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Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae)

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Advertisement calls of the cricket frog, *Acris crepitans*, show statistically significant variation among populations in all call variables measured. Call variables show strong clinal variation resulting in calls of lower frequency, longer duration and slower call rates produced by *A. c. blanchardi* in open habitat in the west of the range, and calls of higher frequency, shorter calls and faster call rates produced by *A. c. crepitans* in the pine forests in the eastern part of the range. This clinal variation does not result from pleiotropic effects of body size or any other morphological characters we measured.

The two subspecies usually reside in different habitats, but some *A. c. blanchardi* reside in an isolated pine forest in central Texas. By comparing the calls of this subspecies in open and forest habitat, and by statistically removing the effects of clinal variation for all populations, we determined that habitat explains some of the variation in call structure; this is not true of subspecies.

Our data reject several hypotheses that purport to explain the evolution of mate recognition signals. (1) We reject the notion of Paterson and others that there is strong stabilizing selection on species-specific mate recognition signals. (2) There is no support for the hypothesis that call variation is primarily due to pleiotropic effects of body size or other morphological characters over the geographic range we examined. (3) There is no evidence for reproductive character displacement. (4) Our data, as well as experimental studies of habitat acoustics, support the hypothesis that some differences in calls among habitats result from environmental selection on call structure to enhance call transmission. We suggest that the latter hypothesis does not explain the strong clinal component of call variation. This might result from the passive effects of gene flow between populations at the extremes of the range under selection generated by habitat acoustics.

KEY WORDS:—*Acris crepitans* – advertisement calls – cricket frogs – habitat acoustics – mate recognition signals – reproductive character displacement – sexual selection – speciation.

CONTENTS

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INTRODUCTION

There is intense selection for individuals of bisexual species to mate with genetically similar individuals in order to promote successful syngamy. Communication systems that discriminate between conspecifics and heterospecifics have such a consequence, and the evolution of ethological isolating mechanisms is an intricate component of the speciation process (Blair, 1958; Mayr, 1963; Littlejohn, 1981; Butlin, 1987; Coyne & Orr 1989b). There has been considerable debate as to the forces responsible for the divergence of mate recognition systems. Dobzhansky (1937) suggested that they evolve as a mechanism to ensure species integrity, and that reinforcement or reproductive character displacement is an outcome of this process. Paterson (1985) emphasized the positive aspects of mate recognition systems, and suggested that there is intense stabilizing selection on such systems to promote homogamic mating. As another alternative, Ringo (1977), Lande (1981) and West Eberhard (1983) discussed how the diversifying effects of sexual selection could lead to divergence of mate recognition systems, and thus promote speciation. Other hypotheses suggest that mate recognition systems might evolve to match the transmission characteristics of the local environment, or as a pleiotropic effect of other changes in the animal's phenotype (e.g. Passmore, 1981; Nevo & Capranica, 1985; Paterson, 1985). These hypotheses need not be exclusive. For example, sexual selection could favour calls that are adapted to local transmission conditions because they are perceived by the female as more intense.

Most studies of mate recognition systems have investigated interspecific interactions (e.g. Blair, 1958; Walker, 1974; Butlin & Hewitt, 1985; Gerhardt, 1988; Littlejohn, 1988; Coyne & Orr, 1989a; Otte, 1989). These studies have been important in demonstrating the efficacy of species isolating mechanisms, and some studies have documented either geographical (Littlejohn, 1976; Otte, 1989) or phylogenetic patterns (Duellman & Pyles, 1982; Coyne & Orr, 1989a) of isolating mechanisms that suggest reproductive character displacement. However, these studies usually examine recognition systems that have already diverged sufficiently to result in species isolation. In general, there is a lack of rigorous investigations of mate recognition systems that may be in the process of divergence. Since most models of speciation are based on divergence and reproductive isolation between conspecific populations, the lack of such data seems an important omission.

We have undertaken a study of intraspecific differences in anuran advertisement calls and the underlying sensory basis for processing such signals (Ryan & Wilczynski, 1988). Here, we report patterns of microgeographic variation in the advertisement call of the cricket frog *Acris crepitans* (Family Hylidae). Nevo & Capranica (1985) examined patterns of call variation in these frogs over their entire geographic range. They showed that calls differ among populations and proposed that this was due to one or more of the following effects: (1) Evolution of calls as a pleiotropic effect of body size; selection against

desiccation favours larger size in the more arid, western portion of the range (Nevo, 1973). (2) Environmental selection on calls that results from the two subspecies usually inhabiting different habitats. (3) Reproductive character displacement between the two subspecies, which they referred to as incipient ecospecies. We tested these hypotheses in *A. crepitans*, but did so with more fine-scaled geographic analysis to emphasize variation among populations for which reproductive interactions were more likely, and to concentrate our samples around the area of parapatry of the two subspecies.

The goals of our study are: (1) to document variation in calls among geographically proximate populations of *A. crepitans*; (2) to compare patterns of variation of call characters with those of morphological characters; (3) to compare the variation in call characters that are used primarily for attracting females with those characters used primarily for male aggressive interactions; and, (4) to examine the degree to which clinal variation, variation associated with habitat type, and variation associated with subspecific status account for variation in call characters. In particular, we will use these data to assess the various hypotheses for the evolution of mate recognition systems: reproductive character displacment, pleiotropy, sexual selection and environmental selection on call characters. These data will allow us to evaluate population-based variation in the species-specific mate recognition system within what is generally accepted to be a good biological species (Dessauer & Nevo, 1969; Salthe & Nevo, 1969). As such, these data should contribute significantly to our understanding of the evolutionary divergence of communication systems that effect species isolation.

The species: Acris crepitans

The two species of cricket frogs (*Acris*), *A. crepitans* and *A. gryllus*, are members of the family Hylidae. The genus is a monophyletic group, but the phylogenetic relationship of *Acris* to other hylids is not resolved (e.g. Hedges, 1986). The genus occurs throughout much of central and eastern North America, as far west and south as Texas and as far east and north as New York (Fig. 1). *Acris gryllus* is restricted to south-eastern North America and a large part of its range overlaps with that of *A. crepitans*.

Acris crepitans consists of two subspecies, *A. c. crepitans* and *A. c. blanchardi*, which are parapatric near the Trinity River in Texas, and in regions of Arkansas and Tennessee (Fig. 1). The geographic distributions of these subspecies coincide with patterns of allozyme variation (Dessauer & Nevo, 1969; Salthe & Nevo, 1969). In Texas, *A. c. crepitans* occurs east of the Trinity River in the pine forests, while *A. c. blanchardi* is found primarily in the hill country and grasslands of central and west Texas, as well as in an isolated pine forest in central Texas (Fig. 1; Blair, 1950).

The call of this species is a short click-like sound, and is repeated in rapid sequence, each bout of which is a call group. Each individual call consists of pulses, and these pulses can also be organized into pulse groups (Fig. 2). Calls increase in length from the beginning to the end of the call group (Table 1). Males increase call length as an aggressive response to other males (Wagner, 1989), and females prefer shorter calls to longer calls (Wagner, unpublished data). This suggests that the call group is graded in both structure (shorter to

longer calls) and function (female attraction to male aggression). Our terminology for descriptions of the call is consistent with that of Ryan & Wilczynski (1988) and Wagner (1989), but differs from Nevo & Capranica (1985).

