Evolution, 50(6), 1996, pp. 2435–2453

ALLOZYME AND ADVERTISEMENT CALL VARIATION IN THE TÚNGARA FROG, *PHYSALAEMUS PUSTULOSUS*

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Abstract.—We analyzed variation in advertisement calls and allozymes in 30 populations along a 5000-km transect throughout most of the range of the túngara frog, *Physalaemus pustulosus*. All 12 call variables measured show significant differences among populations despite the importance of the advertisement call in species recognition. Some call variables exhibited clinal variation, whereas most others differed between the two major allozyme groups that have invaded Panama at different times, perhaps 4–4.5 million yr apart. Call variables that primarily affect discrimination among conspecifics tended to exhibit greater variation than call variables that are crucial for species recognition. The proximate mechanism of production underlying a call variable, however, is a better predictor of its variation. Contrary to predictions of some sexual selection models, call variation exhibits predictable patterns of geographical variation, although a substantial portion of variation among populations is not explained by geographic position. Although allozymes, calls, and geography usually covary, closer populations can have more similar calls independent of allozyme similarity.

Key words.—Advertisement calls, allozyme variation, genetic variation, geographical variation, *Physalaemus pustulosus*, sexual selection, túngara frog.

Received December 1, 1995. Accepted July 3, 1996.

Patterns of geographic variation within species give some indication of both the potential for future evolution and the past history of selection and constraints. Such patterns are particularly interesting when they involve characters that are important in promoting genetic divergence among populations. Mate recognition characters do just that.

The importance of sexual signals in reproductive isolation has been emphasized since Darwin (1859, 1871) and was an important component of the Evolutionary Synthesis (Mayr 1982). Variation among species in mate recognition characters suggests the potential for species recognition. It has been demonstrated experimentally in a variety of taxa that this signal variation can guide a female's mate preference to conspecific over heterospecific males (e.g., crickets: Huber 1990; *Drosophila*: Coyne and Orr 1989; fish: Ryan and Wagner 1987; frogs: Blair 1964; Rand 1988; Ryan 1991; Gerhardt 1994; birds: Marler 1957; Searcy and Andersson 1986). Thus, divergence in mate recognition signals resulting from reinforcement (Dobzhanksy 1937; Butlin 1987; Coyne and Orr 1989) or correlated with more general divergence (Mayr 1963; Blair 1964; Endler 1977; Nevo and Capranica 1985) can be a significant component of the speciation process.

Mate recognition characters are sometimes classified as being involved in either species recognition or sexual selection. Undoubtedly, many mate recognition signals result in conspecific identification, but the signals still may vary within a species with important evolutionary consequences. Field observations combined with experimental manipulations have shown that female preferences among conspecific males are often influenced by such variation (reviewed in Ryan and Keddy-Hector 1992; Andersson 1994). Mate recognition characters, therefore, can result in biased choices both among conspecifics and among different species, regardless of the factors responsible for the origin of such preferences (Ryan and Rand 1993a).

What are sometimes classified as "species recognition signals" can show substantial variation among populations (Ryan and Wilczynski 1991). Lande (1982) and West-Eberhard (1983) suggested that the rapid and arbitrary divergence of mate recognition signals under sexual selection could promote behavioral isolation among populations and, ultimately, the formation of new species (see Young et al. 1994 for empirical support of this hypothesis). Turner and Burrows (1995) further suggested that sexual selection could cause sympatric speciation. Lande's (1982) results are especially germane to our study. He specifically modeled divergence in male signals along a cline of parapatric populations with no barriers to migration, and showed that there can be rapid displacement of a sexually selected signal over space potentially leading to substantial divergence among populations (see fig. 2 in Lande 1982). Even with such displacement, however, gene flow could maintain a cline in similarities among closer populations (i.e., isolation by distance).

In previous studies, we have examined in detail how variation in mate recognition signals within a population influences female mating preferences that generate sexual selection in the túngara frog, *Physalaemus pustulosus* (e.g., Ryan 1980; Rand and Ryan 1981; Ryan 1985; Wilczynski et al. 1995), and how variation in signals among the túngara frog and its closest relatives can result in species recognition and sensory exploitation (Ryan et al. 1990a; Ryan and Rand 1993a,b, 1995). In this study we examine patterns of call variation across the geographic range of the túngara frog in relation to allozyme variation.

We address five major questions: Do advertisement calls show significant intraspecific variation? Is there call divergence between the two major allozyme groups of túngara frogs? Does call function or call production better predict patterns of variation of call components? Are there predict-

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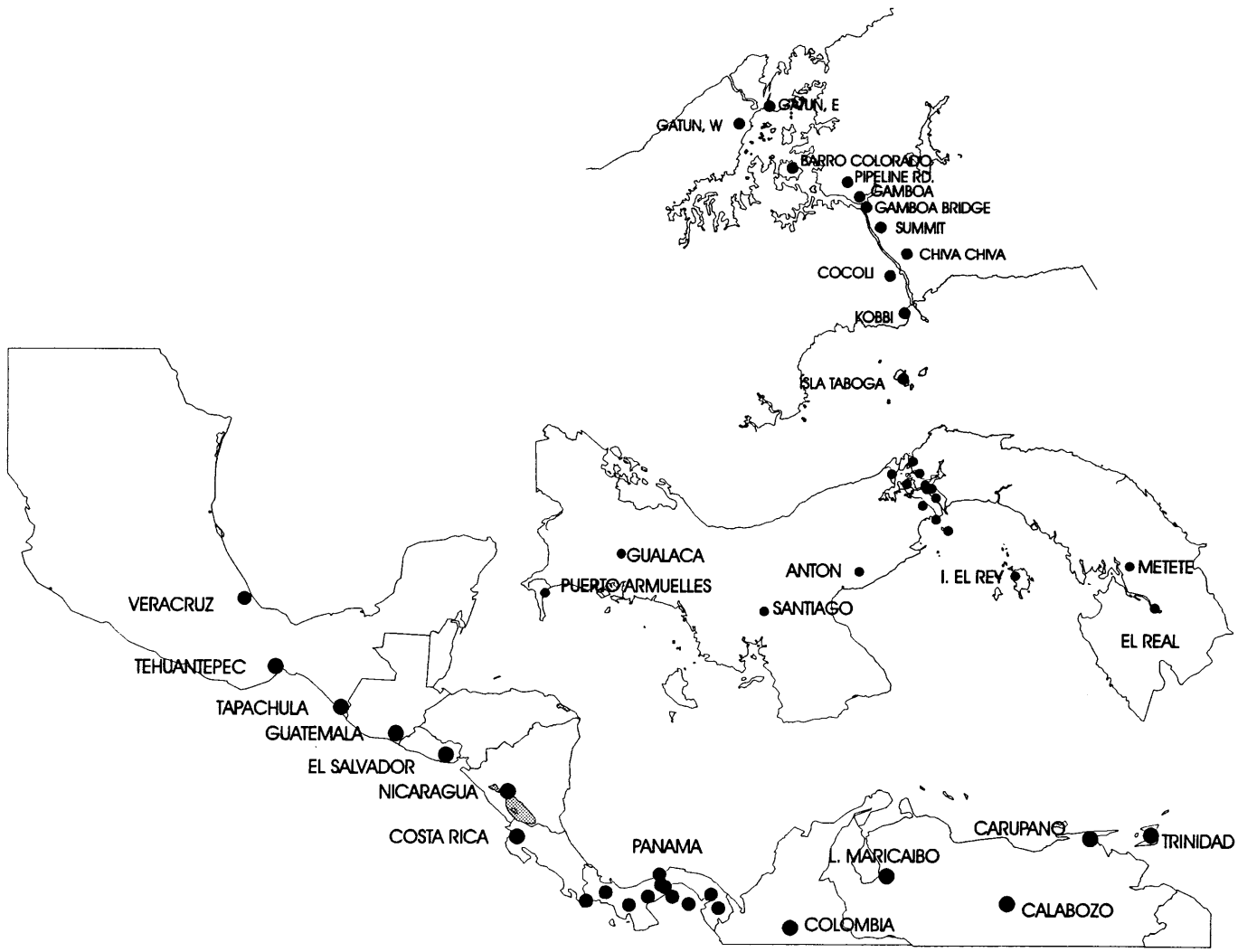

FIG. 1. Localities at which *Physalaemus pustulosus* were sampled across the entire transect (bottom), within Panama (middle), and along the Panama Canal (top).

able patterns of geographic variation in calls? If call variation is geographically predictable, is this the result of isolation by distance?

The System

Taxonomy and Biogeography.—The túngara frog, *Physalaemus pustulosus*, ranges from the Atlantic side of northern Mexico, south through much of lowland Pacific Middle America, into northern Colombia and east through Venezuela to the island of Trinidad and perhaps Guyana (Fig. 1; Table 1). This small frog (ca. 30 mm snout-vent length) is a member of the family Leptodactylidae (Lynch 1971), and together with five or six other taxa, constitutes the *P. pustulosus* species group (Cannatella and Duellman 1984; Cannatella et al., unpubl.). Within the species group, one clade is restricted to areas west of the Andes, while the other clade (which contains *P. pustulosus*, *P. petersi*, and its presently undescribed sister taxon) is restricted to Middle America and South America east of the Andes.

An analysis of allozyme variation suggests that *P. pustulosus* has crossed from South America into Panama on at least two occasions (Rand et al., unpubl.). This multiple invasion is now evidenced by a zone of secondary contact with significant introgression in western Panama. A possible biogeographical scenario is that túngara frogs first invaded Central America about 8 M.Y.B.P., prior to the establishment of the land bridge across the Isthmus, which is thought to have occurred 2.4 M.Y.B.P., and that a subsequent invasion occurred onto this land bridge when it emerged (Rand et al., unpubl.). Although the allozymes show two major genetic groupings within *P. pustulosus*, it seems clear that these groups are monophyletic and we consider them one species (Rand et al., unpubl.).

