

Investigating opposing patterns of character displacement in release and advertisement vocalizations of *Bufo fowleri* and *Bufo americanus* (Anura; Bufonidae)

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Abstract: Anuran release vocalizations function to prevent prolonged amplexus between males, whereas advertisement vocalizations potentially act as a premating isolation mechanism. To facilitate rapid release during heterospecific amplexus, selection should favor convergent character displacement in release vocalizations. Conversely, to promote isolation between sympatric congeners, selection should favor divergent character displacement in advertisement vocalizations. I examined properties of release and advertisement vocalizations of *Bufo fowleri* and *Bufo americanus* that are important in mate recognition (periodicity and dominant frequency) to determine if the two calls exhibit opposing patterns of character displacement. Release vocalizations showed no pattern of displacement. The periodicity of sympatric advertisement vocalizations differed from that of allopatric vocalizations for *B. americanus* but not for *B. fowleri*. However, the results were not consistent with the pattern expected for divergent character displacement because the two species differed in this parameter in allopatry. These results are compared with published reports of character displacement in the *B. americanus* species-group in the context of a possible mosaic hybrid zone for these species.

Résumé : Les cris de libération des anoures servent à empêcher la prolongation de l'amplexus entre mâles, alors que les cris d'avertissement sont peut-être un mécanisme d'isolement avant l'accouplement. Pour faciliter la libération rapide durant un amplexus hétérospécifique, la sélection naturelle devrait théoriquement favoriser la convergence du glissement de niche dans les cris de libération. Inversement, pour faciliter l'isolement des congénères sympatriques, la sélection devrait favoriser la divergence dans le glissement de niche dans les cris d'avertissement. J'ai étudié les propriétés qui sont importantes pour la reconnaissance du partenaire (périodicité et fréquence dominante) dans les cris de libération et d'avertissement de *Bufo fowleri* et *Bufo americanus* dans le but de déterminer si les deux cris ont des patterns opposés de glissement de niche. Les cris de libération ne montrent aucun pattern de glissement. La périodicité des cris d'avertissement de crapauds sympatriques diffère de celle des cris d'avertissement de crapauds allopatriques chez *B. americanus*, mais pas chez *B. fowleri*. Cependant, ces résultats ne correspondent pas au pattern théorique de glissement de niche divergent, parce que ce paramètre est différent chez les deux espèces dans des conditions d'allopatrie. Ces résultats sont comparés aux rapports publiés de glissement de niche chez le groupe d'espèces *B. americanus* dans le contexte d'une zone potentielle de mosaïque d'hybrides chez cette espèce.

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Introduction

Because of their disparate functions, social signals may be subjected to selective pressures that result in opposing patterns of character displacement (Grant 1972; Scott and Foster 2000). For example, Cody (1969, 1970, 1973) reported that territorial signals of birds (plumage color and vocalizations) converge in sympatry to facilitate interspecific communication during heterospecific bouts (Cody 1969, 1970, 1973; Cody and Brown 1970; Rice 1978; and reviews by Grant 1972;

Vadas 1990; Scott and Foster 2000). Conversely, signals used to select conspecific mates (i.e., premating isolation mechanisms) may diverge in sympatry (see Brown and Wilson 1956; Blair 1974; Waage 1979; Butlin 1989; Benedix and Howard 1991; Howard 1993; Hostert 1997) to avoid selective disadvantages imposed by hybridization (Dobzhansky 1937, 1940).

Release and advertisement vocalizations are produced by most North American anurans (Bogert 1960) and, because of their different functions, should exhibit opposing patterns of character displacement. Release vocalizations are typically produced by males in response to mating attempts by other males. Production of release calls by an amplexed male signals the amplexing male to dismount (Aronson 1944; Blair 1947; Brown and Littlejohn 1972). For anuran species that exhibit nondiscriminate amplexic behavior (e.g., bufonids; Aronson 1944; Blair 1947; Volpe 1959; Sullivan and Wagner 1988; Marco et al. 1998), convergent character displacement of release calls may facilitate interspecific communication

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during heterospecific amplexus. Inefficiency of the release signal in initiating dismount could result in prolonged amplexus between males (Blair 1947; Sullivan and Wagner 1988), thus increasing exposure to predation and decreasing time available for breeding opportunities (Blair 1968).

Conversely, advertisement vocalizations function to attract conspecific mates (Blair 1974). Numerous studies on anuran advertisement vocalizations have shown divergent character displacement with respect to this premating isolation mechanism (Blair 1955, 1974; Littlejohn 1965; Fouquette 1975; Loftus-Hills and Littlejohn 1992). However, only one other study has addressed the potential for opposing patterns of character displacement in anuran release and advertisement vocalizations (Leary 2001).