Phonotaxis experiments have shown that female *A. crepitans* discriminate the advertisement calls of males from different species, different subspecies, geographically distant populations (2500 km, Capranica, Frischkopf & Nevo, 1973; Nevo & Capranica, 1985), populations as close as 50 km (Ryan & Wilczynski, 1988; Ryan, Perrill & Wilczynski, in press), and within the same population (Ryan, Wilczynski & Wagner, unpublished data). The tuning

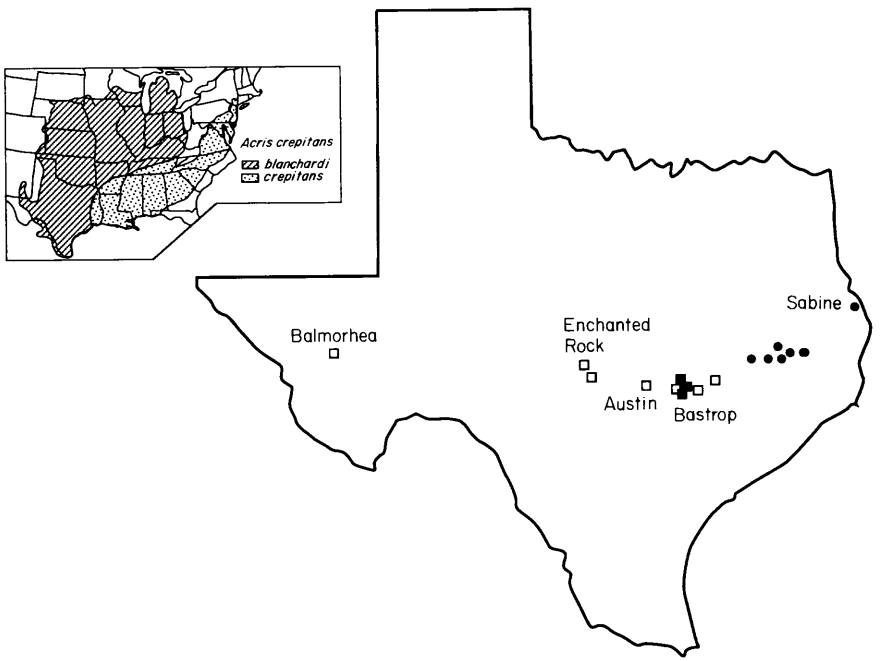

Figure 1. Populations of *Acris crepitans blanchardi* (squares) and *A. c. crepitans* (circles) in open (open figures) and forest (solid figures) habitats sampled in this study. (*Inset*) The distribution of the two subspecies in North America. (Inset after Conant, 1975.)

properties of the auditory nerve are partially responsible for inter- and intraspecific female call preferences. In anurans, much of the filtering necessary for recognizing important spectral information takes place in the auditory periphery (Zakon & Wilczynski, 1988), and in *A. crepitans* the tuning of one of the auditory end organs, the basilar papilla, matches the dominant frequency of the advertisement call. Therefore, as with most anurans tested, the correlated properties of the advertisement call and the auditory system result in conspecific recognition (Zakon & Wilczynski, 1988); however, in *A. crepitans* it also brings about finer discriminations (i.e. population preferences) than have been demonstrated for most other anurans (Capranica *et al.*, 1973; Nevo & Capranica, 1985; Ryan & Wilczynski, 1988; Ryan *et al.*, in press; but also see Ryan *et al.*, 1990).

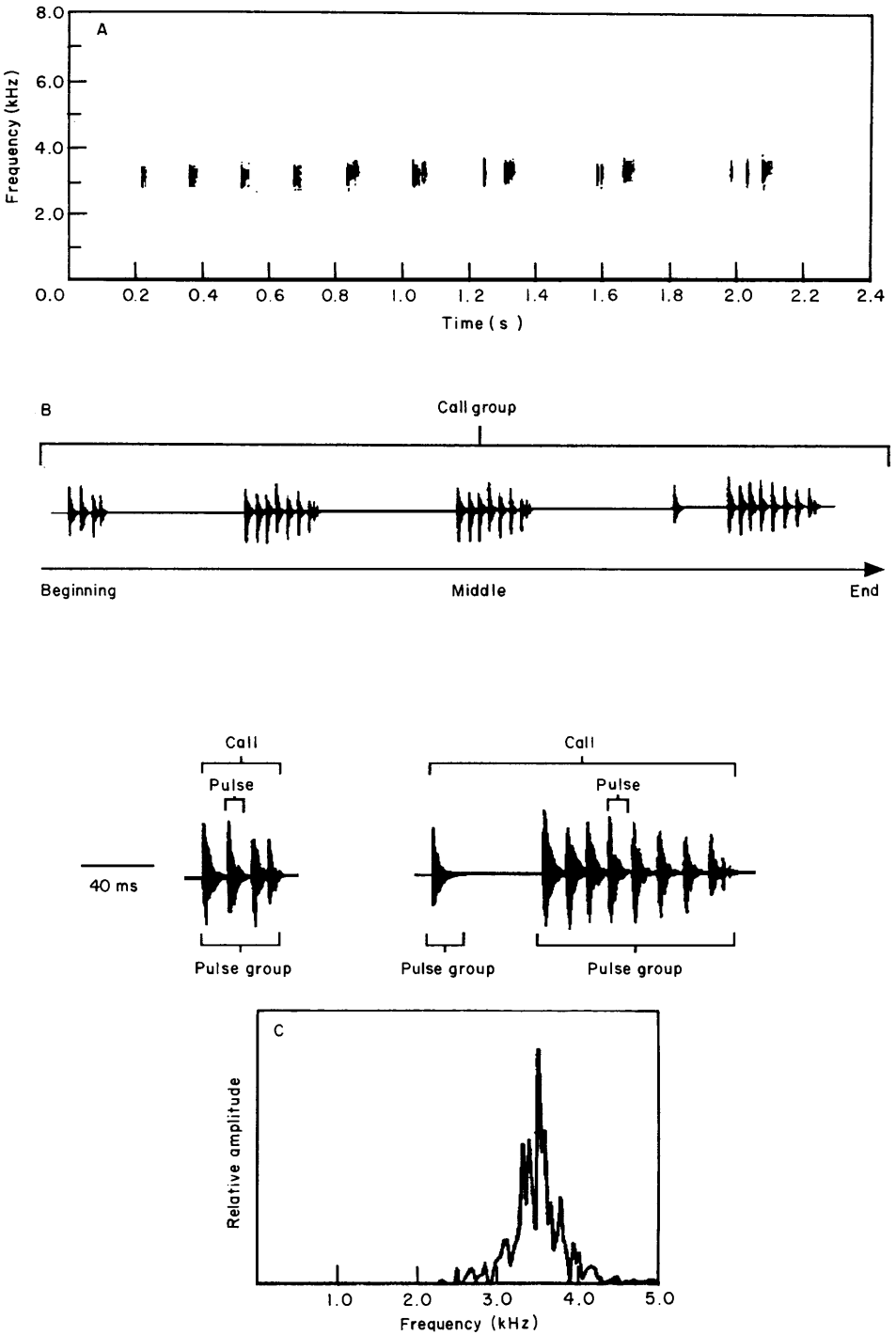

Figure 2. The advertisement call of *Acris crepitans*. A, Sonogram of an entire call group. B, Oscillogram (amplitude versus time) showing how calls progressively change within a call group, and the pulse and pulse group structure of representative calls from the beginning and end of a call group. C, Fourier transform showing the distribution of energy across frequencies for a single call (modified from Wagner, 1989).

TABLE 1. Mean (and standard deviation) of call characters of populations of *Acris crepitans*. Position in call group is indicated as: B = beginning, M = middle, E = end (Dur, duration; DC, duty cycle; SVL, snout-vent length; Tymp, diameter of tympanic membrane; Pect, diameter of pectoral girdle; CV, coefficient of variation. Units of long., degrees; call group duration, s; call duration, ms; morphology, cm)

