INTERSPECIES COMMUNICATION, HETEROSPECIFIC SEX DISCRIMINATION AND CHARACTER DISPLACEMENT OF THE RELEASE AND ADVERTISEMENT VOCALIZATIONS OF BUFO WOODHOUSII, B. AMERICANUS AND B. TERRESTRIS

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VITA

Christopher Joseph Leary, son of Daniel Leary and Gretchen (Baker) Leary, was born November 28, 1968, in Syracuse, New York. He graduated from West Genesee High School in 1987. He attended Herkimer County Community College for one semester, then entered Auburn University in September, 1988, and graduated with a Bachelor of Science degree in Zoology in June, 1992. He entered Graduate School, Auburn University, in January, 1993.

THESIS ABSTRACT

INTERSPECIES COMMUNICATION, HETEROSPECIFIC SEX DISCRIMINATION AND CHARACTER DISPLACEMENT OF THE RELEASE AND ADVERTISEMENT VOCALIZATIONS OF BUFO WOODHOUSII, B. AMERICANUS AND B. TERRESTRIS

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Previous studies utilize an artificial amplexus method to acquire bufonid release vocalizations for spectrographic analyses. Analyses of Bufo americanus release vocalizations induced through artificial amplexus differed significantly ($p < 0.05$; 4 of 20 parameters) from those produced during conspecific amplexus.

Release and advertisement vocalizations of anurans have disparate functions. Release vocalizations prevent prolonged male-male amplexus whereas advertisement vocalizations serve as a premating isolation mechanism. I examined spectrographs of release and advertisement vocalizations of allopatric and sympatric populations of B. woodhousii, B. americanus and B. terrestris to determine if character displacement exists. Numerous instances of convergent and divergent character displacement were found for comparisons between B. woodhousii and B. americanus indicating displacement resulting from hybridization. Release vocalizations of B. woodhousii and B. terrestris were primarily convergent whereas advertisement vocalizations were largely divergent. Thus, selective pressures among these species have resulted in convergent character displacement of release vocalizations and divergent character displacement of advertisement vocalizations thereby facilitating interspecies communication during heterospecific amplexus while maintaining interspecific isolation in sympatry.

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Style manual or journal used Journal of Herpetology (Chapter 1) and Evolution (Chapter 2)

Computer software used Clarisworks 2.0, Canary 1.1.1, SuperANOVA and JMP IN 3

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I. AMPLEXUS-INDUCED VARIATION IN THE RELEASE VOCALIZATIONS OF BUFO AMERICANUS

Anuran release calls are an acoustimechanical response to misdirected male-male mating attempts (Aronson, 1944; Blair, 1947; Bogert, 1960; Brown and Littlejohn, 1972). Production of release calls by an amplexed male anuran signal an amplexing conspecific and/or heterospecific to dismount (Aronson, 1944; Blair, 1947; Bogert, 1960; Brown and Littlejohn, 1972).

Boulenger (1897) noted that release calls of bufonids could be evoked by simulating natural axillary amplexus. This involved gently clasping a toad's thorax with a forefinger and thumb (referred to as "artificial" amplexus throughout this paper). As a result, Boulenger's (1897) method has become the standard for acquiring anuran release calls for spectrographic analyses. However, previous studies have not determined if artificial amplexus accurately induces conspecific amplexus release vocalizations. Therefore, the purpose of this study was to determine if release vocalizations of Bufo americanus evoked through artificial amplexus are different from those produced during conspecific amplexus.

METHODS

Seventy release vocalizations from 35 adult male Bufo americanus were recorded from 4 localities in central New York State (Weedsport, Cayuga County, n=8; Phoenix, Oswego County, n=11; Beaver Lake, Onondaga County, n=9; and Three Rivers Wildlife Refuge, Onondaga County, n=7). All toads were recorded during the breeding period, 1995.

Release vocalizations were evoked artificially from adult male advertising toads by method of Boulenger (1897). Toads were held within 20mm of a Uher Dynamic Microphone (Model M136) while a series of vocalizations were recorded on a Uher 4000 Report IC recorder equipped with Ampex 631 1.5 MIL polyester 1/4" magnetic tape (19 cm/s recording speed). Attempts were made to exert equal and uniform pressure on each subject during artificial amplexus.

Immediately following artificial amplexus recording episodes, toads were "nudged" with a nearby advertising conspecific male (simulating female amplexus initiation behavior [Bogert, 1960]) to induce natural amplexus. The microphone was held directly adjacent to amplexed pairs (within 20 mm) while a series of conspecific release recordings were made. Recordings were also collected in the reverse order (conspecific amplexus followed by artificial amplexus) an approximate equal number of times. This was done to eliminate sequential effects associated with minor changes in body temperature that may have occurred while toads were handled during artificial amplexus (see Brown and Littlejohn, 1972).

Release vocalizations typically consisted of a series of three or more chirps. Measurements were made on the first, middle (one of two middle-most chirps for even-numbered chirp vocalizations) and last chirp for each vocalization. The following twenty parameters were measured from spectrographs and waveforms using Canary 1.1.1 Cornell Bioacoustics software (default settings): interchirp duration (mS) between all chirps averaged per vocalization, chirp duration (mS) of the first, middle and last chirp averaged per vocalization, dominant (midpoint) frequency (kHz) per chirp, maximum and minimum frequency (kHz) per chirp, range of frequency (kHz) per chirp (difference between maximum and minimum frequencies), number of pulses per chirp and periodicity (mS) of all pulses averaged per chirp (see Fig. 1 for illustration of call parameters). A single pulse consisted of any amplitude modulation where the depth of the amplitude fell to 50% or less before the onset of the subsequent pulse (see Gerhardt and Davis, 1988). Periodicity was measured from the onset of rise in amplitude of one pulse to the onset of rise in amplitude of the subsequent pulse.

Two-tailed, paired-design t-tests (Zar, 1984) were used to determine if vocalizations evoked through artificial amplexus differed from conspecific amplexus vocalizations (JMP IN statistical software version 3, SAS Institute Inc.).

RESULTS

Four of 20 measured release vocalization parameters (mean interchirp duration, mean range of frequency of the first chirp, and mean minimum frequencies of the middle and last chirps) produced during artificial amplexus differed significantly ($p < 0.05$) from those produced during conspecific amplexus (Table 1). Mean interchirp duration for artificially evoked vocalizations was significantly longer than that for conspecific amplexus ($p = 0.0001$; Table 1). The mean range of frequency of the first chirp for artificial amplexus was significantly greater than that for conspecific amplexus ($p = 0.01$; Table 1). Additionally, mean minimum frequency of the middle and last chirp for conspecific amplexus were significantly higher than that produced during artificial amplexus ($p = 0.003$ and 0.0007 respectively; Table 1).

DISCUSSION

Statistically significant differences in release vocalizations produced during artificial and conspecific amplexus may have resulted from a larger surface area that a human forefinger and thumb occupy on the thorax during artificial amplexus, presumed greater restriction during artificial amplexus, and/or differences in pressures placed on the toad during artificial versus conspecific amplexus.

Significantly longer mean interchirp duration for artificially evoked release vocalizations indicated that this method prolonged constriction of musculature surrounding the laryngeal aperature (see Martin, 1971). Toads may have responded to artificial amplexus in a defensive manner causing some initial resistence to produce release calls in normal succession.

Martin (1971) determined that the dominant frequency of bufonid vocalizations result primarily from the fundamental frequency of vibrations of the vocal cords which, in turn, is governed, in part, by variation in air pressure passing through a larynx of varying aperture. In fact, two "morphologically identical larynges might produce rather different dominant frequencies because of different activation pressures (air pressures) during call production" (Martin, 1971). Statistically significant differences in mean range of frequency for the first chirp and mean minimum frequency of the middle and last chirps were most likely due to differences in air pressure (resulting from differential amplexus pressures) passing over the laryngeal apparatus during production of conspecific versus artificial amplexus vocalizations.

Extraneous "noise" associated with naturally occurring conspecific release vocalizations often result in poor quality recordings. In particular, interfering advertisement vocalizations of nearby anurans characteristically exceed amplitude levels of release vocalizations. In these instances, artificial amplexus methods may provide clearer spectrographic images. However, significant differences in vocalizations emitted during artificial and conspecific amplexus warrant the use of natural amplexus recordings for spectrographic analyses.

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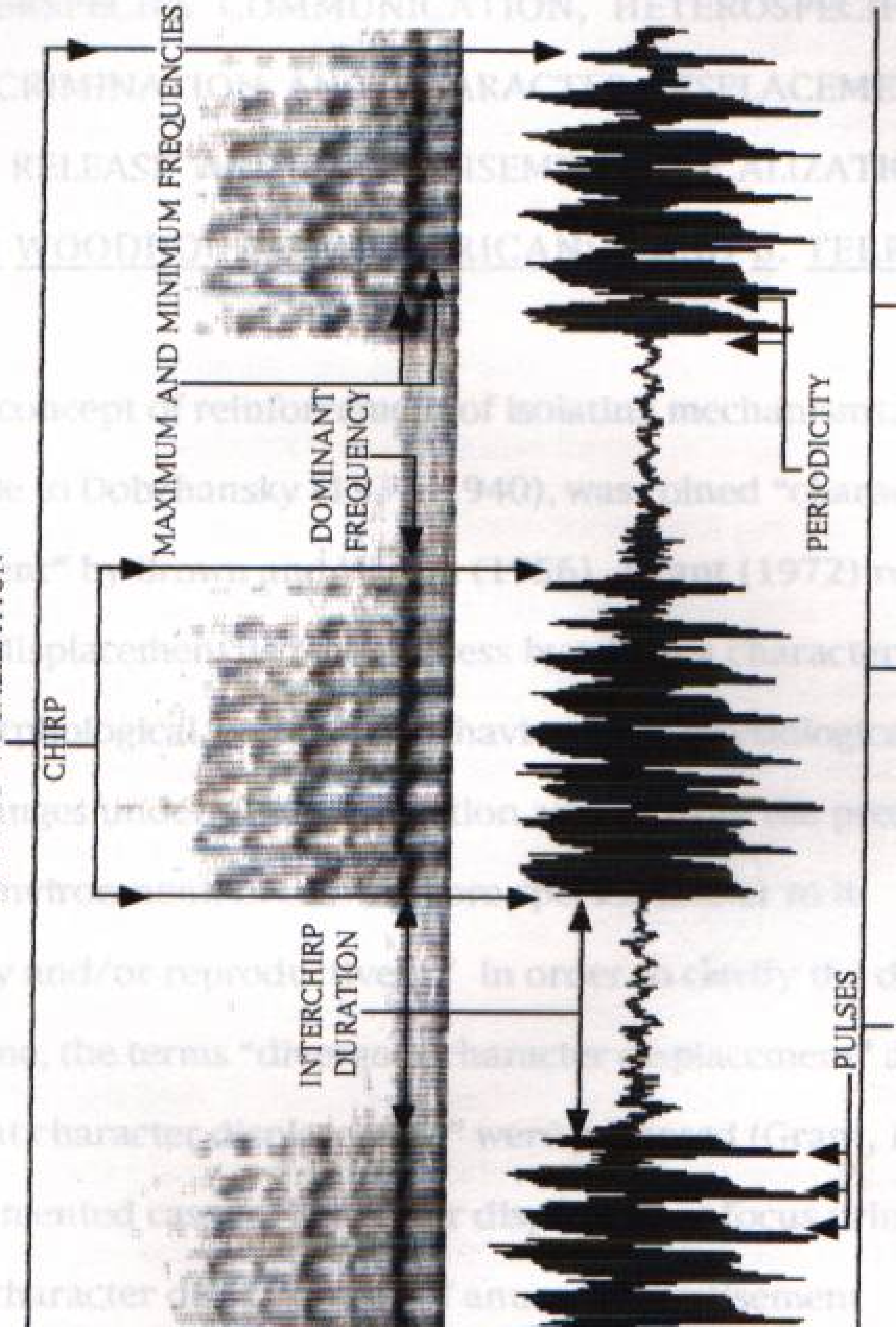
TABLE 1. Means and standard deviations (SD) for release vocalization parameters that were significantly affected (two-tailed p-values; $p < 0.05$) by artificial versus conspecific amplexus.

Parameter	Mean	SD	p-value
Average Interchirp Duration (mS)			
Artificial	133.5	35.0	
Conspecific	96.8	34.0	0.0001
Range of Frequency (kHz) First Chirp			
Artificial	0.80	0.14	
Conspecific	0.74	0.14	0.01
Minimum Frequency (kHz) Middle Chirp			
Artificial	0.92	0.13	
Conspecific	0.97	0.16	0.003
Minimum Frequency (kHz) Last Chirp			
Artificial	0.90	0.12	
Conspecific	0.96	0.14	0.0007

FIGURE LEGENDS

FIG. 1. Release vocalization of B. americanus (A. Spectrograph, B. Waveform) illustrating the parameters measured. Recorded at Beaver Lake, Onondaga County, NY (SVL = 75 mm, 20°C).


A
10
5
kHz
1000
B
0
-1000
mV
mS
0
200
400
600
RELEASE VOCALIZATION
CHIRP
INTERCHIRP DURATION
MAXIMUM AND MINIMUM FREQUENCIES
DOMINANT FREQUENCY
PULSES
PERIODICITY

II. INTERSPECIES COMMUNICATION, HETEROSPECIFIC SEX DISCRIMINATION AND CHARACTER DISPLACEMENT OF THE RELEASE AND ADVERTISEMENT VOCALIZATIONS OF BUFO WOODHOUSII, B. AMERICANUS AND B. TERRESTRIS

The concept of reinforcement of isolating mechanisms, attributable to Dobzhansky (1937, 1940), was coined "character displacement" by Brown and Wilson (1956). Grant (1972) redefined character displacement as "the process by which a character state [either morphological, ecological, behavioural or physiological] of a species changes under natural selection arising from the presence, in the same environment, of one or more species similar to it ecologically and/or reproductively." In order to clarify the direction and outcome, the terms "divergent character displacement" and "convergent character displacement" were proposed (Grant, 1972).

Documented cases of character displacement focus primarily on divergent character displacement of anuran advertisement vocalizations (Gerhardt, 1994) [e.g.; Gastrophryne (W.F. Blair, 1955a; Loftus-Hills and Littlejohn, 1992); Bufo, Hyla, Acris and Scaphiopus (Blair, 1962, 1974; Littlejohn, 1965; Gerhardt, 1994); Pseudacris

(Fouquette, 1975); Litoria (Littlejohn, 1976; Gerhardt and Davis, 1988); Bombina (Sanderson, et al., 1992)]. However, character displacement with respect to anuran release vocalizations has not previously been investigated. This study examines advertisement and release vocalizations of allopatric and sympatric populations of B. woodhousii, B. americanus and B. terrestris with the premise that the two signals have disparate inter/intraspecies communicatory functions, thus, are subject to selectional forces acting in opposing directions. Advertisement vocalizations serve as a premating isolation mechanism, therefore, are subject to divergent character displacement (Blair, 1955). Release vocalizations, on the other hand, function in prevention of prolonged amplexus among mismatched male-male conspecifics and heterospecifics (Aronson, 1944; Blair, 1947; Bogert, 1960; Brown and Littlejohn, 1972), therefore, are subject to convergent character displacement.

Bufo woodhousii, B. americanus and B. terrestris belong to an assemblage of six closely related species (the "Bufo americanus group") based upon artificial hybridization experiments (Blair, 1963a, 1963b). Isolating mechanisms among sympatric members of this group (e.g.; structural and temporal preferences in breeding sites, call discrimination, genetic compatibility, morphological differences and chemoreceptory cues) are apparently insufficient in preventing genetic exchange between species (Blair, 1941, 1942, 1955; Blair, 1956, 1958, 1972a, 1972b; Cory and Manion, 1955; Lowe, 1954; Mount, 1975; Volpe, 1952, 1959; Weatherby, 1982). Geographic ranges of B. americanus, B. woodhousii and B. terrestris are distributed such that distinct allopatric populations exist for each species (Fig. 1, PT I). Sympatric populations are distributed in a manner where B. woodhousii overlaps with both B. americanus and B. terrestris. However, B. americanus and B. terrestris remain parapatric east of the Mississippi-Alabama border (Fig. 1, PT I). The lack of structural and temporal isolation at breeding sites accompanied by distinct allopatric and sympatric distribution patterns provide an ideal basis for an investigation of geographic variation of advertisement and release vocalizations of these species.

The importance of advertisement vocalizations as premating isolating mechanisms for anurans and premise for reinforcement (divergent character displacement) of the signal in zones of sympatry is well established and well not be reviewed (see Blair, 1974). However, the absence of investigations regarding character displacement of anuran release vocalizations necessitates its review.

Male anurans produce release calls in response to male-male mating attempts (Aronson, 1944; Blair, 1947; Bogert, 1960; Brown and Littlejohn, 1972). Calls consist of a chirp (acoustic component) accompanied by vibrations of the flanks (mechanical component) (Aronson, 1944; Blair, 1947; Bogert, 1960; Brown and Littlejohn, 1972). Production of release calls by an amplexed male anuran signals the amplexing conspecific and/or heterospecific to dismount (Aronson, 1944; Blair, 1947; Bogert, 1960; Brown and Littlejohn, 1972). Although release calls are most commonly exhibited by male anurans during male-male amplexus, spent females also produce such calls (Bogert, 1960; Duellman and Trueb, 1986). Therefore, release calls apparently signal an inability to reproduce with the amplexing sex (Bogert, 1960; Duellman and Trueb, 1986).

Female bufonids, in response to male advertisement vocalizations, will approach and subsequently "nudge" a selected mate, thereby initiating amplexus (Bogert, 1960; Blair, 1963c). This tactile stimulus is a powerful releaser for male bufonids. In fact, Bogert (1960) indicated that the amplexus response could be induced by "nudging" a toad with virtually any object. The ease of eliciting the amplexus response and high concentrations of bufonid species at breeding aggregations often result in heterospecifically amplexed pairs (Blair, 1958). Naturally occurring conspecific and heterospecific amplexus imply that it would be advantageous for the release mechanism to be understood within species as well as between species in sympatric conditions (Blair, 1947). Failure of the release mechanism to be understood between sympatric species would result in prolonged amplexus, thus reducing time available for breeding opportunities for male anurans (Brown and Littlejohn, 1972). Blair (1968) assumed that rapid release of an amplexing male is also advantageous in conserving energy, preventing gametic wastage and reducing exposure to predation.

Blair (1947) reported that although males of most toad species generally responded appropriately (i.e. ceasing amplexus) to release vocalizations/vibrations of heterospecifics, release signals of B. woodhousii were ineffective in initiating amplexing B. valliceps to dismount. Sullivan and Wagner (1988) found that B. valliceps males clasped 11 different B. woodhousii males in natural breeding aggregations and "remained amplexed for 1-24 hours in spite of vigorous attempts by the signaling B. woodhousii males to escape". Similar findings have been reported by Rugh (1935) Orton (1951) and Thornton (1955). This lack of an appropriate dismount response suggests that release vocalizations/vibrations of more distantly related heterospecifically amplexed pairs are not as effective in promoting "release" as they are for more closely related species (Blair, 1947).

As a result of the dual (acoustimechanical) nature of release calls, the importance of the signals relative to the receiver (conspecific or heterospecific) has been controversial (Aronson, 1944; Bogert, 1960; Rand, 1988). Aronson (1944) found that release vibrations alone were sufficient to initiate the dismount response. He suggested that release vocalizations reinforced the vibratory component. In other words, release of an amplexing male may be more rapid when the two signals accompany one another. Aronson (1944) based this assumption on Yerkes' (1905) work which revealed that sound stimuli modify tactile stimuli in frogs: anurans responded to touch "more violently" if they first heard a sound. Therefore, the acoustic component of the release call is probably an important signal in initiating dismount (Savage, 1934; Aronson, 1944; Bogert, 1960; Rand, 1988). Consequently, release vocalizations should be subject to selective pressure to become more similar and therefore recognizable by other species in zones of sympatry (Rand, 1988).

Blair (1968) stated that "the release call would be expected to be under no functional pressure to differentiate in sympatric species and would differentiate only inasmuch as it had the same mechanisms of production as the mating call, which is under such pressure." Similarly, Brown and Littlejohn (1972) suggested that there should be uniformity in release call structure among sympatric species. They reasoned that release calls do not function as an isolating mechanism or sex attractant, as do advertisement vocalizations, and should not be exposed to the same divergent selective pressures. Consequently, the release call should be "conservative in its evolution" (Brown and Littlejohn, 1972). Barrio (1964) found that advertisement vocalizations of Odontophrynus occidentalis (Leptodactylidae) were pulsed while those of O. americanus, a sympatric species, were unpulsed. However, release calls of both species in sympatry were unpulsed and similar (Barrio, 1964). Similarly, Brown and Littlejohn (1972) determined that release calls are less divergent than advertisement vocalizations among species comprising the B. americanus group. However, release calls of sympatric species have not been compared to conspecific release calls in allopatry. Therefore, it is not known whether similarities in release vocalizations were the result of "evolutionary conservativeness", as proposed by Blair (1968) and Brown and Littlejohn (1972), or convergent character displacement.

Advertisement vocalizations of these three species have previously been shown to exhibit divergent character displacement (Blair,1962). However, I reinvestigated advertisement vocalizations of these species to insure that instances of convergent character displacement, with respect to release vocalizations, were not the result of hybridization. Grant (1972) contended that "convergence" of character states of sympatric species often result from hybridization and, therefore, are frequently misinterpreted. Convergent character displacement of advertisement vocalizations of B. woodhousii, B. americanus and B. terrestris would be expected to occur only if there was extensive hybridization among sampled populations. Therefore, convergent character displacement of release vocalizations was expected to be accompanied by divergent character displacement or no change in advertisement vocalizations.

CHAPTER II, PART I

BUFO WOODHOUSII AND B. AMERICANUS

METHODS

Sampling and Recording

A total of 253 B. woodhousii and B. americanus were recorded for release vocalizations during the breeding periods in 1994, 1995 and 1996. For B. woodhousii, 46 individuals were sampled from five allopatric localities in Lee County, Alabama, 59 were sampled from six localities of sympatry with B. americanus in Chambers and Lee Counties, Alabama. For B. americanus, 63 individuals were sampled from six allopatric localities in central New York State and 85 were sampled from seven localities of sympatry with B. woodhousii from southern Pennsylvania to central Alabama (Table 1, Fig. 1). Advertisement vocalizations were recorded from 143 individuals (38 allopatric B. woodhousii, 42 sympatric B. woodhousii, 21 allopatric B. americanus and 42 sympatric B. americanus) representing a subset of total individuals sampled for release vocalizations (Table 1).

In order to evoke the release call response, adult advertising male toads were gently grasped behind the forelimbs (a standard method proposed by Boulenger, 1897) and held adjacent to a microphone (within 20 mm) while a series of release vocalizations were recorded. Natural male-male amplexus was not used because of the difficulty involved in inducing conspecific/heterospecific amplexus. Additionally, recordings of naturally occurring release vocalizations were often "noisy" due to advertisement vocalizations of nearby anurans, thus hindering accurate spectrographic analyses. Advertisement vocalization recordings were made by placing a microphone within 30 cm of advertising males.