The purpose of this study was to determine if release vocalizations of two syntopic species, *Bufo fowleri* and *Bufo americanus*, exhibit a pattern of geographic variation consistent with convergent character displacement. Additionally, I examined advertisement vocalizations to determine if there is a pattern consistent with divergent character displacement. Results from previous studies have raised considerable controversy concerning the potential for divergent character displacement of advertisement vocalizations in these species. Volpe (1952) contended that there is no evidence of hybrid inferiority and, therefore, no selection for divergent premating isolation mechanisms in sympatric populations of *B. fowleri* and *B. americanus*. In fact, Volpe (1957) proposed that hybrids may attain new "adaptive peaks" relative to parental types. In contrast, Blair (1962) provided evidence of divergent character displacement in advertisement vocalizations of these species, suggesting a selective disadvantage for *B. fowleri* × *americanus* hybrids. However, Blair (1962) did not analyze allopatric populations of both species, and call parameters were not adjusted for temperature effects. Similarly, Jones (1973) provided evidence of divergent character displacement of advertisement calls, but did not assess the allopatric character state for the two species and did not adjust call parameters for temperature effects (for a critical review of Jones 1973 see Loftus-Hills 1975, but see reply by Jones 1975). To show divergent character displacement, the presumed precontact character state (allopatric character state) and presumed postcontact character state (sympatric character state) must be significantly different for at least one of the species, and the precontact character states of the two species must be similar. The antithesis is true for convergent character displacement (see Grant 1972).

The present study attempts to reassess the evidence for divergent character displacement in advertisement vocalizations in these two species, taking into account the allopatric character states and temperature effects. Release calls are analyzed to determine if the signal converges in sympatry, given the recent evidence reported for *B. fowleri* and *Bufo terrestris* (Leary 2001). Analyses focus on call parameters that are important in mate recognition. These parameters include the periodicity of release vocalizations (a measure equal to the inverse of pulse rate (Gerhardt and Davis 1988) and an estimate of release vibration rate (Martin 1971, 1972)) and the dominant frequency and periodicity of advertisement vocalizations (Aronson 1944; Blair 1947; Blair 1956, 1958; Sullivan 1982, 1992; Sullivan and Leek 1987;

Gerhardt 1988; Howard and Palmer 1995; Howard and Young 1998).

Methods

Bufo fowleri is sympatric with *B. americanus* throughout much of the eastern United States but is allopatric to *B. americanus* and other members of the *B. americanus* group (see Blair 1963a, 1963b, 1972a, 1972b; Sullivan et al. 1996; Gergus et al. 1997) throughout a narrow zone in central Alabama that extends along the Fall Line Hills (Mount 1975; Conant and Collins 1991; Fig. 1).

I recorded release vocalizations of 236 *B. fowleri* and *B. americanus* from 11 allopatric and 13 sympatric populations during 1994–1996 (Table 1, Fig. 1). Advertisement vocalizations were recorded from 143 individuals (Table 1). To evoke release calls, I grasped male toads behind the forelimbs, held them within 20 mm of a microphone, and recorded 1–5 vocalizations (see Leary 1999). I obtained recordings of advertisement vocalizations by placing a microphone within 30 cm of advertising males. Vocalizations were recorded in the field using a Uher 4000 Report IC open-reel recorder (recording speed 19 cm/s), Ampex 631 1.5 MIL polyester 6.35-mm magnetic tape, and a Uher (Model M136) Dynamic Microphone. I recorded the temperature of the substrate where males produced advertisement calls to the nearest 1.0°C by placing a quick-read thermometer immediately adjacent to advertising toads. Substrate temperatures were also recorded near individuals prior to grasping to evoke release calls. Release calls were recorded immediately upon capture to minimize temperature effects associated with handling. Temperatures were used to test for effects on vocal parameters (see Zweifel 1968; Brown and Littlejohn 1972).

I measured dominant frequency and periodicity from spectrograms and waveforms of advertisement vocalizations and periodicity from waveforms of release vocalizations using Canary 1.1.1, Cornell Bioacoustics Workstation software (settings: frame length 256 points, time 5.752 ms, 50% overlap, FFT size 256 points, Hamming filter, and amplitude logarithmic). The dominant frequency of advertisement calls was measured at the midpoint of the dominant frequency band to the nearest 0.1 kHz. Periodicity (used here to reflect release vibration rate; see Martin 1971, 1972) was calculated by measuring the interval between the onset of the rise in amplitude in successive pulses to the nearest 1.0 ms (a measure equal to the inverse of the pulse rate; see Gerhardt and Davis 1988). A single pulse was defined as any amplitude modulation where the depth of amplitude fell to 50% or lower before the onset of a subsequent pulse.