The Call.—The advertisement call of *P. pustulosus* consists of a whine that can be produced alone or followed by up to six chucks (Fig. 2). The whine is necessary and sufficient to elicit female phonotaxis. Within the whine some parts are necessary to elicit phonotaxis, others are not necessary but make the whine more attractive, and still other parts, such as the upper harmonics of the whine, have no influence on

TABLE 1. For all localities we show the latitude (LAT) and longitude (LONG) in tenths of degrees, and means (*SE*) of call characters measured at 30 localities across the range of *Physalaemus pustulosus* (Fig. 1). Abbreviations: AMPL, number of pulses in the amplitude modulated portion of the whine; CHDM, dominant frequency of the chuck; DB12, relative amplitude of first and second harmonic at the onset of the whine; DBCH, the difference in amplitude, in decibels, between the chuck and the whine; DOMH, dominant frequency of the entire call; DRAM, duration of the amplitude modulated portion of the whine; DRCH, duration of the chuck; DURT, duration of the whine to the onset of the chuck; FNLH, final frequency of the whine; INIT, initial frequency of the whine; PAMH, whine frequency immediately after the amplitude modulation; TLDR, total duration of the total call, including the chuck; *N*, sample size (see Fig. 3 for description of call measures). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns $P > 0.05$.

Locality (Lat/Long)	AMPL	CHDM	DB12	DBCH	DOMH	DRAM	DRCH	DURT	FNLH	INIT	PAMH	TLDR	*N*
Veracruz	0.5	2860	21.4	7.1	666	2	46.3	224.4	484	928	918	277.3	10
Mexico	*0.5*	*89.0*	*2.8*	*1.7*	*26.1*	*2.0*	*2.2*	*10.6*	*4.9*	*13.0*	*12.4*	*11.9*	
(19.73/96.43)													
Tehuantepec	1.5	2875	16.5	8.7	691.1	5.5	43	238.7	500	955.5	895.5	294.5	9
Mexico	*0.8*	*101.4*	*2.2*	*1.6*	*20.8*	*2.8*	*3.8*	*9.5*	*10.5*	*25.5*	*23.9*	*12.5*	
(16.35/95.28)													
Tapachula	2.5	2932	14.9	8.4	664	9.8	42.5	282.4	480	1020	960	341.3	10
Mexico	*0.8*	*116.2*	*0.8*	*1.6*	*29.3*	*3.3*	*3.3*	*3.2*	*0*	*20.0*	*29.2*	*7.0*	
(14.86/92.22)													
Taxico	2.1	2976	14.5	9	654	7	30.5	279.2	488	1024	952	328.2	10
Guatemala	*0.8*	*92.9*	*2.0*	*0.9*	*18.3*	*2.9*	*2.5*	*8.2*	*8.0*	*26.1*	*18.6*	*10.4*	
(14.03/90.18)													
San Miguel	1.3	2840	14.7	11.7	738	4.2	34.4	254.2	488	944	930	313.8	10
El Salvador	*0.6*	*78.4*	*1.4*	*1.5*	*33.2*	*2.3*	*1.4*	*4.2*	*8.0*	*16.0*	*16.1*	*9.5*	
(14.49/88.18)													
Tipitapa	1.3	2928	15.6	12.2	698	4.7	37.2	264.8	464	970	918	312.4	10
Nicaragua	*0.7*	*70.7*	*1.5*	*1.5*	*31.3*	*2.5*	*2.9*	*8.7*	*10.6*	*18.6*	*18.4*	*9.8*	
(12.20/86.07)													
Liberia	1.0	3020	12.6	11.1	696	14.2	30.7	251.2	472	1080	910	294.9	10
Costa Rica	*0.8*	*66.7*	*1.7*	*0.5*	*17.3*	*2.8*	*1.0*	*7.5*	*8*	*24.5*	*21.7*	*7.0*	
(10.61/85.45)													
Puerto Armuelles	8.0	2611	6.7	11.5	720	28.7	38	262.8	477.1	1017.1	805.7	327.5	7
Panama	*0.7*	*130.0*	*2.6*	*2.1*	*4.3*	*2.9*	*3.0*	*13.7*	*2.8*	*41.7*	*24.9*	*14.7*	
(8.27/82.86)													
Gualaca	6.5	2616	8.5	15.75	634	27.1	41.5	280.7	470	1000	848	343.9	10
Panama	*1.1*	*57.8*	*1.2*	*1.3*	*26.0*	*4.8*	*1.8*	*9.6*	*4.4*	*26.1*	*22.1*	*10.3*	
(8.53/82.29)													
Santiago	4.3	2714	6.6	12.2	696	17.7	36.5	284.5	496	986	824	321.7	10
Panama	*1.2*	*56.5*	*1.2*	*1.1*	*28.0*	*5.1*	*2.2*	*11.8*	*10.6*	*30.4*	*36.9*	*13.1*	
(8.13/80.98)													
Anton	6.6	2750	3.6	2.9	688	23.2	46.6	273.9	512	1056	920	324.9	10
Panama	*0.6*	*92.6*	*1.3*	*0.7*	*32*	*2.0*	*5.3*	*8.9*	*13.0*	*35.3*	*19.0*	*11.4*	
(8.40/80.24)													
Gatun, west	4.0	2592	8	14.8	672	15.6	47.3	270.6	460	940	830	335	10
Panama	*1.3*	*80.1*	*1.7*	*1.0*	*18.4*	*5.1*	*4.6*	*8.0*	*9.4*	*29.9*	*23.7*	*7.4*	
(9.25/79.95)													
Gatun, east	4.0	2896	3.4	3.3	640	15.9	40.6	368.8	488	1052	902	411.9	10
Panama	*0.8*	*84.9*	*0.4*	*0.9*	*23.0*	*3.3*	*3.2*	*7.1*	*8.0*	*30.2*	*21.1*	*8.7*	
(9.28/79.92)													
BCI	3.8	2630	16.2	7.3	654	15.4	42.3	307.4	480	888	836	375.2	10
Panama	*1.3*	*89.9*	*1.5*	*1.2*	*15.2*	*4.9*	*2.5*	*5.6*	*0*	*14.3*	*15.1*	*5.5*	
(9.17/79.85)													

TABLE 1. Continued.

Locality (Lat/Long)	AMPL	CHDM	DB12	DBCH	DOMH	DRAM	DRCH	DURT	FNLH	INIT	PAMH	TLDR	N
Pipe Line Rd Panama (9.16/79.73)	7.8 *1.8*	2455 *70.3*	14.1 *1.4*	12.5 *1.4*	622.2 *11.2*	29.5 *6.6*	47.5 *2.8*	334.4 *8.3*	457.7 *11.7*	960 *35.1*	788.8 *20.3*	410 *8.8*	9
Gamboa Panama (9.12/79.70)	6.2 *0.8*	2448 *53.5*	11 *1.3*	9 *0.9*	614 *12.6*	29.1 *3.1*	41.9 *2.5*	313.3 *9.5*	458 *11.3*	898 *28.0*	784 *10.6*	369.9 *10.4*	10
Gamboa Bridge Panama (9.11/79.69)	2.3 *0.9*	2676 *75.5*	12.2 *2.2*	13.7 *1.4*	688 *19.5*	8.8 *3.9*	34.5 *2.8*	298 *11.7*	516 *14.2*	1000 *35.6*	900 *20.0*	335.2 *13.3*	10
Summit Panama (9.07/79.65)	6.8 *1.5*	2526 *121.2*	12 *2.8*	8.3 *1.3*	656 *20.1*	23.8 *5.0*	45.4 *3.1*	295.1 *11.2*	478 *12.8*	1014.8 *29.2*	820 *30.4*	350.6 *12.4*	10
Chiva Chiva Panama (9.02/79.59)	3.3 *1.4*	2428 *80.4*	16.7 *1.9*	6.7 *1.4*	716 *24*	10.9 *4.5*	49.2 *3.7*	312.8 *12.8*	468 *21.5*	1010 *39.3*	904 *37.9*	362.9 *12.9*	10
Cocoli Panama (8.97/79.59)	6.4 *2.3*	2580 *83.0*	11.7 *1.4*	10 *1.9*	694 *25.6*	24.6 *8.6*	40.7 *2.7*	331.4 *9.8*	474 *4.2*	1024 *39.7*	854 *28.7*	378.3 *11.5*	10
Kobbe Panama (8.90/79.59)	4.6 *1.5*	2595.5 *115.6*	12.5 *2.5*	10.4 *4.1*	722.2 *24.5*	15.6 *4.8*	39.8 *4.2*	335 *14.0*	482.2 *20.1*	1008.8 *39.1*	868.8 *29.2*	374.5 *15.8*	9
Isla Toboga Panama (8.80/78.45)	5.7 *2.4*	2450 *75.4*	14.3 *3.4*	11.4 *2.2*	700 *27.5*	27.2 *11.0*	41.12 *2.8*	369.8 *15.9*	442.5 *13.3*	940 *28.2*	860 *28.2*	413.1 *15.4*	8
Isla El Rey Panama (8.45/78.85)	0 *0*	2784 *137.4*	16 *1.7*	10.6 *1.4*	696 *29.9*	0 *0*	41 *3.8*	360.2 *7.4*	480 *0*	932 *21.5*	932 *21.5*	425 *13.3*	5
Metete Panama (8.50/77.97)	4.1 *1.7*	2374 *119.9*	18.3 *1.8*	7.3 *0.9*	642 *22.7*	16.1 *6.9*	34.9 *1.4*	350.2 *14.0*	484 *15.1*	948 *32.5*	832 *41.9*	403.5 *13.0*	10
El Real Panama (8.13/77.73)	3.5 *1.5*	2680 *66.6*	17 *1.9*	11.3 *1.6*	714 *31.9*	15.4 *6.3*	44.1 *2.0*	419.6 *12.4*	480 *11.9*	964 *30.6*	912 *22.1*	483.6 *13.0*	10
Mariquita Colombia (5.18/74.90)	0 *0*	2690 *76.2*	12.3 *1.3*	5.6 *1.2*	620 *26.3*	0 *0*	44.5 *1.7*	290 *10.9*	486 *8.4*	910 *12.7*	910 *12.7*	342.9 *12.0*	10
Lago Maracaibo Venezuela (8.56/71.63)	8.6 *1.6*	2572 *130.7*	9 *2.2*	2.4 *1.0*	664 *29.9*	30.4 *5.0*	46.2 *6.1*	296.4 *29.5*	480 *0*	1032 *62.4*	816 *16*	346 *30.4*	5
Calabozo Venezuela (8.98/67.35)	7.3 *1.9*	2464 *61.7*	10 *1.7*	9.5 *1.3*	602 *18.4*	33.2 *8.9*	37.5 *2.4*	267.8 *7.1*	416 *10.6*	926 *35.1*	756 *20.8*	320.7 *6.1*	10
Carupano Venezuela (10.64/63.22)	2.1 *0.8*	2586 *66.6*	14 *1.2*	4.5 *1.0*	588.8 *26.8*	9.4 *3.7*	36.6 *2.6*	258.3 *7.5*	437.7 *13.5*	848.8 *18.5*	775.5 *26.1*	302.4 *6.3*	9
Trinidad (10.63/61.28)	3.75 *1.4*	2400 *117.7*	13 *2.8*	21.25 *0.9*	635 *65.5*	13.5 *5.7*	46.75 *4.7*	248.5 *12.5*	445 *26.2*	855 *55.0*	805 *33.0*	302.75 *13.8*	4

TABLE 1. Continued.

