 7.2. Sonograms (left) and power spectra (right) of (A) a typical advertisement call of a male green treefrog (*Hyla cinerea*), and (B) a synthetic advertisement call that was just as attractive as the natural call in a two-stimulus playback experiment (from Gerhardt 1983).

The mean BEF was always lower than the average carrier frequencies of advertisement calls in the same populations, although only barely so in a population from Bastrop, Texas. Females were tested with synthetic calls that differed only in carrier frequency and represented calls of about average frequency for the population and calls that differed from the average by more than one standard deviation. The results of three of six tests were qualitatively consistent with the neural data: statistically significant proportions of females preferred calls of average frequency to calls of higher frequency and preferred calls of lower-than-average frequency to calls of average frequency. Females from the Austin population surprisingly did not prefer the low-frequency call (3.2 kHz) to the call with a frequency of 3.5 kHz, which was close to the population average (3.6 kHz), although the mean BEF was 2.7 kHz. Females in the Bastrop population did not prefer calls of about average frequency (3.8 kHz) to calls of higher frequency (4.1 kHz), and they preferred the low-frequency call (3.5 kHz) to the call of average frequency (3.8 kHz) even though the mean BEF (3.72 kHz) better matched the latter frequency. By contrast, females from the Indiana population showed preferences in both tests that were predicted by the neural data; moreover, the preferences for the low-frequency call in one test was robust in the face of a 6-dB decrease in the sound pressure level (SPL) of this stimulus relative to the alternative.

### Green Treefrogs

Males of the green treefrog (*Hyla cinerea*) produce a noisy advertisement call with the frequency components around 0.9 and 3 kHz having the greatest relative amplitude (Figure 7.2A). A synthetic call with just three components (0.9, 2.7, and 3.0 kHz) was as attractive to females as typical natural calls having many more components (Figure 7.2B; Gerhardt 1974). In Figure 7.3A, we show the distribution of characteristic frequency in a sample of auditory neurons recorded in the eighth nerve of the green treefrog by Capranica and Moffat (1983). The population of high-frequency neurons is made up of fibers innervating the basilar papilla, and the populations tuned to lower frequencies are representative of neurons innervating the amphibian papilla. In advanced anurans, such as treefrogs, the midfrequency population derives its sensitivity from hair cells found in a caudal extension of the amphibian papilla, whereas the low-frequency population, found in all anurans, derives its sensitivity from hair cells in the rostral part of the papilla (see Narins, this volume). Notice that the most sensitive neurons innervating the amphibian papilla have much lower thresholds than the most sensitive neurons innervating the basilar papilla (Figure 7.3B). The wide range of thresholds is not represen-



**Figure 7.3.** (A) Histogram showing the distribution of characteristic frequency (CF) in a sample of primary auditory neurons recorded from the green treefrog, *Hyla cinerea*. (B) Raw data from which the histogram of A was generated. (C) Audiogram based on evoked potentials recorded from the midbrain (torus semicircularis) of the green treefrog. A and B modified from Capranica and Moffat (1983); C modified from Lombard and Straughan (1974).

tative of any single individual, however, because the data were pooled from multiple recordings from a sample of frogs. What cannot be observed from such a figure is the nonlinear interaction occurring within the amphibian papilla: the response of a low-frequency neuron at its characteristic frequency can be suppressed by the addition of a

second tone of higher frequency (Capranica and Moffat 1983; review in Zakon and Wilczynski 1988).

As discussed above, most estimates of auditory sensitivity are not based on data from single auditory neurons but rather from multiunit recordings or evoked potentials. Such recordings are made using relatively large, low-impedance electrodes that register the spiking activity from many neurons. Typically the electrode is placed in the largest and most complex of the auditory nuclei in the ascending pathway, the torus semicircularis, which is considered a homolog of the inferior colliculus of mammals. As shown in Figure 7.3C, an audiogram derived from evoked potentials is consistent with the single-unit data (Figure 7.3A): lowest thresholds occur in the three frequency bands corresponding to the three populations of primary auditory neurons as defined by characteristic frequency. Moreover the relative sensitivity of the three populations is also correlated with the shape of the midbrain audiogram (Lombard and Straughan 1974).