Periodicity of release vocalizations was measured from the first, middle (one of two middle chirps for even-numbered chirp vocalizations), and last release chirp. Values of the three chirps were then averaged for each vocalization. Periodicity of advertisement vocalizations was measured in a similar manner but was calculated from a middle 192-ms segment of the call (the largest segment that allowed clear resolution of the pulse rate).

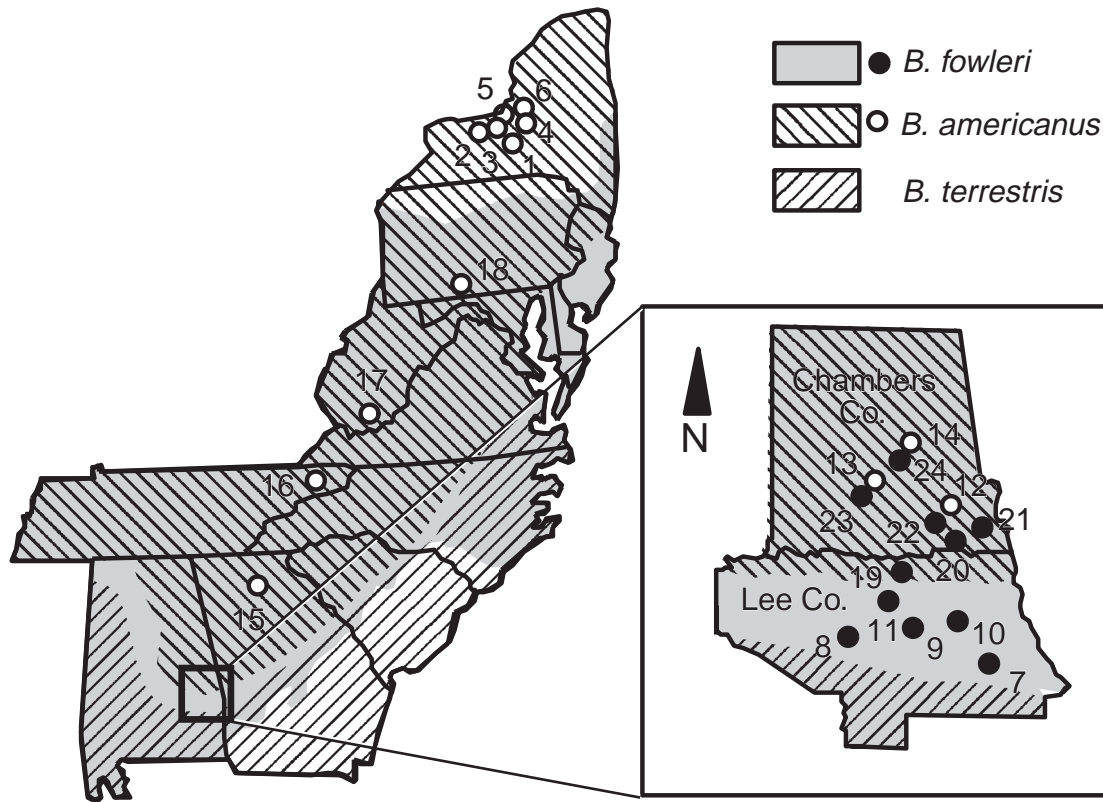
Statistical analyses were performed using JMP IN version 3.0 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

Results

Release vocalizations

Mean periodicity for the combined populations of each species was subjected to nested analyses of covariance to determine if sympatric release calls differed from allopatric calls. I assigned temperature as the covariate and location (allopatry or sympatry) as the grouping factor, and nested population within location. Recording temperatures ranged

Fig. 1. Map of the eastern United States depicting the approximate ranges of *Bufo fowleri* and *Bufo americanus* (Mount 1975; Conant and Collins 1991) and sample localities. The southernmost range of *B. fowleri* overlaps with that of *B. terrestris*, a member of the *B. americanus* group (Blair 1963a, 1963b). For localities and sample sizes see Table 1.



from 16 to 24°C for allopatric *B. fowleri*, from 8 to 20°C for allopatric *B. americanus*, from 18 to 26°C for sympatric *B. fowleri*, and from 10 to 23°C for sympatric *B. americanus*. To test for homogeneity of slopes among locations for the effects of temperature on call periodicity, models were first run with locality and temperature as interaction terms. No significant differences in slopes were found ($p \geq 0.1$; Table 2). Models were then run without interaction terms. Location had no effect on call periodicity for either *B. fowleri* or *B. americanus* ($p \geq 0.2$; Table 2); the possibility of character displacement in release vocalizations can be eliminated.

