Sexual Differences in the Auditory System of the Tree Frog *Eleutherodactylus coqui*

Abstract. *Acoustic playback experiments with calling males in their natural habitat and two-choice orientation experiments with females indicate that males and females of the neotropical tree frog* Eleutherodactylus coqui *respond to different notes in the two-note call of the male. This functional dichotomy of the two notes in the male's call reflects a difference in the distribution of the best excitatory frequencies of primary auditory neurons for the males and females. To the best of our knowledge,* Eleutherodactylus *is the first known example of a vertebrate in which the peripheral auditory sensitivity shows a sexual difference.*

Eleutherodactylus coqui (Thomas) is a neotropical leptodactylid frog whose range is restricted to the island of Puerto Rico and includes several small introduced populations in Miami, Florida, and in St. Thomas and St. Croix, U.S. Virgin Islands (*1*). Males emit a two-note call ("Co-Qui") each evening from sunset until midnight or shortly thereafter (*2*). If a "resident" calling male is approached by a second calling male, the resident will drop the second note of his call and emit only the "Co" note. If the intruder continues to approach to within about 0.6 m of a calling male, the resident will initiate a biting, butting attack on the intruder (*3*).

Acoustic playback experiments were conducted in the high-altitude rain forest near El Yunque Peak (altitude 900 m) in the Luquillo Mountains of eastern Puerto Rico in order to determine which features of the two-note *E. coqui* call were critical in evoking the single "Co" note response observed during male-male interactions. A series of natural and synthetic sounds were played to calling males with a Nagra IV-D tape recorder and a Nagra DH speaker-amplifier. The intensity of the acoustic stimulus was measured with an impulse sound-level meter (Bruel & Kjaer 2204) and was adjusted to a level to which the animal readily responded [80-db sound pressure level (SPL) at 2 m]. Three recorded calls were presented at a rate of one every 4 seconds for 3 minutes: a natural two-note "Co-Qui" call (stimulus a), a synthetic "Qui" (stimulus b), and a synthetic "Co" (stimulus c). These were presented in random order to each male *E. coqui*, and the stimulus and the evoked vocal responses were recorded on a second, identical, tape recorder. A total of 16 males were tested.

In general, the stimuli elicited two classes of responses from a male: a two-note "Co-Qui" call that tended to alternate with the stimulus, and a one-note, short-latency (100-msec) "Co" response that appeared to be time-locked with the stimulus. The number of one-note "Co" responses was divided by the number of natural calls (stimulus a) presented during the 3-minute playback period to yield the percentage of one-note responses, p_a. The p_a values for all of the individuals tested with the natural call were averaged, obtaining $\bar{p}_a$. In a similar fashion, $\bar{p}_b$ and $\bar{p}_c$ of one-note responses were obtained for the other two stimuli. The values of $\bar{p}_b$ and $\bar{p}_c$ were then tested by pairs for differences with $\bar{p}_a$ (2 by 2 χ^2 test for homogeneity).

Histograms of the males' responses and sound spectrogram tracings of the corresponding stimuli (*4*) are shown in Fig. 1. The mean percentage of one-note responses for the natural call, $\bar{p}_a = 42.2$ percent, was used as a standard against which the responses to other stimuli were compared (Fig. 1). The "Co" note of the intruding male's call (or of the synthesized playback of the male's call) is sufficient to evoke the one-note "Co" response from the resident; however, the "Qui" note was relatively ineffective in evoking this response.

Behavioral studies were conducted to determine which notes of the male's call are most attractive to a female (*2*). Adult females were placed in a screened