	AMPL	CHDM	DB12	DBCH	DOMH	DRAM	DRCH	DURT	FNLH	INIT	PAMH	TLDR	*N*
Grand means, standard errors, coefficients of variation													
	4.1	**2673**	**12.6**	**9.5**	**669**	**15.8**	**40.8**	**297**	**476**	**973**	**868**	**350**	275
	0.3	*18.9*	*0.4*	*0.3*	*4.9*	*1.0*	*0.6*	*3.2*	*2.3*	*6.2*	*5.5*	*3.4*	
	—	0.12	0.55	0.60	0.12	—	0.25	0.18	0.08	0.10	0.10	0.16	
among populations													
Kruskal-Wallis	*85.1*	*95.3*	*102.6*	*114.2*	*62.8*	*87.2*	*77.8*	*177.6*	*84.5*	*87.0*	*112.0*	*169.6*	30
P	***	***	***	***	***	***	***	***	***	***	***	***	
among two major allozyme groups													
Mann-Whitney U	*6633*	*12033*	*9566*	*9418*	*9355*	*6408*	*6250*	*3085*	*8929*	*9866*	*11087*	*3614*	2
P	*	***	*	*	*	**	**	***	ns	**	***	***	
correlation with cumulative distance: long transect													
r	*0.349*	*−0.783*	*−0.292*	*−0.089*	*−0.474*	*0.351*	*0.215*	*0.374*	*−0.470*	*−0.435*	*−0.625*	*0.168*	20
P	ns	**	ns	ns	**	ns	ns	*	*	**	**	ns	
correlation with cumulative distance: central Panama transect													
r	*0.187*	*−0.400*	*0.445*	*0.027*	*0.718*	*0.136*	*−0.264*	*0.409*	*−0.136*	*0.219*	*0.273*	*0.227*	11
P	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	

female phonotaxis (Rand et al. 1992; Wilczynski et al. 1995). Female túngara frogs can distinguish between the conspecific whine and the whine produced by all other males of the species group (Ryan and Rand 1993a,b), although not between the conspecific call and what we estimate to be the call of its most recent common ancestor (Ryan and Rand 1995). There can be substantial variation in the whine among males. The degree to which this variation influences female preferences is not known.

The chuck alone does not allow females to identify the call as signaling a conspecific, but it does enhance the call's attractiveness to females. This call component only elicits phonotaxis when produced in concert with a whine, which in nature is always the case. Females prefer calls with chucks (Rand and Ryan 1981; Ryan 1985) and prefer calls of lower frequency chucks (Ryan 1980, 1985), although recent studies (Wilczynski et al. 1995) show that this low-frequency preference is weaker than the earlier studies had suggested. The chuck also increases predation risk because it makes the call more attractive to the frog-eating bat, *Trachops cirrhosus* (Tuttle and Ryan 1981; Ryan et al. 1982). Other sounds can replace the chuck with the same influence on call attractiveness, suggesting that female preference for chucks might be quite permissive (Ryan et al. 1990a; Ryan and Rand 1990, 1993a,b).

The effect or the function of the call in different levels of discrimination differs between the whine and the chuck. Only the whine is involved in discriminating among different species, while the chuck is of obvious importance in discriminating among individuals; we do not know how whine variation influences within-species discrimination.

MATERIALS AND METHODS

Field Collections

We collected túngara frogs and tape recorded their advertisement calls from 30 populations throughout most of the species' range (Fig. 1; Table 1; Appendixes). The eight sites ranging from Veracruz, Mexico, south to Puerto Armuelles, Panama, were sampled in June 1993. Panama was sampled most extensively; 15 sites were sampled in the country with 10 of them being along a more fine-grained transect that paralleled the Panama Canal in central Panama (Fig. 1; Table 1). We sampled these sites in central Panama at various times between 1990 and 1994. Five sites were sampled in South America. The site at Mariquita, Colombia, was sampled in November 1993. We sampled three sites in Venezuela in 1992, and the site in Trinidad was sampled by A. H. Wynn and R. Crombie in 1991.

At each site we recorded advertisement calls of males, and attempted to collect and preserve these same males as voucher specimens. Calls were recorded using a Marantz PMD 420, Sony TCD5M, or a Sony Professional Walkman tape recorder and a Sennheiser ME-80 microphone with K3-U power module on metal tape. Temperatures were recorded at the calling site of each male. Animals were either transported alive to the laboratory or tissues were removed and frozen in the field with liquid nitrogen and then transported in dry ice. We removed the following tissues for electrophoretic analysis after

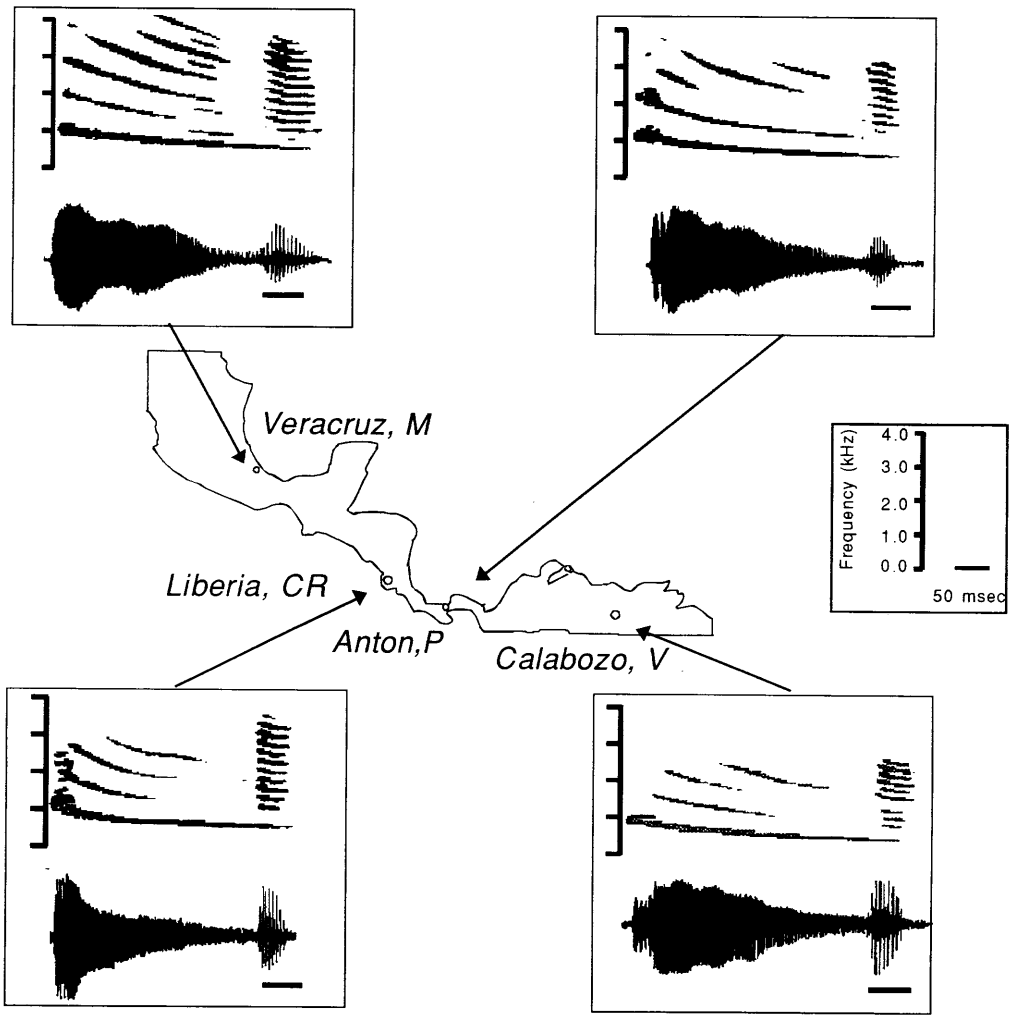

FIG. 2. A sample of advertisement calls, a whine followed by a single chuck, of *Physalaemus pustulosus* (sonograms, top; oscillograms, bottom) illustrating some of the variation among four populations.

the males were euthanized with MS222: liver, thigh muscle, heart, and sometimes eyes.

Call Analysis

Calls were analyzed on a Kay DSP 5500 Sonograph at a sampling rate of 10.24 kHz. One call per individual was measured, and each call had a single chuck. We usually analyzed the first call with a chuck recorded by that individual. Most calls were recorded at temperatures between 25° and 28°C and thus we considered temperature effects on call variables to be trivial. Similarly, a preliminary analysis of 49 individuals from seven populations showed that most call characters were not significantly influenced over the small range of sizes encountered in this survey (mean snout–vent length = 24.5 mm, $CV = 0.053$). Correlation coefficients of body size and call characters ranged from 0.23 to −0.23, with the exception of the whine frequency immediately following the amplitude modulated prefix, which was correlated with body size at $r = -0.33$. But even this factor is only slightly affected by body size ($r^2 = 0.11$). Thus we did not use body size as a covariate in our analyses.