Slope estimates (Table 3) were used to adjust the periodicity of release calls to the mean recording temperature of 19°C (see Appendix Table A1 for unadjusted and adjusted call values). To determine if release calls of the two species differ, the temperature-adjusted periodicity of allopatric populations was combined with that of sympatric populations for each species. The periodicity of *B. fowleri* release calls (mean = 14.8 ms, SE = 0.83 ms, $n = 97$) and *B. americanus* release calls (mean = 31.7 ms, SE = 0.69 ms, $n = 139$) differed significantly ($F_{[1,234]} = 243$, $p < 0.0001$).

Advertisement vocalizations

Substrate temperatures near males producing advertisement vocalizations ranged from 16 to 24°C for allopatric *B. fowleri*, from 18 to 24°C for sympatric *B. fowleri*, from 8 to 16°C for allopatric *B. americanus*, and from 10 to 18°C for sympatric *B. americanus*. To test for homogeneity of slopes between locations for effects of temperature on

periodicity and dominant frequency, nested analyses of covariance were first run with locality and temperature as interaction terms. Slopes did not differ between allopatric and sympatric *B. fowleri* ($p > 0.05$; Table 4). Models for *B. fowleri* were then run without interaction terms. The effect of location was not significant for dominant frequency or periodicity ($p \geq 0.5$; Table 4), therefore there was no evidence that calls of sympatric *B. fowleri* were displaced from allopatry.

It was not possible to run a nested analysis of covariance for advertisement vocalizations of *B. americanus* because of the small sample sizes of allopatric populations ($n = 4-9$; Table 1) that were recorded under a narrow range of temperatures (4 individuals (19%) were recorded at 8°C and the remaining 17 individuals (81%) were recorded at 16°C). Therefore, allopatric populations of *B. americanus* were combined and sympatric populations of *B. americanus* were combined to test for homogeneity of slopes between locations for the effects of temperature on dominant frequency and periodicity of advertisement calls. There was a significant interaction for dominant frequency ($F_{[1,59]} = 6.690$, $p = 0.01$) and periodicity ($F_{[1,59]} = 10.443$, $p = 0.002$). Heterogeneity of slopes for allopatric and sympatric advertisement vocalizations, but not release vocalizations, was likely to be the result of the small sample size of allopatric *B. americanus* and the narrow range of temperatures under which those individuals were recorded (see above). Otherwise, these results suggest that temperature had different physiological effects on the laryngeal musculature of *B. americanus* in

Table 1. Recording sites and sample sizes (number of individuals recorded) for release vocalizations and advertisement vocalizations of *Bufo americanus* and *Bufo fowleri* in allopatry and sympatry.

