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Clinal variation in anuran advertisement calls: basis for acoustic isolation?

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Summary. The calls of male treefrogs (*Eleutherodactylus coqui*) were studied along an altitudinal gradient in the Luquillo Mountains in eastern Puerto Rico. The fundamental frequency of each note in the advertisement call was highly negatively correlated with altitude. Although males responded vigorously to playbacks of calls of other males recorded at their own altitude, males at high altitudes responded less frequently to recorded calls of males from low altitudes, and males at low altitudes responded less frequently to recorded calls of males from high altitudes. These results are discussed in relation to potential isolation in contiguous populations of *E. coqui.*

Introduction

Most species of frogs rely on one or more unique properties of their call (i.e., fundamental frequency, duration, etc.) as a form of conspecific recognition (Bogert 1960). Several factors can influence the spectral and temporal properties of an animal's call, including air temperature and body size (see Littlejohn 1977 for review). For example, fundamental call frequencies and call repetition rates tend to vary inversely with body size (Blair 1958; Nevo and Schneider 1976; Ryan 1980). Some frogs have been shown to alter their diel calling patterns and the temporal properties of their calls in the face of background noise and conspecific interference (Narins 1982a; Schwartz and Wells 1984).

The Puerto Rican coqui, *Eleutherodactylus coqui* (Anura: Leptodactylidae) is an arboreal frog found throughout Puerto Rico at altitudes from sea level to above 1000 m (Drewry 1970). It is an ideal subject for the study of intraspecific variation of call parameters, since much is known of the behavior and acoustic communication of this anuran (Narins and Capranica 1976, 1978; Narins 1982b; Narins and Hurley 1982; Drewry and Rand 1983). Males produce a characteristic two-note advertisement call: "Co-Qui." At the higher altitudes (900 m), the first note, "Co", is a constant-frequency 100 ms tone with a fundamental frequency of about 1.2 kHz which functions in male-male territorial interactions (Narins and Capranica 1976). The second note, "Qui," sweeps upward in frequency from 1.8 kHz to 2.1 kHz in 170 ms; this note functions in the attraction of females (Narins and Capranica 1976, 1978).

Playback of a natural call or a synthetic call of appropriate frequency and intensity to an isolated male of *E. coqui* evokes a characteristic vocal response from that individual (Narins and Capranica 1978). The male drops the second ("Qui") note from the advertisement call and produces the isolated "Co" note. This "Co" note response may be elicited by the playback of either the complete two-note call or the "Co" note alone, and may thus serve as a sensitive bioassay for the detection of sounds by a male coqui in his natural habitat (Narins 1982a, b, 1983). It has been shown that a given male responds best (produces more "Co" note responses) to playbacks of conspecific calls with frequencies near those of his own calls, and has a lower probability of response for call frequencies differing from his own calls (Narins and Capranica 1978; Narins 1983).

It has been observed that there is a positive correlation between body size and altitude in *E. coqui*, and a negative correlation between altitude

and the fundamental frequency of the animal's advertisement call (Drewry and Rand 1983; M. Stewart, unpublished data). In the present study, we examine these relationships in detail and propose some explanations for them. We also demonstrate that males from high-altitude respond less vigorously to calls of males from low-altitudes than they do to calls from males at their own altitude, and vice-versa.

Methods

Study site

This study was conducted in the Luquillo Mountains of the Caribbean National Forest in eastern Puerto Rico during May and June of 1983, and from June to August of 1984. Calls of 174 males of *E. coqui* were recorded between 2000 and 2400 h, Atlantic Standard Time, along a 13 km stretch of Puerto Rico Highway 191, which transects the northeast face of the Luquillo Mountains up to El Yunque Peak (Fig. 1). The calling sites of the males studied ranged in altitude from 18 m to 1000 m above sea level. An AKG CE8 directional microphone with windscreen was placed approximately one meter in front of each calling male and ten to twenty calls were recorded on a cassette recorder (Sony TC-D5M). Following recording, the frog was captured, and its snout-vent length (SVL, the distance from the tip of the animal's snout to its cloacal opening) was measured with a plastic rule to the nearest mm. The altitude was recorded to the nearest 10 m using an altimeter (Thommen 1000-31) calibrated against a United States Geological Survey 20 m contour map. The air temperature and relative humidity were measured at each frog's calling site with a sling psychrometer (Bacharach).

