

Is congener abundance related to vocal adjustments that minimize acoustic interference?

DAVID LUTHER, MIGUEL A. ACEVEDO, MARIA ISABEL HERRERA MONTES,
ALBERTO R. ESTRADA AND T. MITCHELL AIDE

University of Puerto Rico - San Juan

Corresponding author current address: David Luther, George Mason University,
4400 University Dr., MS 3E1, Fairfax VA 22030, dluther@gmu.edu

Current address for all other authors.: PO Box 23360, Department of Biology,
University of Puerto Rico, San Juan PR, 00931-3360

ABSTRACT.—Interactions between heterospecific species with similar sexual signals can lead to errors in mate attraction and species recognition. As a consequence sexual signals might diverge to reduce communication errors. Since high-density populations create more noise than low-density populations we hypothesize that individuals should suffer less acoustic interference from sympatric congeners with low-density populations than higher density populations of congeners. Even though species might be sympatric at multiple sites, only populations at sites with a relatively high abundance of both species should require one species to make vocal adjustments that minimize acoustic interference. To test this hypothesis we studied *Eleutherodactylus coqui* populations in the presence of relatively high numbers of *E. portoricensis* and in the presence of relatively low abundance of *E. portoricensis*. We found subtle differences in advertisement calls of *E. coqui* at locations where *E. portoricensis* had relatively high abundance compared to locations where *E. portoricensis* was absent or had relatively lower abundance. Differences in *E. coqui* call structure were linked to the portion of the call used for mate attraction and did not affect the portion of the advertisement call that is used for territorial interactions. The results indicate that the relative abundance of a congener is an important factor that can promote differentiation amongst vocal signals to increase signal discrimination between the calls of multiple species.

KEYWORDS.—Reproductive character displacement, behavioral plasticity, background noise, acoustic signal, population density

INTRODUCTION

When species with similar signals interact, selection should favor divergence of these signals to minimize interference and to reduce the risk of interspecific hybridization (Coyne and Orr 2004). Therefore, mate recognition systems should diverge as a result of selection against mistakes in species recognition (Wiley 1994). Knowledge of the conditions that facilitate this divergence can aid our understanding of the role of selection in creating reproductive isolation and speciation (Gerhardt and Huber 2002).

Species could alter their signaling behavior to reduce or avoid acoustic interference and minimize communication errors. Such errors include the lack of responses

to appropriate signals, which could result in additional time and risks in finding a mate or confronting a rival, or responses to signals from different species, which could lead individuals to respond to inappropriate rivals or mates (Wiley 1994). If species alter the time of signaling (Cody and Brown 1969, Ficken et al 1974), the location of signaling (Klump 1996), or the structure of the signal (Slabbekorn and Smith 2002, Luther and Wiley 2009) they could reduce acoustic interference and increase communication efficiency.

Previous studies have observed differences in communicatory signals in populations that are sympatric and allopatric with closely related heterospecific species (Amézquita et al 2006, Micancin 2008,

Höbel and Gerhardt 2003, Littlejohn 1969, Kirschel et al 2009). However the consistency and quantity of background noise should influence a species need to alter its signaling behavior for improved communication efficiency (Klump 1996), not just the presence or absence of a noise from a heterospecific species. Therefore the relative abundance of syntopic populations should be an important aspect of communication efficiency and the need for divergent signaling behaviors (Kirschel et al 2009). To test the relationship between abundance and vocal adjustments between heterospecific species we assessed the acoustic signals of *Eleutherodactylus coqui* in the presence of *E. portoricensis* at multiple locations where each species has a different relative abundance in relation to the other species.

Eleutherodactylus coqui and *E. portoricensis* are similar in morphology, habits, and vocal behavior (Drewry 1970; Narins 1983). Furthermore, until 1966 naturalists considered the two taxa to be one species, but Thomas (1966) demonstrated the existence of two separate species, one found only in forested montane regions from roughly 700 m to 1100 m (*E. portoricensis*) the other found from sea level to 1100 m (*E. coqui*). In the field the two species are most reliably differentiated by advertisement call and iris color (Thomas 1966; Drewry 1970).