Population ^a	Species	Sample size ^b	Condition	Locality and approximate latitude and longitude
1	<i>B. americanus</i>	10 (9)	Allopatric	Port Byron, Cayuga Co., N.Y., 43°01'45''N – 076°37'35''W
2	<i>B. americanus</i>	8 (8)	Allopatric	Weedsport, Cayuga Co., N.Y., 43°02'35''N – 076°34'10''W
3	<i>B. americanus</i>	8	Allopatric	Jordan, Onondaga Co., N.Y., 43°03'45''N – 076°30'15''W
4	<i>B. americanus</i>	9	Allopatric	Beaver Lake, Onondaga Co., N.Y., 43°09'30''N – 076°28'30''W
5	<i>B. americanus</i>	14 (4)	Allopatric	Three Rivers National Wildlife Refuge, Onondaga Co., N.Y., 43°12'14''N – 076°19'24''W
6	<i>B. americanus</i>	10	Allopatric	Phoenix, Oswego Co., N.Y., 43°13'30''N – 076°17'35''W
7	<i>B. fowleri</i>	9 (9)	Allopatric	0.4 km E of Co. Rd. 054 on Co. Rd. 433, Auburn, Lee Co., Ala., 32°32'55''N – 085°25'50''W
8	<i>B. fowleri</i>	5 (8)	Allopatric	1.2 km E of junction of Co Rds. 137 and 152, Auburn, Lee Co., Ala., 32°34'45''N – 085°31'50''W
9	<i>B. fowleri</i>	11 (7)	Allopatric	0.8 km N of Co. Rd. 137 on Co. Rd. 053, Auburn, Lee Co., Ala., 32°35'30''N – 085°30'50''W
10	<i>B. fowleri</i>	7 (12)	Allopatric	3.2 km E of State Highway 147 on Glenn Ave, Auburn, Lee Co., Ala., 32°36'15''N – 085°26'45''W
11	<i>B. fowleri</i>	9 (2)	Allopatric	4.8 km NW of State Highway 147 on U.S. 280, Auburn, Lee Co., Ala., 32°41'45''N – 085°31'10''W
12	<i>B. americanus</i>	9 (2)	Sympatric	1.2 km NE of State Highway 147 on Co. Rd. 28, Lee Co., Ala., 32°43'05''N – 085°30'05''W
13	<i>B. americanus</i>	13 (8)	Sympatric	1.6 km N of State Highway 147 on U.S. 431, Chambers Co., Ala., 32°47'50''N – 085°27'50''W
14	<i>B. americanus</i>	8 (6)	Sympatric	Co. Rd. 55, Chambers County Lake, Chambers Co., Ala., 32°49'00''N – 085°20'54''W
15	<i>B. americanus</i>	15 (9)	Sympatric	Buford Dam, Gwinnett Co., Ga., 34°09'15''N – 084°03'20''W
16	<i>B. americanus</i>	19 (9)	Sympatric	Watagua Dam, Carter Co., Tenn., 36°19'21''N – 082°07'20''W
17	<i>B. americanus</i>	7 (8)	Sympatric	New River Gorge, Summers Co., W.V., 37°32'30''N – 080°55'00''W
18	<i>B. americanus</i>	9	Sympatric	Waynesboro, Franklin Co., Pa., 39°45'16''N – 077°30'18''W
19	<i>B. fowleri</i>	15 (11)	Sympatric	1.2 km NE of State Highway 147 on Co. Rd. 28, Lee Co., Ala., 32°43'05''N – 085°30'05''W
20	<i>B. fowleri</i>	7	Sympatric	Halawakee Creek, 10.4 km S of Co. Rd. 55, Chambers Co., Ala., 32°44'55''N – 085°22'00''N
21	<i>B. fowleri</i>	9 (14)	Sympatric	Snapper Creek, 4.8 km S of Co. Rd. 55, Chambers Co., Ala., 32°48'03''N – 085°19'48''W
22	<i>B. fowleri</i>	9 (6)	Sympatric	1.6 km N of State Highway 147 on U.S. 431, Chambers Co., Ala., 32°48'15''N – 085°27'50''W
23	<i>B. fowleri</i>	7 (11)	Sympatric	Co. Rd. 55, Chambers County Lake, Chambers Co., Ala., 32°49'00''N – 085°20'54''W
24	<i>B. fowleri</i>	9	Sympatric	3.2 km SW of U.S. 431 on State Highway 50 Chambers Co., Ala., 32°51'00''N – 085°28'30''W

^aPopulations are arranged chronologically from the southernmost to the northernmost allopatric/sympatric population and correspond to localities shown in Fig. 1.

^bSample sizes for release vocalizations; numbers in parentheses are sample sizes for advertisement vocalizations.

allopatry and sympatry, and furthermore, that temperature affects the same call parameters differently during production of advertisement calls and release calls (which are produced by similar mechanisms; see Martin 1971). I therefore combined allopatric populations with sympatric populations

of *B. americanus* to obtain a single slope for the effects of temperature on dominant frequency and periodicity that were used to adjust call parameters. There was a significant effect of temperature on both parameters ($p \leq 0.0003$; Table 5).

Slope estimates (Table 5) were used to adjust call parameters

Table 2. Results of nested analyses of covariance for the periodicity of release calls of *B. fowleri* ($n = 97$) and *B. americanus* ($n = 139$).

	Location	Population	Temperature	Homogeneity of slopes
<i>B. fowleri</i>	$F_{[1,9]} = 1.429$	$F_{[9,85]} = 1.706$	$F_{[1,85]} = 6.736$	$F_{[1,84]} = 0.09$
	$p = 0.2$	$p = 0.1$	$p = 0.01$	$p = 0.7$
<i>B. americanus</i>	$F_{[1,11]} = 0.377$	$F_{[11,125]} = 1.346$	$F_{[1,125]} = 2.958$	$F_{[1,124]} = 2.336$
	$p = 0.5$	$p = 0.2$	$p = 0.08$	$p = 0.1$

Note: Temperature is the covariate and population is nested within location. See the text for an explanation of the analytical procedure.