Both species were observed calling from land and/or water and were assumed to be in thermal equilibrium with their surroundings (see Fouquette, 1980). Therefore, temperature of the medium in which advertisement calls were being exhibited (air and/or water) was recorded for all toads to the nearest 1.0°C. Snout-vent length (SVL) of each individual was also recorded to the nearest 1.0 mm.

Vocalizations were recorded using a Uher 4000 Report IC open-reel recorder (recording speed 19 cm/s), Ampex 631 1.5 MIL polyester 1/4" magnetic tape and a Uher Dynamic Microphone (Model M136). All vocalizations were analyzed using Canary 1.1.1 Cornell Bioacoustics software (default settings).

Spectrographic Analyses

Release Vocalizations

Release vocalizations typically consisted of a series of 3 or more chirps and frequently exhibited variation in conformation among chirps. Therefore, measurements were made on the first chirp, middle chirp (one of two middle-most chirps for even-numbered chirp vocalizations) and the last chirp for each vocalization. The following twenty parameters were measured from spectrographs and waveforms: interchirp duration (mS, averaged over all chirps per vocalization), chirp duration (mS) of the first, middle and last chirp (averaged per vocalization), dominant (midpoint) frequency (kHz) per chirp, maximum and minimum frequency (kHz) per chirp, range of frequency (difference between maximum and minimum) per chirp (kHz), number of pulses per chirp and the periodicity (mS) of all pulses averaged per chirp. A single pulse was considered any amplitude modulation where the depth of the amplitude fell to 50% or less before the onset of the subsequent pulse (see Gerhardt and Davis, 1988). Periodicity was measured from the onset of rise in amplitude of one pulse to the onset of rise in amplitude of the subsequent pulse (see Fig. 2 for illustration of release call parameters).

Advertisement Vocalizations

Number of pulses, periodicity (mS), dominant (midpoint) frequency (kHz), maximum and minimum frequencies (kHz) and range of frequency (kHz, difference between maximum and minimum frequencies) were calculated from a middle 192 mS segment (the largest segment that allowed clear resolution of periodicity) of the advertisement vocalization (Fig. 3). Pulses and periodicity of advertisement vocalizations were measured in the same manner described for release vocalizations.

Temperature Correction

Recording temperatures ranged from 16 to 24°C for allopatric B. woodhousii; 8 to 20°C for allopatric B. americanus; 18 to 26°C for sympatric B. woodhousii and 10 to 23°C for sympatric B. americanus.

Simple linear regression indicated that interchirp duration, chirp duration, number of pulses per chirp, periodicity, range of frequency and maximum frequency of release vocalizations varied significantly with effective temperature ($p \leq 0.05$) for at least one chirp for one or both species in allopatry and/or sympatry (Table 2). Dominant frequency of the last chirp was significantly correlated with temperature ($p = 0.02$) and dominant frequency of the first chirp was marginally significantly correlated with temperature

($p = 0.07$) when allopatric and sympatric B. woodhousii samples were combined. There were significant correlations ($p \leq 0.05$) for the effect of temperature on all advertisement call parameters for at least one species in either sympatry or allopatry (Table 3).

Separate regression coefficients were calculated for the effects of temperature on all release and advertisement vocalization parameters for each species in allopatry and in sympatry. Regression coefficients were then used to adjust all parameters to the grand mean recording temperature of release vocalizations (21°C) using the method of linear least-squares (Littlejohn, 1965).

SVL Correction

The mean SVL of individuals sampled for release vocalizations was 74±5 mm for allopatric B. americanus; 66±7 mm for sympatric B. americanus; 57±5 mm for allopatric B. woodhousii and 56±4 mm for sympatric B. woodhousii. Analysis of variance revealed displacement with respect to SVL of adult vocalizing toads. Allopatric B. americanus were significantly larger than sympatric B. americanus ($p = 0.0001$). Allopatric B. woodhousii were also significantly larger than sympatric B. woodhousii ($p = 0.02$). Additionally, allopatric B. americanus were significantly larger than allopatric B. woodhousii ($p = 0.0001$) and sympatric B. americanus were significantly larger than sympatric B. woodhousii ($p = 0.0001$). Therefore, statistically significant differences in SVL among allopatric and sympatric samples indicated a net shift that resulted in a greater degree of similarity between sympatric species (i.e.; difference in mean SVL between species in allopatry was 17mm, whereas difference in mean SVL between species in sympatry was only 10mm). Analysis of variance indicated that mean SVL for the subset of individuals sampled for advertisement vocalizations was not significantly different from mean SVL of total individuals sampled for release vocalizations.

SVL has previously been shown to have a significant effect on bufonid vocalizations (McAlister, 1961; Zweifel, 1968; Brown and Littlejohn, 1972), therefore, potentially influence character displacement (Loftus-Hills and Littlejohn, 1992). Simple linear regression, plotting the adjusted values for the effects of temperature against SVL, indicated that SVL had a significant effect ($p \leq 0.05$) on number of pulses, periodicity, dominant frequency and minimum and maximum frequencies for at least one chirp within the release vocalizations for one or both species in allopatry and/or sympatry (Table 2). SVL also had a significant effect ($p \leq 0.05$) on dominant frequency and maximum and minimum frequencies of the advertisement vocalizations for one or both species in allopatry and/or sympatry (Table 3). Periodicity of advertisement vocalizations was significantly correlated with SVL for B. americanus when allopatric and sympatric samples were combined ($p = 0.04$).

To eliminate pleiotropic effects of SVL on release vocalizations, separate regression coefficients were calculated for the effects of SVL on temperature-adjusted values for the number of pulses, periodicity, dominant frequency and minimum and maximum frequencies for each species in allopatry and in sympatry. Regression coefficients were then used to adjust vocalizations to the mean SVL of each species (B. americanus = 69 mm; B. woodhousii = 56 mm) using the method of linear least-squares. Interchirp duration, chirp duration and range of frequency were not significantly affected by SVL ($p > 0.05$) and were not adjusted. All advertisement vocalization parameters, with the exception of the range of frequency, were adjusted to the mean SVL of the total number of individuals sampled for release vocalizations using the same methods. Instances of character displacement that resulted from pleiotropic effects of body size were detected by examining differences between significance levels calculated in analyses of variance using values adjusted for temperature alone versus those adjusted for both temperature and SVL.

The mean, standard deviation, variance and minimum and maximum values of release and advertisement vocalizations corrected for temperature alone and both temperature and SVL for B. americanus and B. woodhousii in allopatry and sympatry are listed in Appendices I and II respectively.

Statistical Analysis

Analyses of variance were performed on all release vocalization parameters for the first, middle and last chirps and all advertisement vocalization parameters adjusted for temperature alone and adjusted for both temperature and SVL. Data for each species were combined for allopatric populations and compared to the combined data for sympatric populations. The following ANOVA comparisons were made to test for character displacement:

1) allopatric B. americanus versus sympatric B. americanus,
2) allopatric B. woodhousii versus sympatric B. woodhousii,
3) allopatric B. americanus versus allopatric B. woodhousii, and
4) sympatric B. americanus versus sympatric B. woodhousii.

Statistically significant instances of convergent character displacement required the following pattern (adopted from Grant, 1972):

A) Sympatric vocalizations of one or both species were significantly displaced from conspecific allopatric vocalizations. Significant differences with respect to both species indicated bilateral convergent character displacement. Significant differences with respect to one species, but not the other, indicated unilateral convergent character displacement.

B) Vocalizations of allopatric B. americanus were significantly different from the vocalizations of allopatric B. woodhousii.

C) Vocalizations of the species in sympatry were not significantly different from each other.

Statistically significant instances of divergent character displacement required that the antithesis of "B" and "C" occur. Vocalization parameters that were significantly displaced from the allopatric character state for one or both species but did not indicate divergent or convergent character displacement (i.e.; were significantly different for all comparisons) were uninformative and required further investigation. Box plots were utilized for this purpose. Parameters whose mean distributions approached one another so as to result in a greater degree of overlap of quantile distributions in sympatry indicated convergent trends. The antithesis indicated a divergent trend. The absence of significant displacement of either species from its allopatric character state precluded the existence of character displacement (see Grant, 1972). Therefore, only those parameters where one or both species were significantly displaced from their allopatric character state were examined for trends.

Parameters that were significantly different for comparisons between the allopatric and sympatric character states of the species corrected for temperature only, but were not significantly different in the same comparison when adjusted for both temperature and SVL indicated that displacement resulted from pleiotropic effects of SVL. Vocalization parameters that indicated the reciprocal were not reliable indicators of character displacement. Results that followed this pattern indicated that displacement occurred only when allopatric and sympatric B. americanus and/or B. woodhousii were adjusted to a common SVL. However, mean SVL of the species in allopatry was significantly different from that in sympatry (i.e.; allopatric B. americanus and B. woodhousii were significantly larger than sympatric conspecifics).

The extent of the geographic distribution of sampled populations potentially influenced instances of character displacement due to clinal variability (see Grant, 1972). Box plots of sampled populations arranged from the southern-most to the northern most population were used to detect clinal variation .

Statistical analyses were performed utilizing JMP IN version 3 (SAS Institute Inc.) and SuperANOVA (Abacus Concepts, Inc.) programs.

RESULTS

Release Vocalizations

Release vocalizations of sympatric B. woodhousii were not significantly different from allopatric B. woodhousii when corrected for temperature alone or when corrected for both temperature and SVL (Table 4, columns 3 and 4). Therefore, B. woodhousii was unaffected by the presence of B. americanus. Hence, any indication of character displacement was due to the unilateral character shift of B. americanus.

Convergent Character Displacement

One parameter, dominant frequency of the middle chirp adjusted for temperature, indicated a statistically significant case of unilateral convergent character displacement (Table 4, columns 1,3,5 and 7; Fig. 4). Convergence was not solely due to displacement of B. americanus. Apparently, vocalizations of sympatric B. woodhousii were much more variable, accounting for a shift in mean distribution (Fig. 4). Unilateral convergent character displacement of dominant frequency of the middle chirp did not remain statisitically significant when corrected for both temperature and SVL (i.e.; allopatric B. americanus was not significantly different from sympatric B. americanus [Table 4, column 2; Fig. 4]). Therefore, neither species diverged from its allopatric character state indicating that convergence of dominant frequency of the middle chirp resulted from pleiotropic effects of body size (Fig. 4).

Divergent Character Displacment

There were three statistically significant instances of unilateral divergent character displacement. Those parameters included number of pulses for the first, middle and last chirps adjusted for temperature (Table 4, columns 1,3,5 and 7; Figs. 5, 6 and 7 respectively). Number of pulses for the middle and last chirps did not remain significantly different between allopatric B. americanus and allopatric B. woodhousii when adjusted for both temperature and SVL (Table 4, compare columns 5 and 6). Therefore, those parameters did not remain statistically significant cases of divergent character displacement. Number of pulses of the first chirp remained a statistically significant case of unilateral character divergence when corrected for both temperature and SVL (Table 4; Fig. 5).

Displacement Trends

Convergent Character Displacement. Box plots of the following parameters indicated a unilateral convergent trend when corrected for temperature; average chirp duration (Fig. 8), dominant frequency of the first and last chirps (Figs. 9 and 10), and maximum frequency of the first and last chirps (Figs. 11 and 12). In these instances, there may be a tendency to conclude that there was bilateral displacement with no net change in comparisons between allopatric B. americanus versus allopatric B. woodhousii and/or sympatric B. americanus versus sympatric B. woodhousii. However, in each instance, allopatric B. americanus was significantly displaced from sympatric B. americanus (represented by the 95% confidence intervals of the mean distributions of the diamonds)(see Table 4, columns 1 and 2). Therefore, there was a net character shift in mean distributions of B. americanus to become more similar to B. woodhousii, resulting in greater degrees of quantile distribution overlap in sympatry.

Maximum frequency of the last chirp indicated a unilateral convergent trend when corrected for temperature only and continued to indicate a unilateral convergent trend when corrected for both temperature and SVL (Table 4, compare columns 1,5 and 7 to columns 2, 6 and 8; Fig. 12). Dominant frequency of the first and last chirps, and maximum frequency of the first chirp did not remain significantly different between allopatric B. americanus and sympatric B. americanus when corrected for both temperature and SVL, thereby indicating convergence due to pleiotropic effects of SVL (Table 4, columns 1 and 2; Figs. 9, 10 and 11 respectively).

Divergent Character Displacement. Although number of pulses for the middle and last chirps were no longer statistically significant instances of unilateral divergent character displacement when corrected for both temperature and SVL, box plots revealed that both continued to show a divergent trend (Figs. 6 and 7).

Clinal Variation

Box plots were arranged from the southern-most to the northern-most B. americanus population for the 9 temperature adjusted parameters that indicated character displacement. Three parameters (chirp duration and number of pulses in the first and middle chirps) indicated abrupt shifts between allopatry and sympatry (e.g.; number of pulses of the middle chirp, Fig. 13). The remaining parameters did not indicate clinal variation.

"Hybrid" Vocalizations

Release vocalizations of some B. americanus were "hybrid" in conformation. In other words, they were comprised of several characteristic shapes (Fig. 14C). Components of the release vocalization that were typical for B. woodhousii appeared to be incorporated into the repertoire of B. americanus (Fig. 14). Alabama populations did not exhibit hybrid vocalizations and only a single B. americanus from the Georgia population produced hybrid calls. Hybrid calls were found in approximately 17% of sympatric populations of B. americanus outside of Alabama and Georgia and were as frequent in allopatric populations of B. americanus.

Advertisement Vocalizations

Advertisement vocalizations of sympatric B. woodhousii were significantly displaced from allopatric B. woodhousii with respect to minimum frequency (Table 5, columns 3 and 4). Dominant frequency, range of frequency and minimum and maximum frequencies of sympatric B. americanus were significantly displaced from the allopatric character state (Table 5, columns 1 and 2). Therefore, both species appeared to be affected by the presence of the other creating the potential for either unilateral or bilateral character displacement.

Character Displacement

Range of frequency of advertisement vocalizations was the only statistically significant case of convergent character displacement (Table 5; Fig. 15). In this instance, B. americanus was displaced from its allopatric state, B. woodhousii was not (Table 5; Fig 15). These results indicate that B. americanus exhibits a more similar range of frequencies with B. woodhousii in sympatry. However, it did not indicate that the two species necessarily overlapped with respect to dominant, minimum or maximum frequencies and was therefore biologically insignificant without further investigation. Analyses of variance results for dominant, minimum and maximum frequencies were non-informative (Table 5) and required further analyses to ascertain convergent/divergent trends.

Displacement Trends

Box plots of dominant frequency adjusted for the effects of temperature alone and both temperature and SVL indicated a unilateral convergent trend resulting from a significant character shift of sympatric B. americanus from its allopatric character state (Fig. 16). Minimum frequency indicated a bilateral convergent trend when corrected for temperature alone and both temperature and SVL (Fig 17). Maximum frequency, adjusted for the effects of temperature, indicated a unilateral convergent trend resulting from a significant character shift of sympatric B. americanus from its allopatric character state (Fig. 18). When corrected for both temperature and SVL maximum frequency was bilaterally convergent (Fig.18). Quantile distributions of box plots for dominant, minimum, and maximum frequencies revealed that these parameters were distinct where the species were allopatric but began to overlap in zones of sympatry (Figs. 16,17 and 18). Therefore, statistically significant convergent character displacement of range of frequency of advertisement vocalizations was reinforced (Fig. 15).

Clinal Variation

Dominant frequency, range of frequency, and minimum and maximum frequencies of B. americanus advertisement vocalizations indicated abrupt shifts in sympatry (i.e.; dominant frequency, Fig. 19). There was no indication of clinal variation.

DISCUSSION

Statistically significant differences in mean SVL of adult vocalizing allopatric and sympatric B. americanus played an important role in displacement of release vocalizations. A total of six release vocalization parameters, including dominant frequency of the first, middle and last chirps, chirp duration and maximum frequency of the first and last chirps, indicated unilateral convergent character displacement. Dominant frequency of all chirps and maximum frequency of the first chirp were convergent due to pleiotropic effects of SVL. Pleiotropic effects of SVL also significantly affected divergent character displacement of the number of pulses in the middle and last release chirps. Statistically significant convergent character displacement of the range of advertisement vocalization frequency resulted in overlapping minimum, maximum and dominant frequencies of the species in sympatry. However, advertisement vocalizations were not affected by differences in SVL. To my knowledge, there is no other evidence confirming pleiotropic effects of SVL account for character displacement in anuran vocalizations.

Loftus-Hills and Littlejohn (1992) reexamined advertisement vocalizations of Gastrophryne carolinensis and G. olivacea to determine the effects of body size (SVL) on divergent character displacement of the vocalizations documented by Blair (1955a). Blair (1955b) reported a clinal trend in SVL's of mature G. carolinensis and G. olivacea but did not determine correlations between SVL and measured vocalization parameters (Blair,1955a). Volpe (1957) suggested that Blair's (1955a) findings may have been the result of indirect pleiotropic effects of body size on vocalizations. However, Loftus-Hills and Littlejohn (1992) found no evidence to support the suggestions of Volpe (1957) in their reexamination of Blair's (1955a) work.

Blair (1947) and Bogert (1960) commented on an apparent overall trend among bufonids where differences in species sizes accounted for differences in release vocalizations (i.e. pulse rates). Differences I report for SVL among sympatric and allopatric B. americanus and B. woodhousii support a convergent trend with respect to body size that theoretically upholds selection for convergence of release vocalizations over divergence of advertisement vocalizations. It seems reasonable to assume that there is a selective trade-off with respect to the two vocalizations. In one instance selection should favor convergence of release vocalizations to facilitate interspecies communication and prevent prolonged amplexus among mismatched heterospecifics. On the other hand, selection should favor divergence of advertisement vocalizations in order to prevent hybridization. Assuming the two vocalizations are not totally independent of one another (i.e. the release vocalization repertoire is to some extent influenced by the advertisement vocalization [Blair, 1968; Bogert, 1960; Martin, 1971; Brown and Littlejohn, 1972]), it is not surprising that there was no clear convergent pattern with respect to release vocalizations if isolation mechanisms (i.e. advertisement vocalizations) among the species are selectively "more important". However, if divergence of advertisement vocalizations were more important I would expect a coinciding divergent trend with respect to SVL among species where they are sympatric. In fact, Lowe (1954) reported that size alone acts as an isolating mechanism in some anurans. This does not appear to be the case here. In fact, adult vocalizing sympatric B. americanus (mean SVL = 66 mm) were more similar in size to sympatric B. woodhousii (mean SVL = 56 mm) than allopatric B. americanus (mean SVL = 74 mm) were to allopatric B. woodhousii (mean SVL = 57 mm). Although Blair (1941) did not find any geographical gradients with respect to body size (SVL) of B. woodhousii and B. americanus, he did not test for character displacement.

ANOVA results indicated statistically significant convergent character displacement of advertisement vocalization frequency ranges that were shown to overlap with respect to dominant, minimum and maximum frequencies in zones of sympatry. These findings indicate hybridization between B. americanus and B. woodhousii. However, I conclude that this explanation is unlikely for the following reasons. Hybridization should result in bilateral character displacement. However, all character shifts in this study resulted in unilateral displacement of B. americanus. Additionally, B. americanus X B. woodhousii hybrids were not observed in extensive collection and observations of these species in Alabama (Mount, 1975) or Georgia (Neill, 1949).

Blair (1941), Volpe (1952), Cory and Manion (1955), Zweifel (1968) and Green (1984) all reported naturally occurring hybridization between B. americanus and B. woodhousii outside of Alabama. Apparently, species isolation mechanisms are maintained in Alabama but breakdown in other sympatric regions. Therefore, it is possible that displacement of release vocalizations of B. americanus, and not B. woodhousii, resulted from differences in sampled ranges.-Bufo woodhousii were collected only in Alabama where there is no evidence of hybridization. However, B. americanus was collected from Alabama to New York State (Fig. 1); a range that includes regions where natural hybrids reportedly occur. "Intermediate" advertisement vocalizations are readily detectable in hybrid-crosses between these species and were not recorded on rare instances they were encountered in this study. However, Blair (1941) found that introgrades from various crosses of these species produced viable offspring. Thus, the effects of introgression may render the detection of hybridization difficult and cannot be eliminated as a possible source of variation in this study.

Zweifel (1968) stated that the pulse rate (periodicity) of *B. americanus* and *B. woodhousii* advertisement vocalizations are a more "sensitive indicator of hybridization than morphology." Although Blair (1962) reported divergent character displacement with respect to advertisement pulse rates of these species, I did not find displacement with respect to this parameter. Weatherby (1982) analyzed external morphology and pulse rates of advertisement and release calls of *B. americanus* and *B. woodhousii* throughout much of Alabama and also reported no evidence of hybridization.

"Hybrid" release vocalizations of *B. americanus* provide evidence for introgression. Hybrid vocalizations were detected in a single *B. americanus* collected from Georgia and were not seen in specimens from Alabama. Hybrid vocalizations were equally common in northern sympatric populations and allopatric populations. Thus, these results agree, in part, with the differing accounts of hybridization in various regions reported by Blair (1941), Volpe (1952), Cory and Manion (1955), Zweifel (1968), Mount (1975), Weatherby (1982) and Green (1984) .

"Hybrid" release vocalizations of B. americanus are, to my knowledge, previously undocumented. The reason for the existence of hybrid calls in allopatric B. americanus can only be speculated upon. Fossil evidence and geographic histories are generally inadequate to ascertain specified geographic shifts in the boundaries of these species over time (Blair, 1963a, 1972a; Tihen, 1972). If these vocalizations resulted from hybridization or introgression between the species they do not represent the characteristic "intermediacy" previously reported in anurans. Brown and Littlejohn (1972) determined that pulse rates (periodicity) of release vocalizations were the most useful criterion for distinguishing hybrids. Brown and Guttman (1970) and Brown and Littlejohn (1972) found that hybrid B. arenarum x B. spinulosus and hybrid B. woodhousii x B. houstonensis respectively, possessed release call pulse rates that were intermediate between parental species. Sullivan and Lamb (1988) reported similar results for hybrid B. microscaphus x B. woodhousii. Spectrographic analyses of release vocalizations in this study also indicated intermediacy with respect to pulse rates. However, pulses were distributed at nonuniform rates. In many instances, "typical" intermediacy may have occurred, but in other instances waveform analyses indicated that pulse rates were partitioned into segments that reflected one species and segments that reflected sympatric species pulse rates (Fig. 14). Apparently, B. americanus has incorporated portions of the B. woodhousii repertoire into its release vocalization. Whether this serves as an adaptation to facilitate interspecies communication or resulted from hybridization and/or introgression cannot be confirmed.

Dominant frequency and minimum and maximum frequencies of advertisement vocalizations were shown to overlap in sympatric zones whereas they remained distinct in allopatry. In the absence of hybridization, advertisement vocalizations are expected to converge only if selective pressures influencing release vocalizations to converge override selective pressures for advertisement vocalizations to diverge. If advertisement and release vocalizations are "coupled", both introgression and convergent character displacement of release vocalizations would be expected to result in "convergence" of advertisement vocalizations. Numerous investigators have revealed that hybrid anurans exhibit advertisement and release vocalizations that are intermediate with respect to parental species (Blair, 1963c; Brown and Guttman, 1970; Brown and Littlejohn, 1972; Sullivan and Lamb,1988). The effects of introgression would be expected to eventually show a decline in vocalization intermediacy after repeated backcrosses to one of the parental species (Zweifel, 1968; Mecham, 1960). Results presented in this study may be indicative of repeated introgression events.