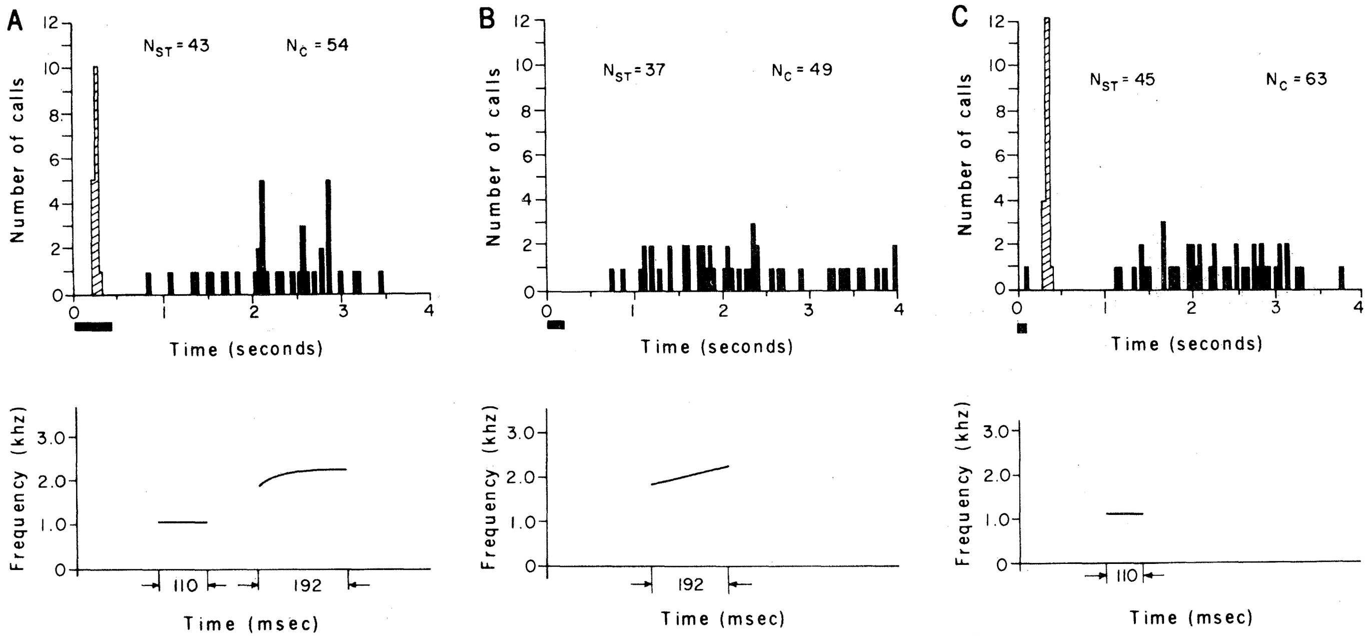

Fig. 1. Representative histograms of vocal responses of male *E. coqui* to acoustic playback of natural and synthetic calls shown in sonagram tracings below each histogram. The solid vertical bars in the histograms represent two-note "Co-Qui" responses; the striped bars represent one-note "Co" responses. The horizontal bars below the histograms represent stimulus duration. Abbreviations: N_{ST}, number of stimulus repetitions presented to the male during the 3-minute test period: N_C, total number of calls given by the male during the same period. The bin width is 50 msec. (A) Responses to the natural call. The mean percentage of one-note responses to the natural call is $\bar{p}_a = 42.2$ percent (based on four males). (B) Responses to the "Qui" note presented alone; $\bar{p}_b = 0.9$ percent (ten males), which is significantly lower ($P < .0005$) than $\bar{p}_a$ for the natural call. Exponentially and linearly swept "Qui" notes were used; they were equally ineffective in evoking a one-note response from a test male. (C) Responses to "Co" note alone; $\bar{p}_c = 39.8$ percent (13 males), which is not significantly different ($P > .05$) from $\bar{p}_a$ for the natural call.

container midway between two loudspeakers (Nagra DH) 4 m apart. Through each loudspeaker a different acoustic stimulus could be broadcast by means of recorded calls played on separate tape recorders (Nagra IV). In some cases only one stimulus was presented (one-choice trial), whereas in other cases stimuli were presented simultaneously through both loudspeakers (two-choice trial). The stimuli used in these trials were identical to those in our study of vocal responses of males.

After a female had settled down, playback was begun and the screened container was removed. The orientation and approach behavior of the female reflected the hierarchy of preferences for the different stimuli. A trial period of 20 minutes was chosen since female *E. coqui* move slowly and may pause five or more minutes between hops. Only trials in which the animals showed deliberate, oriented responses were used; escape attempts were not included when compiling the results. When given a choice between two calls, the females respond equally well to either the two-note call or the "Qui" note alone (Table 1). On the other hand, the "Co" note by itself has relatively little attraction for the female.

Our behavioral studies thus indicate that the two-note call of *E. coqui* conveys a different significance for each sex. The first note functions in male-male interactions and may serve to maintain spacing between adjacent males but does not attract a female. The second note serves to attract conspecific females and apparently plays no role in male-male encounters.