Table 3. Results of linear regression analyses of the periodicity of release calls with temperature as the independent variable.

	Locality	n	b	p	r^2
<i>B. fowleri</i>	Allopatry	41	-0.472	0.03	0.104
	Sympatry	56	-0.317	0.2	0.027
<i>B. americanus</i>	Allopatry	59	-1.618	0.04	0.07
	Sympatry	80	-0.674	0.02	0.067

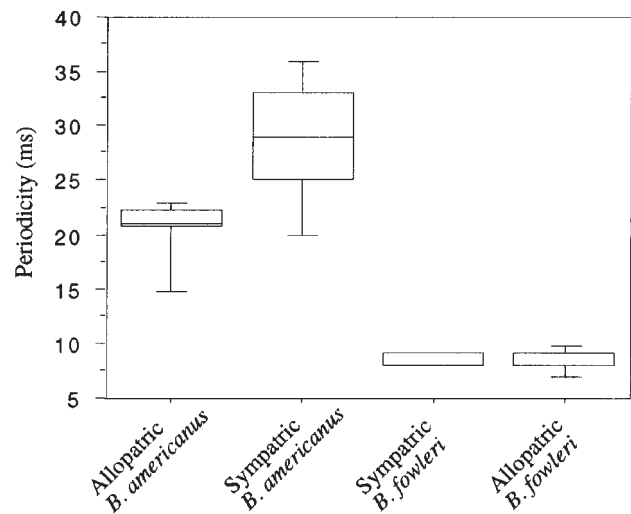
Note: *Bufo fowleri* and *B. americanus* are divided into allopatric and sympatric populations; n is the sample size, b is the regression coefficient, p is the significance of the slope, and r^2 is the coefficient of determination.

ters to 19°C (see Appendix Table A1 for unadjusted and adjusted call values). Nested analysis of variance, using the temperature-adjusted data, indicated that the dominant frequencies for allopatric *B. americanus* and sympatric *B. americanus* did not differ ($p = 0.2$; Table 6). However, the periodicities for allopatric and sympatric *B. americanus* did differ ($p = 0.04$; Table 6). Displacement resulted in greater divergence of advertisement vocalizations in sympatry (Fig. 2). However, the results are not consistent with a pattern expected for divergent character displacement because calls of allopatric *B. americanus* and allopatric *B. fowleri* also differed (Fig. 2).

To determine if clinal variation accounted for displacement of advertisement vocalizations (see Grant 1972), the periodicity of sympatric populations of *B. americanus* (adjusted for temperature) was regressed against latitude (see Benedix and Howard 1991). The results indicated a significant latitudinal cline in sympatry ($n = 42$, $b = 1.02$, $p = 0.04$, $r^2 = 0.10$). However, visual examination of box plots revealed a shift in the allopatric and sympatric character states in the opposite direction to that expected if the results were due to clinal variation (i.e., there is not a continuum in the direction of the cline between allopatry and sympatry) (Fig. 3).

Discussion

Although the periodicity of *B. fowleri* and *B. americanus* advertisement vocalizations differed in sympatry, my results indicate that the calls of the two species were already divergent in allopatry. Therefore, divergent character displacement for advertisement vocalizations of *B. fowleri* and *B. americanus* is not supported. However, greater divergence of calls in sympatry may promote isolation between these

Fig. 2. Box plots of the periodicity of advertisement vocalizations adjusted for temperature for *B. fowleri* and *B. americanus* in allopatry and sympatry. Box plots show divergence of the call in sympatry. For information on box plots see McGill et al. (1978).

species (as reported by Blair 1962 and Jones 1973), regardless of the evidence that calls were divergent prior to contact. Whether differences in allopatric and sympatric advertisement calls affect female mate choice in these species remains to be tested (see Gerhardt 1994).

Leary (2001) reported that the periodicity of release vocalizations of *B. fowleri* and *B. terrestris* exhibits a pattern of geographic variation that is consistent with convergent character displacement. Greater similarity of the release vocalizations in sympatry presumably facilitates interspecific communication during heterospecific amplexus. Results for *B. fowleri* and *B. americanus* reported here indicate that the periodicity of allopatric release vocalizations is not significantly different from that in sympatry for either species, thereby precluding character convergence. My field observations indicated that release signals elicit the dismount response during heterospecific amplexus between *B. fowleri* and *B. americanus*, despite an average difference of 17.0 ms in the periodicity of the calls. However, I have not performed experiments to determine whether the speed at which male *B. fowleri* and *B. americanus* dismount is as rapid as the dismount response during conspecific amplexus. Blair (1947) found that release calls of heterospecific toads were usually effective in initiating release (including tests between

Table 4. Results of nested analyses of covariance for the periodicity and dominant frequency of advertisement calls of *B. fowleri* ($n = 80$).