Further analyses are necessary to determine if release vocalizations of sympatric anurans are under selective pressure to converge and the extent to which advertisement vocalizations are affected and influence release vocalizations. One possible source may include more distantly related species, such as B. woodhousii and B. valliceps. Geographic ranges of these species have distinct allopatric and sympatric demarcations (Conant and Collins, 1991) and hybrids are inviable or infertile (Blair, 1956). Additionally, there are numerous reports of heterospecific amplexus between these species (Rugh, 1935; Blair, 1947; Orton, 1951; Thornton, 1955; Blair, 1956; Sullivan and Wagner, 1988).

Results reported in this study may be attributed to introgression, however, there is contrary evidence suggesting selection for convergence of release vocalizations. Recordings of release and advertisement vocalizations of known hybrid and nonhybrid B. woodhousii and B. americanus are necessary to elucidate the effects of hybridization and introgression on vocalizations. Detailed comparisons between hybrid and nonhybrid zones may provide a clearer understanding of factors responsible for the apparent "breakdown" of premating isolation mechanisms. Further investigation may reveal that convergence of advertisement vocalizations does not significantly affect species-specific responses to conspecific advertisement vocalizations for B. americanus and B. woodhousii. Hence, "convergence" of advertisement vocalizations in zones of sympatry may result from overriding selective pressures for convergent character displacement of release vocalizations without significantly affecting species integrity. Thus, interspecies communication would be facilitated during heterospecific amplexus and interspecies isolation would be maintained.

Table 1. Locations of recording sites and sample sizes (number of individuals recorded) for release vocalizations and advertisement vocalizations of B. americanus and B. woodhousii in allopatry and sympatry. Sample sizes for advertisement vocalizations are within parentheses adjacent to release vocalization sample sizes. Populations are designated with a number that correspond with the locality numbers in Fig. 1.

Population	Species	Sample size	Condition	Locality
1	B. americanus	10(9)	allopatric	Port Byron, Cayuga Co., NY
2	B. americanus	8(8)	allopatric	Weedsport, Cayuga Co., NY
3	B. americanus	9	allopatric	Jordan, Onondaga Co., NY
4	B. americanus	10	allopatric	Beaver Lake, Onondaga Co., NY
5	B. americanus	15(4)	allopatric	Three Rivers National Wildlife Refuge, Onondaga Co., NY
6	B. americanus	11	allopatric	Phoenix, Oswego Co., NY
7	B. woodhousii	9(9)	allopatric	0.4 km E of Co. Rd. 054 on Co. Rd. 433, Auburn, Lee Co., AL
8	B. woodhousii	7(8)	allopatric	1.2 km E of Co Rds. 137 and 152 junction, Auburn, Lee Co., AL
9	B. woodhousii	13(7)	allopatric	0.8 km N of Co. Rd. 137 on Co. Rd. 053, Auburn, Lee Co., AL
10	B. woodhousii	8(12)	allopatric	3.2 km E of State Highway 147 on Glenn Ave, Auburn, Lee Co., AL
11	B. woodhousii	9(2)	allopatric	4.8 km NW of State Highway 147 on US 280, Auburn, Lee Co., AL

12	B. americanus	10(2)	sympatric	1.2 km NE of State Highway 147 on Co. Rd. 28, Lee Co., AL
13	B. americanus	13(8)	sympatric	1.6 km N of State Highway 147 on US 431, Chambers Co., AL
14	B. americanus	11(6)	sympatric	Co. Rd. 55, Chambers County Lake, Chambers Co., AL
15	B. americanus	15(9)	sympatric	Buford Dam, Gwinnett Co., GE
16	B. americanus	19(9)	sympatric	Watagua Dam, Washington Co., TN
17	B. americanus	8(8)	sympatric	New River Gorge, Summers Co., WV
18	B. americanus	9	sympatric	Waynesboro, Franklin Co., PN
19	B. woodhousii	16(11)	sympatric	1.2 km NE of State Highway 147 on Co. Rd. 28, Lee Co., AL
20	B. woodhousii	7	sympatric	Halawakee Creek, 10.4 km S of Co. Rd. 55, Chambers Co., AL
21	B. woodhousii	10(14)	sympatric	Snapper Creek, 4.8 km S of Co. Rd. 55, Chambers Co., AL
22	B. woodhousii	10(6)	sympatric	1.6 km N of State Highway 147 on US 431, Chambers Co., AL
23	B. woodhousii	7(11)	sympatric	Co. Rd. 55, Chambers County Lake, Chambers Co., AL
24	B. woodhousii	9	sympatric	3.2 km SW of US 431 on State Highway 50 Chambers Co., AL

TABLE 2. Effects of temperature (Temp) and snout-vent length (SVL) on release vocalization parameters for B. americanus and B. woodhousii in allopatry and sympatry. A " * " indicates a significant effect ($p \leq 0.05$) and a " 0 " indicates no significant effect ($p > 0.05$) in linear regression analyses. Marginally significant p-values are reported ($0.05 < p \leq 0.07$). Dom. Freq. = dominant frequency, Min. Freq. = minimum frequency and Max. Freq. = maximum frequency.

	B. americanus				B. woodhousii			
	Allopatric		Sympatric		Allopatric		Sympatric	
Call Parameter	Temp	SVL	Temp	SVL	Temp	SVL	Temp	SVL
Interchirp Duration	*	0	*	0	*	0	0	0
Chirp Duration	*	0	*	0	0	0	0	0
Pulses first chirp	*	0	0	0	0	*	0	0
Pulses mid chirp	0.06	*	0	0	0	0	0	0
Pulses last chirp	*	0	0	0	0	*	0	0
Periodicity first chirp	0	0	0	0	0	*	0	0
Periodicity mid chirp	0	*	*	*	0	0	0	0
Periodicity last chirp	0	0	0	0	0	0	0	0
Dom. Freq. first chirp	0	*	0	*	0	0	0	0
Dom. Freq. mid chirp	0	*	0	*	0	0	0	0
Dom. Freq. last chirp	0	*	0	0.07	0	0	0	0
Range first chirp	*	0	0	0	0.06	0	0	0
Range mid chirp	*	0	0	0	0	0	0	0
Range last chirp	0	0	0	0	0	0	*	0
Min. Freq. first chirp	0	0	0	*	0	0	0	0
Min. Freq. mid chirp	0	*	0	*	0	0	0	0
Min. Freq. last chirp	0	*	0	*	0	0	0	0
Max. Freq. first chirp	*	*	0	0	0.06	0	0	0
Max. Freq. mid chirp	0.06	0	0	0	0	0	0	0
Max. Freq. last chirp	0	*	0	0	0	0	*	0

TABLE 3. Effects of temperature(Temp) and snout-vent length (SVL) on advertisement vocalization parameters for B. americanus and B. woodhousii in allopatry and sympatry. A " * " indicates a significant effect ($p \leq 0.05$) and a " 0 " indicates no significant effect ($p > 0.05$) in linear regression analyses.

	B. americanus				B. woodhousii			
	Allopatric		Sympatric		Allopatric		Sympatric	
Call Parameter	Temp	SVL	Temp	SVL	Temp	SVL	Temp	SVL
Number of Pulses	*	0	*	0	*	0	*	0
Periodicity	*	0	*	0	*	0	*	0
Dominant Frequency	0	0	*	*	0	*	*	0
Range	0	0	*	0	0	0	0	0
Minimum Frequency	0	0	*	*	0	*	*	0
Maximum Frequency	0	0	*	0	0	*	*	0

TABLE 4. ANOVA results (actual p-values) comparing the release vocalizations of allopatric B. americanus versus sympatric B. americanus (Ba allo vs. Ba sym), allopatric B. woodhousii versus sympatric B. woodhousii (Bw allo vs. Bw sym), allopatric B. americanus versus allopatric B. woodhousii (Ba allo vs. Bw allo) and sympatric B. americanus versus sympatric B. woodhousii (Ba sym vs. Bw sym). "Temp" columns indicate results for parameters adjusted for temperature only (21°C) and "Temp + SVL" columns indicate results for parameters adjusted for both temperature and mean SVL (mm) (see text). A " * " indicates a significant difference ($p \leq 0.05$) and a " • " indicates no significant difference ($p > 0.05$). Empty cells indicate those parameters that were not significantly effected by SVL and were not adjusted. Dom. Freq. = dominant frequency, Min. Freq. = minimum frequency and Max. Freq. = maximum frequency.

Call Parameter	Ba allo vs. Ba sym 1 Temp	 2 Temp + SVL	Bw allo vs. Bw sym 3 Temp	 4 Temp + SVL	Ba allo vs. Bw allo 5 Temp	 6 Temp + SVL	Ba sym vs. Bw sym 7 Temp	 8 Temp + SVL
Interchirp Duration	0.7 •		0.9 •		0.009 *		0.01 *	
Chirp Duration	0.0001*		0.6 •		0.0001*		0.0001 *	
Pulses first chirp	0.004 *	0.004 *	0.4 •	0.8 •	0.3 •	0.08 •	0.0001*	0.0001*
Pulses mid chirp	0.0001*	0.0001*	0.3 •	0.09•	0.6 •	0.01 *	0.0005*	0.0005*
Pulses last chirp	0.0001*	0.02 *	0.6 •	0.3 •	0.5 •	0.009 *	0.0002*	0.0002*
Periodicity first chirp	0.6 •	0.003 *	0.09•	0.08•	0.001 *	0.6 •	0.0001*	0.0001*
Periodicity mid chirp	0.3 •	0.003 *	0.9 •	0.6 •	0.002 *	0.0001*	0.0001*	0.0001*
Periodicity last chirp	0.1 •	0.3 •	0.2 •	0.2 •	0.0001*	0.0001*	0.0001*	0.0001*
Dom. Freq. first chirp	0.003 *	1.0 •	0.4 •	0.4 •	0.0001*	0.0001*	0.0001*	0.0001*
Dom. Freq. mid chirp	0.002 *	1.0 •	0.7 •	0.7 •	0.0001*	0.0001*	0.2 •	0.2 •
Dom. Freq. last chirp	0.001 *	1.0 •	0.6 •	0.6 •	0.0001*	0.0001*	0.0001*	0.0001*
Range first chirp	0.03 *		1.0 •		0.003 *		0.0002*	
Range mid chirp	1.0 •		0.3 •		0.0001*		0.0001*	
Range last chirp	0.7 •		1.0 •		0.0001*		0.0001*	
Min. Freq. first chirp	0.0001*	0.0001*	0.2 •	0.2 •	0.0001*	0.0001*	0.0001*	0.0001*
Min. Freq. mid chirp	0.0001*	1.0 •	0.1 •	0.1 •	0.0001*	0.0002*	0.0001*	0.0001*
Min. Freq. last chirp	0.0001*	1.0 •	0.1 •	0.09•	0.0001*	0.0001*	0.0001*	0.0001*
Max. Freq. first chirp	0.05 *	1.0 •	0.6 •	1.0 •	0.0001*	0.0001*	0.0001*	0.0001*
Max. Freq. mid chirp	1.0 •	0.06 •	0.6 •	0.6 •	0.0001*	0.0001*	0.0001*	0.0001*
Max. Freq. last chirp	0.0001*	0.04 *	1.0 •	1.0 •	0.0001*	0.0001*	0.0001*	0.0001*

TABLE 5. ANOVA results (actual p-values) comparing advertisement vocalizations of allopatric B. americanus versus sympatric B. americanus (Ba allo vs. Ba sym), allopatric B. woodhousii versus sympatric B. woodhousii (Bw allo vs. Bw sym), allopatric B. americanus versus allopatric B. woodhousii (Ba allo vs. Bw allo) and sympatric B. americanus versus sympatric B. woodhousii (Ba sym vs. Bw sym). "Temp" columns indicate results for parameters adjusted for temperature only (21°C) and "Temp + SVL" columns indicate results for parameters adjusted for both temperature and mean SVL (see text). A " * " indicates a significant difference ($p \leq 0.05$) and a " • " indicates no significant difference ($p > 0.05$). Empty cells indicate those parameters that were not necessary to adjust for the effects of both temperature and SVL (see text).

Call Parameter	Ba allo vs. Ba sym		Bw allo vs. Bw sym		Ba allo vs. Bw allo		Ba sym vs. Bw sym	
	1	2	3	4	5	6	7	8
	Temp	Temp + SVL	Temp	Temp + SVL	Temp	Temp + SVL	Temp	Temp + SVL
Number of Pulses	0.8 ●	0.8 ●	0.4 ●	0.7 ●	0.0001*	0.0001*	0.0001*	0.0001*
Periodicity	0.2 ●	0.3 ●	0.3 ●	0.6 ●	0.0001*	0.0001*	0.0001*	0.0001*
Dominant Frequency	0.0001*	0.0001*	1.0 ●	1.0 ●	0.0001*	0.0001*	0.0001*	0.0001*
Range of Frequency	0.0001*		0.9 ●		0.0001*		0.06 ●	
Minimum Frequency	0.0001*	0.0001*	0.001*	0.0008*	0.0001*	0.0001*	0.0001*	0.0001*
Maximum Frequency	0.0001*	0.0001*	1.0 ●	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

FIGURE LEGENDS

FIG. 1. Map of the eastern United States depicting approximate ranges of B. woodhousii and B. americanus and B. terrestris (Conant, 1975; Mount, 1975) and sample localities. For sample sites where advertisement vocalizations were also obtained see Table1.

FIG. 2. Release vocalization of B. americanus (A. Spectrograph, B. Waveform) illustrating the parameters measured. Recorded at Beaver Lake, Onondaga County, NY (SVL = 75 mm, 20°C).

FIG. 3. Middle 192 mS segment of B. woodhousii advertisement call illustrating vocalization parameters (A. Spectrograph, B. Waveform). A total of 19 pulses are represented in the waveform. Recorded 0.4 km east of Co. Rd. 054 on Co. Rd. 433, Auburn, Lee Co., AL, 17°C, SVL = 60 mm.

FIG. 4. Box plots of the dominant frequency (kHz) of the middle chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (21°C)(A) and both temperature and SVL (mm)(B). The center lines of the means diamonds are the group means. The top and bottom of the diamonds form the 95% confidence intervals

for the means. Rectangles represent quantile boxes where the ends of the boxes are the 25th and 75th quantiles and the line across the middle of the boxes identifies the median sample value. Quantile box width reflects relative sample sizes. Whiskers represent the upper and lower extremes. The horizontal line extending the entirety of the graph indicates the combined sample mean.

FIG. 5. Box plots of the number of pulses of the first chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 6. Box plots of the number of pulses of the middle chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 7. Box plots of the number of pulses of the last chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 8. Box plots of the average chirp duration (mS) of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature. See Fig. 4 for an explanation of the box plots.

FIG. 9. Box plots of the dominant frequency (kHz) of the first chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 10. Box plots of the dominant frequency (kHz) of the last chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 11. Box plots of the maximum frequency (kHz) of the first chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 12. Box plots of the maximum frequency (kHz) of the last chirp of the release vocalization for B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 13. Box plots of the number of pulses of the middle chirp of the release vocalization for B. americanus across the sampling range adjusted for the effects of both temperature and SVL. Plots are arranged chronologically from the southern-most population (left) to the northern-most population (right). Populations 12 through 18 are sympatric with B. woodhousii and populations 1 through 6 are allopatric. Designated population numbers correspond to the localities in Table 1. See Fig. 4 for an explanation of the box plots.

FIG. 14. Spectrographs and waveforms of the release vocalizations of (A) Allopatric B. americanus (recorded from Beaver Lake, Onondaga Co., NY, temperature = 20°C, SVL = 75 mm) (B) Allopatric B. woodhousii (recorded from 0.8 km north of Co. Rd. 137 on Co. Rd. 053, Auburn, Lee Co., AL, temperature = 24°C, SVL = 55 mm) and (C) Sympatric B. americanus (recorded from Waynesboro, Franklin Co., PN, temperature = 23°C, SVL = 70 mm).

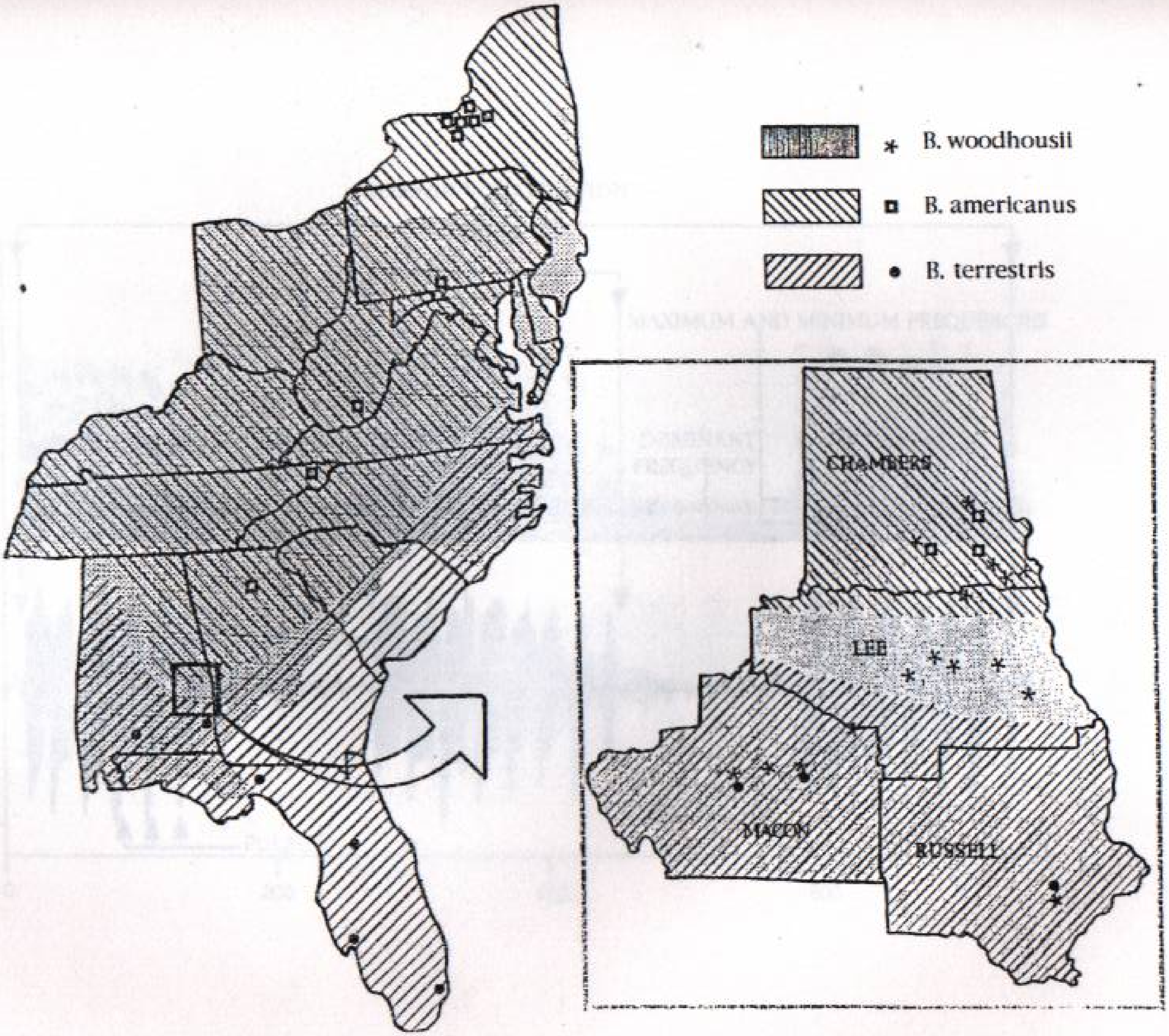
FIG. 15. Box plots of the range of frequency (kHz) of the advertisement vocalization of B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature. See Fig. 4 for an explanation of the box plots.