The following call characters were analyzed (abbreviations refer to those used in Table 1 and Figs. 3, 4): AMPL: the number of pulses in the initial, amplitude-modulated portion of the whine; CHDM: the dominant frequency of the chuck; DB12: the difference in amplitude, in decibels, between the first and second harmonic of the whine at the beginning of the call; DBCH: the difference in amplitude, in decibels, of the chuck second harmonic (which is also the final frequency of the whine) and the chuck dominant; DOMH: the dominant frequency of the entire call, including the chuck; DRAM: the duration of the initial amplitude modulated portion of the whine; DURT: duration of the whine (i.e., to the onset of the chuck); FNLH: the final frequency of the whine, usually this is also the second harmonic of the chuck; INIT: the initial frequency of the whine; PAMH: the frequency of the whine immediately after the amplitude-modulated portion; TLDR: the total duration of the call, including the chuck.

Allozyme Analysis

Tissues were kept frozen at −80°C until used. Approximately equal volumes of heart, liver, eye, and skeletal muscle

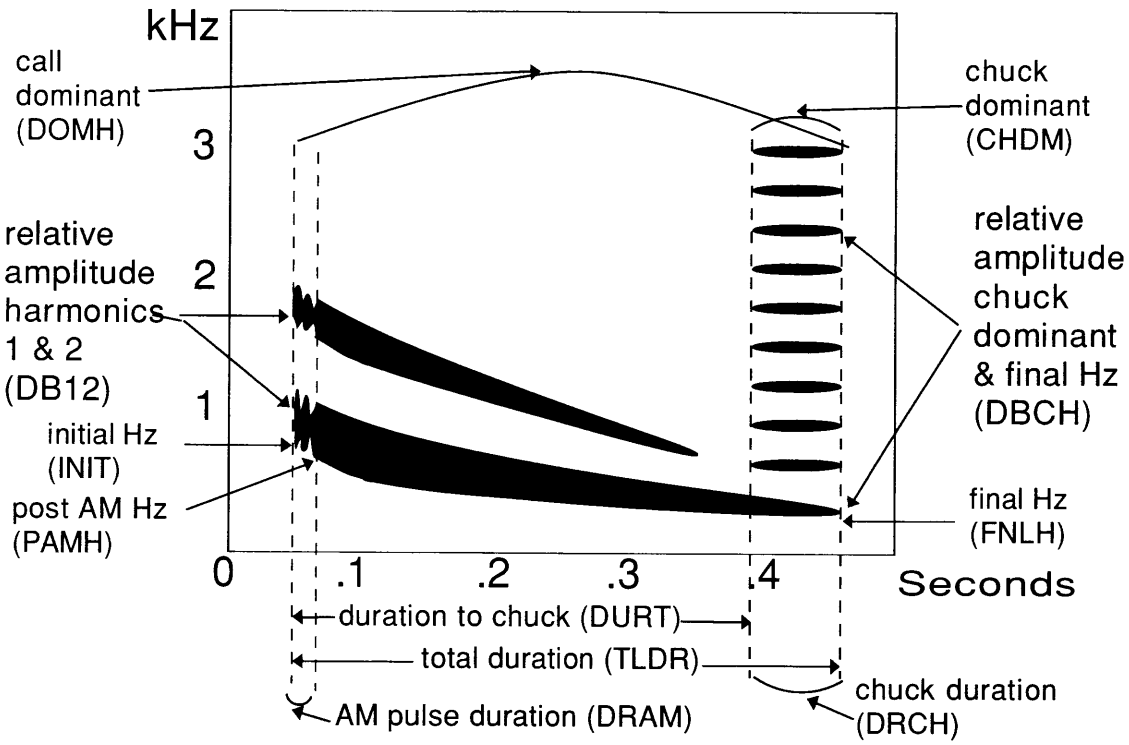

FIG. 3. An illustration of the call of *Physalaemus pustulosus*, a whine followed by a single chuck, showing the call variables measured in the analysis.

(thigh) were separately homogenized in an equal volume of grinding buffer (0.25M sucrose, 2% phenoxyethanol) and centrifuged at 14000 rpm for 10 min. The supernatant was removed and stored at −80°C until used the following day.

Standard horizontal starch gel electrophoresis (Murphy et al. 1990) was performed. Data were analyzed with BIOSYS-1 (Swofford and Selander 1981). Complete results and biogeographical interpretation of the allozyme analyses are presented in Rand et al. (unpubl.).

Statistical Analysis and Hypothesis Testing

Tests of Hypotheses: Univariate analysis.—Standard statistics were calculated for all call variables for all populations and are presented in Table 1. We present box plots (Fig. 4) to allow a visual comparison of call characters among populations. Individual call characters were statistically compared among populations with a Kruskal-Wallis analysis of variance and between the two major allozyme groups with a Mann-Whitney U-test. These data allowed us to address the first two questions: Do advertisement calls show significant intraspecific variation? Is there is any evidence for call divergence between the two major allozyme groups of túngara frogs?

Because allozyme groups within *P. pustulosus* are geographically contiguous, clinal variation could result in statistically significant differences between the two allozyme groups (e.g., Ryan and Wilczynski 1991). Thus, for each call character we determined if there was both significant clinal variation and differences among the two groups. If characters varied both clinally and among groups, we could not assume the differences were due to divergence of the groups per se, but that such differences could be an incidental effect of clinal variation. Grant (1972) discusses the analogous problem in detecting geographic patterns of character displacement (see fig. 3 in Grant 1972).

We calculated the Spearman rank correlation between distance and each call character, and interpreted a statistically significant correlation (i.e., $P < 0.05$) as evidence of clinal variation. In the analysis of the larger transect, the straight-line distance from site to site was measured and the transect length was 5001 km from beginning to end. For each population we noted the cumulative distance, in which the most northern population, Veracruz, Mexico, was zero. We analyzed 20 populations from Veracruz, Mexico, to Trinidad, using only one population, Gamboa, to represent the smaller, central Panama transect (Fig. 1). Thus, in order from northwest to southeast, the populations in this larger transect were: Veracruz, Tehuantepec, and Tapachula, Mexico; Guatemala; El Salvador; Nicaragua; Costa Rica; Puerto Armuelles, Gualaca, Santiago, Anton, Gamboa, I. El Rey, Metete, and El Real, Panama; Colombia; L. Maracaibo, Calabozo, and Carupano, Venezuela; and Trinidad (Fig. 1, Table 1).

We also examined variation across a smaller, more fine-grained transect in central Panama. In order from northwest to southeast the populations were: Gatun west, Gatun east, BCI, Pipeline Road, Gamboa, Gamboa Bridge, Summit Park, Chiva Chiva, Cocoli, Kobbe, and I. Taboga (Fig. 1, Table 1).

Coefficients of variation for all call characters for all populations combined were calculated. This allowed us to compare the amount of variation in measures of the whine and

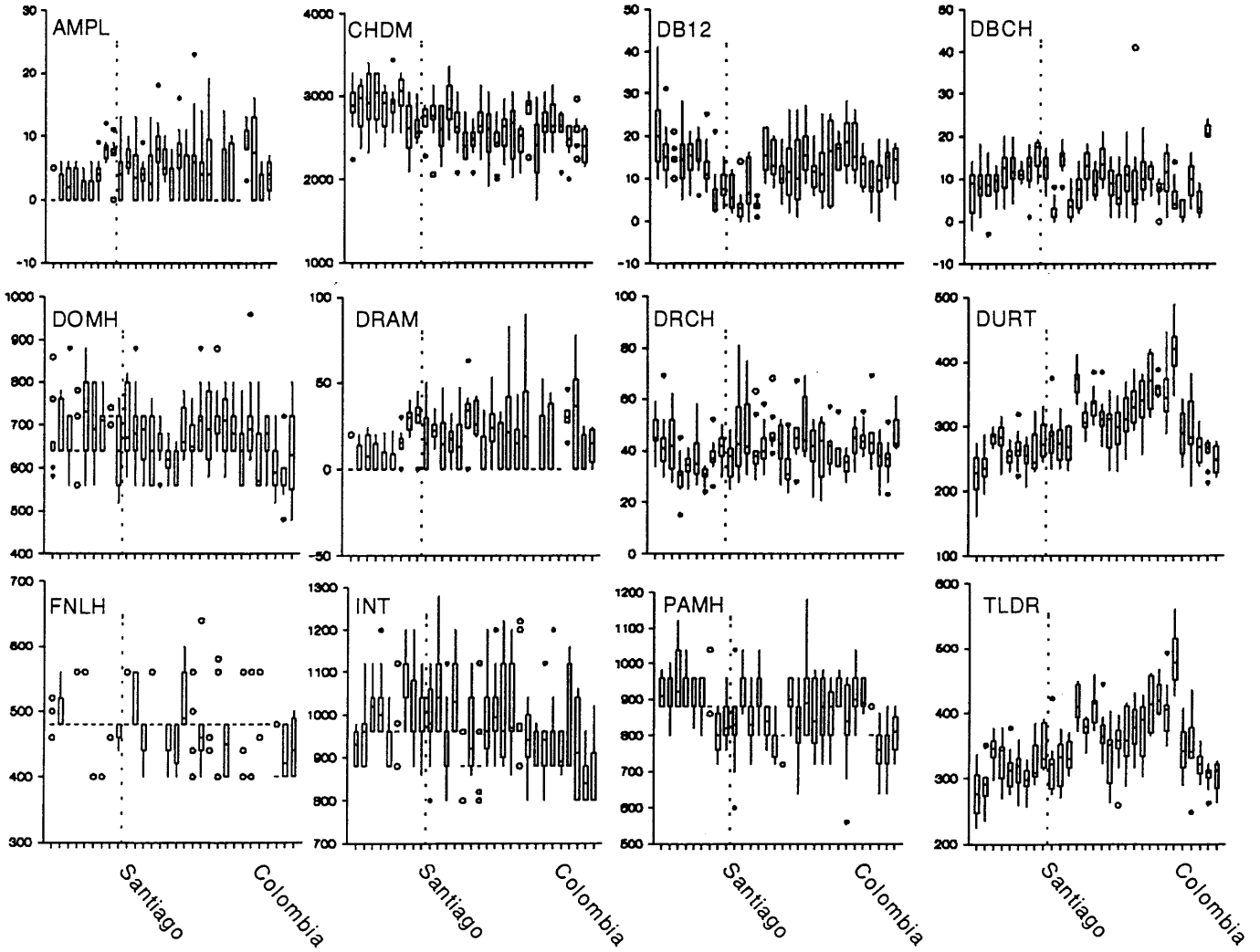

FIG. 4. Box plots, showing the median (bar), quartiles (hinges), range (line), and outliers (circles) for all call variables across all localities sampled. The dashed vertical line on each plot divides the samples into the two major allozyme groups.

