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Animal behaviour

Behavioural tuning in a tropical amphibian along an altitudinal gradient

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Males of the coqui treefrog, *Eleutherodactylus coqui*, produce a distinct two-note 'co-qui' advertisement call from sunset to midnight throughout most of the year. Previous work established that both the spectrotemporal aspects of the call and the frequency of highest inner-ear sensitivity change with altitude above sea level. These variations are such that the frequency of the emitted co-note closely matches the frequency to which the inner ear is most sensitive. Given this parallel variation, we expected that the call-evoked behavioural response of male coqui treefrogs would also show an altitude dependence, and hypothesized that males would produce their most robust acoustical territorial response to advertisement calls that match calls from their own altitude. We tested this hypothesis in the field by studying the vocal response behaviour of coquis to playbacks of synthetic, altitude-dependent conspecific calls, and indeed found that the most robust vocal responses were obtained using stimuli closely matching the calls from the same altitude.

1. Introduction

The neotropical treefrog, *Eleutherodactylus coqui* (Leptodactylidae), is abundant throughout the island of Puerto Rico. Its name is onomatopoeic; male frogs emit a two-note advertisement call consisting of a constant-frequency tone (co-note) followed by a second (qui) note sweeping upward in frequency through about one-third of an octave. The co-note mediates male–male territorial interactions, while the qui-note attracts females [1]. In the Luquillo Mountains, coqui frogs live along an altitudinal gradient from sea level to about 1000 metres above sea level (m.a.s.l.). Both the size of male frogs and the spectrotemporal parameters of their advertisement calls vary systematically along this gradient [2–4]. At low altitudes, small males produce rapid, high-pitched calls, whereas at high altitudes larger males emit slower, lower-pitched calls. Moreover, the spectral peak of the co-note correlates with the frequency of highest inner-ear sensitivity along the same altitudinal gradient [4], in accordance with the matched-filter hypothesis [5]. It has been shown that the male behavioural response primarily depends on the co-note frequency; all other aspects of the calls that also vary with altitude have no (e.g. qui-note frequency) or only very little (e.g. call duration) effect [1]. We therefore hypothesize that the male *E. coqui*'s acoustic territorial defence is tuned to the co-note frequency of frog calls from their local population. To test this hypothesis, we investigated the tuning of vocal response behaviour of male frogs along the same altitudinal transect. At three different altitudes, vocalizing males of *E. coqui* were presented with synthetic calls that mimicked the conspecific calls from different altitudes. We found that the vocal response behaviour of the male frogs is tuned to calls from their own elevation.
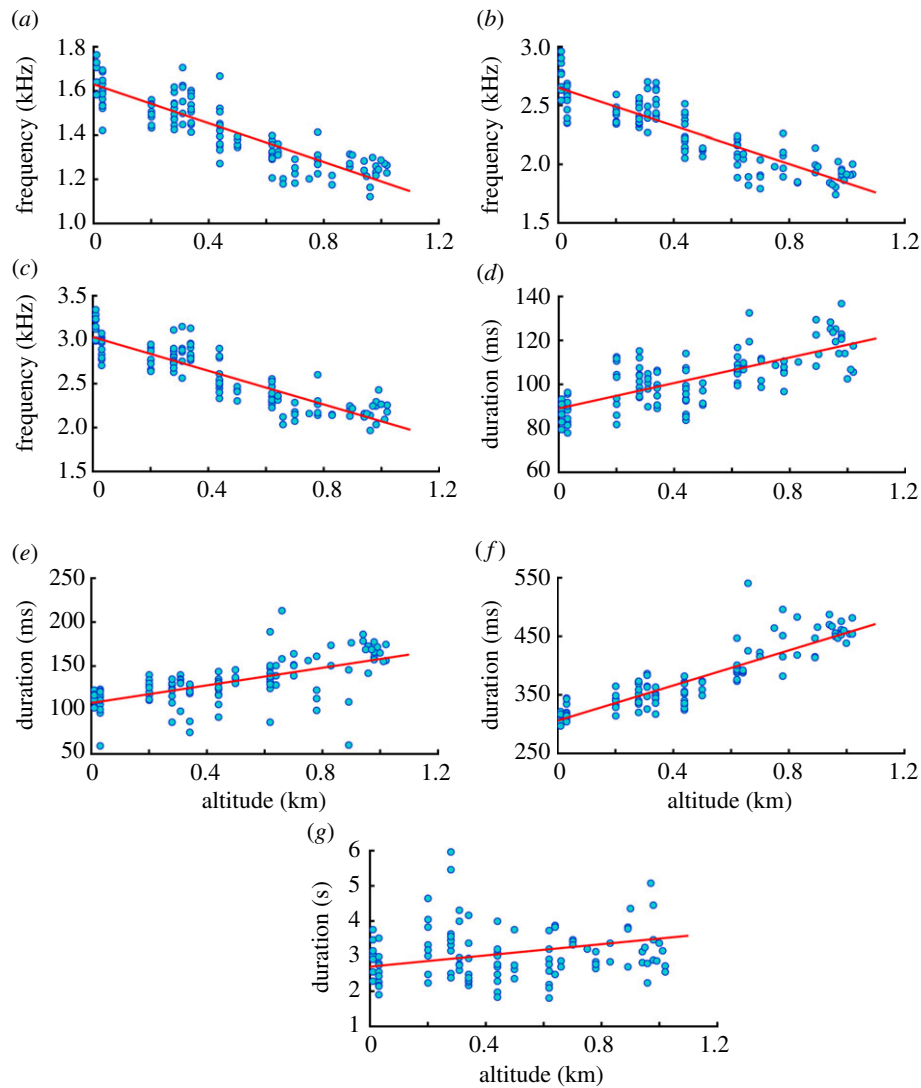
Figure 1. Altitude-dependence of the coqui frog's call. The advertisement call of *E. coqui* varies with altitude above sea level. Using previously acquired recordings [3] (data available in [7]), we quantified this altitude-dependence for seven call variables: (*a*) co-note frequency; qui-note (*b*) onset and (*c*) offset frequencies; (*d*) co-note and (*e*) qui-note durations; (*f*) the call duration, taken from co-note onset to qui-note offset; and (*g*) the interval between consecutive calls. Each variable was fitted with a straight line using least-square minimization (table 1 for results). The coefficients from the regression analysis were used to generate the synthetic, altitude-dependent calls used in the playback experiments. (Online version in colour.)