Call parameter	Location	Population	Temperature	Homogeneity of slopes
Dominant frequency	$F_{[1,7]} = 0.0002$ $p = 0.9$	$F_{[7,70]} = 2.43$ $p = 0.02$	$F_{[1,70]} = 0.289$ $p = 0.5$	$F_{[1,69]} = 3.843$ $p = 0.054$
Periodicity	$F_{[1,7]} = 0.326$ $p = 0.5$	$F_{[7,70]} = 5.804$ $p < 0.0001$	$F_{[1,70]} = 40.637$ $p < 0.0001$	$F_{[1,69]} = 0.093$ $p = 0.7$

Note: Temperature is the covariate and population is nested within location. See the text for an explanation of the analytical procedure.

Table 5. Results of linear regression analyses of periodicity and dominant frequency of advertisement calls with temperature as the independent variable.

	Locality	n	Call attribute	b	p	r^2
<i>B. fowleri</i>	Allopatry	38	Periodicity	-0.262	0.0001	0.471
			Dominant frequency	0.008	0.1	0.049
	Sympatry	56	Periodicity	-0.322	0.0001	0.588
			Dominant frequency	0.018	0.01	0.135
<i>B. americanus</i>	Pooled	63	Periodicity	-1.817	0.0001	0.400
			Dominant frequency	0.022	0.0003	0.197

Note: *Bufo fowleri* is divided into allopatric and sympatric populations, while sympatric and allopatric populations of *B. americanus* were pooled (see the text for an explanation of the analytical procedure); n is the sample size, b is the regression coefficient, p is the significance of the slope, and r^2 is the coefficient of determination.

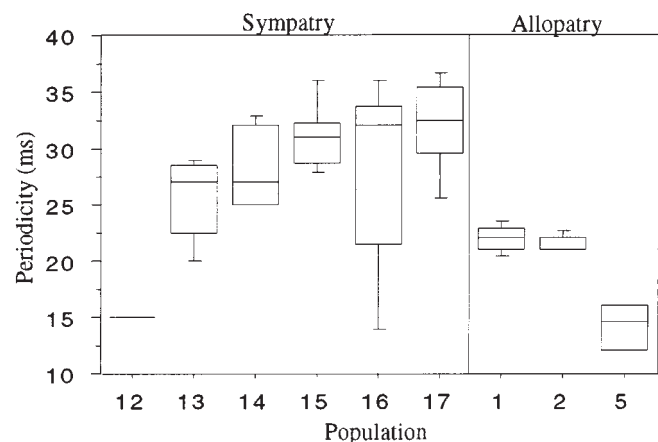
Table 6. Results of nested analyses of variance using temperature-adjusted data for the dominant frequency and periodicity of advertisement vocalizations of *B. americanus* ($n = 63$).

Call parameter	Location	Population
Dominant frequency	$F_{[1,7]} = 1.633$ $p = 0.2$	$F_{[7,54]} = 1.517$ $p = 0.1$
Periodicity	$F_{[1,7]} = 6.151$ $p = 0.04$	$F_{[7,54]} = 6.009$ $p < 0.0001$

Note: See the text for an explanation of the analytical procedure.

species within the *B. americanus* group), but there was evidence that release signals are not effective between pairs with the greatest divergence in release-call pulse rates (see also Sullivan and Wagner 1988).

The results of analysis of release and advertisement vocalizations of *B. fowleri* and *B. terrestris* (Leary 2001) and *B. americanus* suggest that convergent character displacement of release vocalizations and divergent character displacement of advertisement vocalizations are unlikely to co-occur because of the disparate character states required prior to contact between species. For example, establishing the necessary precontact pattern for divergent and convergent character displacement of the two calls would require divergence of release vocalizations and a lack of differentiation in advertisement vocalizations among the incipient species during an allopatric speciation event. Given the conserved nature of the release calls (Brown and Littlejohn 1972) and the similar mechanisms of sound production for the two calls (Martin 1971, 1972), the existence of one pattern probably precludes the other. For instance, in *B. fowleri* and *B. terrestris*, the release and advertisement calls are different prior to contact (Leary 2001). Hence, only release vocalizations

Fig. 3. Box plots of the periodicity of advertisement vocalizations (adjusted for temperature) for sympatric and allopatric populations of *B. americanus* across the sampled range. Populations are arranged from the southernmost latitude to the northernmost latitude. Populations are designated by numbers that correspond to population localities in Table 1. There was no evidence of clinal variation after removal of population 12 ($n = 2$; see Results). For information on box plots see McGill et al. (1978).

can exhibit a pattern consistent with convergent character displacement. A pattern of divergent character displacement in advertisement vocalizations cannot occur in this case because the calls are already different in the precontact state. Similarly, the results presented here indicate that both advertisement and release calls of allopatric *B. fowleri* are significantly different from those of allopatric *B. americanus*. Therefore, only release calls potentially exhibit a pattern consistent with character displacement, though there was no evidence of convergence in sympatric calls.