How well do these estimates of frequency sensitivity predict the frequency preferences of females? At normal breeding temperatures of about 22–26°C, females from eastern Georgia tested at a playback level of 75 dB SPL (sound pressure level, re. 20  $\mu$ Pa) preferred a standard synthetic call with a low-frequency peak of 0.9 kHz, which was close to the mean in the population, to alternatives of 0.7 kHz and 1.1 kHz. Females also preferred a standard synthetic call with a high-frequency peak of 3.0 kHz (close to the mean in the population) to alternatives of 2.1 kHz and 3.6 kHz (Gerhardt 1987). Under these conditions then, female preferences are well correlated with estimates of the frequency sensitivity of the population of midfrequency neurons found in the amphibian papilla and that of the high-frequency neurons in the basilar papilla. These patterns of preference in green treefrogs are consistent with the matched-filter hypothesis, and thus should help females detect conspecific males in mixed-species choruses. These patterns also represent a possible basis for stabilizing selection that should not only result in the rejection of heterospecific calls with different emphasized frequency bands but also some conspecific calls at either end of the range of distribution (Gerhardt 1974, 1987, 1994). As we emphasize below, however, these two generalizations do not hold under other conditions.

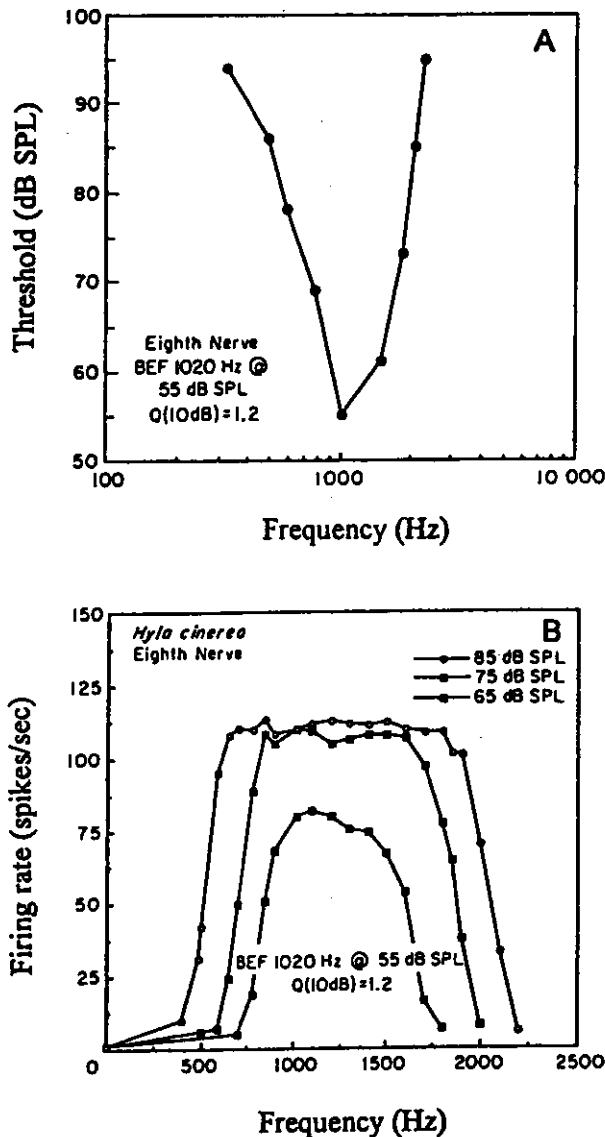
In another respect, neural estimates of frequency selectivity fail to predict the preferences of females in the context of intraspecific communication if we assume that excitation of any primary auditory neuron contributes to the attractiveness of a signal as much as any other primary neuron. For example, a large population of sensitive amphibian-papilla neurons is tuned to frequencies below the low-frequency spectral peak in the advertisement call (Figures 7.2A and 7.3A, B). Yet at normal breeding temperatures fe-

males rejected synthetic calls with a low-frequency spectral peak of 0.5 or 0.6 kHz, which falls into a region to which many of these neurons are sensitive, in favor of the standard call with a low-frequency peak of 0.9 kHz. The estimated numbers of primary neurons tuned to about 0.7–1.2 kHz are about the same as those in the low-frequency population, and the lowest thresholds of the most sensitive neurons in both populations are also similar.