	Call Group				Groups of pulses			Duration			Number of pulses				Morphology				
Long	Calls	Dur	DC	Dom kHz	B	M	E	B	M	E	B	M	E	Call rate	SVL	Foot	Head	Tymp	Pect
103.70	90.6	23.5	0.29	2.71	1.26	1.92	2.82	35.5	62.8	122.2	4.0	4.7	12.0	5.37	2.86	1.89	0.91	0.17	1.00
	(27.9)	(6.4)	(0.09)	(0.25)	(0.39)	(0.56)	(0.68)	(6.0)	(23.0)	(27.8)	(0.5)	(1.2)	(4.1)	(0.78)	(0.08)	(0.33)	(0.03)	(0.02)	(0.02)
99.00	41.5	13.3	0.23	3.58	1.02	1.51	2.22	32.0	58.8	100.3	6.6	8.4	12.6	4.30	2.34	1.72	0.72	0.11	0.81
	(16.4)	(5.2)	(0.10)	(0.13)	(0.06)	(0.57)	(0.95)	(5.6)	(30.5)	(35.2)	(1.4)	(3.6)	(2.8)	(0.80)	(0.13)	(0.08)	(0.05)	(0.01)	(0.05)
98.40	27.3	12.2	0.22	3.52	1.18	1.62	1.88	54.5	88.6	118.2	7.5	9.0	11.1	2.46	2.37	1.73	0.76	0.12	0.82
	(8.8)	(7.2)	(0.10)	(0.20)	(0.45)	(0.66)	(0.71)	(26.9)	(46.6)	(38.6)	(1.8)	(2.6)	(1.9)	(0.98)	(0.08)	(0.10)	(0.02)	(0.01)	(0.03)
98.08	18.1	5.2	0.23	3.56	1.00	1.10	1.33	32.0	55.4	77.6	6.2	8.3	9.7	3.92	2.25	1.78	0.78	0.16	0.83
	(3.7)	(2.4)	(0.10)	(0.15)	(0.00)	(0.19)	(0.45)	(8.7)	(21.2)	(23.8)	(1.4)	(2.4)	(2.1)	(2.02)	(0.09)	(0.05)	(0.04)	(0.02)	(0.03)
97.40	34.6	13.7	0.18	3.59	1.36	1.80	2.24	36.9	70.6	103.3	4.4	6.5	9.9	1.23	2.36	1.73	0.75	0.11	0.83
	(11.3)	(5.9)	(0.08)	(0.11)	(0.43)	(0.62)	(0.68)	(28.1)	(49.0)	(28.8)	(1.6)	(2.8)	(1.9)	(0.64)	(0.15)	(0.08)	(0.03)	(0.01)	(0.05)
97.20	39.6	10.1	0.25	3.66	1.10	1.68	1.66	30.5	63.0	85.7	5.1	7.7	16.1	4.73	2.37	1.78	0.74	0.10	0.84
	(12.2)	(4.2)	(0.07)	(0.14)	(0.32)	(0.67)	(0.44)	(11.9)	(28.0)	(17.0)	(2.3)	(2.4)	(2.9)	(1.27)	(0.06)	(0.04)	(0.02)	(0.01)	(0.04)
97.20	50.8	14.2	0.20	3.77	1.00	1.43	2.11	25.1	48.1	88.7	4.9	7.0	10.2	4.67	2.45	1.85	0.82	0.18	0.88
	(14.7)	(6.3)	(0.06)	(0.11)	(0.00)	(0.86)	(0.61)	(4.6)	(28.1)	(21.1)	(0.8)	(1.9)	(2.5)	(0.73)	(0.12)	(0.08)	(0.03)	(0.02)	(0.08)
97.10	27.3	7.8	0.18	3.80	1.00	1.07	1.58	33.6	43.0	71.3	6.3	6.9	9.1	4.19	2.10	1.72	0.73	0.11	0.81
	(7.8)	(2.7)	(0.05)	(0.15)	(0.00)	(0.20)	(0.65)	(6.8)	(16.4)	(23.3)	(1.1)	(1.4)	(2.0)	(0.69)	(0.10)	(0.13)	(0.04)	(0.01)	(0.03)
96.98	63.7	16.0	0.25	3.53	1.00	1.33	1.96	31.5	44.0	91.6	4.7	4.9	8.5	6.12	2.48	1.83	0.78	0.11	0.87
	(27.1)	(8.5)	(0.11)	(0.13)	(0.00)	(0.5)	(0.7)	(6.6)	(18.9)	(37.6)	(0.9)	(0.7)	(3.5)	(2.3)	(0.07)	(0.10)	(0.02)	(0.01)	(0.03)
96.55	24.5	8.4	0.14	3.73	1.02	1.08	1.22	32.4	41.1	59.4	5.5	6.4	8.1	3.48	2.32	1.72	0.71	0.11	0.78
	(13.0)	(5.0)	(0.04)	(0.12)	(0.06)	(0.25)	(0.39)	(3.34)	(12.0)	(13.8)	(0.6)	(0.8)	(1.2)	(1.0)	(0.09)	(0.09)	(0.04)	(0.01)	(0.07)
95.66	45.8	11.6	0.16	3.52	1.11	1.75	1.94	19.7	33.4	70.0	4.6	5.5	9.8	4.01	2.45	1.91	0.77	0.12	0.83
	(18.8)	(3.2)	(0.08)	(0.20)	(0.2)	(0.9)	(0.7)	(8.5)	(17.8)	(34.5)	(1.3)	(1.5)	(2.7)	(0.55)	(0.05)	(0.10)	(0.02)	(0.01)	(0.04)
95.50	40.5	11.2	0.18	3.52	1.04	1.26	1.78	25.4	43.4	79.4	5.4	7.0	9.1	4.03	2.45	1.88	0.85	0.19	0.91
	(25.2)	(5.2)	(0.08)	(0.11)	(0.13)	(0.41)	(0.56)	(7.4)	(25.8)	(25.6)	(1.2)	(2.3)	(2.6)	(1.31)	(0.09)	(0.09)	(0.03)	(0.02)	(0.07)
95.41	40.8	14.7	0.20	3.79	1.10	1.62	2.19	32.5	58.9	96.9	4.6	6.0	8.3	4.07	2.31	1.81	0.75	0.12	0.82
	(15.7)	(8.0)	(0.10)	(0.15)	(0.17)	(0.76)	(0.74)	(14.8)	(29.8)	(25.4)	(1.1)	(1.6)	(2.4)	(1.24)	(0.16)	(0.11)	(0.05)	(0.01)	(0.04)
95.10	45.3	18.6	0.16	3.73	1.00	1.64	2.20	23.1	48.0	88.9	5.3	5.7	9.1	3.21	2.41	1.74	0.75	0.12	0.82
	(17.7)	(9.0)	(0.09)	(0.17)	(0.00)	(0.61)	(0.73)	(5.8)	(27.1)	(43.5)	(0.6)	(1.4)	(3.2)	(1.27)	(0.15)	(0.12)	(0.04)	(0.01)	(0.04)
95.00	79.7	16.7	0.28	3.78	1.10	1.92	2.20	29.5	63.9	87.0	5.1	6.4	8.4	6.25	2.40	1.80	0.77	0.16	0.83
	(47.4)	(6.3)	(0.10)	(0.19)	(0.28)	(0.58)	(0.67)	(10.0)	(18.8)	(16.1)	(1.6)	(1.4)	(2.4)	(1.80)	(0.09)	(0.09)	(0.03)	(0.03)	(0.03)
94.70	64.4	13.4	0.23	3.85	1.06	1.84	2.44	27.1	51.5	89.0	4.7	5.7	9.7	5.56	2.29	1.80	0.79	0.17	0.83
	(36.5)	(7.7)	(0.13)	(0.18)	(0.19)	(0.57)	(0.51)	(5.0)	(17.9)	(12.5)	(1.1)	(0.9)	(1.8)	(1.97)	(0.09)	(0.09)	(0.03)	(0.01)	(0.03)
93.75	31.9	6.4	0.31	3.99	1.18	1.83	1.96	31.0	68.0	85.7	4.6	5.4	7.7	5.93	2.34	1.82	0.79	0.18	0.83
	(8.4)	(1.5)	(0.06)	(0.11)	(0.27)	(0.41)	(0.41)	(6.9)	(21.1)	(14.7)	(0.7)	(1.4)	(1.7)	(0.40)	(0.09)	(0.05)	(0.02)	(0.01)	(0.02)
Grand mean	44.9	12.8	0.22	3.64	1.1	1.5	2.0	31.6	55.8	89.6	5.3	6.5	9.8	4.25	2.39	1.79	0.78	0.14	0.84
	(27.5)	(7.3)	(0.10)	(0.31)	(0.3)	(0.6)	(0.7)	(13.6)	(29.1)	(30.4)	(1.5)	(2.2)	(2.9)	(1.75)	(0.18)	(0.13)	(0.06)	(0.03)	(0.07)
CV	61%	57%	45%	9%	27%	40%	35%	43%	53%	32%	28%	34%	30%	41%	8%	7%	8%	21%	8%

MATERIAL AND METHODS

Selection of samples

Seventeen populations were sampled along a transect at 30.5° (±0.5°) latitude in Texas. The easternmost population was at Sabine, in the pine forest of east Texas, well within the geographic range of *A. c. crepitans*, but far from the range of sympatry with *A. gryllus* (Fig. 1). Because there has been some speculation about reproductive character displacement between these species (Nevo & Capranica, 1985), we avoided areas of sympatry. Sixteen of the 17 populations were within 500 km of one another, extending from Sabine to Enchanted Rock, located in the hill country of the Edward's Plateau in central Texas (Fig. 1, Table 1). The westernmost population was from Balmorhea in the grasslands of far-west Texas, the westernmost edge of the species' distribution (Fig. 1, Table 1).