Table 1. Results of female orientation experiments. A two-plus (++) response was scored if a female approached within 0.5 m of a speaker (close approach); an orientation, an approach toward a speaker, or both, were scored as a one-plus (+) response if the distance from the female to the speaker at the end of the 20-minute trial period was greater than 0.5 m but less than the initial 2.0 m (weak approach). The U response denotes a preliminary approach to the active speaker followed by a midcourse correction and subsequent escape. For the two-choice trials an underlined response corresponds to an orientation toward the speaker presenting the underlined stimulus; N.S., not significant.

Stimuli	Trials (No.)	Females (No.)	Results	*P*
		One-choice experiment		
Co-Qui	7	5	6++, 1+	<.01*
Co	4	4	2+, 2U	†
Qui	3	2	1++, 2+	†
		Two-choice experiment		
Co,$\underline{\text{Qui}}$	6	6	1+; 3$\underline{+}$, 2$\underline{++}$	<.025‡
$\underline{\text{Co-Qui}}$, Co	7	5	4$\underline{+}$, 2$\underline{++}$; 1+	<.025‡
$\underline{\text{Co-Qui}}$, Qui	7	6	3$\underline{+}$; 2+, 2++	N.S.

*Rayleigh statistic (*16*). †Because of the small number of trials, *P* was not calculated for these stimuli. ‡G-test with a weighted response scheme (*17*).

The peripheral auditory systems of anurans are specialized to detect the major spectral and temporal features of the male's species-specific mating call (*5, 6*). The inner ear contains two separate auditory organs, the amphibian papilla and the basilar papilla (*7*). The different frequency ranges to which these two organs are tuned often correspond to the energy present in sounds of biological significance (*8*). Since *E. coqui* exhibits a sexual difference in its behavioral response to the male's two-note call, we wondered whether there might be a difference in neural sensitivity somewhere in the auditory system. The response properties of auditory fibers were therefore studied electrophysiologically in the eighth cranial nerve of males and females to determine whether this selectivity could occur at the level of the peripheral auditory system.

Adults of both sexes were immobilized with *d*-tubocurarine chloride (Calbiochem) injected intramuscularly (2 μl per gram of body weight; 5 mg/cm^3); additional doses were administered when needed to maintain immobility. The surgical procedure, recording, and stimulus system in our laboratory have been described (*6*). The responses of 563 single units to tone-burst stimuli were recorded extracellularly in the eighth nerve of 29 animals (284 units in 20 males and 279 units in 9 females). Thresholds for these fibers ranged from 17 to 105 db SPL, which is consistent with the wide range for unit thresholds reported in other anuran species (*6, 8*). When a single unit was isolated, its best excitatory frequency (BEF) and threshold were measured (*9*). If the unit could be held long enough, a tuning curve was taken and the unit's response to the addition of a second, inhibitory tone was recorded (*10*).

Three populations of fibers were found (Fig. 2A): one group is inhibitable and has BEF's below about 600 to 700 hertz; a second group is noninhibitable and has BEF's below about 1400 hertz; a third group, noninhibitable, has BEF's distrib-