Call analysis

Recorded calls were analyzed using a high resolution signal analyzer (Bruel and Kjaer 2033). In order to standardize the calls for comparison, the following call parameters were measured: (1) the fundamental frequency of the "Co" note 20 ms after the start of the note, (2) the fundamental frequency of the "Qui" note 20 ms after the start of the sweep, and (3) the total call duration, i.e., from the start of the "Co" note the end of the "Qui" note. For each frog, two representative calls were analyzed, and the values obtained were averaged.

For each locality, the data obtained during 1983 and 1984 were first statistically analyzed separately then pooled. The population means for each call parameter were then compared pairwise by using the z-statistic for large samples (McClave and Dietrich 1982). Environmental parameters (altitude and temperature) were similarly analysed and tested. Regressions of altitude on SVL and altitude on "Co" note frequency, "Qui" note frequency, and call duration were analyzed on an IBM 3033 computer using Statistical Analysis System (SAS 1982). Correlation coefficients of each model were tested for significance using the Student's *t*-test.

Cross-altitude playback experiments

Calls of males from two populations (170 m and 680 m altitude) (Fig. 1) were recorded with a cassette recorder (Marantz C-206LP) on 20-second endless cassette tapes (TDK) and used as stimuli for the cross-altitude playback experiments (Fig. 2). The call amplitudes were measured using a calibrated precision sound level meter (Gen Rad 1982), with the one-octave bandpass filter centered at 1 kHz for the high-altitude males and at 2 kHz for the low-altitude males.

Next, the evoked acoustic responses of 15 males from each site to the playback of the stimulus tapes were determined. After a male was located, 50 spontaneous calls were recorded and its spontaneous calling rate calculated. Five of the males from each site were then presented 50 calls of a male from the same site (Fig. 2), while the other ten males were exposed to 50 calls of a male from the other site. The stimulus tapes of the low-altitude and high-altitude males were broadcast with peak intensities of the "Co" note of 74 and 80 dB SPL at one meter, respectively, each of which corresponds to the average intensity for male *E. coqui* at each of these altitudes (Narins and Hurley 1982). Acoustic responses were categorized as one-note calls ("Co" note only), or advertisement calls ("Co" plus "Qui" notes). After testing, each frog was captured, and its SVL and the ambient temperature at its calling site were measured and recorded.

Results

Comparison of 1983 and 1984 data

Figure 3a shows the relationshhip between an animal's SVL (mm) and the altitude above sea level (km) at which it calls. The regression has a coefficient of determination, $r^2 = 0.65$. These measurements are for calling males, but we have observed a similar trend for females as well. The only variable for which the means differed significantly between the two data sets was air temperature ($z = 7.51$, $P \leq 0.01$, $n = 100$). The mean temperature ($\pm$SD) across all altitudes was 22.2° $\pm$1.4° C ($n = 26$) in 1983, and 23.8°$\pm$1.7° C ($n = 74$) in 1984; this difference can be explained by the relatively few temperature measurements from low altitude sites in 1983 (Fig. 3b). Thus, temperature was not used as an independent variable in the analysis of call parameters and the remaining data from the two observation periods were pooled for subsequent analysis.

To account for the variation in the call frequencies and duration, we performed a multiple linear regression analysis (SAS stepwise procedure) using altitude and SVL as independent variables. For the pooled data, altitude was the best single predictor of any dependent variable, followed by SVL (Table 1). The variation in all call parameters with altitude is continuous, that is, there is no "clumping" of the data (Figs. 4–6). Although the models incorporating more than one independent variable were generally better predictors of the call parameters than were single-parameter models (Table 1), the good correlation between altitude and SVL (Fig. 3a) suggests that the single-parameter models are more statistically meaningful.