Table 1. Regression information for altitude-dependent call variables. Altitude-dependence of seven call variables was determined using linear regression to the data in figure 1. That is, each variable was fitted with the model $Y = \mu \times altitude + Y_0$, using least-square minimization. Here, *altitude* is in km.a.s.l. The coefficient (μ) and the constant (Y_0) are entered as means $\pm$ 95% confidence intervals. Also given are the coefficients of determination (r^2), and the number of frogs (*N*) included in the fit.

	unit	μ	Y_0	r^2	*N*
co-note					
frequency	kHz	−0.44 (0.05)	1.63 (0.03)	0.76	116
duration	ms	29 (5)	89 (3)	0.54	116
qui-note					
freq. at begin	kHz	−0.81 (0.08)	2.65 (0.05)	0.77	116
freq. at end	kHz	−0.96 (0.1)	3.03 (0.05)	0.77	116
duration	ms	50 (10)	108 (7)	0.39	116
call duration					
	ms	150 (10)	306 (8)	0.79	116
call−call interval					
	s	0.8 (0.4)	2.7 (0.2)	0.12	115

2. Material and methods

This study was conducted between 26 April and 6 May 2016 in El Yunque National Forest in eastern Puerto Rico. Data were obtained from frogs at three sites along a 13 km stretch of Highway 191 that transects the northeast face of the Luquillo Mountains from sea level up to El Yunque Peak (1080 m.a.s.l.). These were a low-elevation site (200 m.a.s.l. ($n = 6$ frogs)), a mid-elevation site (530 m.a.s.l. ($n = 6$ frogs)) and a high-elevation site (900 m.a.s.l. ($n = 5$ frogs)). Acoustic playback experiments were performed during the hours of peak calling activity, between sunset and shortly after midnight [1,6]. Each night, one recording altitude was visited, with three or four separate visits per recording site. The order in which the recording altitudes were visited was pseudo-randomized. We did not observe any trends in our data related to the time of recording. The number of frogs studied per night varied between zero and three, and depended on our ability to locate and approach a relatively isolated, vocalizing male coqui.

Previously obtained data [3] were used to quantify the linear relation between seven call variables and altitude (figure 1 and table 1) and generate altitude-dependent synthetic calls [7]. We used linear extrapolation to create fictive calls from beyond the height of El Yunque Peak.