A stimulus containing components of 0.3 Hz and 0.9 kHz, which match the tuning of both populations of neurons, was neither more nor less attractive than a standard call lacking the 0.3 kHz-component (Gerhardt, unpublished data). The excitation of neurons of the low-frequency population was, however, likely to be negated somewhat by the tone-on-tone suppression (mediated by neurons tuned to the 0.9 kHz component) mentioned above. In general the biological function of auditory sensitivity to low-frequency regions of the spectrum that do not match acoustic energy in communication signals remains a mystery. The usual speculation is that such sensitivity serves to detect predators (e.g., Capranica and Moffat 1983).

### Frequency Preferences and Stimulus Intensity: Examples from Studies of Green Treefrogs and Spring Peepers

In Figure 7.4B we show an example from *H. cinerea* of the saturation of an auditory nerve fiber whose tuning curve is shown in Figure 7.4A (Capranica 1992). At 65 dB SPL, about 10 dB above the absolute threshold, the maximum firing rate of this neuron is close to the characteristic frequency of 1020 Hz. At stimulus levels of 75 dB and 85 dB SPL, however, the firing rate of the neuron is uniform over a wide range of frequencies. The wide range of absolute thresholds (called range fractionation) of the population of auditory neurons serves to increase the linear (dynamic) operating range of the auditory system as a whole and hence to reduce its saturation. But clearly at some very high stimulus levels, most neurons would be expected to be saturated, and stimulus frequency could then only be encoded reliably by tonotopy or temporal coding schemes such as the volley principle. Tonotopy is the orderly spatial arrangement of hair cells or neurons according to their frequency sensitivity, and at a gross level, the different tuning of the two auditory organs can be considered an example. Tonotopy has also been documented in the amphibian papilla of advanced anurans (including the Hylidae), but its expression in higher centers such as the torus semicircularis is crude at best (review in Fuzessery 1988). No tonotopy has been described within basilar papilla of anurans. Temporal synchronization codes, in which the stimulus frequency is



**Figure 7.4.** (A) Tuning curve of a primary auditory neuron of green treefrog, *Hyla cinerea*, with a characteristic frequency (CF) of about 1.0 kHz. (B) Iso-intensity functions for the same primary neuron. When stimulus intensity is held constant at a low sound-pressure level and frequency is varied, the highest firing rate is elicited at the neuron's CF. At higher sound-pressure levels the same (saturated) firing rate is observed over a frequency range of 1–2 octaves. Modified from Capranica (1992).

represented by the timing of action potentials, could potentially encode frequencies as high as 900 Hz in anurans (Narins and Hillery 1983).

The question of whether threshold estimates of tuning reliably predict frequency preferences at sound pressure levels well above threshold thus can only be tested by behavioral experiments at biologically realistic levels. Moreover this question requires systematic variation not only in fre-

quency but also in absolute and relative intensity. Such studies have been conducted with green treefrogs and spring peepers, and in the latter species, correlations have been sought between auditory tuning and frequency preferences within the same individual females (Schwartz and Gerhardt 1998).

Behavioral studies of the green treefrog demonstrated changes in preferences with variation in the SPL to which alternative stimuli with different frequencies are equalized (Gerhardt 1987). As expected from the predicted effects of saturation, female green treefrogs became somewhat less selective for differences in frequency in the low-frequency (amphibian papilla) range as the SPL was increased from 65 to 85 dB, although females rejected higher-than-average frequencies at all playback levels (Figure 7.5). Inspection of Figure 7.3B indicates that at a playback level of 85 dB, SPL is 20 dB or more above the threshold of a large proportion of primary neurons innervating the amphibian papilla (Figure 7.3B). As shown in Figure 7.4B some neurons are already saturated at 20 dB above the threshold at the characteristic frequency. Although the distribution of thresholds of primary fibers tuned to the low-frequency spectral peak shows an enormous range of values, including neurons with estimated thresholds in excess of 110 dB SPL, we have already cautioned that this is a sample that was pooled from different animals and therefore exaggerates the dynamic range of any individual. Moreover we suspect that at least some of the thresholds higher than 100 dB reflect a deteriorating preparation rather than a truly insensitive neuron.

Contrary to the prediction that saturation would decrease frequency resolution, female green treefrogs became more selective for differences in frequency in the high-frequency (basilar papilla) range as SPL was increased from 65 to 85 dB (Figure 7.6). Estimates of the thresholds of primary auditory fibers (Figure 7.3B) innervating the basilar papilla are not only higher than those of many fibers innervating the amphibian papilla, but are also somewhat higher in comparison with the basilar papilla sensitivity of other species (compare with estimates for the spring peeper below and in Wilczynski et al. 1984). Thus one possible explanation for the increased selectivity at 85 dB SPL is that higher signal levels are needed to excite a sufficient number of these neurons to discriminate relatively small differences in stimulus frequency. In other words frequency discrimination is probably best at signal levels that fall within the linear operating ranges of the largest proportion of auditory neurons innervating a given inner ear organ.