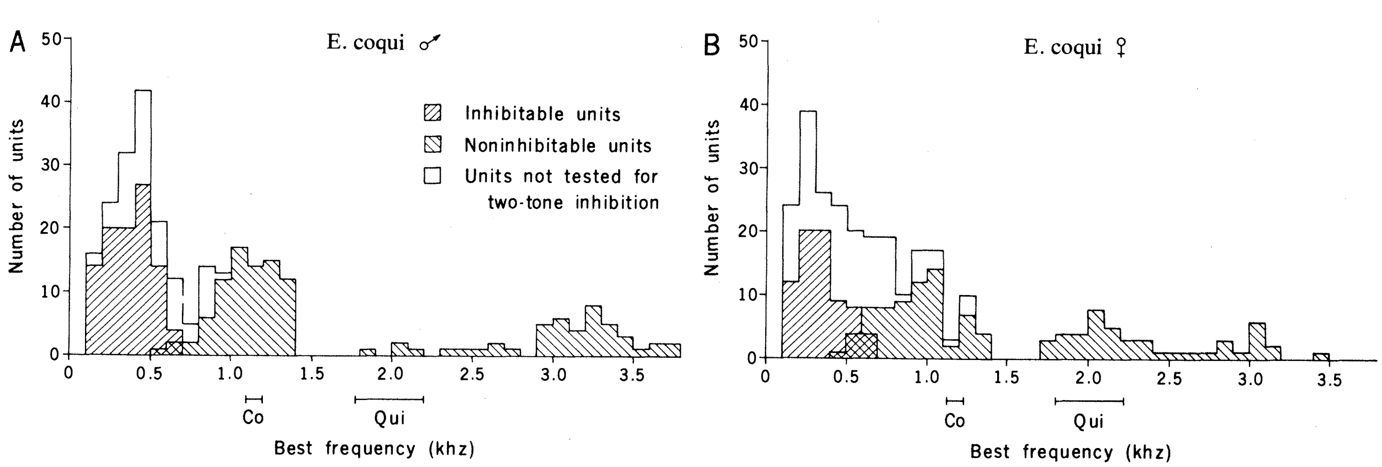

Fig. 2. Histograms of best excitatory frequencies (BEF's) for auditory units recorded from the eighth cranial nerve of *E. coqui*. The striped bars within the histogram represent units tested for two-tone inhibition. The distribution of energy in each of the two call notes based on a sample of 16 males is indicated below the histogram. (A) Results for 284 auditory units of 20 male *E. coqui*. (B) Results for 279 single fibers recorded from nine female *E. coqui*. The three populations are shifted with respect to the corresponding populations in the males' histogram. The high-frequency population is now clustered around the "Qui" note frequency and the relative number of units with BEF's at the "Co" note frequency is reduced.

uted between 1800 and 3700 hertz. The low- and mid-frequency units probably derive from the amphibian papilla, whereas the high-frequency units originate in the basilar papilla (*11*). The histogram reveals a large population of fibers with BEF's in the range of the "Co" note, whereas there are few units with BEF's in the range of the "Qui" note. Neither the low-frequency nor the high-frequency populations in this histogram coincide with the energy in the male's call. Low-frequency sensitivity may play a role in detection of predators for some anuran species (*8*). The role played by the high-frequency fibers is not obvious. Occasionally the male *E. coqui* emits a call whose function is unknown and which contains energy as high as 2700 hertz (*12*). Perhaps the high-frequency fibers provide the basis for detection of this call. The mean BEF of the low-frequency units is not significantly different in males and females ($P > .05$); however, the differences between the mean BEF's of both the mid- and high-frequency units are significantly different for the two sexes ($P < .001$) (*13*).

The sharpness of tuning of an auditory nerve fiber is given by its Q value (the BEF divided by the bandwidth of its tuning curve at 10 db above threshold). The sharpness of tuning of the fibers sensitive to low and middle frequencies was not significantly different in males and females. However, the high-frequency units showed a distinct difference in tuning sharpness between the two sexes; for females, the average Q of the high-frequency fibers was 0.91 whereas in males it was 1.34 (*14*).

The fibers sensitive to high frequencies in females tend to have lower BEF's and broader tuning than those in males. This indicates that the basilar papilla in the inner ear is tuned differently in males and females. Such sexual selectivity in the response properties of the anuran's auditory organs is yet another adaptation for peripheral stimulus filtering. Partitioning the call so that it serves (at least) two functions, and differentially adjusting the peripheral sensitivities to enhance reception of the parameters of the call of interest to each sex, provides a novel mechanism whereby a male can simultaneously communicate with both sexes in a complex sound environment (*15*).