Previous research on *E. coqui* has demonstrated that the body size and call of *E. coqui* change with elevation, where higher elevations lead to larger frogs with slower and lower frequency calls (Narins and Smith 1986). However, these studies have not considered background noise from other species, more specifically *E. portoricensis*, as an additional influence on the call structure of *E. coqui*. Due to the similarity of their calls, and the range overlap of both species, each species has the potential to cause acoustic interference with the transmission and recognition of the other species' signal (Narins 1983). We hypothesize that when *E. coqui* is syntopic and in relatively equal abundance with *E. portoricensis* then *E. coqui* will adjust its call parameters to avoid acoustic interference from the calls of *E. portoricensis*.

MATERIALS AND METHODS

Study Species

E. coqui and *E. portoricensis* are both nocturnal and terrestrial breeders with no aquatic larval stage (Townsend and Stewart 1994; Rivero 1998). Calling for both species peaks between 7 pm and midnight (Drewery and Rand 1983, pers. ob.).

E. coqui and *E. portoricensis* produce similar two-note advertisement calls, 'co-qui'. At an altitude of 350 m, the call of *E. coqui* has a 0.10 s, 1500 Hz pure tone first note 'co', a 0.12 s pause, and then a 0.15 s second note 'qui' sweeping from about 2500 to 2800 Hz, with a total duration of 0.37 s (Figure 1). Analysis of the function of the call of *E. coqui* has shown that the first note is used in male-male interactions, whereas the second note serves to attract females (Narins and Capranica 1976). The *E. portoricensis* call is similar with a 0.09 s 1800 Hz first note followed by a 0.09 s pause and then a second note sweeping from 2800 to 3100 Hz, for a total duration of 0.26 s (Zelick and Narins 1983). In essence *E. coqui* has a lower frequency call that is slower than that of *E. portoricensis*.

Study Sites

We studied *E. portoricensis* and *E. coqui* on El Yunque Mountain in the Caribbean National Forest, northeastern Puerto Rico, at six locations between the elevations of 600 m and 1050 m (Figure 2). All sites occurred in the Subtropical Wet Forest and Lower Montane life zones and the distance between sites were relatively small (100 m to a few km), to avoid geographic differences in species calls, but far enough apart that different individuals were found at each study site. At each site we determined the calling density of each species in 50 x 2 m transects in which we actively searched for all calling male frogs of each species, thus our density estimates are of calling males not the whole populations. Three sites contained both species in approximately equal numbers, one site had three times as many *E. coqui* but still a large number of *E. portoricensis*, we call these sites relatively high abundance *E. portoricensis* sites. One site had only

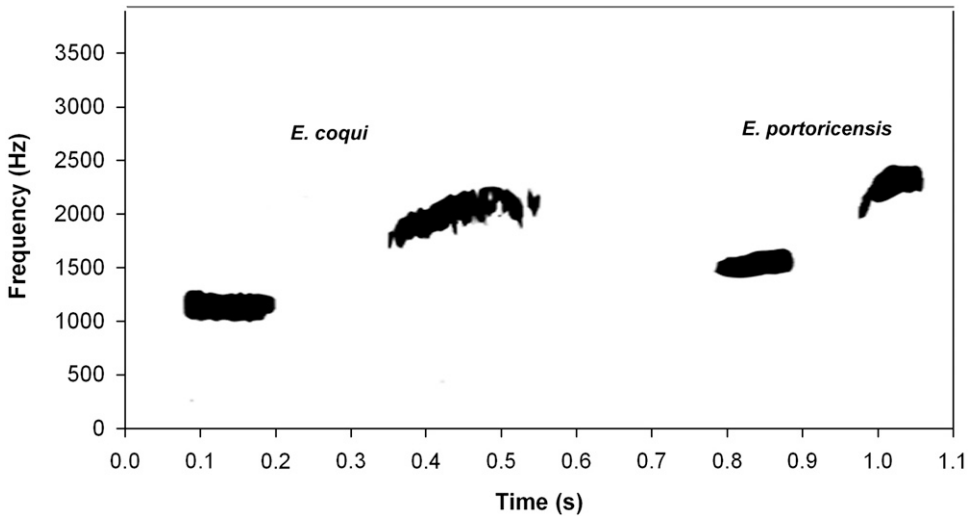


FIG. 1. Sonogram of the calls *E. coqui* and *E. portoricensis*.