In the spring peeper, *Pseudacris crucifer*, males produce nearly sinusoidal calls lasting, on average, about 165 ms; the mean frequency in populations in central Missouri is about 3 kHz (Doherty and Gerhardt 1984; Schwartz and Gerhardt

1998). In Figure 7.7A we show an audiogram based on data from 66 females. The frequency range of conspecific calls matches the maximum sensitivity of the basilar papilla, within which there is no evidence for tonotopy (e.g., Wilczynski et al. 1984). Notice that the lowest average thresholds are around 50 dB SPL over the range of about 3.2–3.6 kHz (median, 3.4 kHz). Moreover the bandwidth at which the average threshold was as low as 60 dB SPL was approximately 1.8 kHz and fully encompasses the range of variation in carrier frequency in the population. The  $Q_{10dB}$  value, a dimensionless measure of the sharpness of tuning, is about 1.91. ( $Q_{10dB}$  is calculated by dividing the BEF by the bandwidth at 10 dB above threshold.)

In Figure 7.7B we show a series of above-threshold estimates of frequency selectivity in the form of iso-intensity

plots; these are the analogs of the iso-intensity spike-rate plots of Figure 7.4B. Here we measured the average size of the multiunit spike activity in the midbrain as stimulus frequency was varied and stimulus SPL was held constant. These plots roughly mirror the audiogram at levels below 85 dB SPL, and although the plot is much less peaked than at lower SPLs, the maximum activity at 75 dB SPL was elicited at about 3.3 kHz, which is very close to the median BEF. Notice however that at 85 dB SPL the iso-intensity function is very flat. If frequency preferences depend on differences in maximum activity elicited by many auditory neurons, and midbrain recordings accurately estimate such activity, these data predict that females should show few, if any, preferences between stimuli falling within the range of about 2.2 and 3.6 kHz at a playback level of 85 dB SPL.