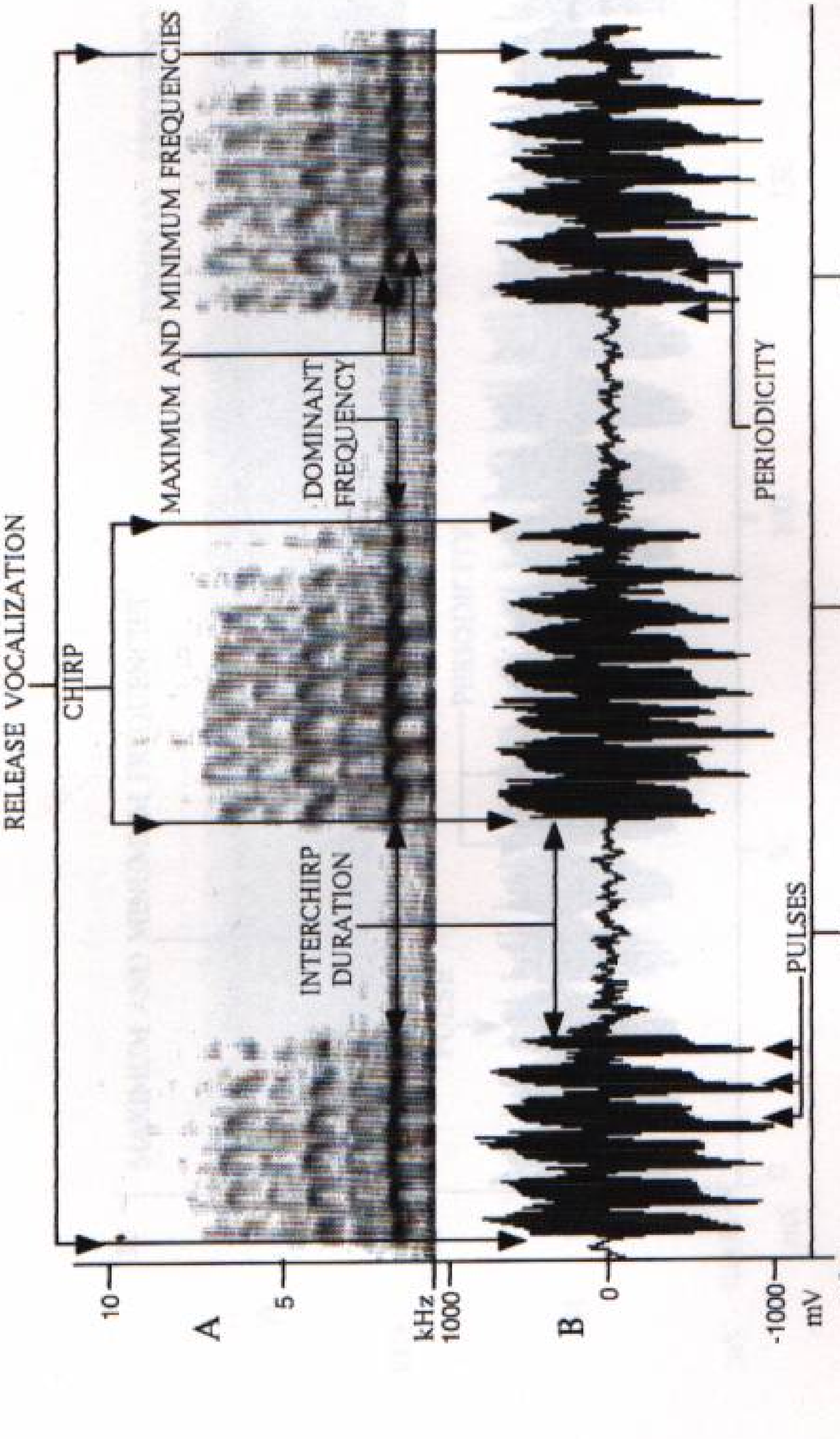
FIG. 16. Box plots of the dominant frequency (kHz) of the advertisement vocalization of B. americanus and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

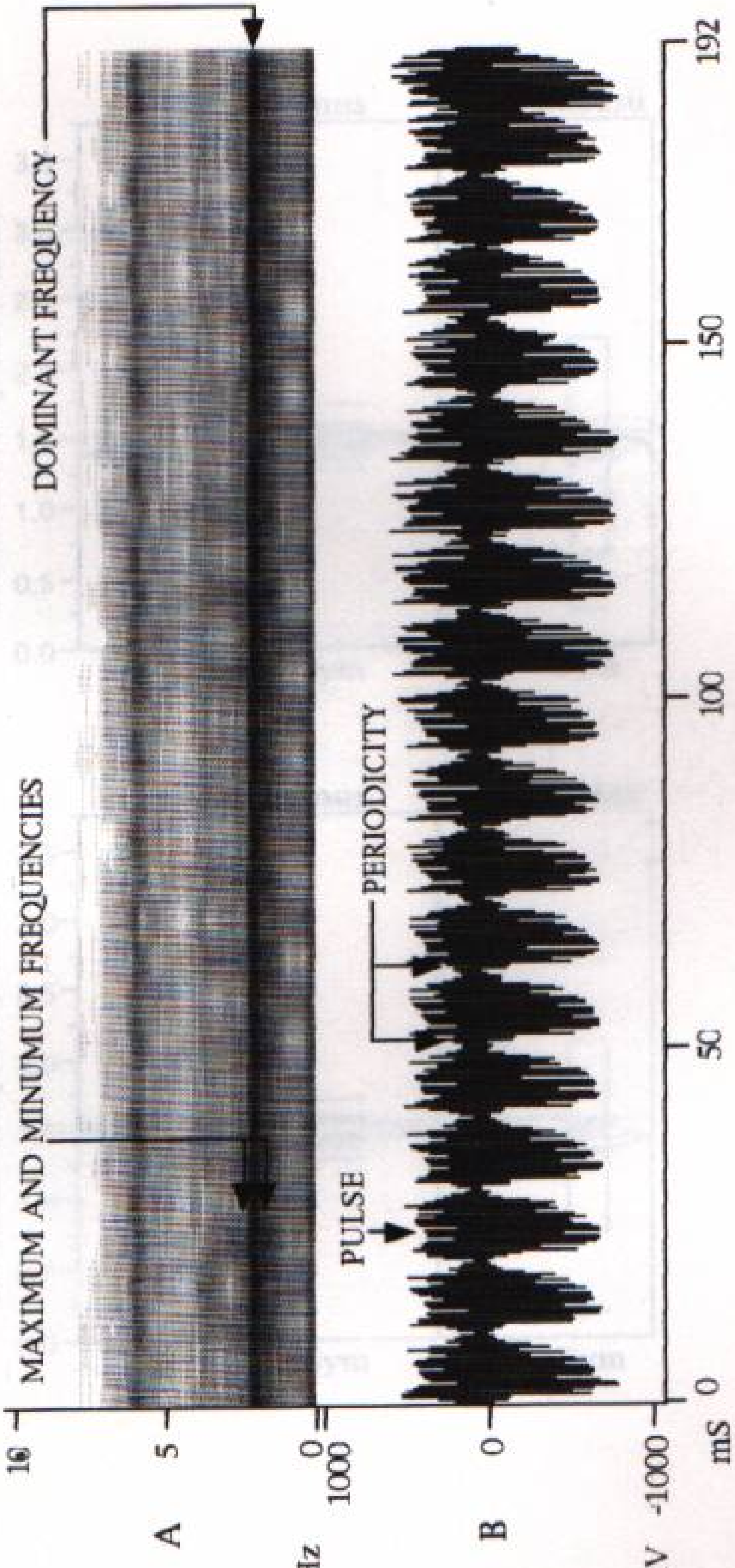
FIG. 17. Box plots of the minimum frequency (kHz) of the advertisement vocalization of *B. americanus* and *B. woodhousii* in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

FIG. 18. Box plots of the maximum frequency (kHz) of the advertisement vocalization of *B. americanus* and *B. woodhousii* in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 4 for an explanation of the box plots.

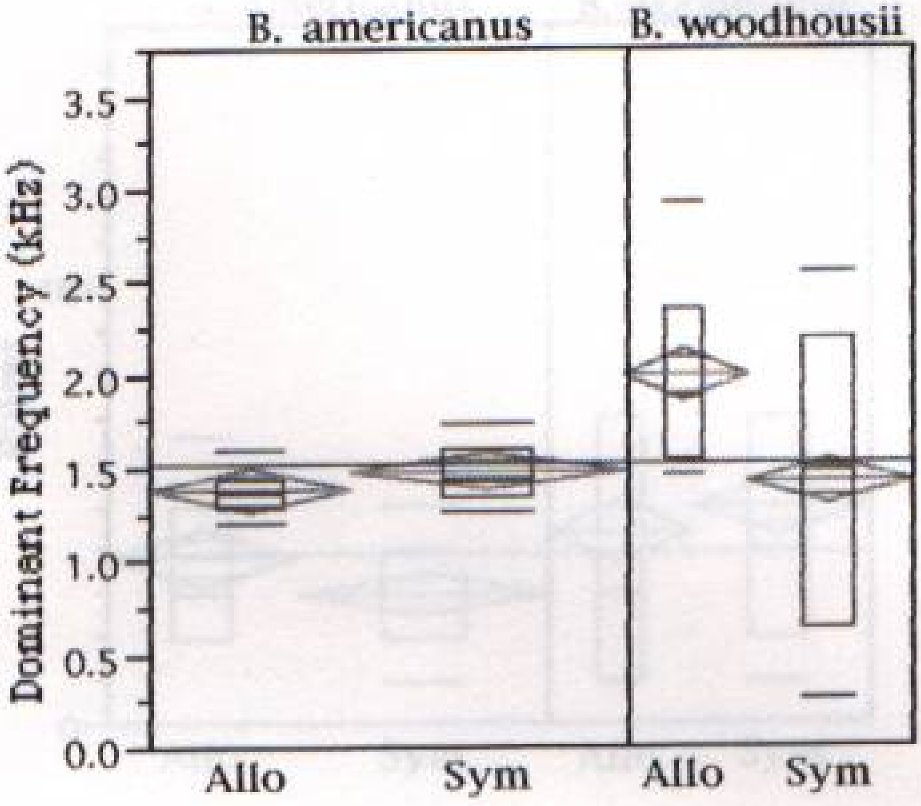
FIG. 19. Box plots of the dominant frequency of the advertisement vocalization for *B. americanus* across the sampling range adjusted for the effects of temperature. Plots are arranged chronologically from the southern-most population (left) to the northern-most population (right). Populations 12 through 17 are sympatric with *B. woodhousii* and populations 1, 2 and 5 are allopatric. Designated population numbers correspond to the localities in Table 1. See Fig. 4 for an explanation of the box plots.


B. woodhousii
B. americanus
B. terrestris
CHAMBERS
LEE
MACON
RUSSELL

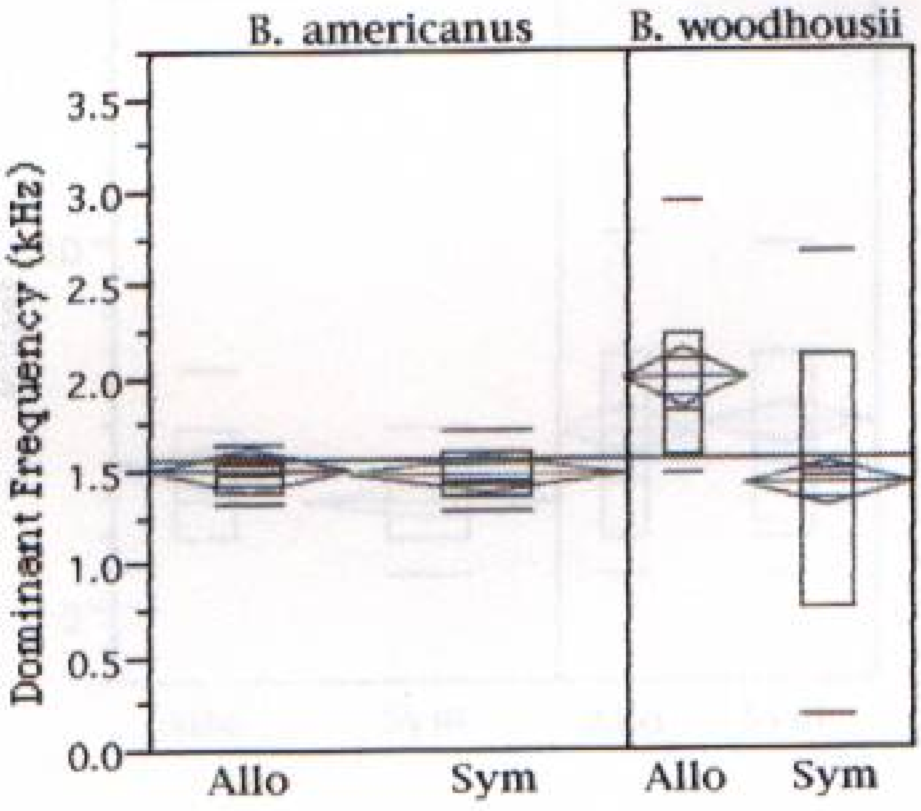

RELEASE VOCALIZATION
CHIRP
MAXIMUM AND MINIMUM FREQUENCIES
DOMINANT FREQUENCY
INTERCHIRP DURATION
PULSES
PERIODICITY
A
B
10
5
kHz
1000
0
-1000
mV
mS 0
200
400
600


A
B
kHz
mV
10
5
0
1000
0
-1000
mS
0
50
100
150
192
MAXIMUM AND MINUMUM FREQUENCIES
DOMINANT FREQUENCY
PULSE
PERIODICITY

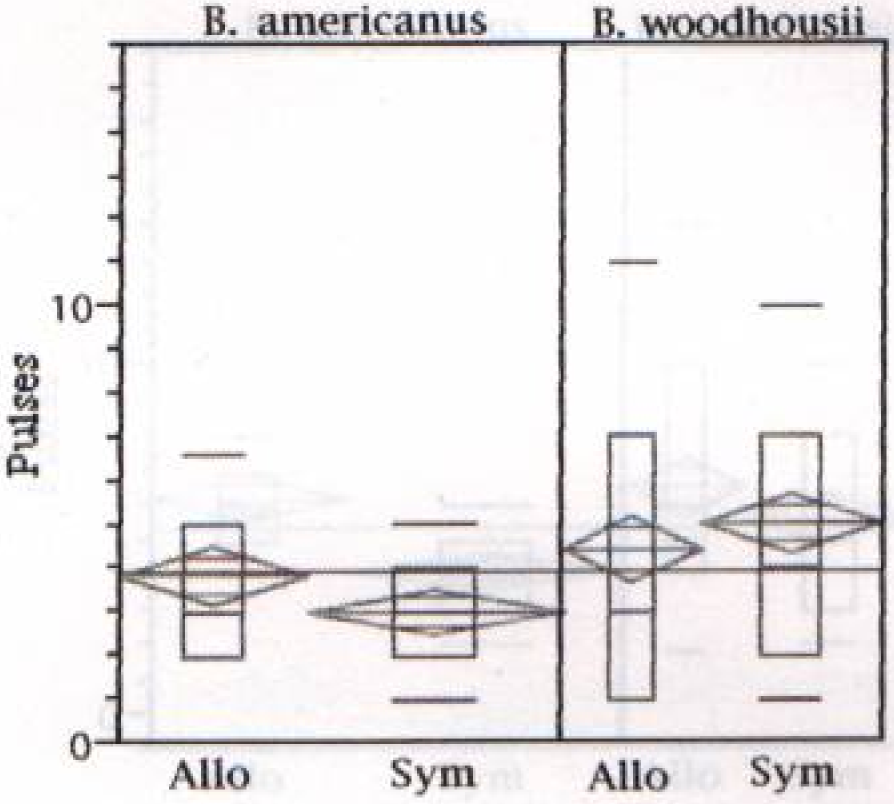
A


B. americanus
B. woodhousii
Dominant Frequency (kHz)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Allo
Sym
Allo
Sym

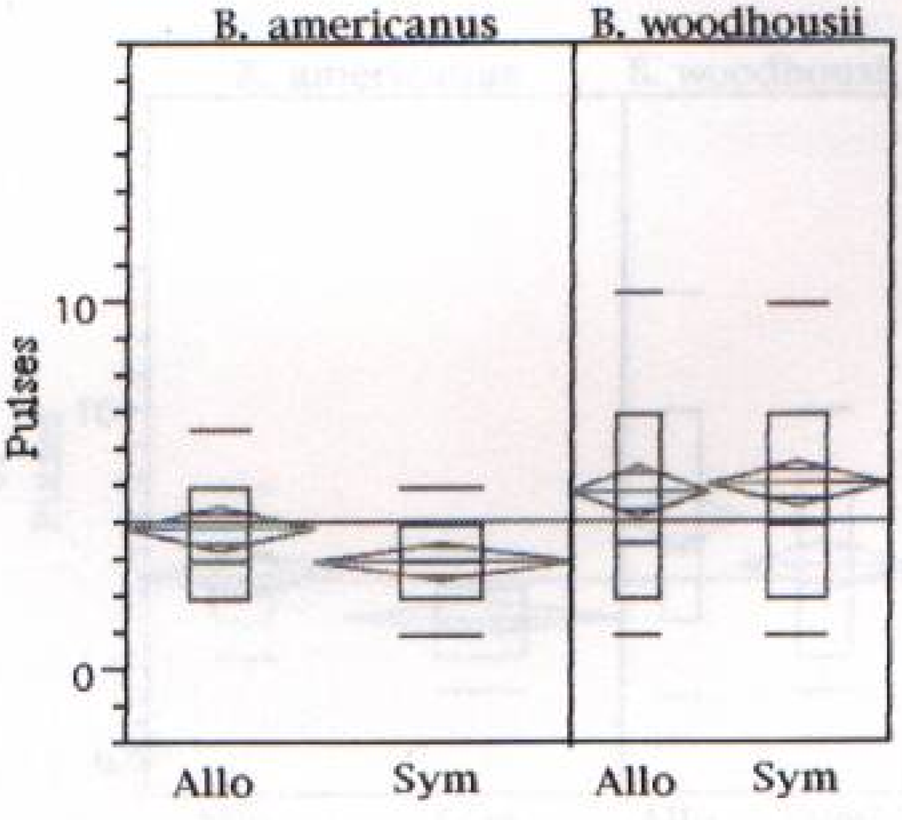
B


B. americanus
B. woodhousii
Dominant Frequency (kHz)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Allo
Sym
Allo
Sym