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References and Notes

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4. Call-note durations, internote intervals, and repetition rates used in acoustic stimuli for male playback experiments were chosen from recordings of typical calls of males from the same population. These were recorded and supplied by G. E. Drewry.
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9. The best excitatory frequency of an auditory nerve fiber is the tonal frequency to which the fiber is most sensitive (that is, has the lowest threshold).
10. A unit is inhibitable if its response to an excitatory tone within its tuning curve can be totally suppressed by the simultaneous addition of a second tone of higher frequency and appropriate intensity (up to 30 db above the excitatory tone) outside the unit's excitatory tuning curve.
11. In the bullfrog (*Rana catesbeiana*) the auditory nerve fibers which innervate the amphibian papilla are sensitive to low- and mid-frequency tones and the fibers which innervate the basilar papilla are sensitive to higher-frequency tones [A. S. Feng, P. M. Narins, R. R. Capranica, *J. Comp. Physiol.* **100**, 221 (1975)]. A similar organization is presumed to be the case for *E. coqui*.
12. G. E. Drewry, in *Puerto Rico Nuclear Center Rain Forest Project Annual Report 1970* (Puerto Rico Nuclear Center, San Juan, 1970), pp. 16–63.
13. The test used was the Kolmogorov-Smirnov two-sample test [S. Siegel, *Non-Parametric Statistics* (McGraw-Hill, New York, 1956)]. A measurement of nine morphological characters in 20 male and female *E. coqui* revealed a pronounced sexual dimorphism in size of all nine characters; for example, mean snout-to-vent length: male, 37.2 mm and female, 52.2 mm; mean longitudinal tympanum diameter: male, 2.0 mm and female, 2.9 mm [R. Thomas, *Q. J. Fla. Acad. Sci.* **28**, 375 (1966)]. The shift in frequency sensitivity between males and females might be related to sexual dimorphism, such as a size difference in the auditory organs of the two sexes. In addition, the characteristics of the mating call of male *E. coqui* vary with the elevation within the rain forest (G. E. Drewry, personal communication; P. M. Narins and R. R. Capranica, unpublished data). Furthermore, males at higher elevations are larger and produce lower-pitched calls than those at lower altitudes. This raises the interesting question of whether the frequency sensitivity of the inner ear in an animal of this species varies with its body size.
14. The Q values for the high-frequency fibers in males are significantly different from those in the females ($P < .001$, Kolmogorov-Smirnov two-sample test). This test also revealed that the Q values for the low- and mid-frequency units were not significantly different in males and females ($P > .05$).
15. At least eight sympatric species of eleutherodactylids contribute to the sonic background in the El Yunque rain forest [A. Schwartz, *Stud. Faun. Curacao Other Caribb. Is.* **91**, 1 (1967)].
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17. The ++ response was a stronger indication of female preference than a + response. The linear distance from the release point to the midpoint of the ++ response area is 1.75 m; the distance to the midpoint of the + response area is 0.75 m. The ratio of these two distances is 2.33. Assuming responsiveness to be a linear function of distance traveled by the female, we assigned a weight of 2.33 to each ++ response and a weight of 1 to each + response.
18. We thank G. E. Drewry, J. Colón, and D. Narins for providing live *E. coqui*; P. Capranica for aid with the field experiments; the Puerto Rico Nuclear Center for the use of its field facilities; and the members of our laboratory for comments on the manuscript. Supported by NIH research grant NS-09244 to R.R.C. and a Sigma Xi grant-in-aid of research to P.M.N.

21 November 1975; revised 29 December 1975

Incentive Contrast in Honey Bees

Abstract. *Bees trained to come to the laboratory for a 20 percent sucrose solution accept it readily, but bees trained with a 40 percent sucrose solution and tested with the 20 percent solution show a pattern of interrupted feeding that may last for several minutes. Bees trained with 20 percent and tested with 40 percent sucrose are undisturbed. When the animals are offered two samples of the 20 percent solution simultaneously, they drink to repletion from whichever they first taste on each visit, but if both a 20 percent and a 40 percent drop are offered the 20 percent solution is rejected after a single experience of the 40 percent solution. Although these results are analogous in many respects to incentive contrast effects found in mammals, they can be understood in sensory terms and do not require the assumption of learning about reward.*

In diverse mammalian species, experience with a preferred food may reduce the acceptability of a less favored food. Monkeys will eat lettuce from a cup they have earlier seen baited with lettuce but not from one earlier seen baited with banana (*1*). Rats trained with bran mash in a maze or a problem box will not accept sunflower seeds substituted for the mash, although sunflower seeds are readily accepted by rats trained with them from the outset (*2*). Goats will normally eat grain, but not in a situation in which they have previously found both grain and bread (*3*). These results have been cited in support of the view that rewards do not serve simply to strengthen response tendencies as reinforcement theorists have proposed (*4*), but that rewards may be learned about, and anticipation of their specific properties may play an important role in instrumental behavior (*5*). Incentive contrast does not, however, necessarily imply learning about reward. In honey bees (*Apis mellifera*), I have found contrast effects, di-