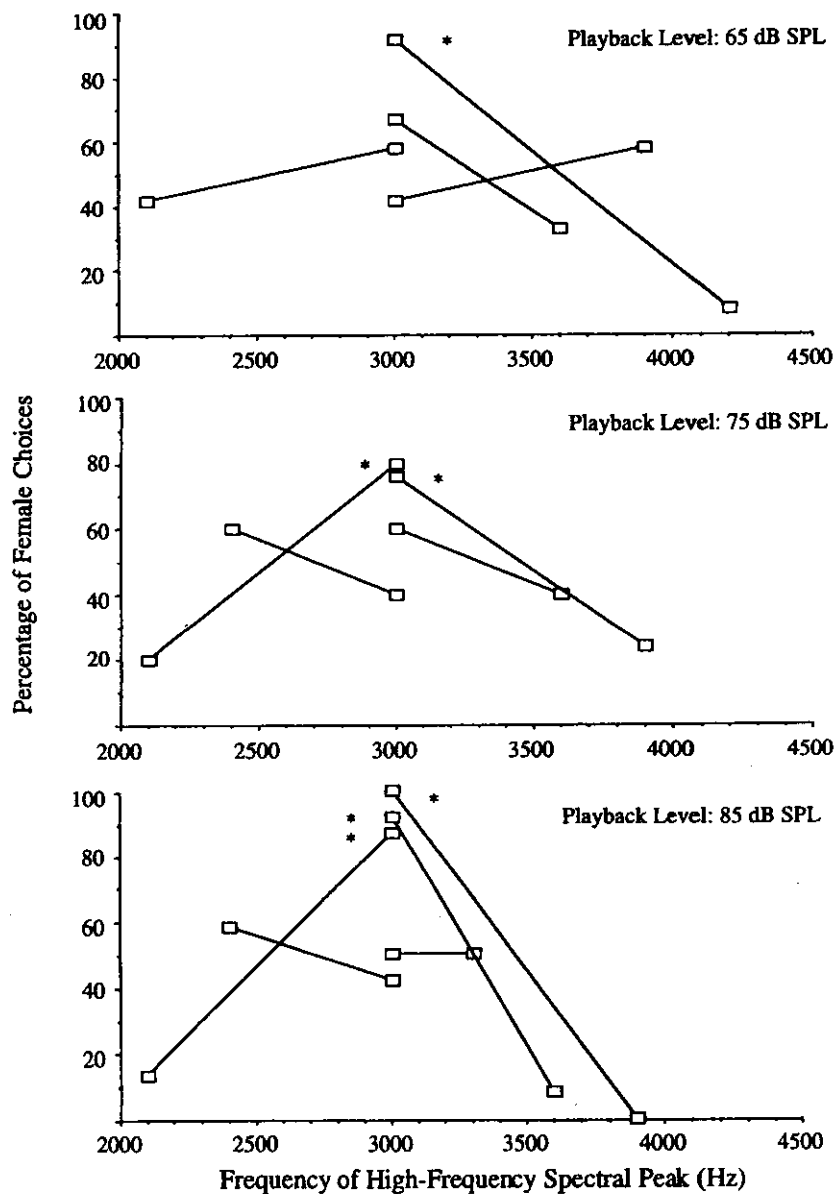
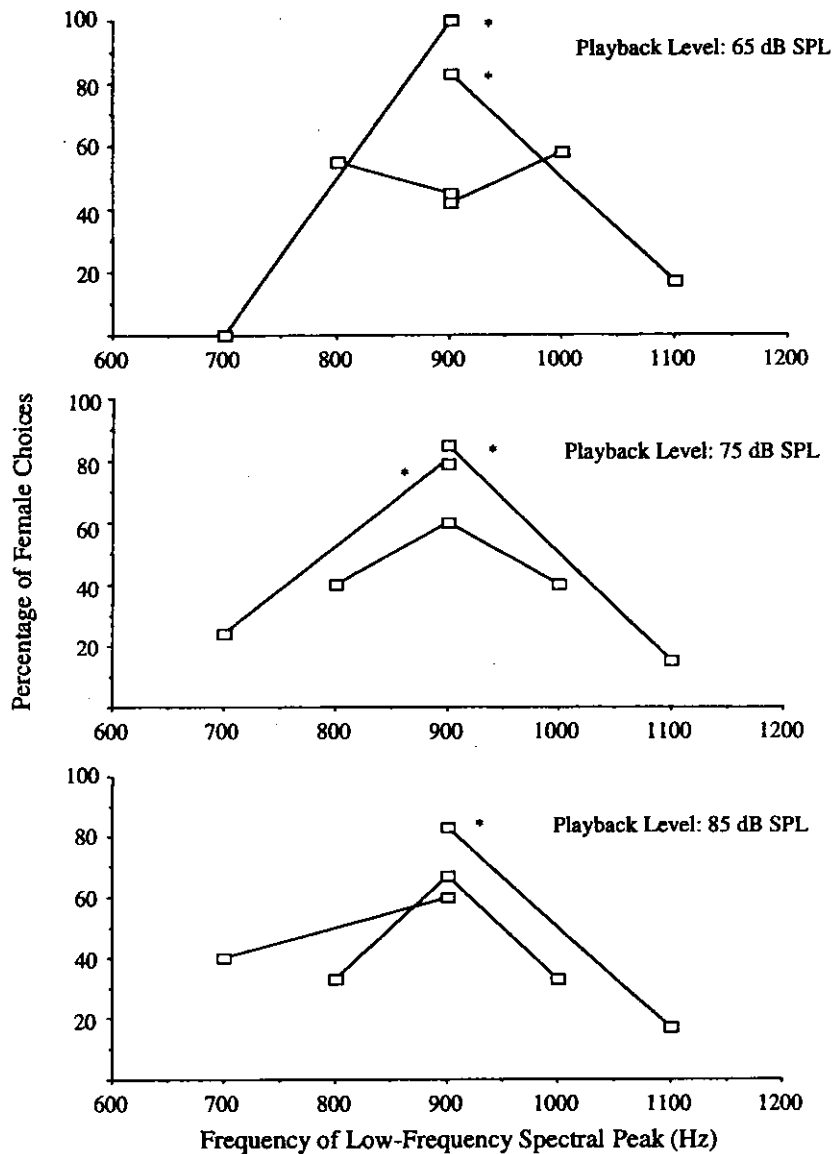


Figure 7.5. Effects of playback level on the behavioral selectivity of female green treefrogs for pairs of synthetic calls that differ in frequency in the high-frequency (basilar papilla) range. Each line connects pairs of points showing the percentages of females responding to two alternative calls with a different frequency. The symbol \* indicates statistically significant ( $p < 0.05$ , two-tailed binomial tests) preferences for the standard high-frequency (3.0 kHz) peak.