Although *A. c. blanchardi* resides primarily in the hill country and grasslands of central and west Texas ('open' habitat; Fig. 1), this subspecies also resides in the 'lost pines' of Bastrop in central Texas (Fig. 1). This forest is a relict of the forest which once covered much of Texas but is now restricted to the eastern region of the state (Blair, 1950). Allozyme analysis reveals that these populations are genetically more similar to other *A. c. blanchardi* than they are to *A. c. crepitans*, thus we treat these Bastrop forest frogs as *A. c. blanchardi* (Morizot, Ryan & Wilczynski, unpublished data). The existence of these populations allows us to avoid totally confounding habitat type with both subspecies and longitude.

We sampled six populations of *A. c. crepitans* and eleven populations of *A. c. blanchardi*. Eight of the *A. c. blanchardi* populations resided in open habitat and three in the pine forest of Bastrop. There were populations of *A. c. blanchardi* in open habitat to both the east (three populations) and the west (five populations) of the populations in the forest (Fig. 1).

We attempted to record the calls of ten males from each population. The sample size for most populations was ten, and was at least nine for every population except one, which was seven. Calls were recorded with either a Sony TCD-5M or a Marantz PMD 420 tape recorder, and a Sennheiser ME 80 microphone with a K3U power module and MZW 415 windscreen on metal tape. The temperature at the male's calling site was recorded, and all call variables were corrected for temperature according to the equations derived by Wagner (1989) for this species. Males were collected and preserved in 70% ethanol after being fixed in formalin. Standard morphological measurements were taken on the preserved specimens.

Undoubtedly, social factors such as chorus density and nearest neighbour distance influenced some call parameters, such as call group duration (Wagner, 1989). These variables could not be controlled in data collection and analysis, but we do not suspect this introduced any non-random bias into the results.

Call analysis

Fifteen calls were analysed for each male. Since calls differed across call groups in temporal features, we analysed five calls each from the beginning, middle and end of each call group. For temporal features, the five calls from each part of the call group were averaged for each male. Dominant frequency

does not vary across call group, thus all data were averaged to determine the dominant frequency for each male.

Spectral components were analysed by fast fourier transform with a DATA 6000 digital waveform analyser. The sampling rate was 10 kHz. The maximum frequency of each call was usually less than 4 kHz, below the Nyquist frequency for this sampling rate (5 kHz). We recorded the dominant frequency of each call group, which was defined as that frequency with the greatest amount of energy.

Gross temporal structures, the number of calls per call group and call repetition rate, were analysed with a Uniscan real time analyser. We also calculated the duty cycle for each call group, which is the proportion of time during the call group that a call is produced. Fine temporal structures were analysed with the digital waveform analyser. These included: call duration, the number of pulses in each call and the numbers of groups of pulses in each call (Fig. 2).

Statistical analysis

The purpose of our analyses was to examine patterns of variation in call and morphological characters among populations. First, we asked if there were significant differences among populations in single characters (univariate analysis) or among groups of characters (multivariate analysis). Second, we compared patterns and degrees of variation between call characters and morphological characters, and between call characters of different function (female attraction and male aggression). Third, we examined the geographical patterns of variation. There are four likely patterns of variation that might be expressed by characters in this study: (1) Random: populations differ but there is no discernible pattern of geographic population variation. (2) Clinal: characters vary in a continuous manner across longitudes. (3) Subspecies: substantial variation is partitioned among subspecies. (4) Habitat: substantial variation is partitioned among habitats. Populations were selected to allow us to examine the latter two patterns of variation independently of one another, as discussed above. Clinal variation could generate significant differences among subspecies and habitats, although the reverse is not necessarily true. The relative importance of these patterns have important implications for the causes of intraspecific call divergence.

Many characters covaried. Therefore, although we present results of univariate analyses of these characters, we did not attempt to interpret differences in the number of characters that vary clinally, between subspecies, or between habitats, as indicating the overall importance of any of these factors. Multivariate analyses, however, allowed for such interpretations.

We used an analysis of variance to compare population means and a Student's *t*-test to compare single variables between subspecies or habitats. A Pearson product moment correlation was used to examine the relationship between various call and morphological characters and longitude. In computing the correlation of characters with longitude, the population mean was treated as a single datum. We also used an analysis of covariance to compare the dominant frequency among populations after removing the effect of body size.

Discriminant function analysis determined how well call and morphological characters predicted group membership by subspecies and by habitat. We used

the average discriminant function score for each population to examine geographical patterns of variation of calls and morphology in multivariate space, and the residual of those scores when regressed against longitude, to remove the effects of clinical variation.

RESULTS

Univariate analysis

All call characters, with the exception of dominant frequency, showed high levels of variation among populations. Coefficients of variation for all other call characters were 27% or greater, while the coefficient of variation for dominant frequency was only 9% (Table 1). Morphological characters, however, showed relatively less variation among populations, all being 8% or less except for the diameter of the tympanic membrane, which had a coefficient of variation of 21%. There was no significant correlation between body and tympanum size ($r = 0.001$, $P > 0.05$). Thus the relative variation of most morphological characters and the dominant frequency of the call was similar, while the relative variation in most of the call characters and the diameter of the tympanic membrane was similar. There are no apparent trends in the relative variation of calls from the beginning to the end of the call group (Table 1).

All call characters and morphological characters showed statistically significant differences among populations (Table 2; Fig. 3). Only dominant frequency exhibited significant variation at all three levels of analysis: clinal, habitat and subspecific. Three call characters, dominant frequency, duration of calls in the end of the call group, and the number of pulses in calls of the end of the call group, showed clinal variation, as indicated by their significant correlations with longitude. Three call characters, the duration of calls from the beginning and middle of the call group, and call rate, and one morphological character, tympanum diameter, differed among both subspecies and habitat without showing clinal variation.

More population variation was attributed to subspecies alone than to either of the other effects in isolation (i.e. clinal, habitat). Four call characters, groups of pulses in calls in the middle of the call group and the number of pulses in the beginning, middle and end of the call group differed only among subspecies. None of the morphological characters differed only among subspecies. Only one character, snout-vent length, differed only among habitats. Twelve of the 14 call characters were significantly correlated with snout-vent length with dominant frequency having the highest correlation (Table 2).

In summary, calls varied in a predictable manner across longitude and between subspecies and habitat types. Calls were of lower frequency, longer and produced at a slower rate in the west and of higher frequency, shorter and produced at a faster rate in the east. These differences are responsible for the perception that the calls of *A. c. crepitans* and the calls produced in forests, in general, are higher pitched and have a rattling quality relative to the calls of *A. c. blanchardi* produced in open habitat (Nevo & Capranica, 1985).

Dominant frequency exhibited the strongest clinal variation of any call character (Fig. 3); this was also true if the far west population of Balmorhea was excluded from the analysis. This is especially important since this call character