For the experiments, a speaker (JBL model Clip+) was placed approximately 1 m from a relatively isolated, calling male *E. coqui* located in his natural habitat. To establish a threshold stimulus level, 10 low-level co-qui calls, with parameters corresponding to the altitude of the male under test, were broadcast and the number of one-note, synchronized co-note responses was recorded. This territorial defence response serves as a convenient metric for the detectability of the stimulus [8]. The stimulus level was then increased in 3 dB steps until a 40% threshold response rate was reached. Next, multiple series of 50 synthetic calls were presented to the focal male and the number of co-note responses to each series recorded. The calls within a series all represented one altitude, while different altitudes were randomly assigned to consecutive series. All stimuli were presented at 3 dB above the initially established threshold stimulus level. As such, the absolute stimulus levels varied considerably between individuals. However, the median absolute stimulus levels were within 6 dB of each other across the three recording sites. The number of presented series was not fixed. In general, new series were presented while the tested animal remained engaged in the experiment (e.g. the subject had not moved away from its calling site, or had not redirected its responses to a nearby male). For every animal, each completed series is represented as a single datum in figure 2. For each series, the co-note response rate was calculated and normalized relative to the maximum response rate for that animal. We excluded three data points from figure 2 because they corresponded to a zero-response rate during the presentation of the final series of calls. In these instances, it was unclear whether the animal was truly non-responsive or had disengaged from the experiment.

In the chosen stimulus paradigm, there are multiple variables that vary with altitude. Their dependence on altitude has been documented [2–4], and in principle, any of them can be offered to explain any observed effects. However, it has been shown that it is the co-note frequency in the call that determines the probability of evoking a male acoustic response [1]. For this reason, we use co-note frequency as the independent variable in the analysis of the results.

3. Results and discussion

Normalized response rates for animals from the three recording altitudes are shown in figure 2. Overall, the data [7] support our hypothesis that the territorial defence response
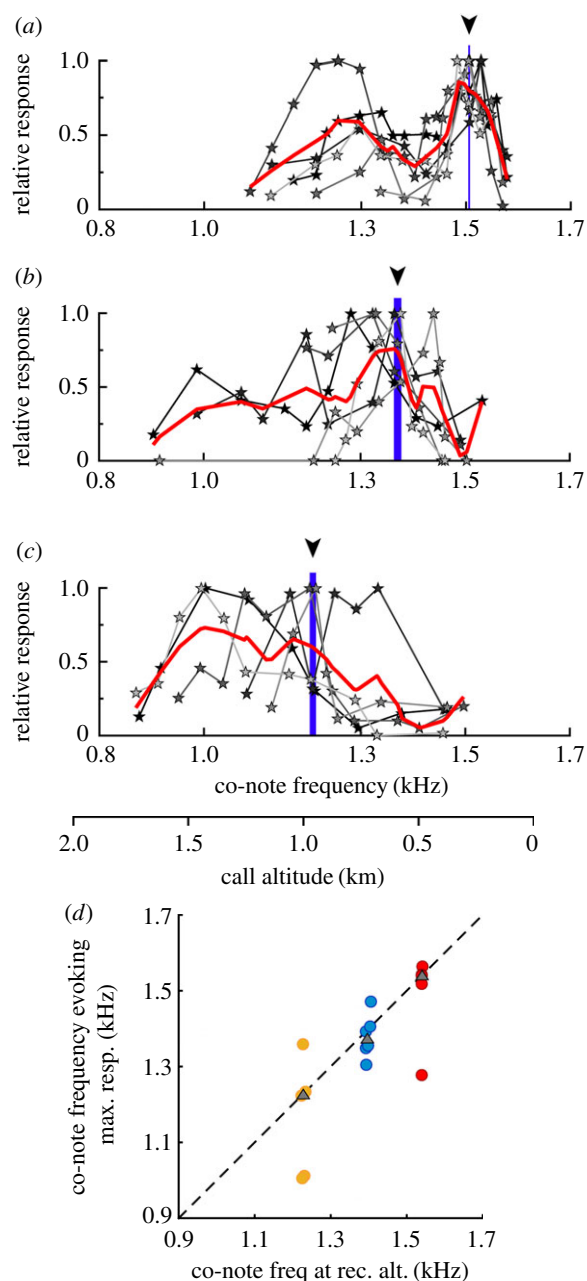