the chuck, call components that differ in the level of mate discrimination in which they function. Only the whine is involved in species recognition, whereas the chuck has an important role in discriminating among individuals. We also compared the coefficients of variation between call characters that appear to be under passive control, due to their strong dependence on morphology, rather than those variables that appear to be actively regulated by behavioral-physiological mechanisms (Martin 1972; Ryan 1988). These comparison allow us to answer the third question: Does call function or call production better predict patterns of variation of call components?

Tests of Hypotheses: Multivariate Analysis.—We used a Mantel test and a partial Mantel test (Smouse et al. 1986) to evaluate various hypotheses regarding factors that might influence intraspecific call variation (see Douglas and Endler 1982 for an analogous approach). The Mantel test calculates correlational relationships among similarity/dissimilarity and distance matrices. The partial Mantel (Smouse et al. 1986) calculates the relationship between two matrices after controlling for covariation with a third matrix. Calculations were computed for all populations and for three smaller subsets of the data with the R-package (Legendre and Vaudor 1991). Tests of significance were computed by running 1000 iterations of the data set. The significance levels determined by this method were usually quite similar to that from the assumption that the Mantel statistic approximates a *t*-distribution as sample sizes increase (Legendre and Vaudor 1991).

The dissimilarity matrix for the calls is a matrix of Euclidean distances between means of all call variables computed using Systat (Wilkinson 1990; Appendixes). The matrix of genetic dissimilarity is from Nei's genetic distances (Nei 1978) between each pair of populations (Appendixes). For ease of discussion, test results are considered in terms of call and allozyme similarity rather than dissimilarity.

The matrix of geographic distances was calculated in the R-package from the latitudes and longitudes for each site (Table 1; Appendixes). These distances are the shortest between all pairs of populations and thus might span water, as opposed to the distances used above in the univariate correlational analysis, which were calculated from nearest-neighbor distances along the transect. The distance matrices

constructed from both techniques give very similar results (r-values within 0.01).

We used the Mantel test, as well as the univariate method described above, to address the second question: Is there call divergence among the two major allozyme groups of túngara frogs? We did this by coding pairs of populations as being in the same or different allozyme groups and compared that matrix with the call dissimilarity matrix (see also Douglas and Endler 1982). For this analysis, the populations from Veracruz to Gualaca are considered the northern allozyme group, whereas the other populations constitute the southern allozyme group; introgression of some allozyme morphs near Gualaca make assignment of that population rather arbitrary (Rand et al., unpubl.). We also used the Mantel test to evaluate the fourth and fifth questions we proposed: Are there predictable patterns of geographical variation in calls? If call variation is geographically predictable, is this the result of isolation by distance?

Covariation of the three variables under study would suggest that the observed patterns of call variation result from patterns of gene flow that are strong enough to mask selection effects, although there could be alternative explanations such as an evolutionary response to clinally varying selection (also see Endler 1977). Lack of clinal variation, as suggested by lack of a significant Mantel correlation between geographic distance or allozyme similarity and overall call similarity, would be consistent with the hypothesis that sexual selection (or other selective forces) has induced patterns of local variation that disrupt clinal variation across the species' range (Lande 1982). Clinal variation would not exclude the possibility of sexual selection, but would suggest that it had not been strong enough to disrupt patterns of clinal variation.

Covariation of all variables analyzed (i.e., geography, allozymes, and calls) would confound the interpretation of the Mantel correlations of each geography and allozymes with call similarity. Partial Mantel correlations, however, allow correlated variation to be controlled by using either geographic distance or allozyme similarity as a covariate.

The Mantel analyses were conducted for all sites combined. We also conducted the same analyses for three subsets of the data. Two subsets of populations represented the two major allozyme groups. One subset was from Veracruz to Gualaca (the northern allozyme group). The second group was from Isla El Rey to Trinidad (Fig. 1). The third subset consisted only of the sites across central Panama—this represents the most fine-grained analysis (Fig. 1). Partitioning the data into smaller subsets allowed us to determine concordance of patterns among levels of variation.

RESULTS

Do Advertisement Calls Show Significant Intraspecific Variation?

The box plots presented in Figure 4 show that most call characters exhibit substantial variation across the transect. Furthermore, a Kruskal-Wallis analysis of variance revealed significant and quite substantial variation among populations in all call characters examined (all $P < 0.001$; Table 1).

Is There Call Divergence between the Two Major Allozyme Groups of Túngara Frogs?

Eleven of the 12 call characters showed significant variation among the two major allozyme groups. Of these 11, five also showed clinal variation, and only one call character showed clinal variation with no variation between the two groups. Thus most of the call differences between the two allozyme groups (six of 11) are not merely the result of clinal variation (Table 1).

In accord with the above results, a Mantel test shows a significant correlation between allozyme group and call similarity ($r = 0.20$, $P < 0.001$). As expected, allozyme groups are correlated with overall allozyme similarity ($r = 0.80$, $P < 0.001$).

Does Call Function or Call Production Better Predict Patterns of Variation of Call Components?

The coefficients of variation (CV) for all call variables ranged from 0.08–0.60. Two variables, the number of pulses in the amplitude modulated portion preceding the whine (AMPL) and the duration of this portion of the call (DRAM), were eliminated from the analysis because of the large number of zero values.

The CVs tended to be higher for variables associated with the chuck (CHDM, DBCH, DRCH, range 0.12–0.60, mean = 0.32) than for variables of the whine (DB12, DOMH, FNHZ, INIT, PAMH; range 0.08–0.55, mean = 0.19; Table 1). The differences in the coefficients of variation between chuck and whine variables were in the direction expected if selection on the chuck had a more diversifying effect than selection tending to preserve the species-specific nature of the whine, but the differences were not statistically significant (Mann-Whitney $U = 3.5$, $P = 0.15$).

There was a significant difference between CVs of call variables when they were partitioned into those under active versus passive control (Mann-Whitney $U = 33$, $P = 0.016$). The variables that we assumed were under active control, usually temporal characters (AMPL, DRAM, DURCH, DURT, TLDUR), had a mean CV of 0.53 (range 0.25–1.00; Table 1) compared to spectral call variables, which we assumed are more likely to be under passive control (DB12, DOMH, FNLH, INIT, PAMH) that had a mean CV 0.18 (range 0.08–0.55).

Is There Predictable Geographic Variation in Calls?

A Mantel test shows a significant correlation between our estimates of call similarity and the geographical distances among the 30 populations we sampled ($r = 0.49$, $P < 0.001$). This same pattern was apparent for the three subsets of the data: northern allozyme group ($r = 0.67$, $P = 0.002$), southern allozyme group ($r = 0.62$, $P = 0.007$), and the central Panama group ($r = 0.23$, $P = 0.046$). These analyses reject the hypothesis that call divergence is random among populations, and reinforces the intuition of some overall patterns in some call characters from visual examination of the box plots of individual call variation (Fig. 4). Although there is a statistically significant pattern of geographical variation, geography only predicts 24%, 45%, 38%, and 5% of the variation

among populations for the total data set, northern group, southern group, and the central Panama group, respectively.

If Call Variation Is Geographically Predictable, Is This the Result of Isolation by Distance?

Mantel tests show that for the entire data set there is a significant relationship between geographic distance and allozyme similarity ($r = 0.71$, $P < 0.001$) and between call similarity and allozyme similarity ($r = 0.43$, $P < 0.001$). A partial Mantel test shows that there is still a strong and statistically significant relationship between calls and geography after controlling for the covariation between allozymes and geography (partial $r = 0.29$, $P = 0.003$). When geographical distance is used as the covariate, however, the relationship between call and allozyme similarity is only barely statistically significant (partial $r = 0.13$, $P = 0.045$).

In the northern allozyme group, there is a significant correlation between calls and geography ($r = 0.67$, $P = 0.002$), and allozymes and calls ($r = 0.67$, $P = 0.002$), but not between allozymes and geography ($r = 0.25$, $P = 0.10$). The partial Mantel tests show that there remains a significant partial correlation between calls and geography when controlling for allozymes (partial $r = 0.71$, $P < 0.001$) and between calls and allozymes when controlling for geography (partial $r = 0.70$, $P = 0.001$).

In the southern allozyme group, there is a significant correlation between all three variables: calls and geography ($r = 0.62$, $P < 0.001$), allozymes and calls ($r = 0.45$, $P = 0.009$), and allozymes and geography ($r = 0.74$, $P < 0.001$). Although there is a significant partial correlation between calls and geography when controlling for allozymes (partial $r = 0.47$, $P = 0.01$), there is not a significant correlation between calls and allozymes when controlling for geography (partial $r = -0.13$, $P = 0.47$).