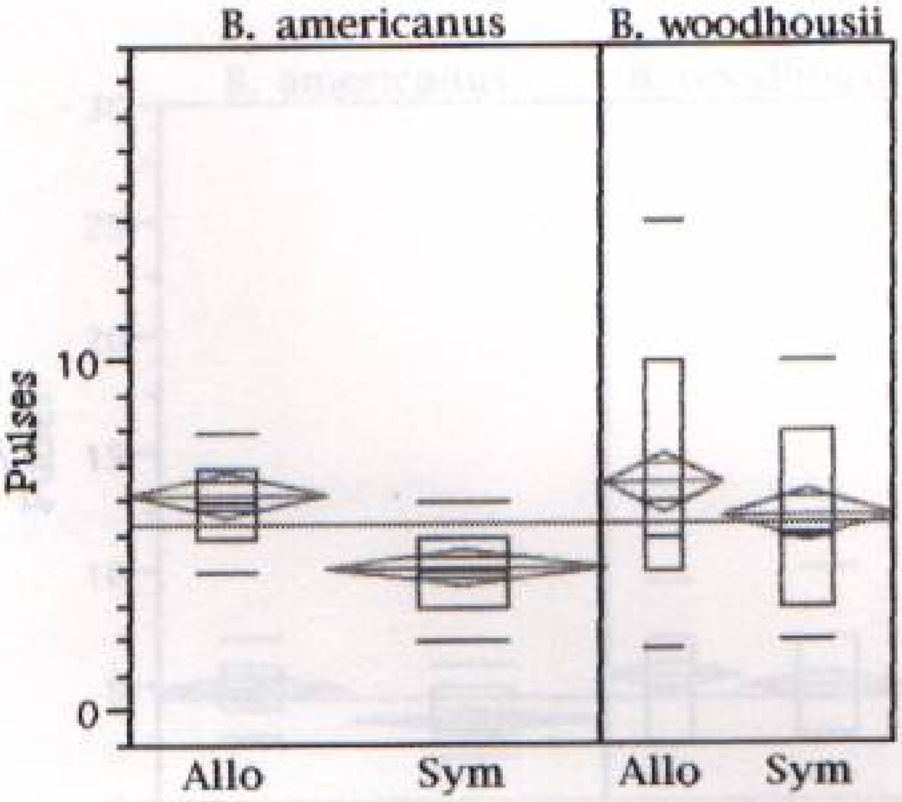
A


B. americanus
B. woodhousii
Pulses
10
0
Allo
Sym
Allo
Sym

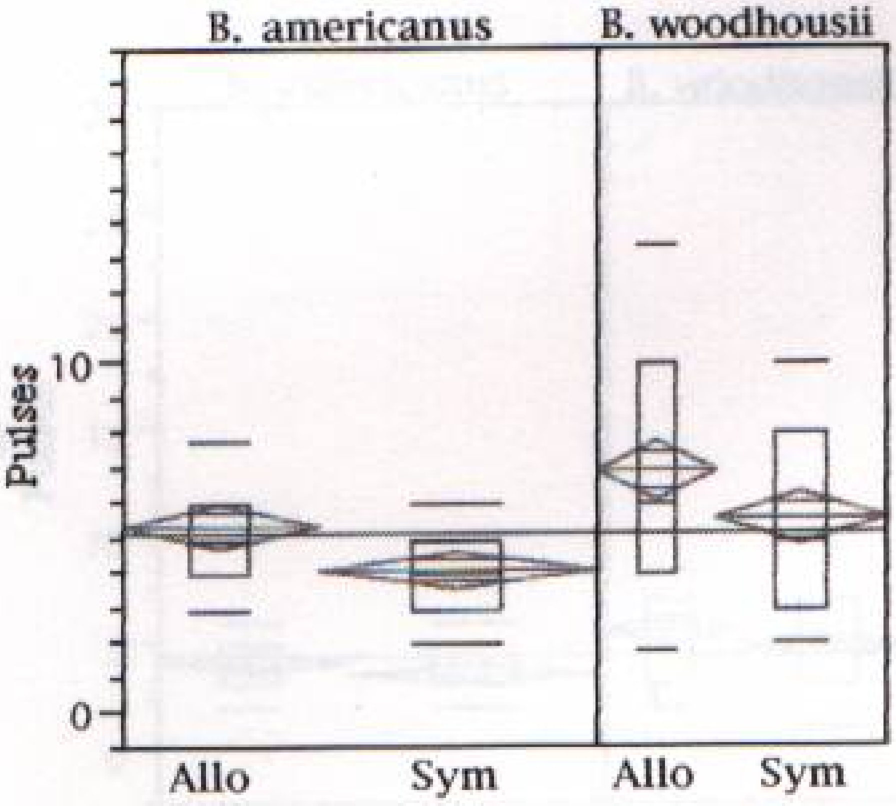
B

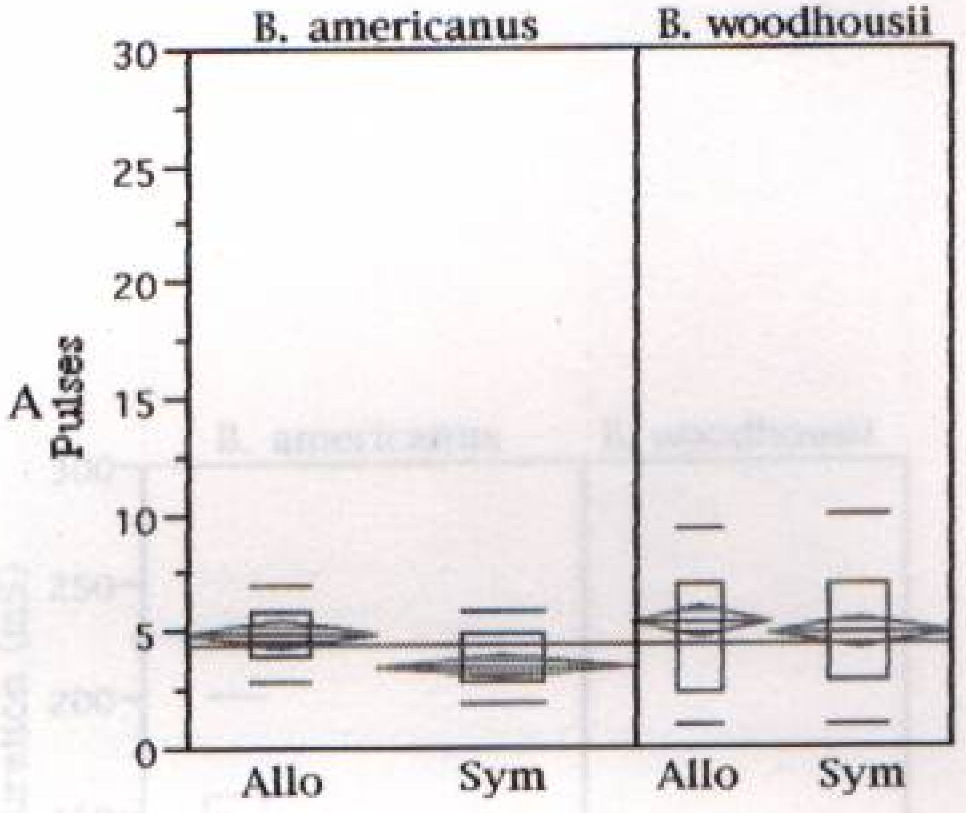

B. americanus
B. woodhousii
Pulses
10
0
Allo
Sym
Allo
Sym

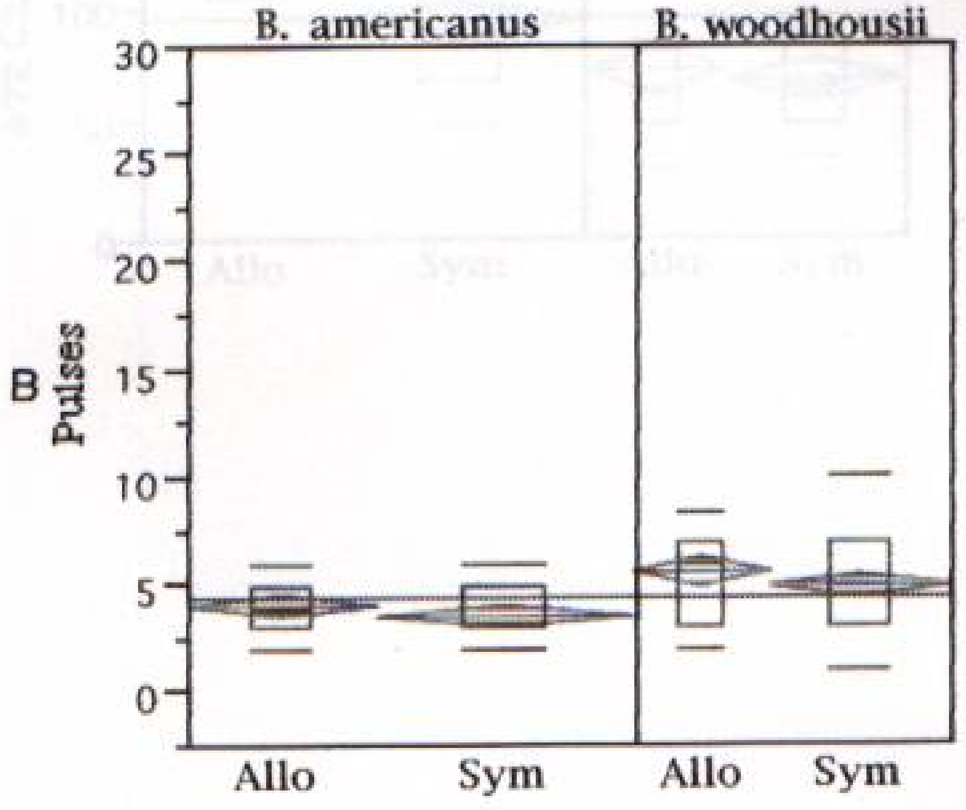
A

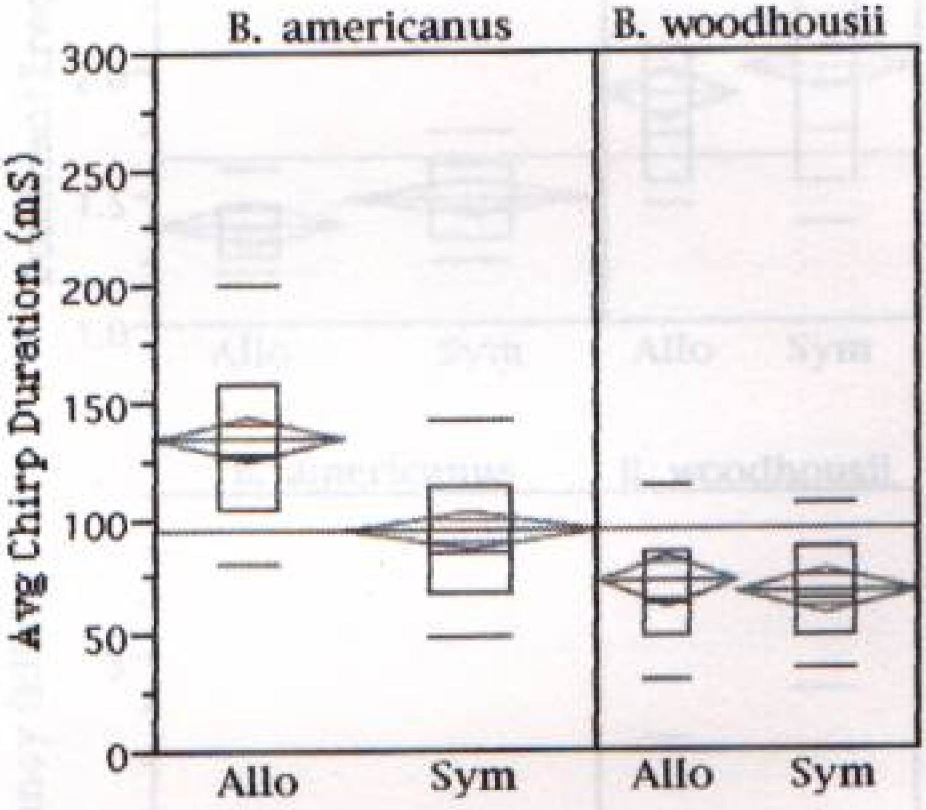

B. americanus
B. woodhousii
Pulses
10
0
Allo
Sym
Allo
Sym

B

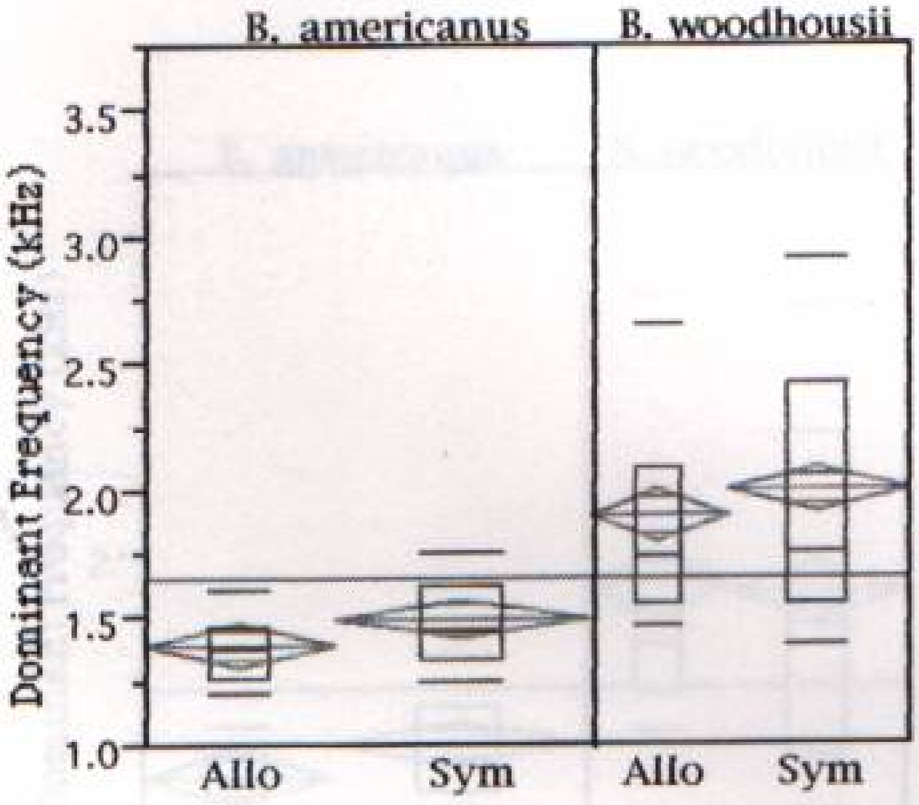

B. americanus
B. woodhousii
Pulses
10
0
Allo
Sym
Allo
Sym


B. americanus
B. woodhousii
A
Pulses
30
25
20
15
10
5
0
Allo
Sym
Allo
Sym

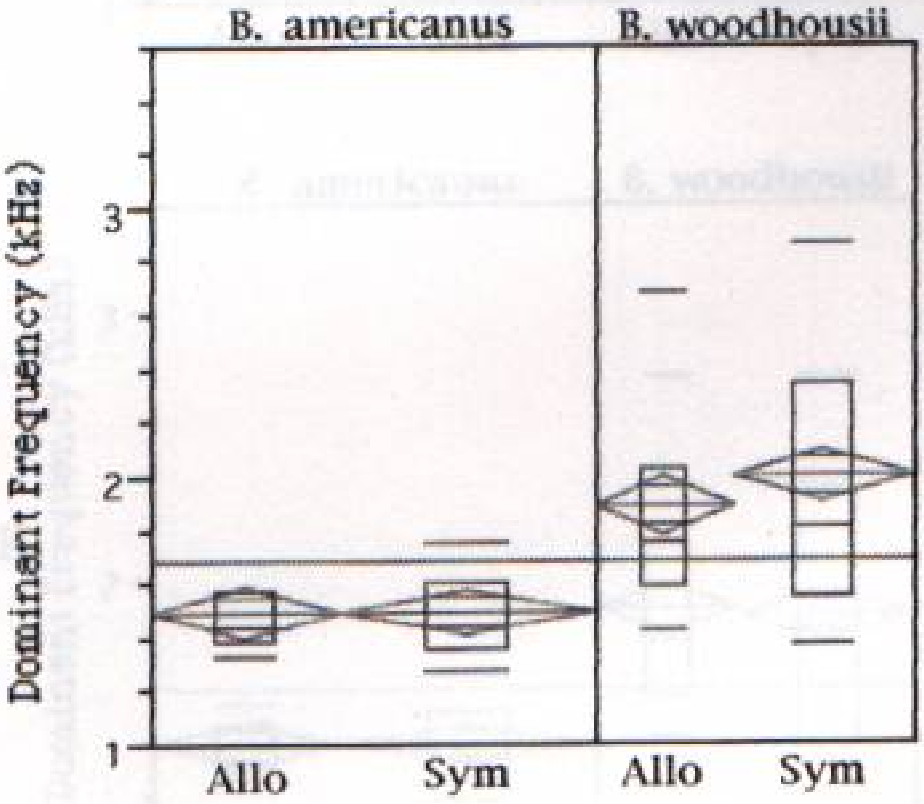

B. americanus
B. woodhousii
B
Pulses
30
25
20
15
10
5
0
Allo
Sym
Allo
Sym

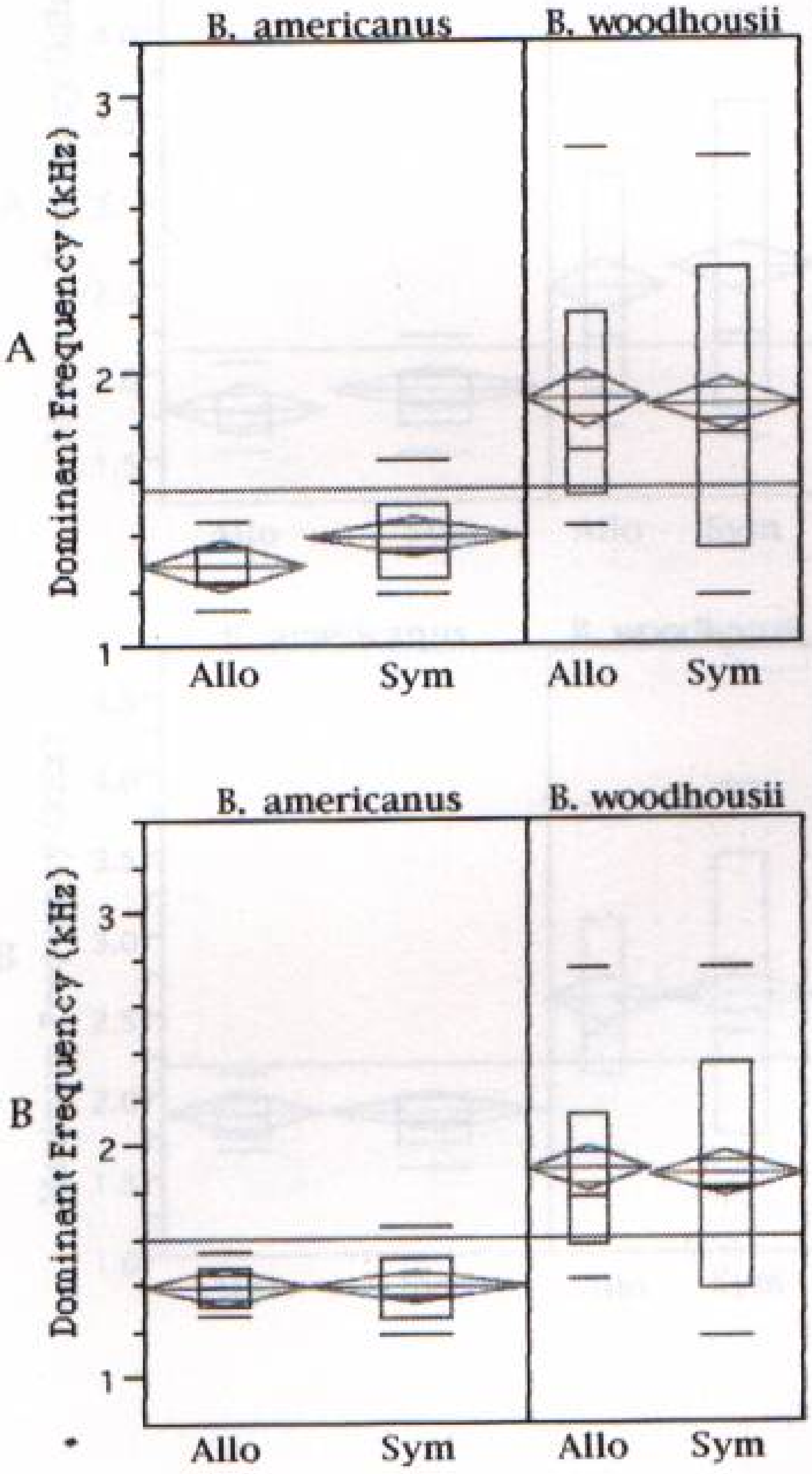

B. americanus
B. woodhousii
Avg Chirp Duration (mS)
300
250
200
150
100
50
0
Allo
Sym
Allo
Sym

A

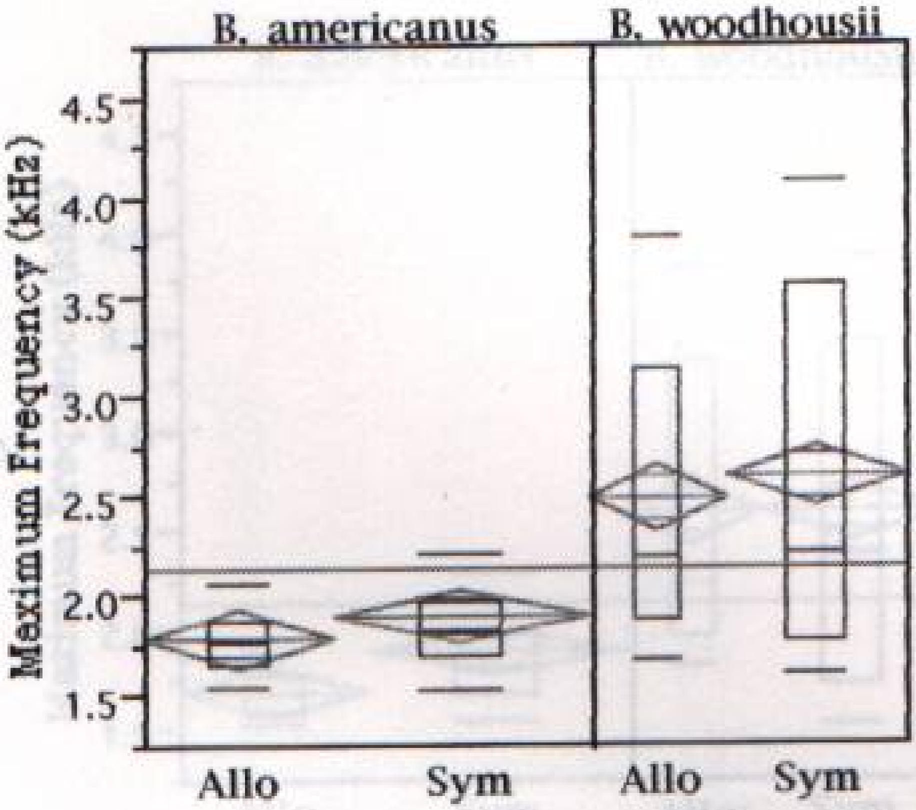

B. americanus
B. woodhousii
Dominant Frequency (kHz)
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym

B

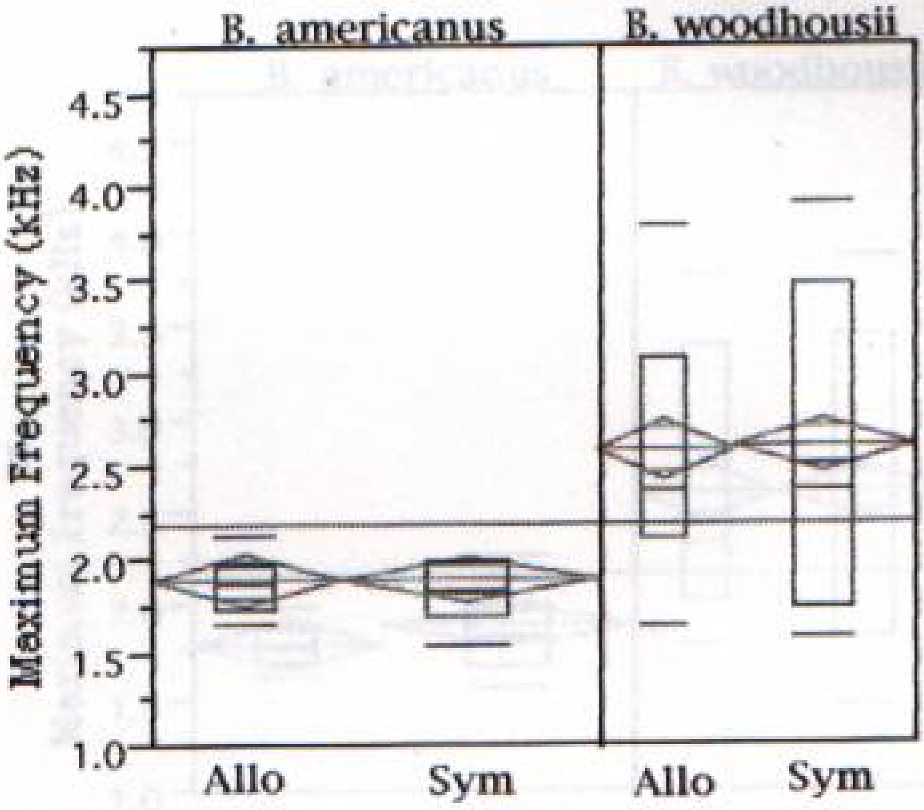

B. americanus
B. woodhousii
Dominant Frequency (kHz)
3
2
1
Allo
Sym
Allo
Sym


A
B. americanus
B. woodhousii
Dominant Frequency (kHz)
3
2
1
Allo
Sym
Allo
Sym
B
B. americanus
B. woodhousii
Dominant Frequency (kHz)
3
2
1
Allo
Sym
Allo
Sym

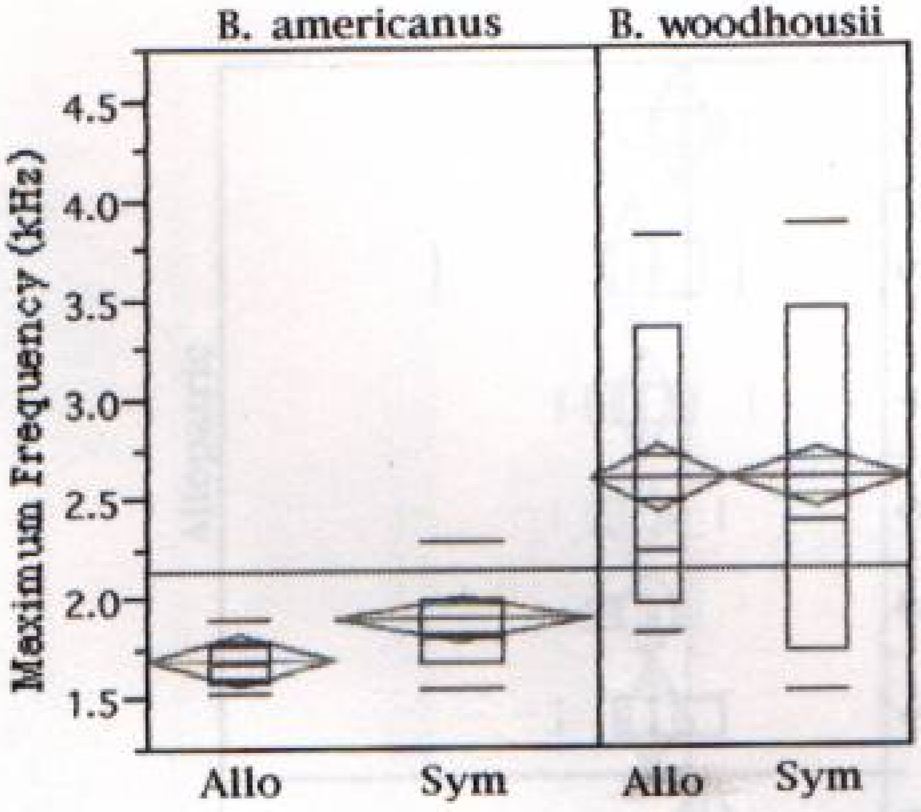
A


B. americanus
B. woodhousii
Maximum Frequency (kHz)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Allo
Sym
Allo
Sym