E. coqui and at one site over 95% of the individuals were *E. coqui*, we call both of these low *E. portoricensis* sites (Table 1).

To investigate whether the relative abundance of *E. portoricensis* has affected the acoustic signals of *E. coqui*, we recorded calls of *E. coqui* and calls of *E. portoricensis* males at the aforementioned sites. Fieldwork took place between April and July 2008. We located calling males at night with a headlamp and recorded their calls using a Marantz PMD 670 with a Sennheiser K6-ME67 microphone. Calls were recorded at 48 kHz and saved as wav files. We also noted the calling location and height from the ground of each individual. After recording 10 to 20 consecutive calls of an individual we captured it and measured the snout-vent length (SVL), of each male to the nearest 0.1 mm, with a caliper.

We analyzed the recorded calls using Raven pro 1.2 (a sound analysis program). We measured the bandwidth, maximum, minimum, and dominant frequency of the 'co' and the 'qui' notes, and the overall call bandwidth for each individual of both species (frequency resolution 172 Hz, temporal resolution 5.8 ms). The bandwidth was calculated by subtracting the minimum frequency from the maximum frequency of a call. For each male we calculated mean call values based on the analysis of 10 calls.

Statistical Analysis

We performed linear regression analyses of call properties on body size (represented by snout-vent length SVL) to assess the effect of body size on call parameters. Because of the influence of body size on call parameters and the elevation differences between our sites we tested for a correlation between body size and elevation, which has been reported for *E. coqui* in other studies (Narins and Smith 1986).

Since noise with the same or similar frequencies is the primary cause of acoustic interference our analysis only focused on frequency parameters. We compared the dominant frequencies of calls of *E. coqui* between sites that had relatively high abundance of *E. coqui* compared to a low abundance of *E. portoricensis* and sites that had relatively high abundance populations for both species by calculating a nested analysis of variance (ANOVA) where study site was nested within high density and low density population categories. Because body size has a large influence on the dominant frequency of calls we included it as an interaction term with site, to control for any difference in the size of individual frogs among sites.

Due to the similarity of the frequencies occupied by each species call we also focused on the frequency bandwidth of