**Figure 7.6.** Effects of playback level on the behavioral selectivity of female green treefrogs for pairs of synthetic calls that differ in frequency in the low-frequency (amphibian papilla) range. The symbol \* indicates statistically significant ( $p < 0.05$ , two-tailed binomial tests) preferences for the standard low-frequency (0.9 kHz) peak.

One possibility is that the flattening of the iso-intensity functions on the low-frequency side of the BEF is caused in part by stimulating some of the more sensitive neurons innervating the amphibian papilla. However the flattening of these curves is pronounced on both sides of the BEF. Moreover inspection of scatter plots of characteristic frequency versus threshold and typical tuning curves in *P. crucifer* from New York shows that the most sensitive midfrequency (amphibian papilla) fibers have characteristic frequencies below about 0.9 kHz and sharp cutoffs on the high-frequency side (Wilczynski et al. 1984). It is thus unlikely that these fibers would be excited by stimulation with frequencies of 1.8 kHz and higher, even at 85 dB SPL.

Female spring peepers from central Missouri were tested at 75 dB SPL with pairs of synthetic calls that differed only in frequency (Doherty and Gerhardt 1984). Females chose a

standard call with a frequency just below the mean frequency in the calls of males in the population (2.88 kHz) over a lower-frequency alternative with a frequency falling just within the range of variation (2.60 kHz); they also chose the standard call over a high-frequency alternative (4.0 kHz) with a frequency above the population range of variation. Females did not, however, prefer the standard call to any higher-frequency alternative within the range of variation. Moreover when the SPL of the preferred ("average") stimulus was reduced by 6 dB, females failed to show preferences unless alternatives had frequencies well beyond the range of variation (1.8 and 4.6 kHz, respectively). Consistent with the trend for the iso-intensity plots to flatten with increasing SPL, more recent experiments conducted at a playback level of 85 dB SPL found no preferences between any alternatives with frequencies that fell within the range of variation

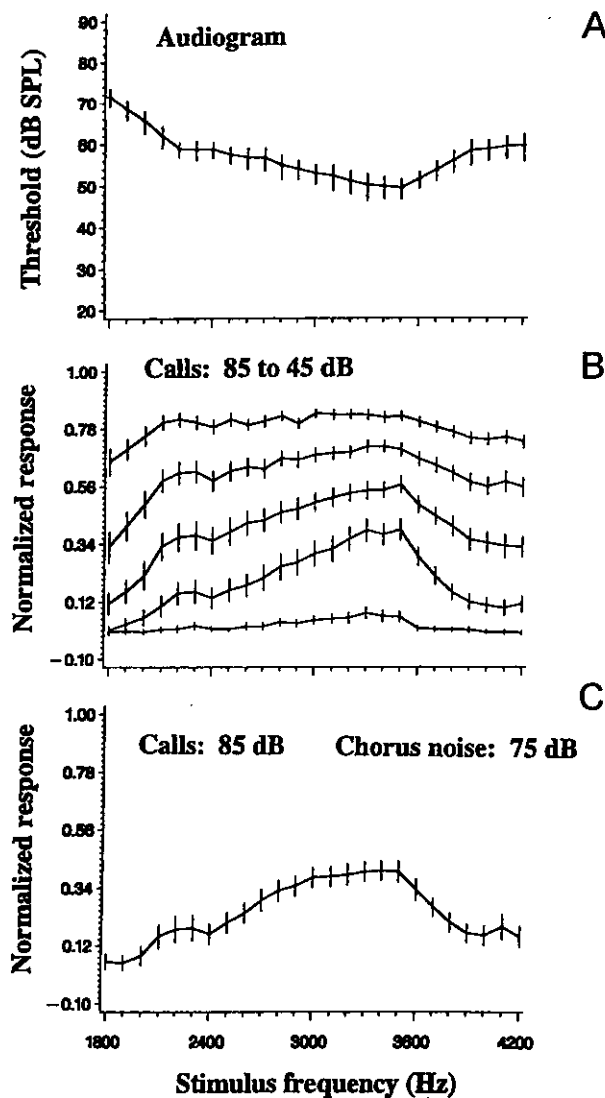


Figure 7.7. (A) Audiogram based on multiunit activity recorded from the torus semicircularis of 66 females of the spring peeper, *Pseudacris crucifer*. (B) Iso-intensity functions (see text) recorded from 65 females (top to bottom: 85, 75, 65, 55, and 45 dB SPL). (C) Iso-intensity function (85 dB SPL) recorded from 47 females that were simultaneously exposed to simulated chorus noise at 75 dB. All data are presented as means  $\pm$  2 SE.