In the central Panama group, call similarity is correlated with both geographical distance ($r = 0.23$, $P = 0.046$) and allozyme similarity ($r = 0.24$, $P = 0.048$), but allozyme similarity and geographic distance are not significantly related ($r = 0.14$, $P = 0.14$). Since allozymes and geography are not highly correlated, using them as covariates in analyses of call variation has less influence on the results than in the above analyses. Calls and geography are similarly correlated with one another when allozyme similarity is used as a covariate ($r = 0.22$, $P = 0.057$), as are calls and allozymes similarly correlated when geography is used as a covariate ($r = 0.22$, $P = 0.056$).

Discussion

Call Variation in Acoustic Mate Recognition Signals

The anuran advertisement call contains species-specific information that guides female phonotaxis towards conspecific calls (Blair 1964; Rand 1988; Ryan 1991; Gerhardt 1994). The species-specific nature of some signals or signal components has suggested to some that there should be relatively little variation in calls within the species (Paterson 1985), despite the suggestion that reproductive character displacement could result in signal variation in response to sympatric heterospecifics (Dobzhansky 1937; Butlin 1987). We might also expect that females would be quite sensitive to experimentally induced variation in such signals. This seems to be the case, as phonotaxis experiments showed that preferences for whine variation are much more restrictive than preferences for chuck variation (Wilczynski et al. 1995). The first question we addressed was: Is there significant intraspecific variation in advertisement calls of the túngara frog? Our study shows substantial variation among populations of *P. pustulosus* in all call variables; coefficients of variation ranged from 8–100% and all call variables significantly differed among populations.

These results are consistent with other recent studies assessing population variation in anuran advertisement calls. For example, Ryan and Wilczynski (1991; see also Wilczynski and Ryan in press) compared calls of 17 populations of cricket frogs, *Acris crepitans*, along a transect that traversed two subspecies (*A. c. crepitans* and *A. c. blanchardi*) and in which 16 of the populations were within 500 kms. Coefficients of variation were similarly high for call variables (8–61%) relative to morphological characters (8–21%), all call variables differed among populations, there was clinal (east to west) variation in two of 14 call variables, and some variables (three of 14) differed among two habitat types (forest versus open habitat) and two subspecies (seven of 14). Nevo and Capranica (1985) showed a similar range of variation throughout the entire range of *A. crepitans*, which covers much of the eastern and central United States.

Substantial population variation in mate recognition signals has also been found in other taxa. For example, there has been considerable attention given to variation in bird song (e.g., Baker and Cunningham 1985; Young et al. 1994), as well as intraspecific variation in acoustic signals of some insects (e.g., planthoppers: Claridge and Morgan 1993; tettigoniids: Ritchie 1991, 1992). Such levels of variation in mate recognition signals are not restricted to acoustic cues, having also been reported in visual (e.g., fish courtship: Houde and Endler 1990; Travis 1994; Endler and Houde 1995) and chemical cues (e.g., moth pheromones: Löfstedt 1993). Variation in species-recognition signals might be the rule rather than the exception, but there are few data to evaluate this assertion. Most studies that have examined geographic variation in acoustic mate-recognition signals have concentrated on variation across hybrid zones (e.g., grasshoppers: Ritchie at el. 1992; fire-bellied toads: Sanderson et al. 1992; leptodactylid frogs: Littlejohn and Robertson 1975) rather than within species.

Variation among Call Variables

As noted above, some researchers have predicted that a species recognition function might restrict intraspecific variation (Paterson 1985), while sexual selection is noted for its diversifying effects on male signals (Darwin 1871; Andersson 1994). Previous phonotaxis studies in the túngara frog, reviewed above, have shown that the whine alone, but not the chuck alone, can result in preferences for conspecifics over heterospecifics. We also know that the presence or absence of the chuck, the number of chucks, and the chuck's frequency structure can all have effects of varying importance on a female's preference for male calls. Although there is

substantial intraspecific and intrapopulational variation in the whine, we do not know how this variation influences preferences. Thus, the effect or function of the call at different levels of discrimination varies between the two call components. In addition, preference for the chuck was rather permissive in that a variety of other sounds experimentally substituted for the chuck increased call attractiveness (Ryan and Rand 1993b), while the experimental manipulation of the whine was more likely to disrupt its ability to elicit female phonotaxis (Wilczynski et al. 1995).

These findings lead to the prediction that the chuck should be more variable than the whine: thus, we asked if call components that function in species recognition and sexual selection exhibit similar patterns of variation. The variables of the chuck tended to exhibit greater variation than those of the whine, but the difference was not statistically significant.

The proximate mechanisms controlling call variation might be a better predictor of degrees of variation among call variables. Ryan and Wilczynski (1991) attempted to explain patterns of population variation among call components in cricket frogs from this perspective. Their data showed that call variables under passive control (Martin 1972), due to their close relationship to morphology (e.g., larynx size and dominant frequency; McClelland et al. 1996), have lower coefficients of variation, as is true for morphological characters. Alternatively, those call variables under active control (due to behavioral-physiological regulation, such as number of pulses) have higher coefficients of variation. We found the same pattern in this study, as well as in interspecific comparisons of species of the neotropical genus *Smilisca*, the African genus *Kassina* (Ryan 1988), and some North American treefrogs (*Pseudacris*), but not North American toads (*Bufo*; Cocroft and Ryan 1995).

Geography and Allozymes as Predictors of Call Variation

Given significant intraspecific call variation in the túngara frog, to what extent can this variation be ascribed to differences among allozyme groups and clinal variation? Although some of the call variation among populations was clinal, more of the call variables exhibited variation that was not clinal, but could be partitioned between the two major allozyme groups within *P. pustulosus*. This was also true in comparisons of overall call similarity. This result is not surprising if the biogeographical scenario proposed by Rand et al. (unpubl.) is correct, suggesting that the two groups were isolated for approximately 4–4.5 million yr before the southern allozyme group invaded Panama from the south with the closure of the Panamanian land bridge about 2.4 M.Y.B.P. This would also suggest, therefore, that secondary contact and any gene flow between these two groups over the last 2.4 million yr has not been sufficient to eradicate all the call differences that arose due to the period of extended geographic isolation.

We were also interested in the extent that the diversifying effects of sexual selection could result in random patterns of call variation among populations. Lande (1982) modeled the evolution of sexually selected characters in a cline in which there were no barriers to migration. He showed that sexual selection could induce local changes in a male's signal, and migration among populations would then result in that change being exhibited, albeit to a lesser degree, in other populations. Such a situation occurring in only one population at one end of a transect could result in smooth clinal variation across the species' range. Alternatively and perhaps more likely, if sexual selection resulted in such changes in a population at another location besides the end of a transect, or among several populations randomly located across a cline, or if the direction of character change varied among populations, then sexual selection would disrupt smooth clinal variation. In the extreme case, sexual selection could generate a random relationship between calls and distance.

In our study, calls do not diverge randomly across space but instead exhibit statistically predictable patterns of geographical variation. This is true for all data combined, and for the three subsets of the data: northern allozyme group, southern allozyme group, and central Panama. It must be remembered, however, that geography only predicts a small amount of call variation. Thus there is still substantial residual variation that could be due to random sexually selected driven variation. Although our data do not offer strong support for the extreme prediction of the sexual selection divergence hypothesis, as would have been the case if there were no predictable pattern of variation, we can not reject that hypothesis based on our statistically significant, but nonetheless weak pattern of geographical variation. A recent study by Young et al. (1994) reported that an acoustic cue known to be important in mate choice has diverged in an isolated population of sage grouse, and this is offered as support of the sexual-selection divergence hypothesis. Studies by Houde and Endler (1990) and Endler and Houde (1995) showed that there is geographical variation in female preference for orange coloration in guppies, thus suggesting strongly that there is spatial variation in sexual selection on male traits. These studies provide especially compelling evidence that sexual selection, combined with natural selection due to variation in predation pressure (Endler 1983), has generated substantial variation in guppy signals.

The observed pattern of geographical variation in túngara frog calls might not merely be the incidental consequence of more proximate populations sharing more genes in common. Although calls, geography, and allozymes covary in the combined data set, and tend to do so in each of the subsets of data, all the partial correlation analyses were consistent in showing that calls and geography are significantly correlated (or nearly so) after the effects of allozyme similarity were controlled. (The initially paradoxical result of geographic distance and allozyme similarity not being significantly correlated in two of the data sets might be due to the isolating effects of the Sierra Madres [northern allozyme group] and the Chagres River [central Panama group] on geographically close populations.) It is possible, however, that the partial correlation analyses did not adequately control for covariation between geographical distance and call similarity due to the influence of unmeasured variables. For example, allozymes are only an estimate of true genetic similarity and some aspects of call variation might also be correlated with genetic variation; calls, in fact, could be a better indicator of true genetic similarity than allozymes. If so, the call similarities among closer populations could be maintained by gene flow, but allozyme variation is not the most accurate

indicator of such a phenomenon. Alternatively, call similarities among proximate populations might have originated through a common ancestor, but maintenance of these call similarities might be for reasons other than gene flow—similar selection pressures, for example. Environmental selection on calls would be a candidate for such a selection pressure, as has been shown in cricket frog calls (Ryan et al. 1990b).

Our results are in some ways comparable to studies of dialect variation in song birds, although we address quite different issues. Dialects, which are learned, are defined as discontinuous geographical variation in song. Nottebohm (1969) suggested that learning local dialects by both males (for later song production) and females (for mate recognition) might lead to allozyme structuring of populations. His analyses of allozyme variation among dialects did not allow a clear resolution of this question (e.g., Nottebohm and Selander 1972; Handford and Nottebohm 1976). Although some studies suggested that allozyme variation was greater among than within dialects (reviewed in Baker and Cunningham 1985; Balaban 1988), others have questioned that conclusion (e.g., Zink and Barrowclough 1984). More recent analyses and discussions (Lougheed and Hanford 1992) have further suggested a lack of any strong relationship between allozyme variation and dialect variation, unlike the strong relationship between allozymes and presumably inherited call variation in our study.

Although we have documented spatial variation in a mate recognition trait in some detail, this study leaves open the question of how this variation influences female phonotaxis as well as the roles of selection, drift, and morphological constraints in generating this variation. But the patterns we uncovered do lead to testable predictions about the interaction of population variation in signals and receivers that will be addressed in future studies.