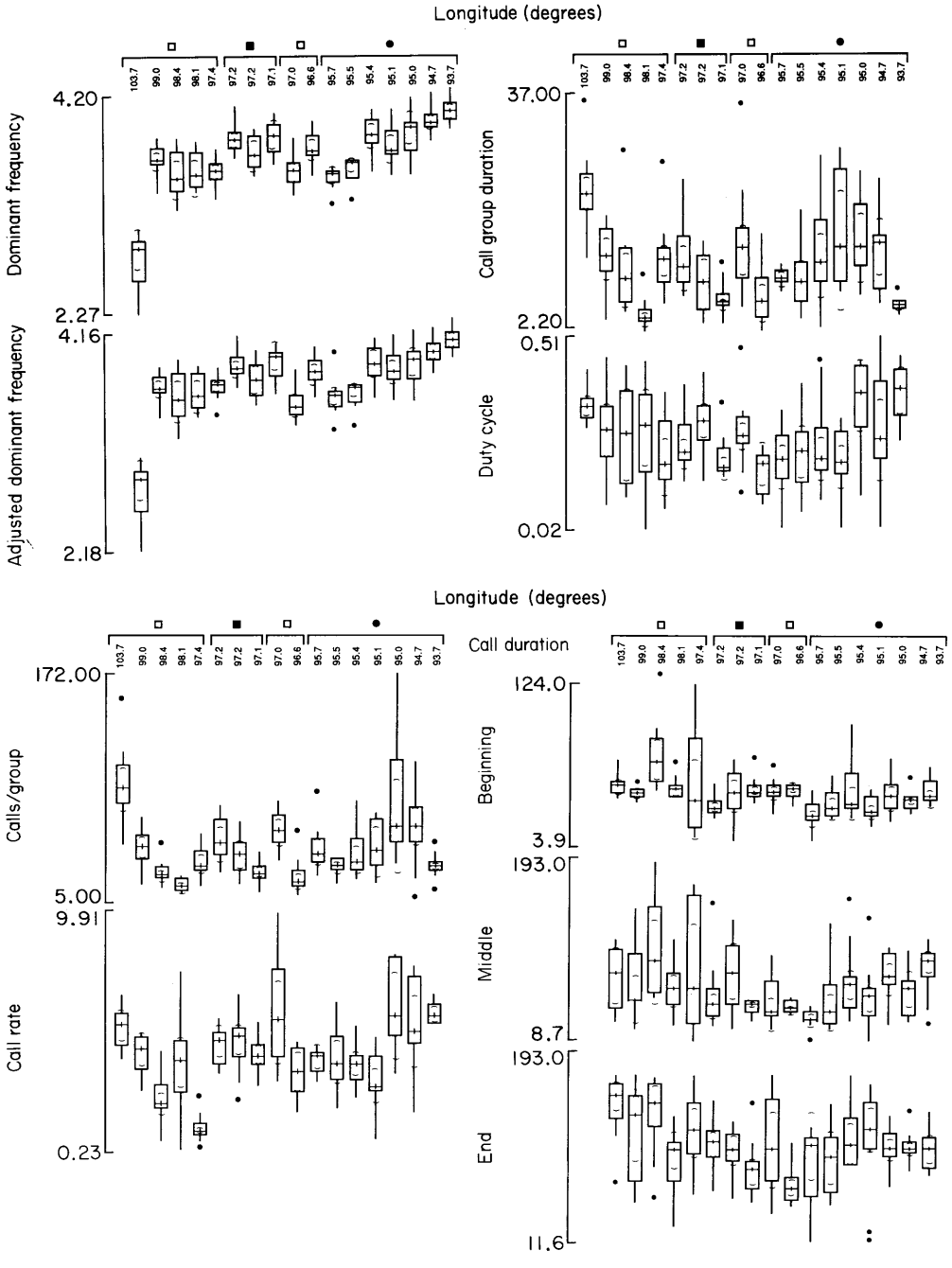

Figure 3. Box plots of various call and morphological characters for populations of *Acris crepitans* sampled in this study. (For each box: cross bars = median, hinges of box = quartiles, parentheses = 95% confidence intervals, line segments = range, dots = outliers. Squares = *Acris crepitans blanchardi*, circles = *A. c. crepitans*, open figures = open habitat, solid = forest habitat. Units for each variable are as in Table 1. Although outliers are represented in the box plots they were included in all statistical analyses.)

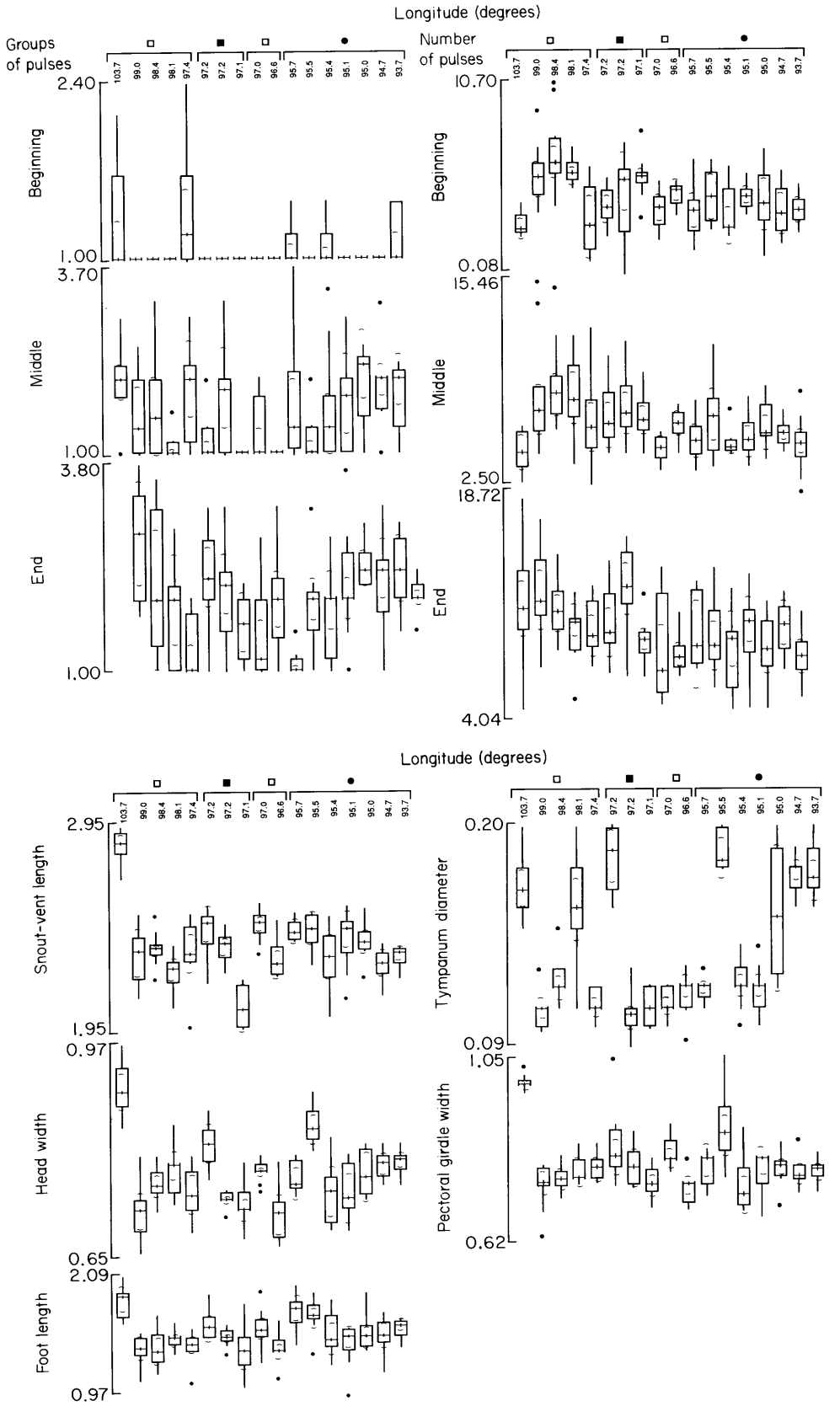
Longitude (degrees)
Groups of pulses
Number of pulses
Beginning
Middle
End
2.40
1.00
3.70
1.00
3.80
1.00
10.70
0.08
15.46
2.50
18.72
4.04
Longitude (degrees)
Snout-vent length
Head width
Foot length
Tympanum diameter
Pectoral girdle width
2.95
1.95
0.97
0.65
2.09
0.97
0.20
0.09
1.05
0.62

TABLE 2. Results of univariate comparisons (analysis of variance, F, Student's t-test , t) of characters between populations, subspecies, and habitat, and the correlation of characters (Pearson's product-moment correlation, r) with snout-vent length (SVL) and longitude. The latter analysis utilized population means. Abbreviations are as in Table 1. Probability levels: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; NS not significant ('—', there was no variance among many populations)

	Call group			Dom	Groups of pulses			Duration			Number of pulses			Call	Morphology				
	Calls	Dur	DC	Hz	B	M	E	B	M	E	B	M	E	rate	SVL	Foot	Head	Tymp	Pect
By population (F)	8.5 (***)	5.8 (***)	2.8 (***)	30.7 (***)	—	2.5 (**)	3.9 (***)	3.5 (***)	2.3 (**)	3.3 (***)	5.0 (***)	4.1 (***)	3.6 (***)	10.0 (***)	20.7 (***)	2.6 (**)	19.3 (***)	43.1 (***)	14.2 (***)
With longitude (r)	0.26 (NS)	0.40 (NS)	0.20 (NS)	−0.86 (**)	0.29 (NS)	−0.01 (NS)	0.26 (NS)	0.43 (NS)	0.25 (NS)	0.57 (*)	0.11 (NS)	0.16 (NS)	0.52 (*)	−0.11 (NS)	0.36 (NS)	−0.07 (NS)	0.25 (NS)	−0.15 (NS)	0.39 (NS)
By sub-species (t)	1.6 (NS)	0.9 (NS)	0.8 (NS)	5.7 (***)	0.4 (NS)	2.0 (*)	1.9 (NS)	2.4 (*)	0.1 (NS)	0.6 (NS)	2.1 (*)	2.5 (**)	3.6 (***)	2.6 (**)	1.0 (NS)	1.3 (NS)	1.3 (NS)	5.7 (***)	0.8 (NS)
By habitat (t)	0.6 (NS)	0.4 (NS)	0.4 (NS)	8.1 (***)	1.4 (NS)	0.9 (NS)	0.5 (NS)	3.0 (**)	0.7 (NS)	1.6 (NS)	1.6 (NS)	1.0 (NS)	1.9 (NS)	3.0 (**)	3.1 (**)	0.7 (NS)	0.2 (NS)	4.1 (***)	0.8 (NS)
With SVL (r)	0.46 (**)	0.38 (**)	0.17 (*)	−0.66 (**)	0.16 (*)	0.16 (*)	0.32 (**)	0.06 (NS)	0.05 (NS)	0.30 (**)	−0.16 (*)	−0.22 (**)	0.17 (*)	0.18 (*)		0.50 (**)	0.73 (**)	0.30 (**)	0.74 (**)