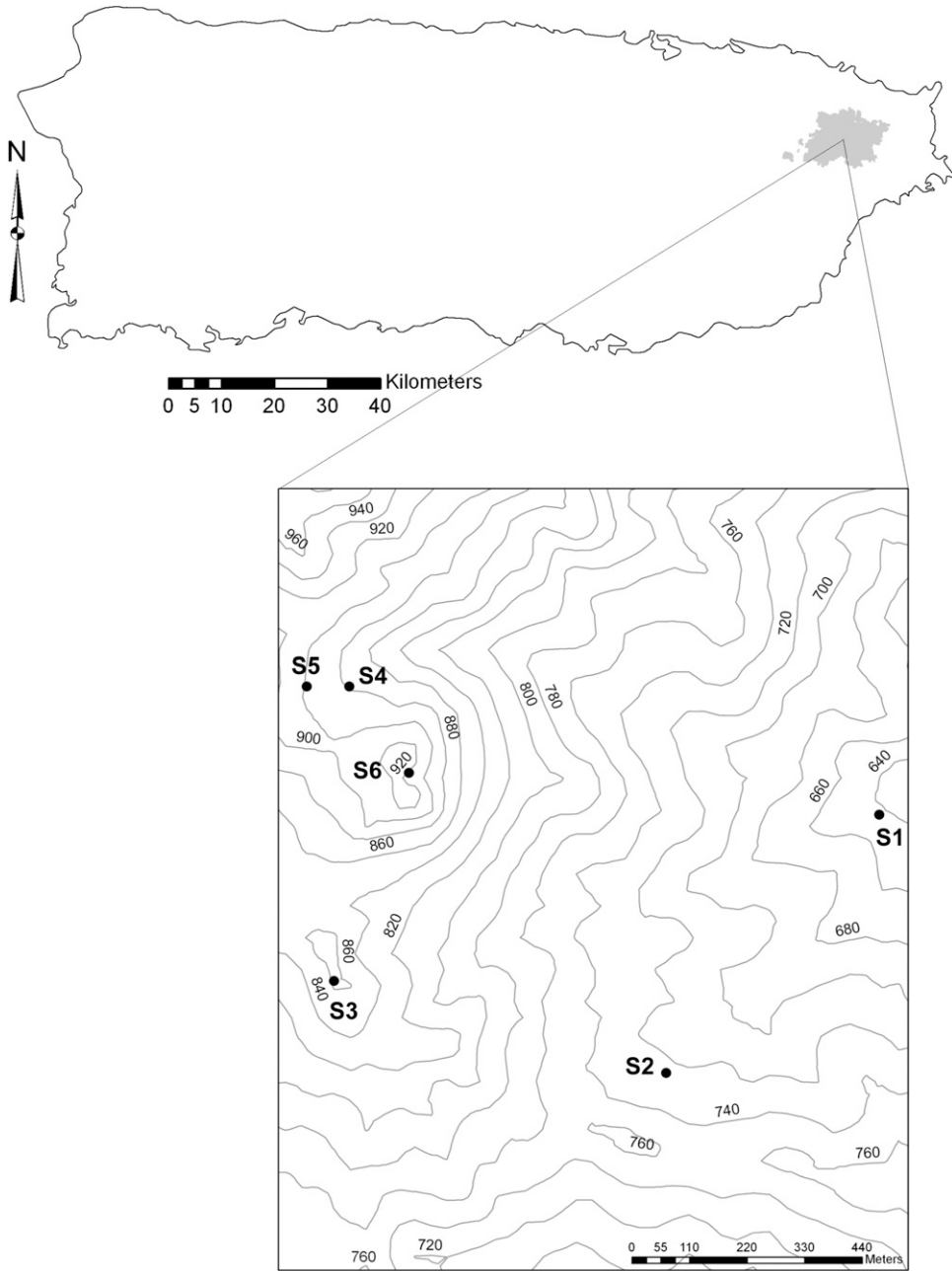


FIG. 2. Map of the island of Puerto Rico. Shaded area is El Yunque National forest. Survey sites are represented by closed black circles and isoclines represent elevation.

the call, which could be adjusted to reduce acoustic interference between the species. Body size is not known to affect frequency bandwidth thus we conducted a one-way

ANOVA of the frequency bandwidth of the call of *E. coqui* with different abundances relative to the abundance of *E. portoricensis*. Since spatial separation between a signaler

TABLE 1. Location of study and the number of individuals that were observed and recorded at each site. Mean and standard deviation call parameters for each species at each site. The abbreviation Elco and Elpo refer to *E. coqui* and *E. portoricensis*, respectively. Number of Elco and Elpo refer to the number of individuals found during the density transects at each site.

Species	Site	Number of Elco	Number of Elpo	Elpo Abundance Category	Dominant Frequency (Hz) co	Dominant Frequency (Hz) qui	SVL Length (mm)	Perch Height (m)	Call Bandwidth
elco	S1	18	0	Low	1247.71 (±62.06)	2217.53 (±110.44)	38.90 (±3.08)	1.52 (±0.45)	1750.35 (±116.32)
elco	S2	22	1	Low	1201.28 (±112.22)	2075.07 (±102.14)	39.67 (±2.51)	1.58 (±0.67)	1733.81 (±95.06)
elco	S3	9	11	High	1295.73 (±57.54)	2182.85 (±90.12)	36.82 (±2.35)	1.43 (±0.66)	1747.54 (±120.92)
elco	S4	11	13	High	1246.99 (±76.20)	2129.85 (±104.91)	38.03 (±2.99)	1.63 (±0.81)	1673.23 (±128.12)
elco	S5	29	8	High	1207.10 (±87.33)	1990.75 (±131.64)	39.66 (±4.89)	1.83 (±0.47)	1647.78 (±119.68)
elco	S6	12	14	High	1190.18 (±36.37)	2040.49 (±49.09)	39.51 (±2.87)	2.04 (±0.34)	1649.67 (±109.29)
elpo	S2	22	1	Low	1584.86 (±77.05)	2461.83 (±155.57)	31.64 (±2.32)	1.35 (±0.82)	1698.76 (±58.74)
elpo	S3	9	11	High	1631.26 (±100.65)	2551.18 (±170.94)	31.96 (±2.18)	1.11 (±0.53)	1688.56 (±155.35)
elpo	S4	11	13	High	1548.08 (±13.90)	2467.11 (±87.40)	32.20 (±2.80)	0.89 (±0.53)	1658.36 (±85.52)
elpo	S5	29	8	High	1573.90 (±46.99)	2372.58 (±99.44)	31.08 (±3.13)	0.95 (±0.49)	1536.16 (±65.42)
elpo	S6	12	14	High	1525.20 (±29.10)	2267.72 (±44.99)	34.53 (±1.83)	1.65 (±0.93)	1535.29 (±71.07)