Jones (1973) contended that divergence of *B. fowleri* and *B. americanus* advertisement vocalizations is a recent phenomenon that occurred over a 30-year period. The argument is based on a decrease in hybrid frequencies found in a population in Bloomington, Indiana, by Blair (1941) and subsequently by Jones (1973). Numerous other investigators have reported extensive naturally occurring hybridization between these two species (Blair 1941; Volpe 1952; Cory and Manion 1955; Zweifel 1968; Green 1984), while others have reported that the two species maintain their integrity in sympatry (Neill 1949; Mount 1975; Weatherby 1982). Differences in the frequencies of hybrids among populations may be due to the presence or absence of divergent premating isolation mechanisms (i.e., advertisement vocalizations) as suggested by Jones (1973), and (or) the extent of anthropogenic disturbance to preferred habitats (see Blair 1941; Volpe 1952; Cory and Manion 1955). *Bufo americanus* reportedly prefers heavily wooded areas, whereas *B. fowleri* prefers open grasslands (Blair 1941; Volpe 1952; Cory and Manion 1955). Alteration of habitat (e.g., removal of vegetation adjacent to breeding sites) may produce syntopic conditions for species that were otherwise structurally isolated (see Blair 1941, 1942; Cory and Manion 1955; for similar findings in *Hyla gratiosa* and *Hyla cinerea* see Mecham 1960). Based upon these reports and evidence provided by Green and Parent (2000), populations of *B. fowleri* and *B. americanus* may exhibit a pattern of geographic variation in vocalizations that is indicative of a mosaic hybrid zone.

Mosaic hybrid zones are typically attributed to variation in sympatric habitats accompanied by differing habitat requirements for the incipient species (Harrison 1986). The result is the formation of patches of habitat where only one species occurs and intermediate habitats where both species occur (and hybridize) (Harrison 1986). Hence, regions of sympatry where one taxon is semi-isolated from the other may result in a mosaic of divergent characters in disparate populations (Howard 1993). The results presented here are based upon nested allopatric and sympatric populations, and all populations examined were from highly disturbed regions (e.g., farm ponds, roadside ditches, and bodies of water in recreational parks and adjacent to dams). To elucidate the process of "reinforcement" in advertisement calls of *B. fowleri* and *B. americanus*, in future investigations, calls from undisturbed and disturbed sympatric regions should be examined and isolated populations within the mosaic hybrid zone compared (see Howard 1993).

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Appendix.

Table A1. Mean (\bar{x}), standard error (SE), and range of the dominant frequency and periodicity of release and advertisement vocalizations before and after adjustment for the effects of temperature for *Bufo fowleri* and *Bufo americanus* in allopatry and sympatry.

	<i>B. fowleri</i>				<i>B. americanus</i>			
	Allopatric		Sympatric		Allopatric		Sympatric	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Release vocalizations								
Periodicity (ms)								
\bar{x}	13.9	14.9	13.1	14.8	32.2	30.5	34.3	32.5
SE	0.84	0.79	0.58	0.57	1.33	1.27	1.16	1.12
Range	7.5–28.2	9.0–30.0	6.8–25.2	8.0–27.0	18.7–65.6	17.0–62.0	9.4–60.2	10.0–60.0
Advertisement vocalizations								
Periodicity (ms)								
\bar{x}	8.2	8.3	7.3	8.3	28.6	20.3	37.1	28.2
SE	0.22	0.17	0.16	0.10	0.65	0.74	1.38	0.96
Range	5.6–12.2	7.0–12.0	6.0–9.8	7.0–10.0	25.5–36.3	11.0–24.0	16.5–53.3	10.0–38.0
Dominant frequency (kHz)								
\bar{x}	1.9	1.9	1.9	1.9	1.5	1.6	1.5	1.6
SE	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.01
Range	1.6–2.2	1.7–2.2	1.7–2.2	1.7–2.1	1.2–1.7	1.3–1.8	1.2–1.8	1.4–1.9