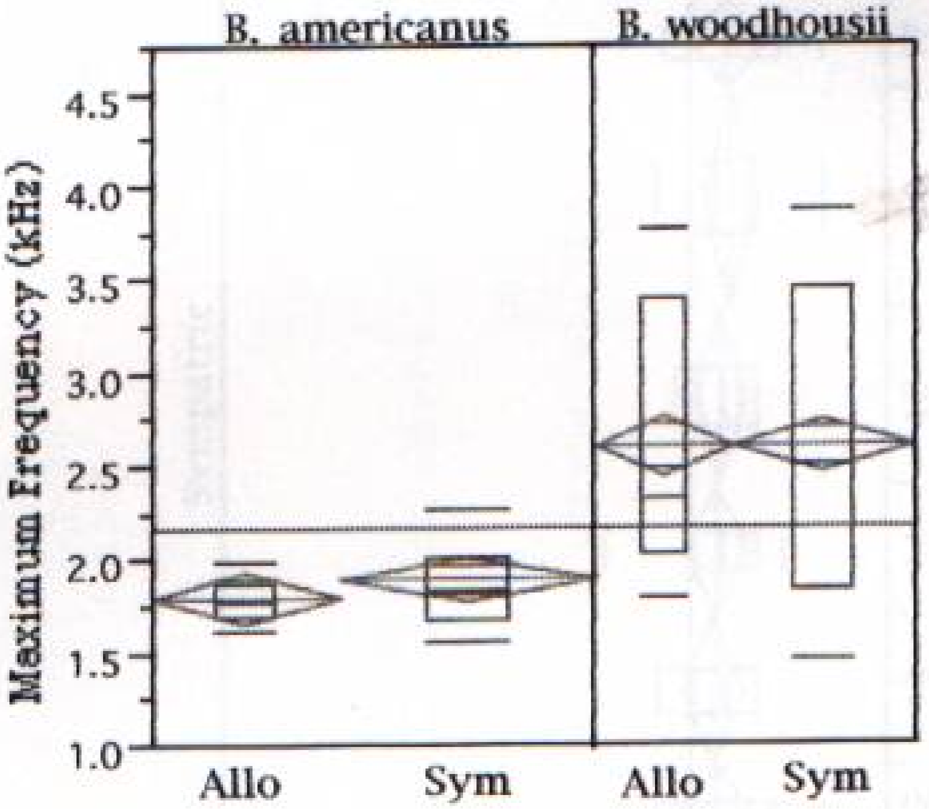
B

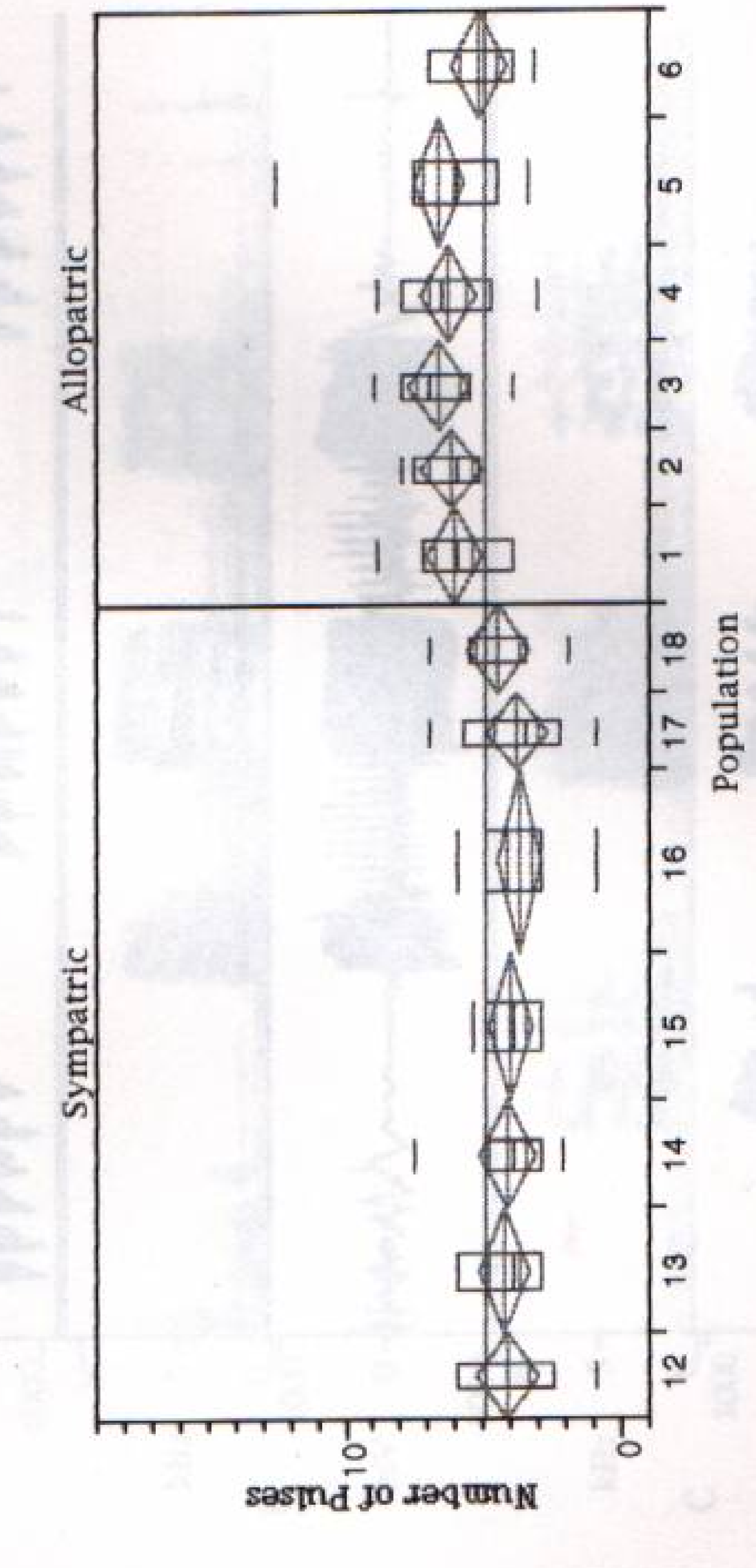

B. americanus
B. woodhousii
Maximum Frequency (kHz)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym

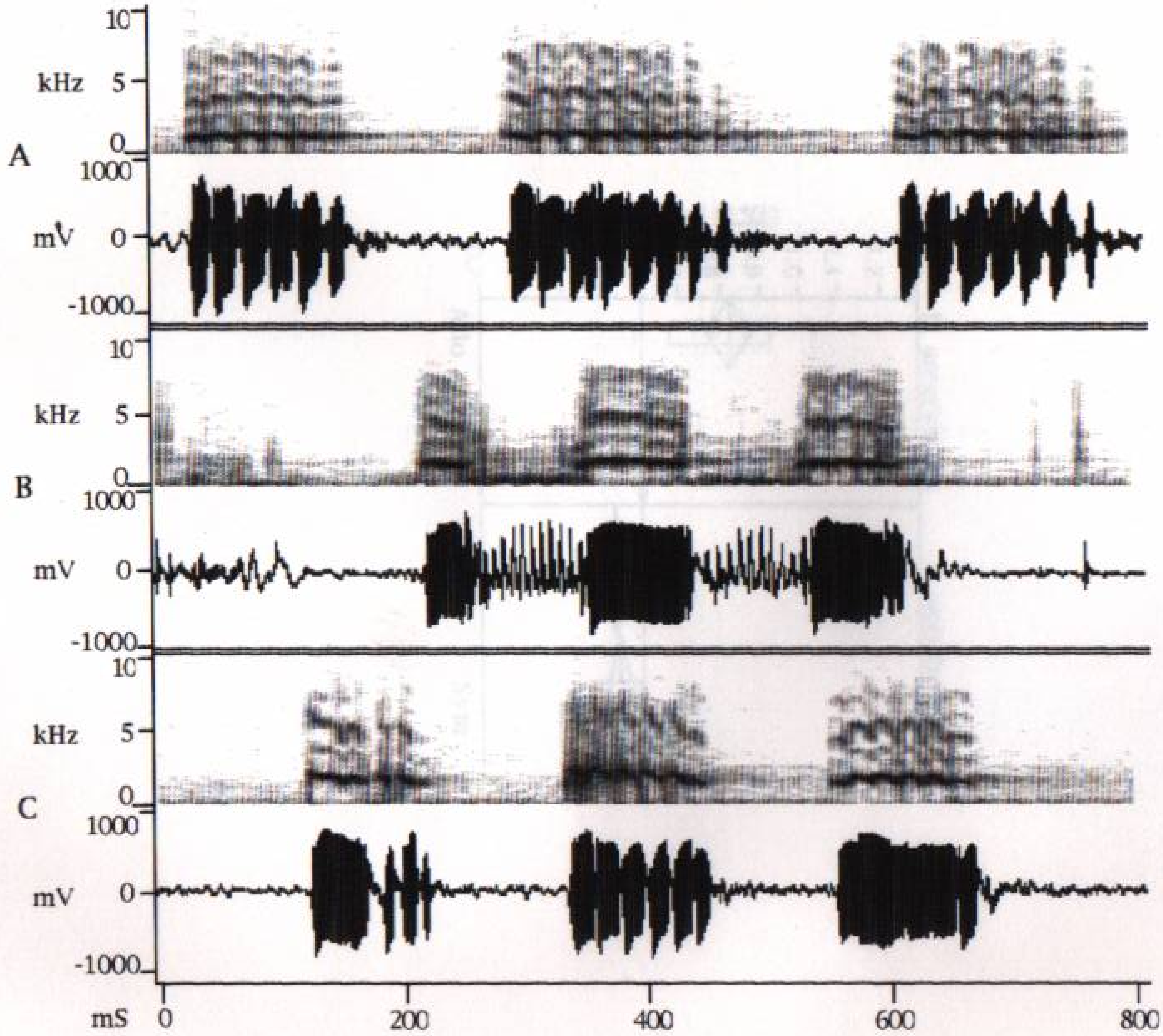
A

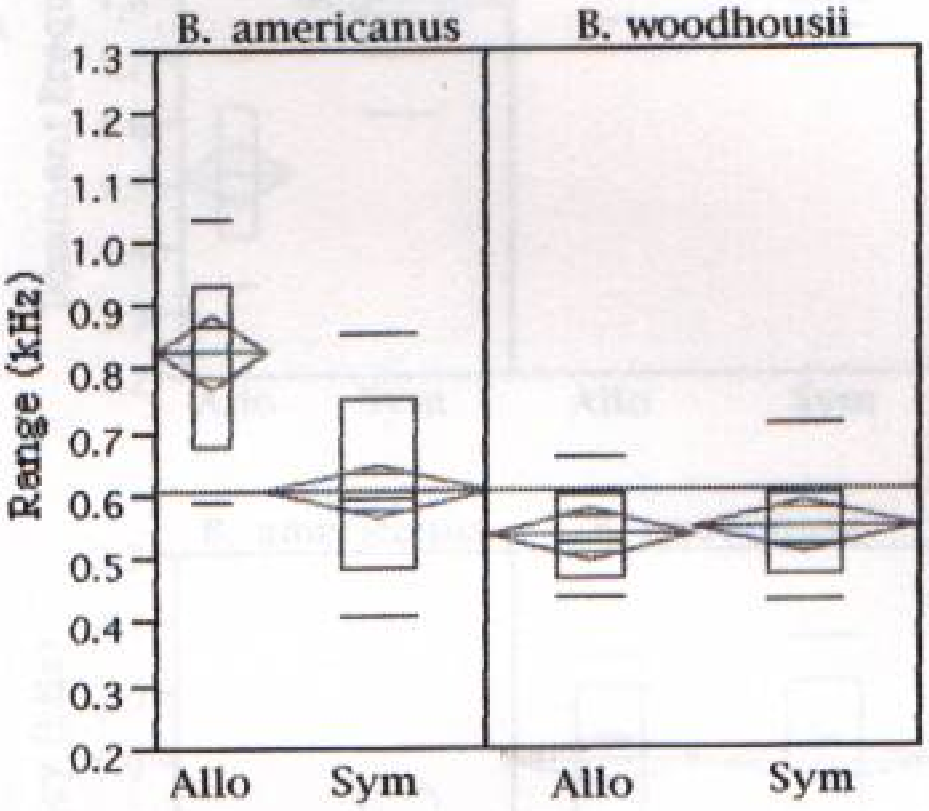

B. americanus
B. woodhousii
Maximum Frequency (kHz)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Allo
Sym
Allo
Sym

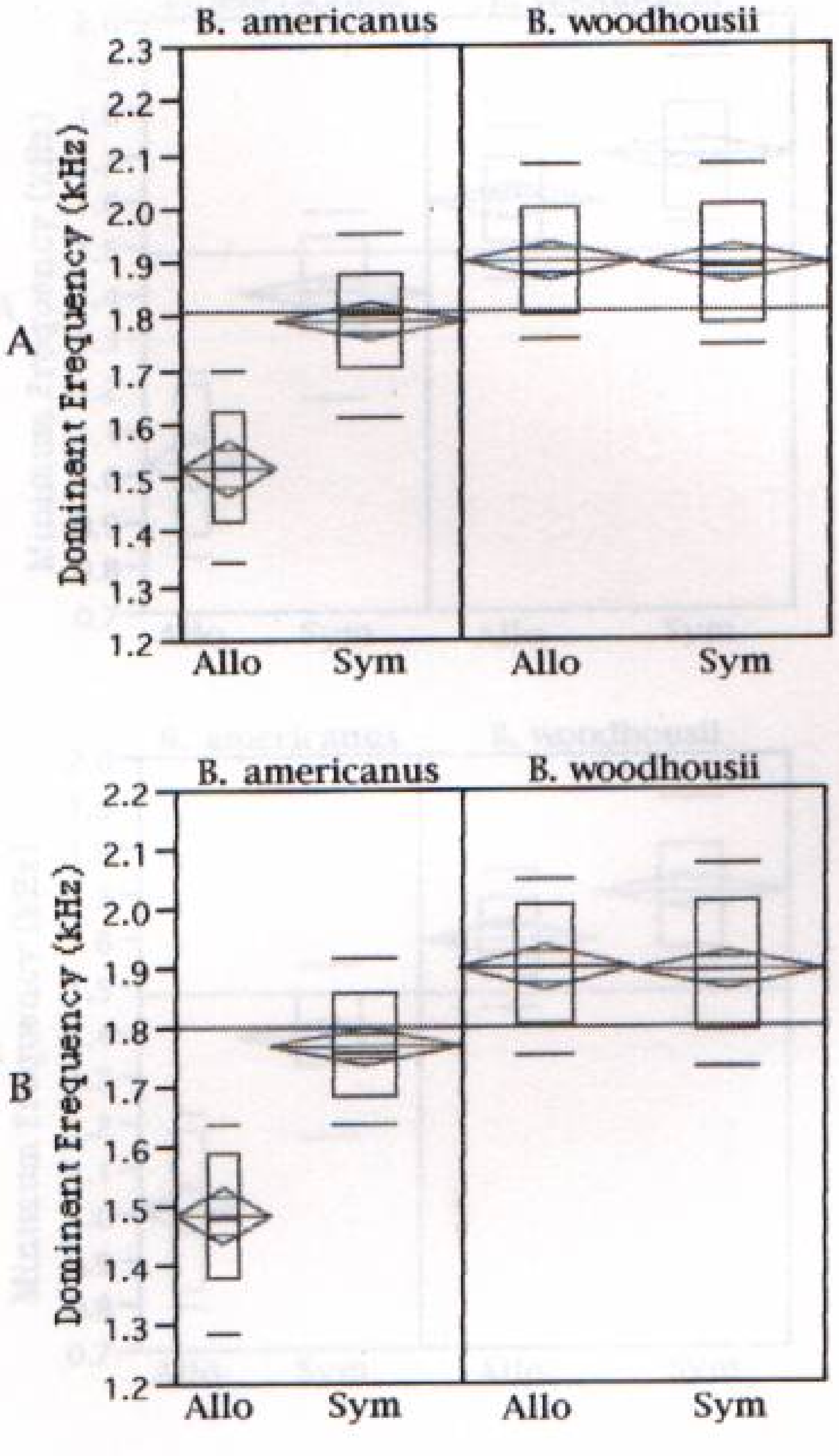
B


B. americanus
B. woodhousii
Maximum Frequency (kHz)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym

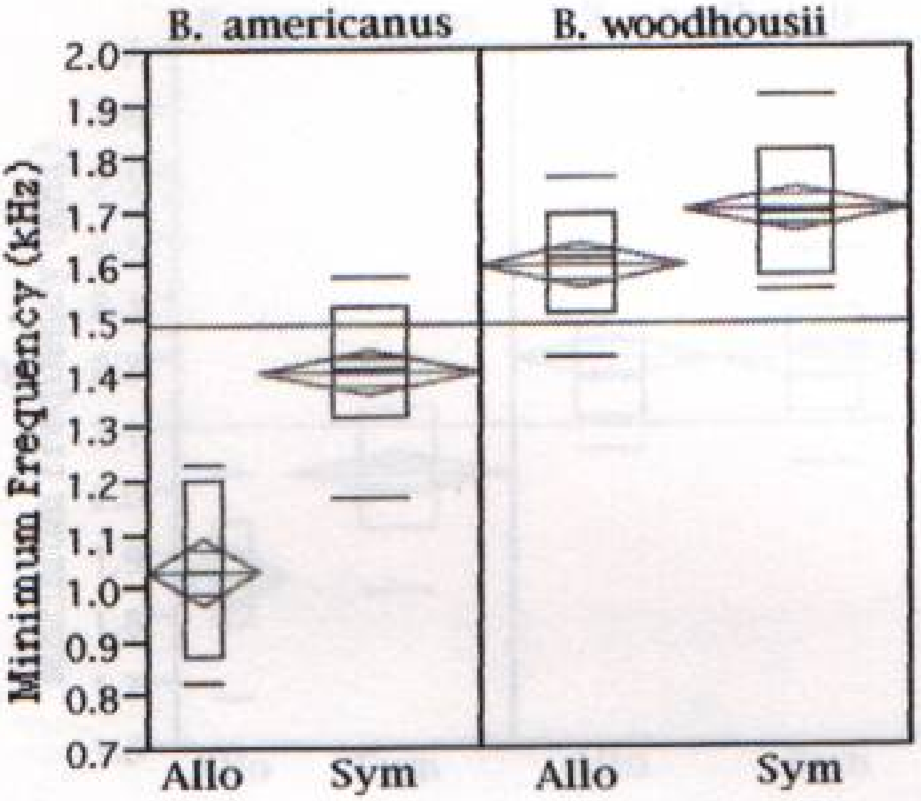

Sympatric
Allopatric
Number of Pulses
0
10
Population
12
13
14
15
16
17
18
1
2
3
4
5
6


A
kHz
10
5
0
mV
1000
0
-1000
B
kHz
10
5
0
mV
1000
0
-1000
C
kHz
10
5
0
mV
1000
0
-1000
mS
0
200
400
600
800

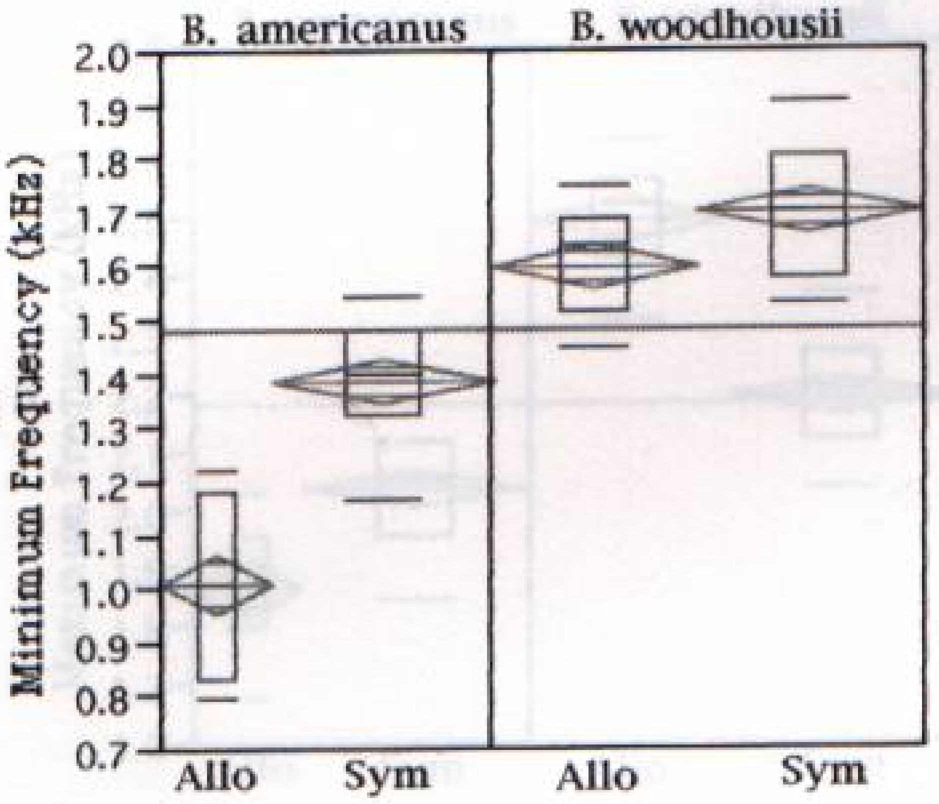

B. americanus
B. woodhousii
Range (kHz)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Allo
Sym
Allo
Sym

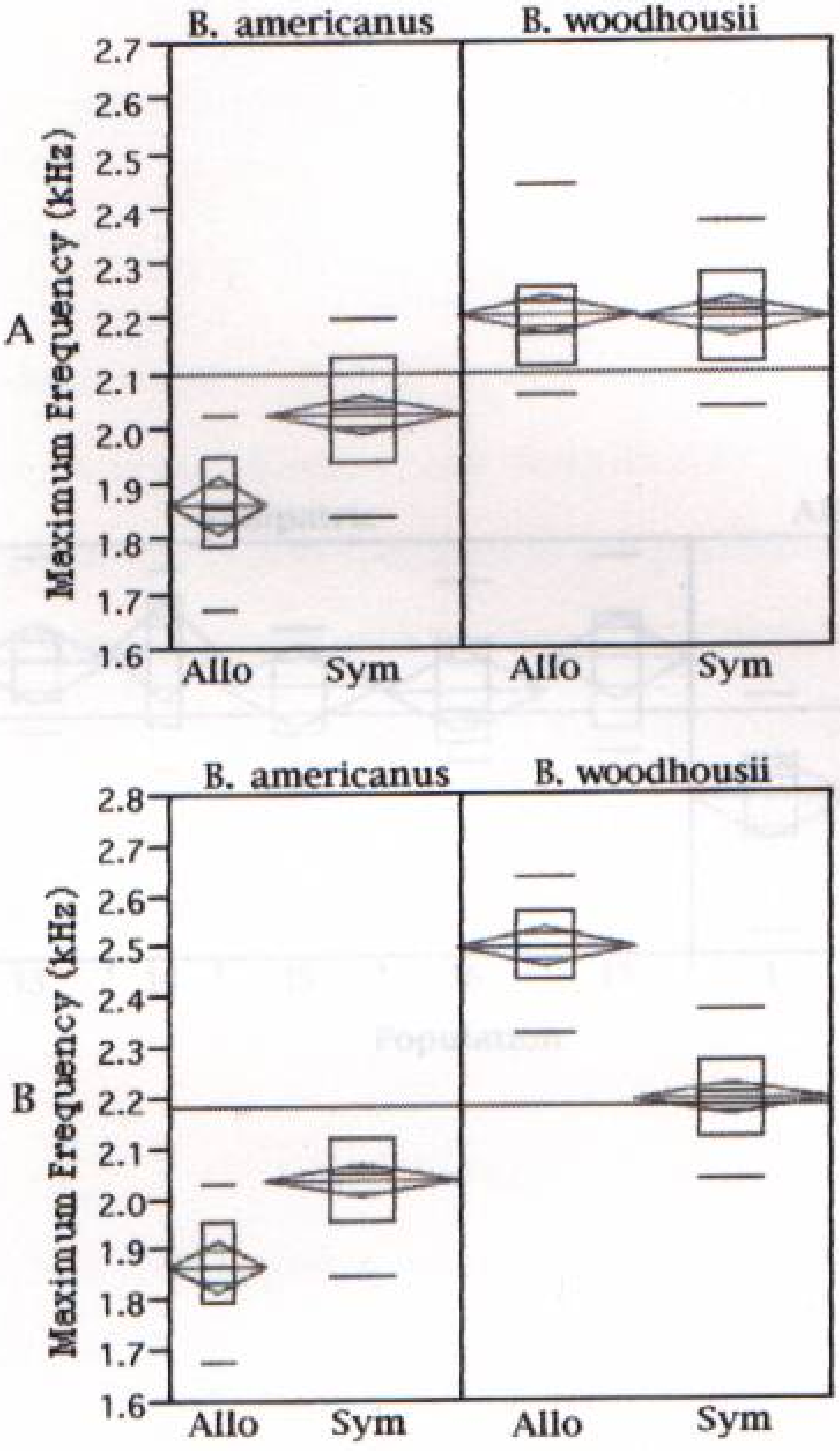

A
B. americanus
B. woodhousii
Dominant Frequency (kHz)
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
Allo
Sym
Allo
Sym
B
B. americanus
B. woodhousii
Dominant Frequency (kHz)
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
Allo
Sym
Allo
Sym