and noise can reduce acoustic interference (Klump 1996) we assessed the call perch height of *E. coqui*, with a one-way ANOVA, to test if equal abundance populations called from different perch heights than high-low abundance populations. All call properties were normally distributed except for the dominant frequency of the 'co' and 'qui' portions of the calls. Since outliers were not an issue for these two variables and ANOVAs are relatively robust to the assumption of normality we did not transform the variables before we conducted ANOVAs. All statistical analyses were performed using JMP 7.0 software.

RESULTS

Body Size Effects

Body size increased with elevation for *E. coqui*, however there was not a significant

relationship between elevation and body size ($R^2 = 0.019$, d.f. = 55, $P = 0.31$). These results could be explained by the fact that we only assessed sites between the elevations of 600 m and 1050 m. Presumably, if we included sites from lower elevations we would find a significant relationship, like the one found by Narins and Smith (1986).

There was a significant negative relationship between body size and the dominant frequency of the 'co' ($R^2 = 0.23$, d.f. = 55, $P = 0.0002$), and the 'qui' ($R^2 = 0.21$, d.f. 55, $P = 0.0003$) portions of the *E. coqui* call (Figure 3). The 'co' portion of the call declined from approximately 1600 Hz in small individuals (SVL - 30 mm) to 1200 Hz in larger individuals (SVL - 40 - 45 mm). A similar decrease was observed in the 'qui' portion of the call with the dominant frequency of smaller individuals around 2600 Hz, and 2000 Hz in larger individuals.

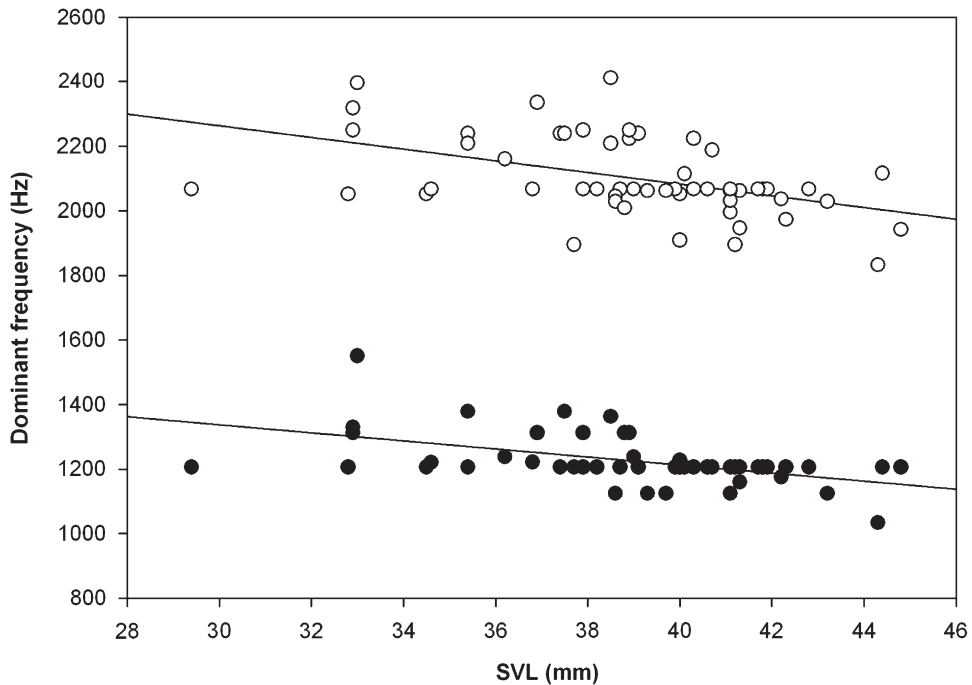


FIG. 3. Dominant frequency of the call of *E. coqui* plotted against body size. The black circles represent the 'co' and the open circles represent the 'qui' portion of the call of *E. coqui*.