Figure 2. Behavioural response rate as a function of co-note frequency. Synthetic, altitude-dependent calls were generated and played back to frogs at three different ranges of altitudes above sea level: (*a*) 200 – 205 m.a.s.l. ($n = 6$ frogs), (*b*) 510 – 540 m.a.s.l. ($n = 6$), (*c*) 900 – 925 m.a.s.l. ($n = 5$). In each panel, the blue area and vertical arrowhead indicate this range. The abscissa refers to the altitude-dependent co-note frequency that was used to generate the calls. A secondary abscissa is shown below (*c*) that gives the corresponding altitudes. For each frog, the response rate is given by a continuous grey line. The bold (red online) line is a trend line fitted to the data (loess using 30% of the data range). (*d*) Scatter plot of co-note frequency that elicited the maximal behavioural response versus the mean co-note frequency produced by frogs at the recording altitude. Grey triangles are the medians for data from the different recording altitudes. The diagonal dashed line indicates equality. (Online version in colour.)

of males is tuned (figure 2*a–c*) to synthetic calls that used parameters from their recording altitude (figure 2*d*). That is, the neurophysiological and behavioural tuning of coquis are matched to communicate best with conspecifics from their own local population. To quantify this tuning, we expressed the dependent variable relative to the co-note frequency associated with the recording altitude, and then

pooled all data. For this combined dataset, we calculated the ratio of the mean response rate for all observations within a frequency band centred around the altitude-matched co-note frequency and the mean response rate for response rates outside this band. If the response rate is not tuned, this ratio will be unity, while for altitude-matched tuning and off-altitude tuning it will be larger and smaller than unity, respectively. For example, when using a bandwidth of 0.02 octave, we calculated this ratio to be 1.64. Bootstrapping ($N = 10\,000$) was used to verify that such a ratio is unlikely ($p < 0.001$) to occur if the behavioural responses were not tuned, or were tuned to a non-matched call altitude. The particular choice of bandwidth did not affect this result nor its significance. It was previously found that the behavioural responses result from same-altitude calls, but that study [9] was for coqui frogs located around 700 m.a.s.l. only. Our findings confirm this result, and show that it extends over the entire altitudinal range. We note that some of the response curves obtained at the lowest recording site (figure 2a) exhibited a secondary, lower-frequency peak. This peak is not explained by responses to the qui-note portion of the calls, and its origin is still under study.

The frog inner ear contains two auditory end organs, the amphibian papilla (AP) and basilar papilla (BP). In males of *E. coqui*, the AP is sensitive to frequencies from 100 to 1400 Hz, while the BP behaves as a single auditory filter that is tuned to frequencies above 2000 Hz [8]. It is therefore likely that the AP is responsible for the transduction of the co-note within the call [9]. The low-altitude animals in our study exhibit the highest sensitivity to co-note frequencies of approximately 1500 Hz, which falls outside the reported AP sensitivity range, which was obtained from animals occupying the highest altitudes [8]. Thus, the frogs from the lower altitudes appear to have extended the frequency range of highest AP sensitivity to include the co-note frequencies from low altitudes. Indeed, preliminary anatomical data indicate that the AP caudal extension, which decodes the highest frequencies in this organ, constitutes a higher proportion of the total AP length in the smallest males of *E. coqui* [10].

The origins of the observed altitudinal gradients are unclear. Since body size, inner-ear tuning and several call characteristics vary with altitude, the observed gradient may result from differential selection on any of these characteristics [11]. Given the lack of evidence for genetic structuring with altitude [12], it is likely that environmental factors contribute to the observed altitudinal gradients [13–15]. For example, selection against the frequency contents of the emitted calls may be driven by the amount of attenuation due to the structure of the habitat [15], or by vocal competition with other species [16]. Auditory tuning may match the frequency contents of the calls [5], but be additionally shaped by the specifics of the acoustic environment [13]. Alternatively, the animal's body size may simply vary due to the temperature-dependent development rate of different tadpole stages [17], which results in larger animals at lower temperatures. The larger overall body size could 'automatically' result in slower, low-frequency acoustic production and detection, without any selection pressure acting upon these properties.

In general, the very smooth variation of these characteristics with altitude suggests that there are no discrete factors, limited to a distinct altitude range, that may explain the observed altitudinal effects.

In its simplest form, communication requires a message from a sender that is effectively processed by a receiver. Here, the receiver is engaged with the message at each elevation because the frequency of the call and of the highest inner-ear sensitivity are matched. This results in a robust, altitude-dependent, one-note territorial call that is tuned to ward off same-altitude (local) males.

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