is known to influence inter- and intraspecific call preferences in this species (Capranica *et al.*, 1973; Nevo & Capranica, 1985: Ryan & Wilczynski, 1988; Ryan *et al.*, in press). Regression analysis showed that 74% of the variation in dominant frequency among populations was explained by longitude. Body size and dominant frequency were highly correlated (Table 2). Although body size, like dominant frequency, showed longitudinal variation in this species, with larger males in the west (Nevo, 1973), this trend was not statistically significant in our sample ($P = 0.156$). After adjusting for differences in body size with an analysis of covariance, longitude explained an even greater portion of the variation in dominant frequency among populations (77%; Fig. 3). Thus clinal variation in dominant frequency was not due to a pleiotropic effect of body size.

Multivariate analysis

We performed a discriminant function analysis to determine the extent to which call variables can discriminate subspecies. The fourteen call characters significantly discriminated subspecific status ($F = 6.2$, $d.f. = 14,147$, $P < 0.001$). The most important variables in the discrimination, as indicated by the standardized canonical coefficients, were call rate (0.87), call group duration (0.80) and dominant frequency (0.74). The discriminant function analysis assigned 79% of the 164 individuals to the correct subspecies. Although the analysis significantly discriminated subspecies, there was a strong clinal component to call variation. When we examined the average discriminant function score for each population, we found that longitude explained a substantial portion of this multivariate measure of call variation ($r^2 = 0.57$; Fig. 4).

The five morphological characters also significantly discriminated among subspecies ($F = 10.6$, $d.f. = 14,147$, $P < 0.000$). The diameter of the tympanic membrane was by far the most important variable in the discrimination (standardized canonical coefficient = 1.13), and the analysis assigned 73% of the 164 individuals to the correct subspecies. Although the call analyses assigned more individuals to the correct subspecies, this difference between the call and morphological analyses was not statistically significant ($\chi^2 = 1.07$, $P = 0.30$). Unlike the analysis based on call characters, however, there was not a strong clinal component to the multivariate measure of morphology among populations ($r^2 = 0.19$).

We performed the analogous analyses to determine the degree to which calls and morphology could discriminate the habitat in which individuals resided. Calls were a significant discriminator ($F = 7.1$, $d.f. = 14,147$, $P < 0.001$). The important variables were similar to the analysis that discriminated subspecies in that call rate (standardized canonical correlation = 0.97) and dominant frequency (0.92) were important discriminators, but in this analysis the duration of calls in the middle of the call group (0.83) rather than call group duration was one of the important variables in the discrimination. Again similar to the analysis discriminating subspecies, this call analysis assigned 78% of the individuals to the correct habitat, and there was a strong clinal component to this measure of multivariate call variation among populations ($r^2 = 0.62$).

Morphology also significantly discriminated individuals among habitats ($F = 9.36$, $d.f. = 14,147$, $P < 0.001$), and as when discriminating among subspecies,

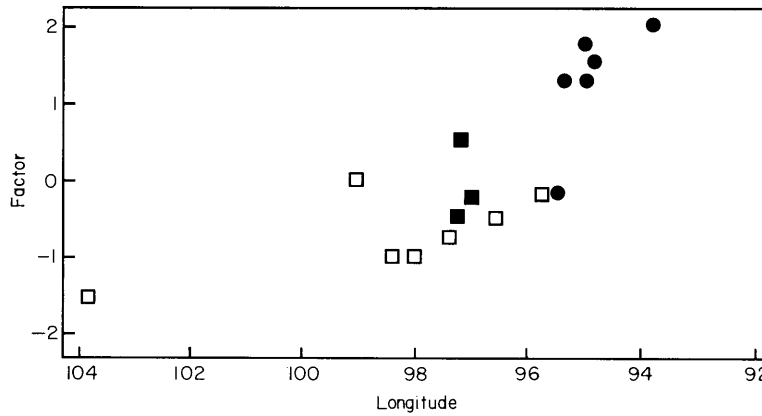

Figure 4. The mean discriminant function score for each population from the analysis using call variability to discriminate among individuals of each subspecies (squares = *Acris crepitans blanchardi*, circles = *A. c. crepitans*, open figures = open habitat, solid = forest habit).

the diameter of the tympanic membrane was again of overwhelming importance (standardized canonical coefficient = 0.90). Less of the variation in morphology was explained by longitude than in the call analysis ($r^2 = 0.27$). This analysis correctly assigned 69% of the individuals to the correct habitat, and there was no significant difference in the number of individuals assigned to the correct habitat between the call and the morphological analyses ($\chi^2 = 1.24$, $P = 0.26$), or when comparing the number of individuals correctly assigned in the four discriminant function analyses (i.e. subspecies and habitat by call and morphology; $\chi^2 = 3.8$, $P = 0.28$).

The multivariate analyses show that although variation in calls was strongly associated with subspecies and habitat, there was a strong clinal component to call variation. This effect could generate the association of call variation with subspecies and habitat. Therefore, we further examined differences in the multivariate call descriptors after removing the effects of clinal variation. We calculated the residual for each population after regressing the mean call score against longitude for the discriminant function analyses based on both subspecies and habitat. In the analysis that used call variables to discriminate subspecies, there were no significant differences between populations of different subspecies after the variation due to longitude was removed ($t = 1.76$, $d.f. = 6,11$, $P = 0.099$). In the analysis that used call variables to discriminate habitat, the difference in the call factors between populations in different habitats was nearly statistically significant ($t = 1.97$, $d.f. = 8,9$, $P = 0.068$; Fig. 5). Also, the residuals were not random as a function of habitat; they tended to be negative for populations in open habitats and positive for forest populations. One population each from the open and forest habitats had residuals of approximately zero, but five of the seven remaining open habitat populations had negative residuals while seven of the remaining nine forest populations had positive residuals ($\chi^2 = 5.40$, $P = 0.020$). However, it should be noted that there are two striking exceptions to this pattern, one population in open habitat and one in forest habitat (Fig. 5).

Finally, we used a multivariate analysis to examine call differences among individuals in the two habitats, open and forest, within the subspecies *A. c.*

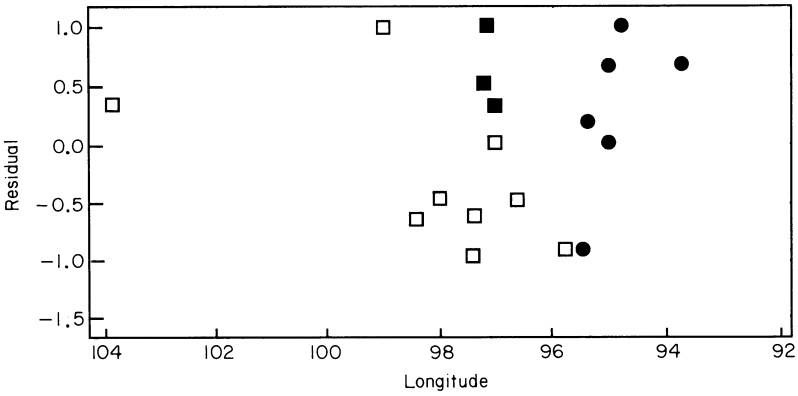

Figure 5. Residuals of the mean discriminant function score for each population from the analysis using call variability to discriminate among individuals residing in each habitat regressed against longitude (squares = *Acris crepitans blanchardi*, circles = *A. c. crepitans*, open figures = open habitat, solid = forest habitat).

blanchardi. Both dominant frequency ($F = 24.0$, $d.f. = 1,104$, $P < 0.001$) and, to a lesser degree that was not statistically significant, call rate ($F = 3.03$, $P = 0.08$) differed among individuals in the two habitats. The multivariate analysis significantly discriminated the two different habitats ($F = 3.1$, $d.f. = 14,91$, $P = 0.001$). Eighty-seven percent of the 107 individuals were assigned to the correct habitat. Animals also differed in snout-vent length between the two habitats ($F = 8.40$, $P = 0.005$), and the multivariate analysis of morphology significantly discriminated habitat ($F = 6.30$, $P = 0.001$), assigning 69% of the individuals to the correct habitat. Although the difference was not statistically significant, call variation was a better indicator of habitat than was morphology ($P = 0.08$). Thus when controlling for either clinal geographic variation or for subspecies, habitat had an effect on the multivariate descriptors of call variation.