Comparison of High and Low Abundance Populations

The dominant frequency of the 'co' portion of the call of *E. coqui* did not differ between populations that were syntopic with relatively high or low abundance populations of *E. portoricensis*, 1227.25 ± 12.8 Hz and 1221.47 ± 19.8 Hz, respectively. The 'qui' portion of the call did differ significantly between populations (Table 2, Figure 4a). Populations of *E. coqui* that were syntopic with relatively high abundance populations of *E. portoricensis* had lower dominant frequencies for the 'qui' portion of the call (2075.98 ± 20.1 Hz) when compared with populations of *E. coqui* syntopic with low abundance populations of *E. portoricensis* (2137.01 ± 26.3 Hz) (nested ANOVA $F_{1,44} = 5.39$, $P < 0.0001$, Table 2).

The mean bandwidth of *E. coqui* that were syntopic with relatively high abundance populations of *E. portoricensis* (1672.72 ± 19.59 Hz) was significantly smaller than the mean bandwidth of calls of individuals that were syntopic with less abundant pop-

ulations of *E. portoricensis* (1741 ± 23.47 Hz) ($F_{1,54} = 4.89$, $P = 0.0297$) (Figure 4b). On average *E. portoricensis* called from a mean perch height of 1.07 ± 0.10 m. *E. coqui* that were syntopic with high abundance populations of *E. portoricensis* called from a higher perch height (1.77 ± 0.11 m) in comparison with individuals in populations syntopic with low abundance populations of *E. portoricensis* (1.55 ± 0.12 m), although the difference was not statistically different.

DISCUSSION

Our results suggest that high congener abundance of *E. portoricensis* has influenced male advertisement calls of *E. coqui*. The second note, 'qui', of the call of *E. coqui*, which is used for mate attraction, has a lower dominant frequency when the two species are both relatively abundant, whereas the first note, 'co', of the call of *E. coqui*, which is used in male-male interactions, did not change whether *E. portoricensis* had high or low abundant populations. In addition the bandwidth of the call of *E. coqui*

TABLE 2. Results of the nested ANOVA of the dominant frequency of the 'qui' portion of the call of *E. coqui*. Study site is nested within the category equal abundance populations and high-low abundance populations and body size (SVL) is an interaction term with study site, to control for its influence among individuals at different sites.

Whole model					
Source	DF	Sum of Squares	Mean Square	F	P
Model	11	474706.72	43155.2	5.39	<0.001
Error	44	352193.66	8004.4		
C. Total	55	826900.38			
Effects Test					
Source	DF	Sum of Squares	F	P	
Species location overlap	1	72078.57	9.00	0.004	
SVL (mm)	1	93269.72	11.65	0.001	
Site[Species location overlap]	4	157234.97	4.91	<0.001	
Site*SVL (mm)	5	36373.58	0.91	0.484	