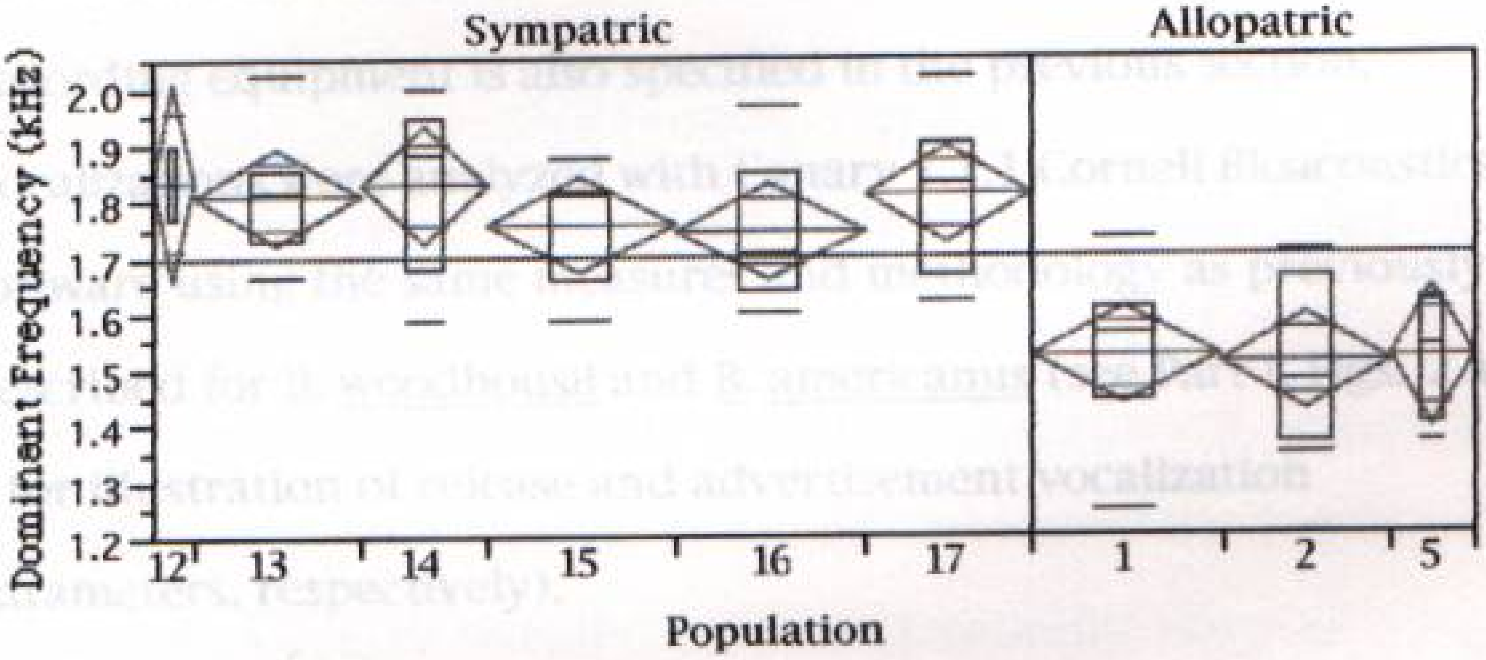
A


B. americanus
B. woodhousii
Minimum Frequency (kHz)
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
Allo
Sym
Allo
Sym

B


B. americanus
B. woodhousii
Minimum Frequency (kHz)
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
Allo
Sym
Allo
Sym


A
B. americanus
B. woodhousii
Maximum Frequency (kHz)
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
Allo
Sym
Allo
Sym
B
B. americanus
B. woodhousii
Maximum Frequency (kHz)
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
Allo
Sym
Allo
Sym


Sympatric
Allopatric
Dominant Frequency (kHz)
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
12
13
14
15
16
17
1
2
5
Population

CHAPTER II, PART II
BUFO WOODHOUSII AND B. TERRESTRIS

METHODS

Methodology and recording procedures for release vocalizations and advertisement vocalizations are described in Chapter II, Part I. Recording equipment is also specified in the previous section. Vocalizations were analyzed with Canary 1.1.1 Cornell Bioacoustics software using the same measures and methodology as previously described for B. woodhousii and B. americanus (see Part I, Figs. 2 and 3 for illustration of release and advertisement vocalization parameters, respectively).

Sampling

A total of 206 B. woodhousii and B. terrestris were recorded for release vocalizations during the breeding periods in 1994, 1995 and 1996. For B. woodhousii, 46 individuals were sampled from five allopatric localities in Lee County, Alabama, 62 were sampled from 5 localities of sympatry with B. terrestris in south-central Alabama. For B. terrestris, 41 individuals were sampled from 4 allopatric localities throughout Florida, 57 were sampled from 5 localities of sympatry with B. woodhousii throughout south Alabama (Table 1, Fig. 1 PT I). Advertisement recordings were obtained from108 individuals (38 allopatric B. woodhousii, 45 sympatric B. woodhousii, 11 allopatric B. terrestris and 14 sympatric B. terrestris) representing a subset of total individuals sampled for release vocalizations (Table 1).

Temperature Correction

Recording temperatures ranged from 16 to 24°C for allopatric B. woodhousii; 21 to 27°C for allopatric B. terrestris; 21 to 27°C for sympatric B. woodhousii and 19 to 26°C for sympatric B. terrestris.

Simple linear regression indicated that interchirp duration, number of pulses, periodicity, dominant frequency, range of frequency, and minimum and maximum frequencies of release vocalizations varied significantly ($p \leq 0.05$) with effective temperature for at least one chirp for one or both species in allopatry and/or sympatry (Table 2). Chirp duration was marginally significantly correlated with temperature ($p = 0.07$) for sympatric B. woodhousii (Table 2). There were significant correlations for effects of temperature ($p \leq 0.05$) on number of pulses and periodicity of advertisement vocalizations for allopatric and/or sympatric

B. woodhousii (Table 3). Dominant frequency of sympatric B. woodhousii was marginally significantly correlated with temperature (p = 0.08, Table 3). Maximum frequency was significantly correlated with temperature when allopatric and sympatric B. woodhousii samples were combined (p = 0.02, Table 3).

Separate regression coefficients were calculated for the effects of temperature on all release vocalization parameters for each species in allopatry and in sympatry. Regression coefficients were then used to adjust all parameters to the grand mean recording temperature of release vocalizations (21°C) using the method of linear least-squares (Littlejohn, 1965). Separate regression coefficients were calculated for effects of temperature on all advertisement vocalization parameters for allopatric and sympatric B. woodhousii using the same methods. All advertisement vocalizations for allopatric B. terrestris were recorded at 21°C and ranged from 20 to 22°C for sympatric B. terrestris. Therefore, recording temperatures for B. terrestris deviated no more than 1°C from the grand mean temperature adjustment value. Hence, advertisement vocalization data for B. terrestris was not corrected for the effects of temperature.

SVL Correction

Mean SVL of individuals sampled for release vocalizations was 54±7 mm for allopatric B. terrestris; 56±3 mm for sympatric B. terrestris; 57±5 mm for allopatric B. woodhousii and 55±3 mm for sympatric B. woodhousii. Analyses of variance indicated statistically significant unilateral convergent character displacement with respect to mean SVL of adult vocalizing toads. SVL of allopatric B. terrestris was not significantly different from sympatric B. terrestris ($p = 0.1$). However, allopatric B. woodhousii were significantly larger than sympatric B. woodhousii ($p = 0.0008$). Additionally, allopatric B. woodhousii were significantly larger than allopatric B. terrestris ($p = 0.02$) while sympatric B. woodhousii were not significantly different from sympatric B. terrestris ($p = 0.06$). Analyses of variance indicated that mean SVL of the subset of individuals sampled for advertisement vocalizations was significantly different ($p < 0.05$) from the total sampled for release vocalizations. However, analyses of variance indicated that the subset of individuals exhibited the same unilateral convergent pattern.

Simple linear regression, plotting adjusted values for the effects of temperature against SVL, indicated that SVL had a significant effect ($p \leq 0.05$) on number of pulses, periodicity and minimum frequency for at least one release chirp for one or both species in allopatry and/or sympatry (Table 2). When allopatric and sympatric B. woodhousii samples were combined the maximum frequency of the first chirp was significantly affected by SVL ($p = 0.04$) and dominant frequency of the first chirp was strongly correlated with SVL ($p = 0.09$). SVL also had a significant effect ($p \leq 0.05$) on the number of pulses, periodicity, dominant frequency, minimum frequency and maximum frequency of advertisement vocalizations for at least one species in either allopatry and/or sympatry (Table 3)

To eliminate pleiotropic effects of SVL on release vocalizations, separate regression coefficients were calculated for the effects of SVL on temperature-adjusted values for the number of pulses, periodicity, dominant frequency and minimum and maximum frequencies for each species in allopatry and in sympatry. Regression coefficients were then used to adjust vocalizations to the mean SVL of each species (B. woodhousii = 56 mm, B. terrestris = 55 mm) using the method of linear least-squares. Interchirp duration, chirp duration and range of frequency were not significantly affected by SVL ($p > 0.05$) and were not adjusted (Table 2). All advertisement parameters, with the exception of range of frequency, were adjusted to the mean SVL sampled for release vocalizations using the same method. Instances of character displacement that resulted from pleiotropic effects of body size were detected by examining differences between significance levels calculated in analyses of variance using values adjusted for temperature alone versus those adjusted for both temperature and SVL.

The mean, standard deviation, variance and minimum and maximum values of release and advertisement vocalization parameters corrected for temperature (raw data for B. terrestris advertisement vocalizations) and both temperature and SVL for B. woodhousii and B. terrestris are listed in Appendices III and IV respectively.

Statistical Analysis

Analyses of variance were performed on all release vocalization parameters for the first, middle and last chirps and all advertisement vocalization parameters adjusted for temperature alone and adjusted for both temperature and SVL. Data for each species were combined for allopatric populations and compared to combined data for sympatric populations. The following ANOVA comparisons were made to test for character displacement:

1) allopatric B. terrestris versus sympatric B. terrestris,
2) allopatric B. woodhousii versus sympatric B. woodhousii,
3) allopatric B. terrestris versus allopatric B. woodhousii, and
4) sympatric B. terrestris versus sympatric B. woodhousii.

Statistically significant instances of convergent character displacement required the following pattern (adopted from Grant, 1972):

A) Sympatric vocalizations of one or both species were significantly displaced from conspecific allopatric vocalizations. Significant differences with respect to both species indicated bilateral convergent character displacement. Significant differences with respect to one species, but not the other, indicated unilateral convergent character displacement.

B) Vocalizations of allopatric B. terrestris were significantly different from the vocalizations of allopatric B. woodhousii.

C) Vocalizations of the species in sympatry were not significantly different from each other.

Statistically significant instances of divergent character displacement required that the antithesis of "B" and "C" occur. Vocalization parameters that were significantly displaced from the allopatric character state for one or both species but did not indicate divergent or convergent character displacement (ie; were significantly different for all comparisons) were uninformative and required further investigation. Box plots were utilized for this purpose. Parameters whose mean distributions approached one another so as to result in a greater degree of overlap of quantile distributions in sympatry indicated convergent trends. The antithesis indicated a divergent trend. The absence of significant displacement of either species from its allopatric character state precluded the existence of character displacement (see Grant, 1972). Therefore, only those parameters where one or both species were significantly displaced from their allopatric character state were examined for trends.

Parameters that were significantly different for comparisons between allopatric and sympatric character states of the species corrected for temperature only, but were not significantly different in the same comparison adjusted for both temperature and SVL indicated that displacement resulted from pleiotropic effects of SVL. Vocalization parameters that indicated the reciprocal were not reliable indicators of character displacement. Results that followed this pattern indicated that displacement occurred only when allopatric and sympatric B. terrestris and/or B. woodhousii were adjusted to a common mean SVL. However, mean SVL of allopatric B. woodhousii was significantly larger than sympatric B. woodhousii.

The extent of geographic distribution of sampled populations potentially influenced instances of character displacement due to clinal variability (see Grant, 1972). Box plots of sampled populations arranged from the southern-most to the northern-most population were used to detect clinal variation.

Statistical analyses were performed utilizing JMP IN version 3 (SAS Institute Inc.) and SuperANOVA (Abacus Concepts, Inc.) programs.

RESULTS

Release Vocalizations

Significant displacement of sympatric release vocalizations from the allopatric character state for B. terrestris and B. woodhousii (Table 4, columns 1,2,3 and 4) introduced the potential for unilateral as well as bilateral character displacement.

Convergent Character Displacement

Five release vocalization parameters (number of pulses of the middle chirp, periodicity of the middle chirp, range of frequency of the first and last chirps and maximum frequency of the last chirp), adjusted for the effects of temperature, indicated statistically significant instances of convergent character displacement (Table 4, columns 1,3,5 and 7). Convergent character displacement of range of frequency of the first chirp was bilateral [Table 4, columns 1, 2, 3, and 4; Fig. 1]). Convergence of number of pulses of the middle chirp, periodicity of the middle chirp, range of frequency of the last chirp and maximum frequency of the last chirp was unilateral (only sympatric B. terrestris was significantly displaced from its allopatric state [Table 4, çolumns 1, 2, 3 and 4; Figs. 2,3,4 and 5 respectively]).

All parameters remained statistically significant instances of convergent character displacement when corrected for both temperature and SVL (Table 4; Figs. 1 through 5).

Divergent Character Displacement

Five release vocalization parameters indicated statistically significant divergent character displacement when adjusted for the effects of temperature. Those parameters included dominant frequency of the first and middle chirps, minimum frequency of the first and last chirps and maximum frequency of the first chirp (Table 4, columns 1,3,5 and 7). Dominant frequency of the middle chirp (Fig. 6) and minimum frequency of the first and last chirps (Figs. 7 and 8 respectively) were unilaterally divergent resulting from significant displacement of sympatric B. woodhousii from its allopatric character state (Table 4, columns 1, 2, 3 and 4). Dominant frequency of the first chirp (Fig. 9) and maximum frequency of the first chirp (Fig. 10) were bilaterally divergent (Table 4, columns 1, 2, 3 and 4). Dominant frequency of the first and middle chirps (Figs. 9 and 6 respectively) and minimum frequency of the first and last chirps (Figs. 7 and 8, respectively) remained statistically significant cases of divergent character displacement when corrected for both temperature and SVL (Table 4,). Maximum frequency of the first chirp did not remain statistically significant when corrected for both temperature and SVL (Table 4, Fig. 10).

Displacement Trends

Convergent Character Displacement. Number of pulses of the last chirp indicated a unilateral convergent trend (Fig. 11). In this instance, sympatric B. terrestris was significantly displaced from allopatric B. terrestris (p = 0.007)(Table 4, column 1), but was not significantly different between the species in allopatry (p = 0.2; Table 4, columns 5 and 6) or in sympatry (p = 0.9; Table 4, columns 7 and 8). However, there was a greater degree of similarity (reflected in p-values) among species in sympatry (p = 0.9) versus allopatry (p = 0.2)(Fig. 11). Number of pulses of the last chirp continued to exhibit a unilateral convergent trend when adjusted for both temperature and SVL (Fig. 11).

Box plots for range of frequency of the middle chirp adjusted for temperature also indicated a unilateral convergent trend (Fig.12). In this instance, sympatric B. terrestris was significantly displaced from its allopatric character state (p = 0.01) and was more similar to B. woodhousii in sympatry (p = 0.03) than in allopatry (p = 0.003) (Table 4, columns 1,3,5 and 7; Fig. 12).

Minimum frequency of the middle chirp adjusted for temperature alone indicated a bilateral convergent trend (Fig. 13). In this instance, both species were significantly displaced from their allopatric character state (Table 4, columns 1 and 3). Minimum frequency of the middle chirp adjusted for both temperature and SVL continued to exhibit a bilateral convergent trend (Fig. 13).

Divergent Character Displacement. Maximum frequency of the first chirp did not remain a statistically significant instance of divergent character displacement when corrected for the effects of both temperature and SVL, but continued to exhibit a divergent trend (Fig. 10).

Clinal Variation

Box plots were arranged from the southern-most to the northern-most population for both species (where bilaterally displaced) or one species (where unilaterally displaced) for temperature adjusted release vocalization parameters that indicated character displacement. There was no indication of clinal variation for either species.

Advertisement Vocalizations

All advertisement vocalization parameters were significantly displaced ($p \leq 0.05$) from the allopatric character state for one or both species when corrected for temperature and/or both temperature and SVL (Table 5, columns 1,2,3 and 4). Therefore, unilateral and/or bilateral displacement potentially existed.

Convergent Character Displacement

Minimum frequency corrected for temperature alone and both temperature and SVL indicated statistically significant unilateral convergent character displacement (Table 5). In this instance, only sympatric B. woodhousii was significantly displaced from its allopatric character state (Table 5, columns 3 and 4)(Fig. 14).

Displacement Trends

Divergent Character Displacement. Maximum frequency of the advertisment call indicated a unilateral divergent trend resulting from significant displacement of sympatric B. woodhousii from its allopatric character state (Fig. 15).

Box plots of number of pulses and periodicity of advertisement vocalizations indicated a unilateral divergent trend (resulting from significant displacement of B. woodhousii) when adjusted for temperature only and a bilateral divergent trend when adjusted for both temperature and SVL (Figs. 16 and 17 respectively).

There was a unilateral divergent trend with respect to dominant frequency of advertisement vocalizations corrected for the effects of temperature and both temperature and SVL resulting from significant displacement of sympatric B. woodhousii from its allopatric character state (Table 5, columns 1, 2, 3 and 4)(Fig. 18).

Clinal Variation

Box plots were arranged from the southern-most to the northern-most population for temperature adjusted advertisement vocalization parameters that indicated unilateral character displacement. Mean periodicity of allopatric populations of B. woodhousii was the only parameter that indicated clinal variation (Fig. 19). However, mean periodicity of sympatric populations did not indicate clinal variation (Fig. 19).

DISCUSSION

A significant character shift with respect to mean SVL of sympatric B. woodhousii resulted in unilateral convergent character displacement of body size. However, statistically significant differences between sympatric and allopatric conspecific vocalizations did not exist for either species. Therefore, pleiotropic effects of body size did not significantly affect character displacement.

The reason for convergence of body size is perplexing and does not support an expected divergence of body size that could potentially serve as an isolating mechanism between sympatric species (Lowe, 1954). Although Brown (1969) and Mount (1975) and Weatherby (1982) reported instances of hybridization between B. terrestris and B. woodhousii in Alabama, the largely divergent trend of advertisement vocalizations lends strong evidence denouncing hybrid sampling.

Unilateral convergent character displacement with respect to SVL of adult vocalizing B. woodhousii potentially supports a pleiotropic adaptation for selection of a uniform release vocalization. Convergence of body size could theoretically result in convergence of various release vocalization components, thus facilitating interspecies communication. However, there is a concomitant selective tradeoff where isolation mechanisms, namely advertisement vocalizations, must diverge in order to maintain species integrity. Sympatric B. terrestris and B. woodhousii appear to have successfully converged with respect to release vocalizations while simultaneously diverging with respect to advertisement vocalizations. A total of 8 release vocalization parameters were either statisitically significant cases of convergent character displacement or indicated a convergent trend. Those parameters included number of pulses of the middle and last chirps, periodicity of the middle chirp, range of frequency of the first, middle and last chirps, minimum frequency of the middle chirp and maximum frequency of the last chirp. A total of 5 release vocalization parameters were statistically significant cases of divergent character displacement. Those parameters included dominant frequency of the first and middle chirps, minimum frequency of the first and last chirps and maximum frequency of the first chirp. Therefore, convergence of range of frequency for the first chirp resulted from divergence of composite parameters. In this instance, minimum and maximum frequencies shifted for both species, resulting in similar differences between the average minimum and maximum frequencies (range). Divergent shifts in minimum frequency of the last chirp resulted in similar convergent effects. While release vocalizations were both convergent and divergent in sympatry, advertisement vocalizations were largely divergent.

Bogert (1960) Martin (1971) and Brown and Littlejohn (1972) found release vocalizations and advertisement vocalizations to be similar in many respects. In fact, Savage (1932) suggested that release vocalizations are modified advertisement vocalizations, differentiated by modifications in respiratory movements. Blair (1968) stated that "the release call would be expected to be under no functional pressure to differentiate in sympatric species and would differentiate only inasmuch as it had the same mechanisms of production as the mating call, which is under such pressure". Thus, advertisement vocalizations and release vocalizations are apparently not completely independent of one another. If release vocalizations are influenced by selective pressures affecting advertisement vocalizations, it is not surprising that there was no clear convergent pattern with respect to release vocalizations if selective pressures for advertisement vocalizations to diverge are "more important". It seems reasonable to assume that there is a selective trade-off with respect to the two vocalizations. In one instance selection should favor convergence of release vocalizations to facilitate interspecies communication and prevent prolonged amplexus among mismatched heterospecifics. On the other hand, selection should favor divergence of advertisement vocalizations to prevent hybridization. Maintaining isolation mechanism integrity (i.e. divergence of advertisement vocalizations) among sympatric species is likely to directly affect displacement of release vocalizations.

Number of pulses, periodicity, dominant frequency and maximum frequency of advertisement vocalizations indicated divergent character displacement. In accordance with Blair's (1968) statement, advertisement vocalization parameters that are analogous to release vocalization parameters (i.e; periodicity, dominant frequency, minimum frequency and maximum frequency) would be expected to reflect similar divergent trends. Indeed, several parameters indicated divergent character displacement with respect to advertisement vocalizations and analogous release vocalization parameters. Those parameters included dominant frequency of the first and middle chirps, minimum frequency of the first and last chirps and maximum frequency of the first chirp. However, periodicity of the middle chirp and maximum frequency of the last chirp were convergent whereas analogous advertisement vocalization parameters were divergent. Thus, it appears as though release vocalizations have converged despite selective pressures for analogous advertisement vocalization parameters to diverge.

Although advertisement and release vocalizations are subject to selective forces acting in opposing directions, the two signals appear to largely influence one another. Thus, convergent and divergent character displacement of the two signals are not mutually exclusive. This study provides evidence of character displacement counterbalanced by selectional forces acting in opposing directions on "coupled" species signals. However, selective pressures among B. woodhousii and B. terrestris have resulted primarily in convergent character displacement of release vocalizations and divergent character displacement of advertisement vocalizations. Thus, interspecies communication during heterospecific amplexus is facilitated while interspecific isolation is maintained in sympatry.

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Table 1. Locations of recording sites and sample sizes (number of individuals recorded) for release vocalizations and advertisement vocalizations of B. terrestris and B. woodhousii in allopatry and sympatry. Sample sizes for advertisement vocalizations are within parenthesis adjacent to release vocalization sample sizes. Populations are designated with a number that correspond with the locality numbers in Figure 1.