Acknowledgments

We thank the following people. For technical assistance: E. Gomez, A. Velarde, C. Jaramillo; for assistance with analysis: S. McCafferty, H. Lessios, B. Poulin, P. Legendre, B. Trainor; for field assistance: P. Backwell, C. D'Orgeix, M. C. Fandino, C. Jaramillo, M. Jenions, R. Ibanez, H. Luedecke, H. Moreno, P. Rand, S. Rodriguez, E. Smith, Z. Tarano, M. Vaira; for loaning material: USNM, R. Heyer; for permit and logistical help: M. Leone, M. Dix, M. Dix, F. Bolanos, M. Machado, J. Cavelier; for comments and suggestions on the manuscript: J. Endler, H. Lessios, G. Rosenthal, R. Zink; for financial support: STRI Molecular Systematics and Evolution Program, SI Scholarly Studies grant, Reeder Fellowship and Hubbs Professorship to MJR (University of Texas), and the National Science Foundation (IBN 93-16185). We are especially grateful to M. Vaira for sharing the ride from Texas to Panama, and M. Berkman for sharing the ride back.

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Corresponding Editor: T. Markow

APPENDIX A

The shortest distance between pairs of populations (km) along the earth's curvature (also see Fig. 1, Table 1). Abbreviations: VERC: Veracruz, Mexico; TEHU: Tehuantepec, Mexico; TAPA: Tapachula, Mexico; GUAT: Taxisco, Guatemala; ESAL: San Salvador, El Salvador; NCA: Tipitapa, Nicaragua; CRIC: Liberia, Costa Rica; PARM: Puerto Armuelles, Panama; GUAL: Gualaca, Panama; SANT: Santiago, Panama; ANTO: Anton, Panama; GATW: Gatun west, Panama; GATE: Gatun east, Panama; BCIC: Barro Colorado Island, Panama; PRPH: Prince Phillip Highway (Pipeline Road), Panama; GAMB: Gamboa, Panama; GBRG: Gamboa, Panama; SUMM: Summit, Panama; CHIV: Chiva Chiva Road, Panama; COCO: Cocoli, Panama; KOBE: Fort Kobbe, Panama; ITAB: Isla Taboga, Panama; IREY: Isla Rey, Panama; METE: Metete, Panama; ELRE: El Real, Panama; MARI: Mariquita, Colombia; LMAR: Lago Maracaibo (Santa Elena), Venezuela; CALA: Calabozo, Venezuela; CARU: Carupano, Venezuela; TRIN: Trinidad.

	Geographical Distance														
	VER	TEHU	TAPA	GUAT	ESAL	NICA	CRIC	PARM	GUAL	SANT	ANTO	GATW	GATE	BCIC	PRPH
VERC	0	394.787	701.308	918.747	1052.175	1387.148	1553.433	1937.744	1965.447	2104.47	2149.11	2119.495	2120.347	2133.781	2144.86
TEHU		0	366.85	605.575	788.537	1094.17	1239.616	1619.263	1655.244	1800.964	1855.001	1838.775	1840.344	1852.744	1864.64
TAPA			0	239.173	436.543	727.892	873.689	1254.87	1289.408	1434.521	1488.153	1472.467	1474.097	1486.395	1498.35
GUAT				0	220.84	488.738	638.859	1022.154	1054.097	1197.815	1250.001	1233.34	1234.956	1247.281	1259.218
ESAL					0	341.565	523.346	901.524	921.546	1055.62	1097.4	1067.905	1068.883	1082.158	1093.392
NICA						0	189.807	560.199	580.484	717.323	764.192	744.618	746.221	758.578	770.492
CRIC							0	384.312	415.72	561.934	620.839	620.734	623.332	633.704	646.366
PARM								0	68.993	207.885	288.473	338.118	342.725	345.81	357.874
GUAL									0	151.139	225.769	269.561	274.109	277.617	289.821
SANT										0	86.255	167.867	173.019	169.087	178.103
ANTO											0	99.389	104.049	95.187	100.862
GATW												0	4.918	14.254	25.931
GATE													0	14.917	24.492
BCIC														0	12.825
PRPH															0
GAMB															
GBRG															
SUMM															
CHIV															
COCO															
KOBE															
ITAB															
IREY															
METE															
ELRE															
MARI															
LMAR															
CALA															
CARU															
TRIN															

APPENDIX A
Extended.

	Geographical Distance														
	GAMB	GBRG	SUMM	CHIV	COCO	KOBE	ITAB	IREY	METE	ELRE	MARI	LMAR	CALA	CARU	TRIN
VERC	2150.444	2151.737	2158.132	2166.116	2169.322	2174.148	2283.714	2269.513	2346.034	2390.547	2835.786	2942.459	3345.439	3695.88	3895.659
TEHU	1869.937	1871.172	1877.353	1885.248	1887.792	1891.631	2008.616	1986.993	2070.712	2112.44	2545.15	2707.112	3135.126	3519.525	3725.385
TAPA	1503.616	1504.847	1511.005	1518.885	1521.375	1525.137	1642.679	1620.437	1704.688	1746.124	2178.32	2347.371	2781.589	3177.208	3384.927
GUAT	1264.495	1265.726	1271.892	1279.776	1282.287	1286.089	1403.508	1381.428	1465.519	1507.003	1940.236	2110.527	2547.997	2950.076	3158.767
ESAL	1098.934	1100.218	1106.585	1114.566	1117.667	1122.358	1233.741	1217.828	1296.109	1339.918	1783.658	1918.957	2347.624	2740.663	2948.423
NICA	775.771	777.01	783.186	791.079	793.625	797.505	914.845	892.923	976.814	1018.285	1453.89	1630.022	2076.442	2495.25	2705.87
CRIC	651.049	652.158	657.817	665.315	666.853	669.24	792.882	762.136	852.858	890.076	1308.43	1531.926	1990.89	2428.314	2639.965
PARM	360.166	360.757	364.108	369.335	368.14	366.5	488.783	441.971	539.104	565.162	943.955	1236.13	1707.116	2169.794	2380.733
GUAL	292.314	292.951	296.522	302.012	301.073	299.849	423.518	378.813	475.675	504.001	896.985	1172.649	1642.859	2103.623	2314.722
SANT	178.089	178.171	179.2	181.959	178.997	174.813	287.76	236.937	333.826	357.698	747.263	1029.792	1501.457	1967.438	2177.916
ANTO	99.267	98.998	98.459	99.583	95.82	90.524	202.054	153.505	250.632	278.331	690.235	947.695	1418.666	1882.799	2093.47
GATW	31.182	32.399	38.725	46.656	49.864	55.38	172.262	150.047	233.139	273.668	717.34	917.296	1383.554	1838.245	2049.682
GATE	30.157	31.414	37.904	45.872	49.523	55.641	169.929	149.385	231.107	272.134	716.829	913.959	1379.955	1834.249	2045.707
BCIC	17.318	18.532	24.59	32.548	35.532	41.088	159.184	135.743	219.654	259.734	703.006	905.719	1372.569	1828.366	2039.737
PRPH	5.819	7.295	13.558	21.437	25.649	32.622	146.54	124.923	207.244	247.797	692.398	892.839	1359.729	1815.723	2027.076
GAMB	0	0	7.93	15.707	19.795	26.937	141.843	119.257	202.282	242.508	686.708	888.944	1356.124	1812.654	2023.973
GBRG	0	0	6.598	14.422	18.532	25.649	140.747	117.952	201.118	241.278	685.397	888.01	1355.254	1811.901	2023.211
SUMM			0	7.93	12.047	19.672	135.19	111.5	195.272	235.123	678.942	883.152	1350.679	1870.866	2019.139
CHIV				0	5.387	13.91	127.889	103.537	187.653	227.265	670.972	876.445	1344.253	1802.007	2013.237
COCO					0	8.229	126.902	100.188	186.009	224.91	667.471	876.193	1344.337	1802.7	2013.886
KOBE						0	125.888	95.593	183.824	221.595	662.27	875.88	1344.502	1803.773	2014.887
ITAB							0	58.44	62.364	108.019	561.05	750.245	1219.513	1681.192	1892.024
IREY								0	97.149	128.097	567.471	794.193	1265.226	1730.53	1940.978
METE									0	48.287	500.665	697.11	1168.079	1633.943	1844.257
ELRE										0	453.167	672.873	1145.103	1615.444	1825.043
MARI											0	520.771	933.669	1421.53	1616.718
LMAR												0	427.478	950.153	1157.184
CALA													0	488.56	689.567
CARU														0	211.77
TRIN															0

APPENDIX B

Call dissimilarities among pairs of populations as estimated by Euclidean distances (see Appendix A for definitions of abbreviations).

	Call Dissimilarity														
	VERC	TEHU	TAPA	GUAT	ESAL	NICA	CRIC	PARM	GUAL	SANT	ANTO	GATW	GATE	BCIC	PRPH
VERC	0	0.595	0.925	1.344	1.064	0.995	1.59	1.852	1.701	1.654	1.79	1.505	1.945	1.078	1.782
TEHU		0	0.726	1.054	0.775	0.754	1.224	1.419	1.416	1.149	1.41	1.273	1.616	0.974	1.683
TAPA			0	0.78	1	0.715	0.99	1.467	1.285	1.246	1.271	1.338	1.233	1.154	1.625
GUAT				0	0.953	0.806	0.683	1.571	1.486	1.247	1.617	1.711	1.382	1.418	1.975
ESAL					0	0.57	1.003	1.367	1.528	1.095	1.687	1.439	1.765	1.275	1.955
NICA						0	0.849	1.365	1.308	1.138	1.7	1.234	1.614	1.144	1.695
CRIC							0	1.269	1.393	1.138	1.605	1.637	1.505	1.633	1.984
PARM								0	0.873	0.59	1.302	1.005	1.5	1.336	1.351
GUAL									0	0.866	1.476	0.714	1.444	1.174	0.898
SANT										0	1.233	0.981	1.267	1.146	1.419
ANTO											0	1.546	0.999	1.611	1.851
GATW												0	1.623	1.044	0.964
GATE													0	1.54	1.747
BCIC														0	1.017
PRPH															0
GAMB															
GBRG															
SUMM															
CHIV															
COCO															
KOBE															
ITAB															
IREY															
METE															
ELRE															
MARI															
LMAR															
CALA															
CARU															
TRIN															

APPENDIX B
Extended.