In summary, calls showed a very strong clinal component in its variation, but this was not true for morphology. When the effects of clinal variation were removed statistically there appeared to be some influence of habitat on call variation, but little or no effect of subspecies. Also, when the populations of *A. c. blanchardi* residing in open and forest habitat were compared, there was an effect of habitat on call variation.

DISCUSSION

Variation among calls within a call group

As has been found by Nevo & Capranica (1985), Ryan & Wilczynski (1988) and Wagner (1989), temporal characteristics of calls differ according to their position within a call group. Calls increase in duration, number of pulses and number of pulse groups from beginning to end. Our results confirm this same pattern of variation. Wagner (1989) has shown that males increase all of these call characters in aggressive response to the vocalizations of other males. Furthermore, females prefer the shorter calls that typify the beginning of the call group over the longer calls found at the end of the call group (Wagner,

unpublished data). Thus the continuum in call structure within a call group reflects a continuum in call function from female attraction to male aggression.

There are no discernible patterns in the relative variation of characters of calls within a call group. This suggests that although female mate attraction and male aggression favour different call durations (e.g. shorter versus longer calls), these selective agents do not differentially affect the variance of the trait. This is not necessarily to be expected. Barlow (1977) suggested that mate attraction signals should be more stereotyped (less variant) than those used in threat displays. If so, this should be reflected in *A. crepitans* since males increase call duration in a graded response during aggressive interactions (Wagner, 1989), as do other frogs (reviewed in Wells, 1988). But these expectations are also not met in some other systems. For example, Crane (1966) showed that threat displays are more stereotyped than mate attraction displays in a fiddler crab, but Hazlett (1972) came to the opposite conclusion from his studies of other species of fiddler crabs.

Variation among populations

Despite the fact that an important consequence of the anuran advertisement call is species recognition (e.g. Blair, 1958; Gerhardt, 1988; Rand, 1988), there is substantial variation in call characters among populations. Of course, not all of these call characters are known to be functional (i.e. result in discrimination), but this is true for some characters (see below).

The magnitude of variation is high for most call characters. Barlow (1977) reviewed the variation in modal action patterns (stereotyped, species-specific displays) of many taxa, and showed a wide range of coefficients of variation. The values for *A. crepitans* exceed most of the values reported by Barlow. However, the level of analyses in the studies summarized by Barlow are heterogeneous; some represent species samples, while other analyses are restricted to the population level.

In *A. crepitans*, dominant frequency has a much lower coefficient of variation than all other call characters. Dominant frequency is an important variable in conspecific and population preferences in *A. crepitans* (Capranica *et al.*, 1973; Nevo & Capranica, 1985; Ryan & Wilczynski, 1988; Ryan *et al.*, in press). This might suggest stabilizing selection on this character because of its role in species recognition. However, Nevo & Capranica (1985) showed that temporal characters are also important in species recognition, and all of these characters have high coefficients of variation. There is another possible explanation for differences in the variation of temporal and spectral call characters. Our results are consistent with phylogenetic patterns of call evolution that Ryan (1988a) uncovered in two genera of frogs, *Smilisca* (Family Hylidae) and *Kassina* (Family Hyperoliidae). Temporal characters were more evolutionarily labile (had higher unit character consistencies) than spectral characters. Ryan suggested that perhaps this reflects greater lability in characters primarily under behavioural–physiological control (as are temporal characters) than those primarily under morphological control (as is dominant frequency). Interestingly, the coefficient of variation of dominant frequency is quite similar to the same measure for most other morphological characters. Thus spectral characters might be inherently more conservative than temporal characters both within and among species.

Most morphological characters had much lower coefficients of variation than most call characters. One surprising exception is that the coefficient of variation in tympanum size is so high. One might have expected its size to vary inversely with dominant frequency, but this clearly is not always the case in *A. crepitans* (Table 1). Furthermore, tympanum size variation is much greater than dominant frequency variation. Although there has been some discussion about how tympanum size might influence call detection, this issue has not been resolved owing in part to the complex nature of tympanic membrane vibration (Pinder & Palmer, 1983; Jaslow, Hetherington & Lombard, 1988). Moreover, it is not tympanic size alone that determines the transfer of energy into the inner ear, but rather its size in relation to middle ear structures and the oval window, its mass and tension, and any coupling between the two middle ear cavities over which it lies (Saunders & Johnstone, 1972; Wilczynski & Capranica, 1984; Eggermont, 1988). None of these factors could be addressed in this study.

Our measures of population variation in call characters allow us to contrast two views of the evolutionary dynamics of mate recognition signals. Paterson (1985) emphasized strong stabilizing selection on such characters, and suggested that this strong selection greatly diminishes the potential for reproductive character displacement and sexual selection to contribute to divergence of mate recognition signals (also see Templeton, 1979; Gerhardt, 1982). Ringo (1977), Lande (1981), West Eberhard (1983) and Ryan (1990) suggested that the random nature of sexual selection might result in population differences in mate attraction signals. Our data clearly reject the contention of Paterson and others. For every call variable, there is a statistically significant difference among populations. Not only have these characters diverged among populations, but they have done so at a much greater rate than morphological characters, even though Nevo (1973) has presented evidence of ecological selection on body size. The results of the univariate analyses are consistent with, but do not necessarily strongly support, the hypothesis of call divergence among populations due to sexual selection. This hypothesis will be considered further in concert with the multivariate analysis.

Geographic patterns of population variation

When single call and morphological characters are considered, there are obvious non-random patterns of geographic variation. Clinal variation, and variation partitioned among subspecies and among habitats, each characterize the patterns of variation of some call characters. Other call characters have patterns of variation that can be characterized by combinations of these patterns. The univariate analysis clearly shows that not only do populations differ in call and morphological characters, but these differences are not random over geographic space. All three patterns of geographic variation, either by themselves or in concert, explain patterns of variation of different call characters. In general, there is an east-west trend in calls becoming longer, having more pulses, lower frequencies and slower call rates. These trends are consistent with those found by Nevo & Capranica (1985) over a much larger geographic range. This supports the perception that in east Texas the calls of *A. c. crepitans* are higher pitched and have a rattling quality when compared to the lower pitched and longer calls, and slower call rates of *A. c. blancardi* in central and west Texas.

Among the univariate characters, dominant frequency deserves special attention because of its importance in species, population and individual discrimination (Capranica *et al.*, 1973; Nevo & Capranica, 1985; Ryan & Wilczynski, 1988; Ryan *et al.*, in press), and its interesting pattern of geographic variation. Dominant frequency differs among habitats and subspecies, and also shows clinal variation. Because clinal variation can generate differences among subspecies and habitats, we discuss it first.

For many frogs, including *Acris* (Capranica *et al.*, 1973; Nevo & Capranica, 1985), body size covaries with dominant frequency. Nevo (1973) showed an east-west clinal increase in body size in *A. crepitans*. He suggested that these differences in body size were due to selection favouring larger frogs in the west; they can withstand the more arid conditions there because of the decreased surface to volume ratio. Nevo (1973) presented experimental evidence supporting this hypothesis. Nevo & Capranica (1985) also found an east-west clinal decrease in dominant frequency; they suggested that this was a pleiotropic effect due to the covariance between size and frequency.

Although there is no doubt that differences in body size can account for differences in frequency among species, populations and individuals (e.g. Ryan, 1988a; Zakon & Wilczynski, 1988), this is not the only cause of such differences. Ryan & Wilczynski (1988) showed that two populations of *A. crepitans* separated by only 50 km had statistically significant differences in dominant frequency (and in the best excitatory frequency of the basilar papilla) even after the effects of body size were removed. In this study, there is a strong clinal component to variation in dominant frequency, 74% of the variation explained by longitude, as was found by Nevo & Capranica (1985), but there was no significant clinal variation in body size. Therefore, variation in size does not explain this pattern of variation in dominant frequency over the geographic range we sampled (Fig. 3). In fact, clinal variation in dominant frequency is slightly more pronounced (r^2 = 77%) when the effects of body size are removed. Since dominant frequency does not covary with any other morphological characters we measured, this suggests that either the call has evolved in response to selection that varies clinally across longitude, or that the clinal variation represents the effects of gene flow between a high frequency and low frequency population, and that the intermediate frequencies are due to the passive effects of gene flow rather than response to selection. It is interesting to note that at the level of anuran communities, Owens & Dixon (in press) showed that there are no discrete biomes, but rather that communities tend to change gradually with a strong east-west component due, they suggest, to gradual rather than abrupt habitat variation.