(Schwartz and Gerhardt 1998). Forester and Czarnowsky (1985) reported that female spring peepers from Maryland, tested at 90 dB SPL, preferred calls with a frequency just below the mean value in the population to an alternative that was more than a standard deviation above the mean. Inspection of sonograms (Forester and Czarnowsky 1985) and oscillograms (Forester and Harrison 1987) of the natural calls used in these tests, however, indicates that the alternatives also differed significantly in their amplitude-time en-

velopes. These temporal differences could have mediated the choices of females either directly or in conjunction with the differences in frequency.

The results of Schwartz and Gerhardt (1998) just discussed were based on population-level analyses: median values of lowest thresholds in audiograms or maximum activity in iso-intensity plots were compared with the observed proportions of females that chose synthetic calls of one frequency over alternatives of another frequency. For this reason Schwartz and Gerhardt (1998) analyzed neural and behavioral data from the same individuals to determine whether the lack of selectivity at the population level could be masking significant between-female variation. For small differences in frequency within the conspecific range of variation, neither audiograms nor iso-intensity plots reliably predicted the preferences of the same individuals tested at 85 dB SPL. Although the neural estimates might have been improved by recording from multiple sites in the torus, recordings of single basilar-papilla neurons show very little variation in BEF. Similarly, Diekamp and Gerhardt (1992) found that electrode position had little effect on audiograms based on multiunit recordings from the midbrain. Repeated testing of females indicated that many individuals were inconsistent in their choices of synthetic calls that differed in frequency. Estimates of frequency preferences could perhaps be improved by testing females multiple times. Nevertheless the presently available neural and behavioral data both suggest that tuning in *P. crucifer* is broad and weak.

In summary audiograms and iso-intensity plots have been only partially successful in predicting frequency preferences and the effects of stimulus intensity in population-level studies. Future studies are likely to be more successful if better estimates of the selectivity of the entire central auditory system (e.g., by use of multiple electrodes) can be correlated with behavioral preferences in the same individuals. The chances for success will also be increased by picking species that show reliable preferences based on relatively small differences in frequency that fall within the range of variation in the population.

### Chorus Noise and Frequency Preferences in Spring Peepers

Whereas midbrain iso-intensity plots did not reliably predict preferences when frequency differences were relatively small, the usefulness of such plots when differences in frequency were more substantial has been supported by a counter-intuitive result. Because spring peepers, like many other anuran species, communicate in large choruses of conspecifics, background noise levels as well as signal levels

are well above threshold. Moreover the conspecific chorus is potentially a far more serious source of noise than that generated by other species, because its spectrum will more broadly overlap that of any particular conspecific signal (Gerhardt and Klump 1988). Thus we might predict that by adding simulated chorus background noise females would not only be less able to detect individual males but would also be less capable of discriminating between signals that differ in carrier frequency. In fact the single discrimination task that females accomplished at a playback level of 85 dB SPL—a preference for 3.5 kHz over an alternative of 2.6 kHz—occurred only in the presence of chorus background noise. In Figure 7.7C we show the mean iso-intensity function at 85 dB SPL recorded in the presence of simulated chorus noise played back at 75 dB SPL (range, 75–86 dB in natural choruses). Clearly this plot shows significantly higher activity at 3.5 kHz than at 2.6 kHz. Correlations between iso-intensity plots (85 dB SPL) in the presence of noise and the preferences of individuals tested at the same level with a chorus-noise background were also highly significant (Schwartz and Gerhardt 1998).

Speculation about the mechanisms underlying the improved frequency discrimination and more-peaked iso-intensity response plots center on temporary elevations of threshold in response to background noise (Schwartz and Gerhardt 1998). Threshold shifts in eighth nerve fibers of *Eleutherodactylus coqui* have also been reported by Narins (1987). This phenomenon changes the linear operating range of the auditory system to a higher range of signal intensity so that the intensity at which saturation occurs is also elevated.