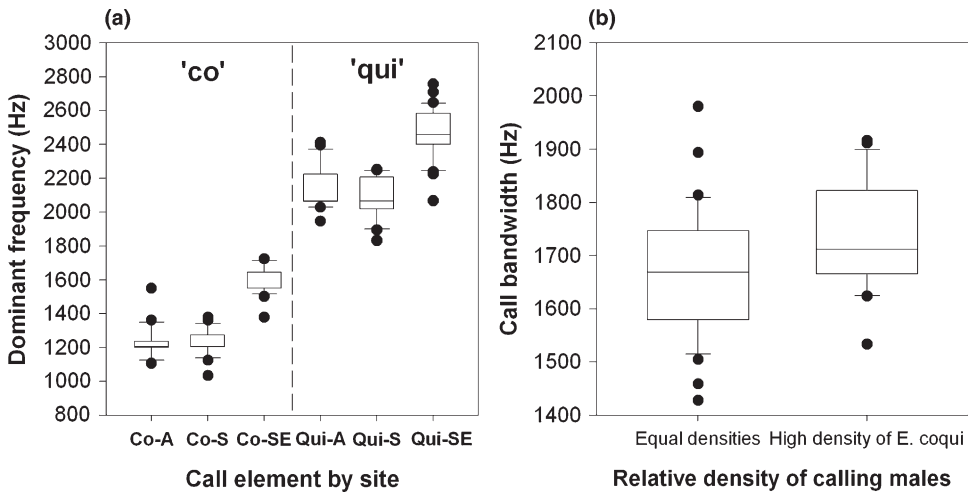


FIG. 4. Boxplots comparing the (a) dominant frequency of the 'co' and 'qui' notes and (b) call bandwidth in the calls of *E. coqui* and *E. portoricensis*. (a) Co-A and Qui-A represent calls of *E. coqui* at sites with a relatively high abundance of *E. coqui* and a low abundance of *E. portoricensis*, Co-S and Qui-S represent calls of *E. coqui* at sites with a relatively high abundance of calling males of both species. Co-SE and Qui-SE represent calls of *E. portoricensis*. The line in the middle of the box represents the median, the lower and upper boundaries of the boxes represent 25th and 75th percentiles, respectively, and the lower and upper bars are the 10th and 90th percentiles, respectively.

was smaller in the presence of high abundance populations of *E. portoricensis*.

The differences between calls of populations of *E. coqui* frogs were most evident in the part of the call used for mate attraction but not in the portion of the call used for male-male interactions. The downward shift in the dominant frequency of the 'qui' portion of the call generates a larger difference between the dominant frequencies of the

'qui' portion of the calls of both species. Greater differences between the two species calls should reduce acoustic interference as well as incorrect responses from females of the wrong species (Wiley 1994).

The smaller bandwidth of the call of *E. coqui* in the presence of high abundance populations of *E. portoricensis* should further reduce acoustic interference. A narrower frequency bandwidth allows receivers to

optimize correct detections by concentrating on a narrower range of frequencies. The narrower frequency bandwidth of the call of *E. coqui* in the presence of high abundance populations of *E. portoricensis* should reduce acoustic interference because it results in less frequency overlap between the signals of the two species (see Figure 4a).

Our study took place at very small spatial scales (kms) and it is unknown if the population of each species remains local from year to year or if species move from site to site. Woolbright (1985) found that *E. coqui* move an average of 3 – 4.5 m per night, but how far they disperse is still unknown. If individuals do not move between sites then reproductive character displacement could arise due to a stronger selection for conspecific recognition than that of gene flow from individuals entering these sites from other populations. However, if individuals move between sites the observed adjustment in call parameters is most likely an example of phenotypic plasticity in which the individuals adjust their calls to avoid acoustic interference in the local sound environment. Burmeister et al (1999) observed that cricket frogs (*Acris crepitans*) adjust the timing and frequency components of their calls in relation to male-male interactions. Similarly our findings might be the result of fine scale adjustments due to an individual's immediate circumstances.

Our study did not evaluate receiver preference. Zelick and Narins (1982) concluded that *E. coqui* males detect the vocalizations of *E. portoricensis*. If the males can detect the calls of *E. portoricensis* presumably the females do as well (Gerhardt and Huber 2002), in which case call selection should act on variation in receiver selectivity (Waage 1979; Höbel and Gerhardt 2003; Amézquita et al 2006, Hödl et al. 2006). Capranica et al. (1973) described that the frequency of maximum sensitivity in the midbrain of *Acris gryllus* varies geographically and is correlated with frequencies present in local call dialects. *E. coqui* could be similar, which would explain why a shift in the 'qui' portion of the call of *E. coqui* would be useful for correct signal detection in the presence of similar calls from *E. portoricensis*.

In fact, male *E. coqui* respond more frequently to calls of males from local populations than calls from non-local populations (Narins and Smith 1986). Future studies should conduct phonotaxis experiments to examine the response of *E. coqui* females to confirm that the lower frequency of the 'qui' in the presence of background noise from *E. portoricensis* does in fact improve female responses to the correct species vocalization.

In conclusion we assessed fine scale vocal adjustments in the call of *E. coqui* populations in the presence of background noise from relatively high abundance and low abundance populations of *E. portoricensis*. Our findings revealed that signal divergence does occur in the call of *E. coqui* when it is syntopic with relatively high abundance populations of *E. portoricensis*, but only in the portion of the call that is used for mate attraction. The signal divergence observed in this study could be explained by reproductive character displacement or behavioral plasticity.

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