Consideration of multivariate measures of call variation also rejects the notion that more complex measures of call variation evolved primarily as a pleiotropic effect of body size and other morphological characters, even though many call characters are correlated with size. More of the variation among populations in the mean discriminant function scores of call variability is explained by longitude than is the analogous measure of morphological variation in the analyses for both subspecies (57% vs 19%) and habitat (62% vs 27%). Also, in the analysis of individuals in different habitats within the subspecies *A. c. blanchardi*, calls assigned more individuals to the correct habitat than morphology (87% vs 69%).

When the effects of longitude were removed in the multivariate analysis of calls there were still some residual differences among populations that reside in different habitats. Any such effect for subspecies was less apparent. The potential role of the habitat in call divergence is also consistent with the analysis restricted to the subspecies *A. c. blanchardi*. Only this subspecies has populations in both habitats, and calls discriminated individuals between these habitats. Clinal variation was not a factor in that analysis because the forest populations are bordered by populations in the open habitat to both the east and the west. In summary, there is a strong clinal component to call variation, and this is not due to pleiotropic effects of morphology. However, after the effects of clinal variation are removed, there is some lesser effect of habitat on call variation.

Causes of intraspecific call divergence

As noted above, our study does not support Paterson's (1985) notion of the specific mate recognition system. His theory emphasizes stabilizing selection on such signals. Despite the fact that advertisement calls are important in species recognition, we have shown that there is a great deal of variation within the species. In fact, variation in characters from the beginning of the call group, which function mainly in mate attraction, is similar to variation of characters at the end of the call group, which function mainly in male-male interactions. Thus it appears that signals used in species recognition need not be temporally static as a result of stabilizing selection, but instead are dynamic, continually evolving systems. It is becoming clear that although Paterson criticizes Dobzhansky and others for being typological, his concept suffers from this same notion (Ryan, 1990). Although there is no doubt that mate recognition systems bring about conspecific matings, in many instances these systems will also be characterized by significant geographic variation which can result in population-based preferences (e.g. Claridge, den Hollander & Morgan, 1988; Ryan, 1990). Whether these population preferences represent adaptations (e.g. Shields, 1982) or are merely consequences of variation in signals and receivers (Lande, 1981; West Eberhard, 1983; Ryan & Wilczynski, 1988) is a matter of debate.

It appears that divergence of call characters is not a mere consequence of general divergence of populations in allopatry. Morphological characters show much less variation than call characters. At the multivariate level, they do not differ among individuals of the same subspecies residing in the same habitat, and they show little or no significant clinal variation. This also is consistent with the prediction of West Eberhard (1983) that characters under ecological selection should exhibit much less divergence than characters under social selection. Her rationale is that ecological characters might approach an optimum, while this is less likely for social characters since their evolution often is characterized by patterns of coevolution similar to an arms race. As noted above, our data also reject the hypothesis that call evolution is a pleiotropic effect of morphological evolution.

Our results also do not support Nevo & Capranica's (1985) suggestion that reproductive character displacement might be occurring in the area of parapatry of the two subspecies. First, clinal variation explains most of the variation in calls among populations. Second, when the clinal component to call variation is removed habitat rather than subspecies tends to explain more of the remaining

variation. It is understandable why Nevo & Capranica (1985) suggested the possibility of reproductive character displacement. After removing the effects of clinal variation (Fig. 5), our data would tend to support the hypothesis of reproductive character displacement if we had not sampled populations of *A. c. blanchardi* in the forest habitat. When considering these populations, however, the primacy of habitat over subspecies in explaining the call variation becomes evident. It is interesting to note that in the westernmost populations of *A. c. blanchardi* there is a tendency for the calls to become more similar to those of *A. c. crepitans*.

The patterns of geographic variation that we report in call characters are consistent with the notion that sexual selection can generate population differences in mate recognition signals. However, random divergence under sexual selection does not predict determinant geographic patterns in differences among populations. These patterns could be accommodated if the mate recognition signals are under direct environmental selection. For example, the optimal properties of an acoustic signal for long-distance transmission are influenced by differences in habitats (e.g. Morton, 1975; Wiley & Richards, 1982; Ryan, 1986; Ryan & Sullivan, 1989). Sexual selection should favour signals that reach the female at a greater intensity (Ryan, 1988a, b). Thus clinal variation in habitat could result in similar patterns of variation in signals. Other results support the hypothesis that some call differences have evolved in response to environmental selection on call structure.

Ryan, Cocroft & Wilczynski (1991) measured the degree of call degradation of calls of the two subspecies in open and forest habitat. Both calls showed very little degradation in the open habitat. Degradation was much more severe in the forest, and the calls of *A. c. crepitans* performed much better in this habitat than did the calls of *A. c. blanchardi* (Fig. 6). There was a significant interaction effect (subspecies by habitat), which supports the hypothesis that environmental selection on call structure is responsible for some of the call variation.

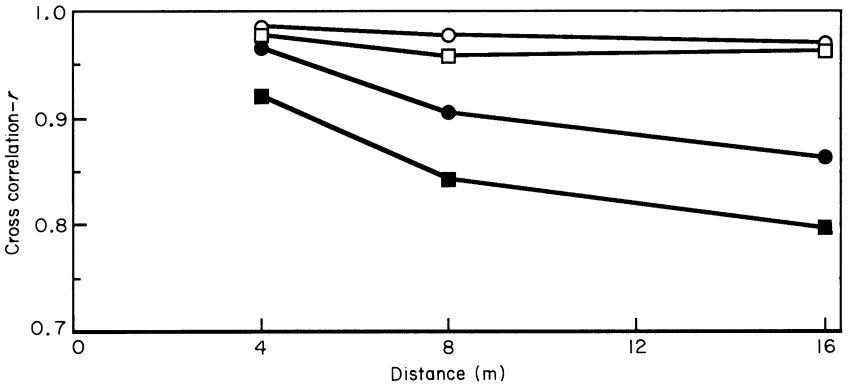

Figure 6. The mean cross-correlation coefficient, as a function of distance from the source, for calls of *A. crepitans crepitans* (circles) and *A. c. blanchardi* (squares; calls were from a population in forest habitat) that were broadcast and then recorded in open habitat (open figures) and forest habitat (solid figures). Higher values represent less degradation of calls (from Ryan, Cocroft & Wilczynski, 1991).

Other evidence supporting the role of habitat acoustics in call evolution comes from examining the calls of *A. c. blanchardi* in the lost pines of Bastrop in central Texas. The calls of these frogs are significantly different from the calls of the same subspecies in open habitats in two univariate characters (dominant frequency and call rate), both of which tend to be the important characters discriminating between the two subspecies, and at the multivariate level.

Our results support the hypothesis that environmental selection on call structure is responsible for some of the call differences between cricket frogs that reside in open and forest habitats. Although Nevo & Capranica (1985) suggested a contribution of reproductive character displacement to call divergence, they also hypothesized that environmental selection on calls and pleiotropic effects of body size were of major importance. Since subspecies and habitat usually covary, this selection force also generates some of the differences between subspecies. However, we doubt that clinal variation in habitat structure across Texas (Owens & Dixon, in press) generates clinal selection on calls due to habitat acoustics, which in turn is responsible for the strong clinal component in call variation. For one, the differences within major habitat types in habitat acoustics might be too subtle, if they exist at all, to generate differential selection on calls within a habitat, and second, our transmission studies show that within open habitat environmental selection on calls is greatly relaxed (Fig. 6). Therefore, it seems more likely that environmental selection on calls might generate call differences between habitat types, and that the clinal variation within habitats is due to the effects of gene flow.

Although the role of mate recognition signals in speciation has made an important contribution to modern evolutionary biology, this study and others show that species recognition is only one consequence of such signals. It is obviously necessary to move away from the typology that results from a species bias, to a consideration of the rich intraspecific variation in mate recognition signals (Ryan, 1990).

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