## Other Nonlinear Effects on Frequency Selectivity

### Interaction between the Two Spectral Peaks in the Gray Treefrog

Gerhardt and Doherty (1988) reported that females of the gray treefrog, *Hyla versicolor*, tested at 85 dB SPL preferred synthetic calls with a frequency of 1.9 kHz to those of 2.2 kHz, which is close to the mean in the calls of males in the same population in central Missouri. In these experiments the synthetic calls had a single carrier frequency, whereas natural calls have a secondary band that is, on average, about 4–12 dB lower in relative amplitude. More recently females were tested with synthetic calls that had both spectral peaks, and at the same playback level females preferred a stimulus of 1.1 kHz (–6 dB) + 2.2 kHz to an alternative of 0.95 kHz (–6 dB) + 1.9 kHz (Tanner and Gerhardt, in preparation). Further research is required to define the neu-

ral bases for the difference in the results of these two experiments, but the assumption that the frequency selectivity of the two auditory organs is independent of one another is clearly unwarranted.

### A Cross-Level Mismatch: Temperature Effects on Frequency Preferences in the Green Treefrog

Temporal properties such as pulse rate are highly temperature-dependent in the calls of anurans. Preferences based on differences in pulse rate change with temperature in a parallel fashion to that in the calls of males, a phenomenon that has been termed temperature coupling (Gerhardt 1978). In contrast typical changes in carrier frequency with temperature in anurans are relatively small (Gerhardt and Mudry 1980). Because the characteristic frequency of the primary auditory fibers innervating the amphibian papilla are temperature-dependent (for a review see Lewis and Lombard 1988; Narins, this volume), the possibility exists that the match between low-frequency auditory tuning and the carrier frequency of conspecific calls might be poorer at some temperatures than at others.

Behavioral correlates of these physiological properties were demonstrated in the green treefrog (Gerhardt and Mudry 1980). Although females prefer synthetic calls with a low-frequency peak of 0.9 kHz to an alternative of 0.6 kHz at normal breeding temperatures (22–26°C), females tested at about 18°C reversed their preference. Indeed, in this temperature range, females even preferred an alternative of 0.5 kHz, which falls within the range of variation of the sympatric congener, the barking treefrog, *H. gratiosa*. Preferences based on differences in the high-frequency peak showed relatively little temperature dependence, as did the characteristic frequencies of neurons innervating the basilar papilla (Narins, this volume).

The shift in frequency preferences is not matched by a parallel drop in the frequency of the low-frequency spectral peak in the advertisement call of *H. cinerea*. Males produced calls with nearly the same low-frequency peaks as they did at normal breeding temperatures after they were acclimated to lower temperatures over the same time course as females that changed their frequency preferences (Gerhardt and Mudry 1980). Thus the maximum sensitivity of the auditory system at low temperatures occurs at frequencies nearly an octave lower than the frequencies emphasized in the advertisement call. Field studies suggest that although males sometimes call at these low temperatures, few females arrive for mating (Richard E. Daniel, personal communication). Whereas females are thus unlikely to prefer the calls of *H. gratiosa* to conspecific males (there are also

behaviorally relevant, fine-temporal differences in the calls; Gerhardt 1981), females breeding at somewhat lower-than-average temperatures might be expected to show a bias for conspecific calls with lower-than-average frequencies. Additional field studies are needed to test this prediction.

## Conclusions

Enhanced regions of auditory sensitivity correspond to one or two frequency bands emphasized in the long-range acoustic signals of anurans, thus supporting the matched filter hypothesis at the interspecific level of analysis. Such tuning is broad and relatively weak but could also mediate frequency preferences that represent stabilizing or directional selection, depending on how well the tuning matches the average carrier frequency or frequencies in conspecific calls. This conclusion must be qualified by considering changes in frequency selectivity under different conditions. In some species, frequency selectivity (iso-intensity plots and behavior) is reduced as stimulus intensity increases; in other species, the opposite trend can occur. Additional nonlinear effects that confound predictions about frequency preferences from threshold estimates include: (1) the enhancement of selectivity based on relatively large differences in frequency by the addition of chorus noise; (2) interactions between preferences mediated by the two inner ear organs; and (3) temperature effects. These phenomena, as well as behavioral studies designed to explore the significance of enhanced sensitivity to frequencies that do not occur in communication signals, deserve much additional research.

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