	Call Dissimilarity														
	GAMB	GBRG	SUMM	CHIV	COCO	KOBE	ITAB	IREY	METE	ELRE	MARI	LMAR	CALA	CARU	TRIN
VERC	1.807	1.461	1.45	1.263	1.581	1.486	1.804	1.164	1.587	1.612	0.974	1.725	2.179	1.652	1.838
TEHU	1.682	0.979	1.211	1.191	1.248	1.085	1.655	0.986	1.446	1.417	0.94	1.48	2.104	1.71	1.804
TAPA	1.692	1.06	1.174	1.114	1.098	1.035	1.526	0.909	1.477	1.25	0.97	1.394	2.041	1.811	1.968
GUAT	1.895	0.93	1.539	1.675	1.348	1.253	1.785	1.156	1.478	1.584	1.295	1.75	2.143	1.855	2.23
ESAL	1.885	0.847	1.532	1.421	1.298	1.024	1.595	0.953	1.53	1.426	1.338	1.812	2.196	1.898	1.916
NICA	1.69	1.006	1.357	1.311	1.175	1.01	1.429	0.844	1.462	1.327	1.682	1.683	1.92	1.656	1.713
CRIC	1.962	1.1	1.53	1.719	1.26	1.215	1.762	1.453	1.721	1.727	1.676	1.644	2.058	2.032	2.236
PARM	1.292	1.192	0.904	1.44	0.747	0.883	1.254	1.637	1.48	1.679	1.491	1.05	1.503	1.83	1.723
GUAL	0.973	1.192	0.773	1.449	0.816	1.063	1.164	1.607	1.356	1.509	1.402	1.171	1.255	1.609	1.264
SANT	1.31	0.761	0.96	1.463	0.797	0.775	1.353	1.311	1.324	1.444	1.356	1.193	1.683	1.724	1.693
ANTO	1.822	1.445	1.194	1.474	1.249	1.311	1.885	1.725	1.877	1.768	1.468	0.977	2.247	2.255	2.42
GATW	1.028	0.354	0.858	1.222	0.979	1.059	1.134	1.354	1.482	1.457	1.231	1.255	1.372	1.501	0.953
GATE	1.678	1.451	1.296	1.644	1.199	1.288	1.711	1.484	1.66	1.537	1.36	1.174	2.063	2.015	2.438
BCIC	0.875	1.225	0.856	1.094	0.978	0.986	1.056	0.992	0.8	1.154	0.814	1.172	1.47	1.096	1.427
PPPH	0.632	1.682	0.753	1.319	0.991	1.264	0.929	1.61	1.168	1.399	1.491	1.144	1.28	1.467	1.224
GAMB	0	1.677	0.874	1.516	1.096	1.345	1.021	1.655	1.052	1.604	1.362	1.121	0.81	1.046	1.285
GBRG		0	1.243	1.427	1.029	0.826	1.537	1.036	1.229	1.297	1.279	1.576	2.121	1.957	1.877
SUMM			0	0.946	0.564	0.826	1.046	1.372	1.048	1.293	1.177	0.579	1.353	1.493	1.531
CHIV				0	0.946	0.861	1.11	1.107	1.274	1.049	1.238	1.191	1.953	1.867	1.786
COCO					0	0.417	0.784	1.157	1.013	0.979	1.382	0.855	1.506	1.702	1.682
KOBE						0	0.905	0.896	1.039	0.826	1.039	1.284	1.788	1.801	1.742
ITAB							0	1.263	1.071	0.961	1.577	1.342	1.368	1.632	1.502
IREY								0	1.232	0.25	0.987	1.693	2.141	1.778	1.82
METE									0	1.228	1.259	1.363	1.563	1.345	1.756
ELRE										0	1.445	1.573	2.149	2.074	1.95
MARI											0	1.363	1.868	1.3	1.698
LMAR												0	1.521	1.691	2.012
CALA													0	1.087	1.471
CARU														0	1.564
TRIN															0

APPENDIX C

Genetic dissimilarities among pairs of populations as estimated by Nei's genetic distance (see Appendix A for definitions of abbreviations).

	Genetic Distance													
	VERC	TEHU	TAPA	GUAT	ESAL	NICA	CRIC	PARM	GUAL	SANT	ANTO	GATW	GATE	BCIC	PRPH
VERC	0	0.038	0.038	0.041	0.049	0.047	0.079	0.181	0.104	0.225	0.273	0.252	0.285	0.298	0.287
TEHU		0	0.005	0.014	0.011	0.015	0.03	0.159	0.089	0.171	0.209	0.223	0.241	0.25	0.245
TAPA			0	0.017	0.008	0.007	0.033	0.181	0.097	0.223	0.279	0.26	0.301	0.308	0.294
GUAT				0	0.023	0.018	0.045	0.146	0.062	0.203	0.241	0.207	0.24	0.257	0.245
ESAL					0	0.001	0.013	0.212	0.124	0.222	0.283	0.268	0.296	0.303	0.291
NICA						0	0.16	0.214	0.118	0.247	0.309	0.27	0.31	0.328	0.311
CRIC							0	0.246	0.162	0.227	0.284	0.279	0.305	0.316	0.305
PARM								0	0.036	0.181	0.18	0.195	0.25	0.214	0.207
GUAL									0	0.161	0.17	0.154	0.2	0.193	0.179
SANT										0	0.014	0.068	0.092	0.03	0.029
ANTO											0	0.064	0.077	0.025	0.028
GATW												0	0.028	0.029	0.02
GATE													0	0.038	0.044
BCIC														0	0
PRPH															0
GAMB															
GBRG															
SUMM															
CHIV															
COCO															
KOBE															
ITAB															
IREY															
METE															
ELRE															
MARI															
LMAR															
CALA															
CARU															
TRIN															

APPENDIX C
Extended.

	Genetic Distance														
	GAMB	GBRG	SUMM	CHIV	COCO	KOBE	ITAB	IREY	METE	ELRE	MARI	LMAR	CALA	CARU	TRIN
VERC	0.266	0.251	0.278	0.27	0.272	0.286	0.297	0.298	0.273	0.283	0.41	0.331	0.392	0.355	0.392
TEHU	0.227	0.213	0.233	0.208	0.216	0.221	0.251	0.275	0.233	0.238	0.368	0.305	0.356	0.324	0.343
TAPA	0.273	0.263	0.29	0.279	0.282	0.295	0.304	0.305	0.281	0.294	0.416	0.337	0.401	0.361	0.401
GUAT	0.227	0.209	0.234	0.226	0.23	0.247	0.253	0.253	0.229	0.239	0.358	0.269	0.327	0.296	0.324
ESAL	0.272	0.261	0.284	0.275	0.274	0.293	0.304	0.306	0.279	0.289	0.418	0.338	0.391	0.364	0.402
NICA	0.289	0.276	0.304	0.298	0.299	0.321	0.315	0.317	0.296	0.307	0.434	0.333	0.407	0.37	0.405
CRIC	0.283	0.272	0.296	0.279	0.28	0.296	0.322	0.325	0.294	0.303	0.434	0.359	0.406	0.375	0.405
PARM	0.196	0.201	0.216	0.202	0.22	0.201	0.216	0.22	0.194	0.2	0.236	0.235	0.262	0.242	0.248
GUAL	0.168	0.167	0.185	0.186	0.186	0.193	0.182	0.179	0.162	0.175	0.187	0.201	0.264	0.231	0.263
SANT	0.026	0.045	0.047	0.044	0.036	0.025	0.106	0.056	0.041	0.056	0.119	0.183	0.191	0.176	0.224
ANTO	0.028	0.041	0.038	0.029	0.029	0.011	0.074	0.063	0.033	0.046	0.104	0.154	0.176	0.164	0.201
GATW	0.023	0.023	0.022	0.045	0.048	0.047	0.053	0.027	0.028	0.042	0.133	0.098	0.175	0.143	0.213
GATE	0.046	0.02	0.011	0.018	0.02	0.037	0.069	0.077	0.059	0.056	0.163	0.159	0.217	0.203	0.251
BCIC	0.001	0.005	0.002	0.014	0.009	0.007	0.046	0.018	0.009	0.019	0.105	0.12	0.153	0.151	0.211
PRPH	0	0.008	0.006	0.025	0.017	0.016	0.048	0.007	0.006	0.021	0.09	0.111	0.152	0.143	0.212
GAMB	0	0.006	0.008	0.024	0.016	0.017	0.054	0.008	0.009	0.023	0.096	0.115	0.155	0.146	0.208
GBRG		0	0	0.007	0.004	0.013	0.042	0.026	0.018	0.024	0.126	0.112	0.163	0.157	0.202
SUMM			0	0.003	0.002	0.009	0.038	0.031	0.016	0.021	0.117	0.114	0.161	0.158	0.208
CHIV				0	0.001	0.001	0.049	0.066	0.034	0.031	0.143	0.144	0.178	0.177	0.202
COCO					0	0.004	0.063	0.05	0.031	0.033	0.121	0.157	0.183	0.184	0.224
KOBE						0	0.055	0.055	0.026	0.03	0.129	0.147	0.17	0.165	0.197
ITAB							0	0.073	0.03	0.034	0.147	0.051	0.154	0.145	0.18
IREY								0	0.019	0.04	0.095	0.111	0.163	0.151	0.235
METE									0	0.004	0.091	0.085	0.134	0.131	0.192
ELRE										0	0.114	0.102	0.15	0.158	0.197
MARI											0	0.201	0.229	0.231	0.283
LMAR												0	0.049	0.053	0.051
CALA													0	0.028	0.062
CARU														0	0.049
TRIN															0