Population	Species	Sample size	Condition	Locality
1	B. terrestris	9	allopatric	Miami, Dade Co., FL
2	B. terrestris	12(11)	allopatric	Tampa, Hillsborough Co., FL
3	B. terrestris	9	allopatric	Ocala National Forest, Marion Co., FL
4	B. terrestris	11	allopatric	Tallahassee, Leon Co., FL
5	B. woodhousii	9(9)	allopatric	0.4 km E of Co. Rd. 054 on Co. Rd. 433, Auburn, Lee Co., AL
6	B. woodhousii	7(8)	allopatric	1.2 km E of Co Rds. 137 and 152 junction, Auburn, Lee Co., AL
7	B. woodhousii	13(7)	allopatric	0.8 km N of Co. Rd. 137 on Co. Rd. 053, Auburn, Lee Co., AL
8	B. woodhousii	8(12)	allopatric	3.2 km E of State Highway 147 on Glenn Ave, Auburn, Lee Co., AL
9	B. woodhousii	9(2)	allopatric	4.8 km NW of State Highway 147 on US 280, Auburn, Lee Co., AL

10	B. terrestris	10	sympatric	Conecuh National Forest, Covington Co., AL
11	B. terrestris	10	sympatric	Eufaula National Wildlife Refuge, Barbour Co., AL
12	B. terrestris	5	sympatric	Junction US Highway 431 and State Highway 169, Russell Co., AL
13	B. terrestris	11	sympatric	Tuskegee Lake, Macon Co., AL
14	B. terrestris	21(14)	sympatric	Tuskegee National Forest, Macon Co., AL
15	B. woodhousii	7	sympatric	Junction US Highway 431 and State Highway 169, Russell Co., AL
16	B. woodhousii	19(19)	sympatric	Tuskegee Lake, Macon Co., AL
17	B. woodhousii	18(6)	sympatric	Tuskegee National Forest, Macon Co., AL
18	B. woodhousii	9(11)	sympatric	Euphapee Creek, Macon Co., AL
19	B. woodhousii	9(9)	sympatric	US Highway 29 at Lee and Macon Co. border, AL

TABLE 2. Effects of temperature (Temp) and snout-vent length (SVL) on release vocalization parameters for B. terrestris and B. woodhousii in allopatry and sympatry. A " * " indicates a significant effect ($p \leq 0.05$) and a " 0 " indicates no significant effect ($p > 0.05$) in linear regression analyses. Marginally significant p-values are reported ($0.05 < p \leq 0.07$). Dom. Freq. = dominant frequency, Min. Freq. = minimum frequency and Max. Freq. = maximum frequency.

Call Parameter	B. terrestris				B. woodhousii			
	Allopatric		Sympatric		Allopatric		Sympatric	
	Temp	SVL	Temp	SVL	Temp	SVL	Temp	SVL
Interchirp Duration	*	0	0	0	*	0	0	0
Chirp Duration	0	0	0	0	0	0	0.07	0
Pulses first chirp	0	0	0	0	0	*	0	0
Pulses mid chirp	*	0	0	0	0	0	0	0
Pulses last chirp	0	0	0	0	0	0.05	0	0
Periodicity first chirp	0	0	0	0	0	*	0	0.06
Periodicity mid chirp	*	0	0	0	0	0	0	0
Periodicity last chirp	0	0	0.07	*	0	0	0	0
Dom. Freq. first chirp	*	0	*	0	0	0	*	0
Dom. Freq. mid chirp	0	0	0	0	0	0	0	0
Dom. Freq. last chirp	*	0	0	0	0	0	0	0
Range first chirp	*	0	*	0	0.06	0	0	0
Range mid chirp	*	0	0	0	0	0	0	0
Range last chirp	*	0	0	0	0	0	0	0
Min. Freq. first chirp	*	0	0	0	0	0	0	0
Min. Freq. mid chirp	0	0	0	*	0	0	*	0
Min. Freq. last chirp	0	0	0	*	0	0	*	0
Max. Freq. first chirp	*	0	*	0	0.06	0	*	0
Max. Freq. mid chirp	*	0	0	0	0	0	0	0
Max. Freq. last chirp	*	0	0	0	0	0	0	0

TABLE 3. Effects of temperature (Temp) and snout-vent length (SVL) on advertisement vocalization parameters for B. terrestris and B. woodhousii in allopatry and sympatry. A " * " indicates a significant effect ($p \leq 0.05$) and a " 0 " indicates no significant effect ($p > 0.05$) in linear regression analyses. Marginally significant p-values are reported ($0.05 < p \leq 0.08$). Temperature adjustments for B. terrestris were not necessary (see text).

	B. terrestris				**B. woodhousii**			
	Allopatric		Sympatric		Allopatric		Sympatric	
Call Parameter	Temp	SVL	Temp	SVL	Temp	SVL	Temp	SVL
Number of Pulses		0		0	*	0	*	*
Periodicity		0		0	*	0	*	*
Dominant Frequency		0		0	0	*	0.07	0
Range		0		0	0	0	0	0
Minimum Frequency		0		0	0	*	0	0
Maximum Frequency		*		0	0	*	0	0

TABLE 4. ANOVA results (actual p-values) comparing the release vocalizations of allopatric B. terrestris versus sympatric B. terrestris (Bt allo vs. Bt sym), allopatric B. woodhousii versus sympatric B. woodhousii (Bw allo vs. Bw sym), allopatric B. terrestris versus allopatric B. woodhousii (Bt allo vs. Bw allo) and sympatric B. terrestris versus sympatric B. woodhousii (Bt sym vs. Bw sym). "Temp" columns indicate results for parameters adjusted for temperature only (21°C) and "Temp + SVL" columns indicate results for parameters adjusted for both temperature and mean SVL (see text). A " * " indicates a significant difference ($p \leq 0.05$) and a " • " indicates no significant difference ($p > 0.05$). Empty cells indicate those parameters that were not necessary to adjust for the effects of both temperature and SVL (see text). Dom. Freq. = dominant frequency, Min. Freq. = minimum frequency and Max. Freq. = maximum frequency.

Call Parameter	Bt allo vs. Bt sym		Bw allo vs. Bw sym		Bt allo vs. Bw allo		Bt sym vs. Bw sym	
	1	2	3	4	5	6	7	8
	Temp	Temp + SVL	Temp	Temp + SVL	Temp	Temp + SVL	Temp	Temp + SVL
Interchirp Duration	0.08 •		0.8 •		0.1 •		0.4 •	
Chirp Duration	0.1 •		0.2 •		0.4 •		0.2 •	
Pulses first chirp Pulses mid chirp Pulses last chirp	0.7 • 0.0001* 0.007 *	0.5 • 0.0001* 0.02 *	0.3 • 0.8 • 0.6 •	0.7 • 0.9 • 0.8 •	0.9 • 0.008 * 0.2 •	0.4 • 0.002 * 0.07 •	0.5 • 0.7 • 0.9 •	0.5 • 0.5 • 0.6 •
Periodicity first chirp Periodicity mid chirp Periodicity last chirp	1.0 • 0.0001* 0.2 •	0.8 • 0.0001* 0.06 •	0.9 • 0.6 • 0.9 •	0.6 • 0.8 • 0.9 •	0.9 • 0.01 * 0.07 •	0.9 • 0.004 * 0.05 *	1.0 • 0.8 • 0.5 •	0.6 • 0.6 • 0.4 •
Dom. Freq. first chirp Dom. Freq. mid chirp Dom. Freq. last chirp	0.002 * 1.0 • 0.009 *	0.002 * 1.0 • 0.008 *	0.0004* 0.002 * 0.05 *	0.0003* 0.002 * 0.05 *	0.3 • 1.0 • 0.3 •	0.2 • 1.0 • 0.3 •	0.001 * 0.0001* 0.2 •	0.001 * 0.0001* 0.2 •
Range first chirp Range mid chirp Range last chirp	0.0001* 0.01 * 0.02 *		0.008 * 0.6 • 1.0 •		0.04 * 0.003 * 0.05 *		0.1 • 0.03 * 1.0 •	
Min. Freq. first chirp Min. Freq. mid chirp Min. Freq. last chirp	1.0 • 0.0001* 1.0 •	1.0 • 0.0001* 1.0 •	0.01 * 0.0002* 0.003 *	0.01 * 0.0002 * 0.003 *	1.0 • 0.0004* 0.1 •	1.0 • 0.0004* 0.1 •	0.0004* 0.0002* 0.03 *	0.0004* 0.0002* 0.03 *
Max. Freq. first chirp Max. Freq. mid chirp Max. Freq. last chirp	0.002 * 0.1 • 0.02 *	0.002 * 0.1 • 0.02 *	0.0003* 0.1 • 0.5 •	0.002 * 0.03 * 0.5 •	0.2 • 0.09 • 0.05 *	0.04 * 0.08 • 0.05 *	0.006 * 0.006 * 0.4 •	0.005 * 0.0006* 0.4 •

TABLE 5. ANOVA results (actual p-values) comparing advertisement vocalizations of allopatric B. terrestris versus sympatric B. terrestris (Bt allo vs. Bt sym), allopatric B. woodhousii versus sympatric B. woodhousii (Bw allo vs. Bw sym), allopatric B. terrestris versus allopatric B. woodhousii (Bt allo vs. Bw allo) and sympatric B. terrestris versus sympatric B. woodhousii (Bt sym vs. Bw sym). "Temp" columns indicate results for parameters adjusted for temperature only (21°C) and "Temp + SVL" columns indicate results for parameters adjusted for both temperature and mean SVL (see text). A " * " indicates a significant difference ($p \leq 0.05$) and a " • " indicates no significant difference ($p > 0.05$). Empty cells indicate those parameters that were not necessary to adjust for the effects of both temperature and SVL (see text).

Call Parameter	Bt allo vs. Bt sym		Bw allo vs. Bw sym		Bt allo vs. Bw allo		Bt sym vs. Bw sym	
	1	2	3	4	5	6	7	8
	Temp	Temp + SVL	Temp	Temp + SVL	Temp	Temp + SVL	Temp	Temp + SVL
Number of Pulses	0.1 •	0.01 *	0.0001 *	0.0001 *	0.0001 *	0.0001 *	0.0001 *	0.0001 *
Periodicity	0.08 •	0.005 *	0.0001 *	0.0001 *	0.0001 *	0.0001 *	0.0001 *	0.0001 *
Dominant Frequency	0.4 •	0.4 •	0.0004 *	0.0002 *	0.02 *	0.0001 *	0.0001 *	0.0001 *
Range of Frequency	0.04 *		0.2 •		0.0001 *		0.0001 *	
Minimum Frequency	0.09 •	0.2 •	0.002 *	0.001 *	0.0003 *	0.008 *	0.3 •	0.09 •
Maximum Frequency	0.8 •	0.02 *	0.001 *	0.0001 *	0.0001 *	0.3 •	0.0001 *	0.0001 *

FIGURE LEGENDS

FIG. 1. Box plots of the range of frequency (kHz) of the first chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (21°C). The center lines of the means diamonds are the group means. The top and bottom of the diamonds form the 95% confidence intervals for the means. Rectangles represent quantile boxes where the ends of the boxes are the 25th and 75th quantiles and the line across the middle of the boxes identifies the median sample value. Quantile box width reflects relative sample sizes. Whiskers represent the upper and lower extremes. The horizontal line extending the entirety of the graph indicates the combined sample mean.

FIG. 2. Box plots of the number of pulses of the middle chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 3. Box plots of the periodicity (mS) of the middle chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 4. Box plots of the range of frequency (kHz) of the last chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature. See Fig. 2 for an explanation of the box plots.

FIG. 5. Box plots of the maximum frequency (kHz) of the last chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 6. Box plots of the dominant frequency (kHz) of the middle chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 7. Box plots of the minimum frequency (kHz) of the first chirp of the release vocalization for *B. terrestris* and *B. woodhousii* in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 8. Box plots of the minimum frequency (kHz) of the last chirp of the release vocalization for *B. terrestris* and *B. woodhousii* in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 9. Box plots of the dominant frequency (kHz) of the first chirp of the release vocalization for *B. terrestris* and *B. woodhousii* in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 10. Box plots of the maximum frequency (kHz) of the first chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 11. Box plots of the number of pulses of the last chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 12. Box plots of the range of frequency (kHz) of the middle chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature. See Fig. 2 for an explanation of the box plots.

FIG. 13. Box plots of the minimum frequency (kHz) of the middle chirp of the release vocalization for B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 14. Box plots of the minimum frequency (kHz) of the advertisement vocalization of B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

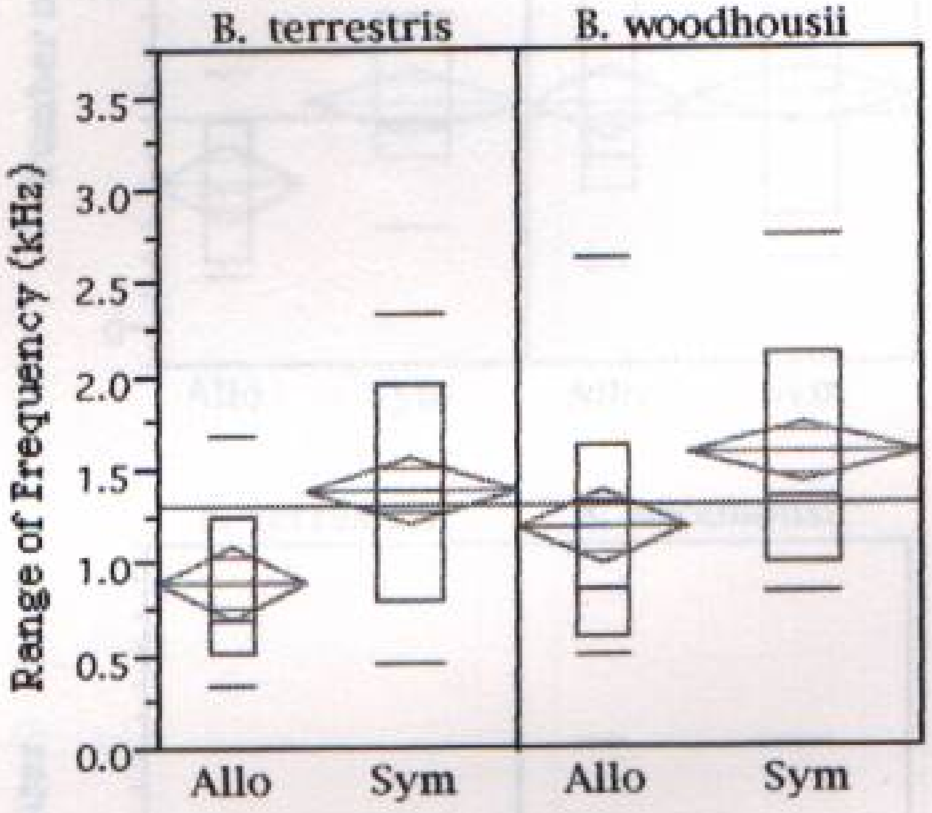
FIG. 15. Box plots of the maximum frequency (kHz) of the advertisement vocalization of B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

FIG. 16. Box plots of the number of pulses of the advertisement vocalization of B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

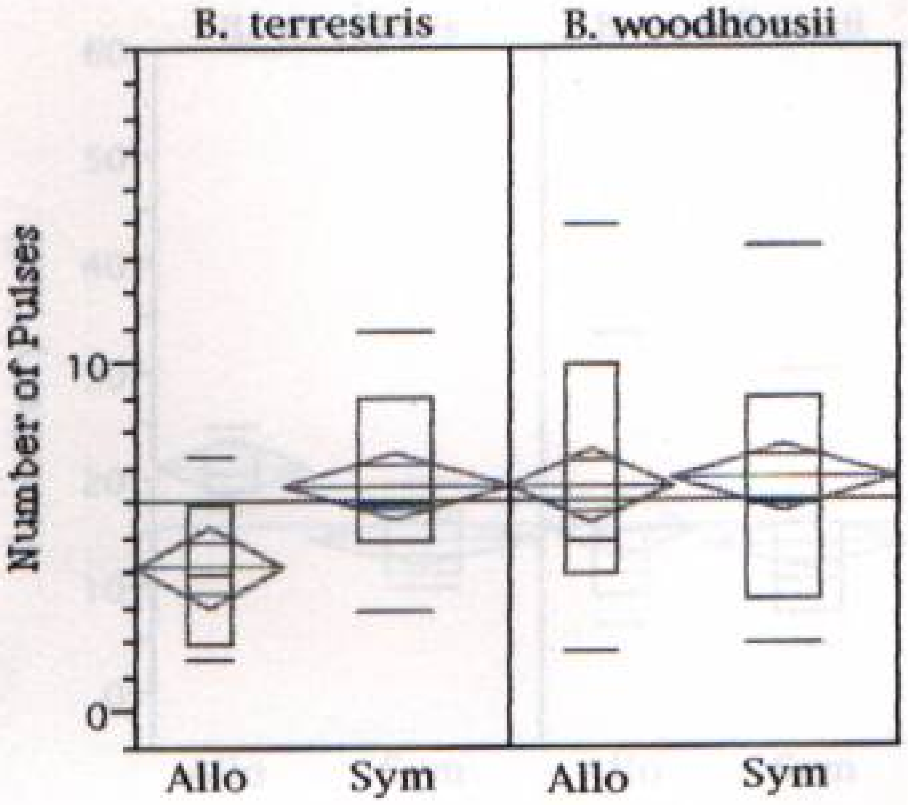
FIG. 17. Box plots of the periodicity (mS) of the advertisement vocalization of B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (mm)(B). See Fig. 2 for an explanation of the box plots.

FIG. 18. Box plots of the dominant frequency (kHz) of the advertisement vocalization of B. terrestris and B. woodhousii in allopatry (allo) and in sympatry (sym) adjusted for the effects of temperature (A) and both temperature and SVL (B). See Fig. 2 for an explanation of the box plots.

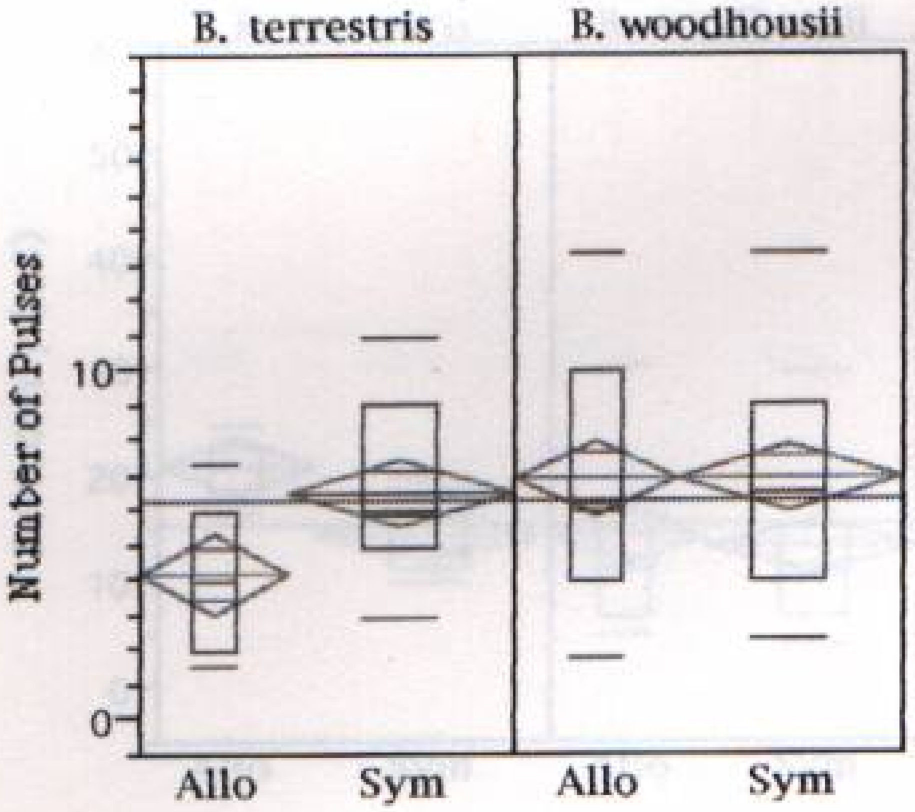
FIG. 19. Box plots of the periodicity of the advertisement vocalization for B. woodhousii across the sampling range adjusted for the effects of temperature. Plots are arranged chronologically from the southern-most population to the northern-most population. The center lines of the means diamonds are the group means. The top and bottom of the diamonds form the 95% confidence intervals for the means. Whiskers represent the standard deviation.


B. terrestris
B. woodhousii
Range of Frequency (kHz)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Allo
Sym
Allo
Sym

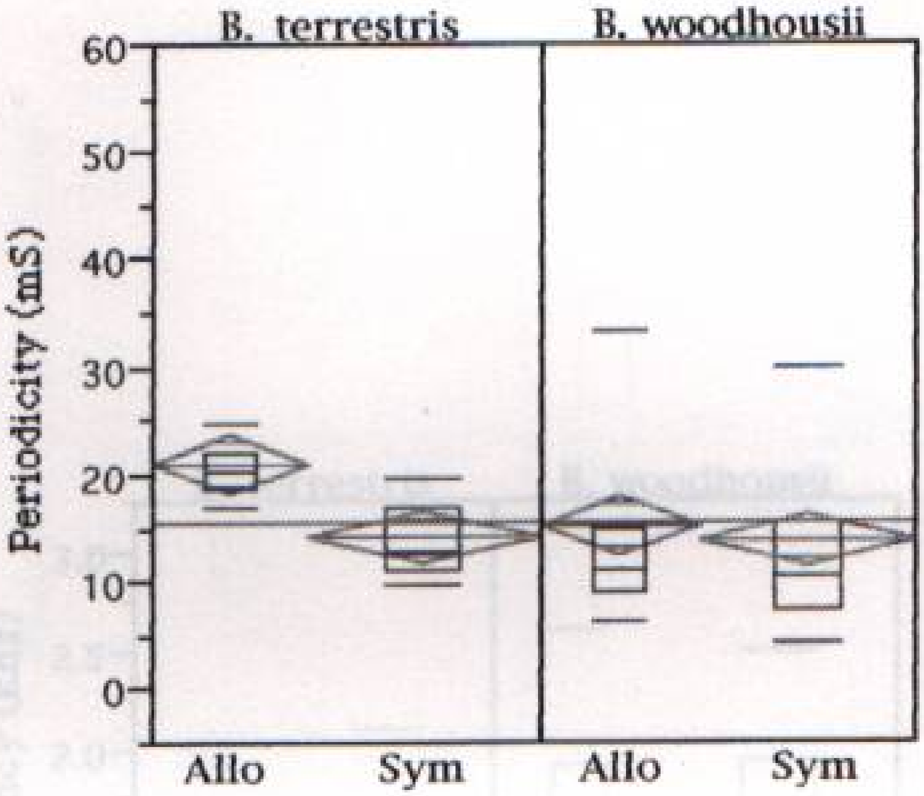
A


B. terrestris
B. woodhousii
Number of Pulses
10
0
Allo
Sym
Allo
Sym

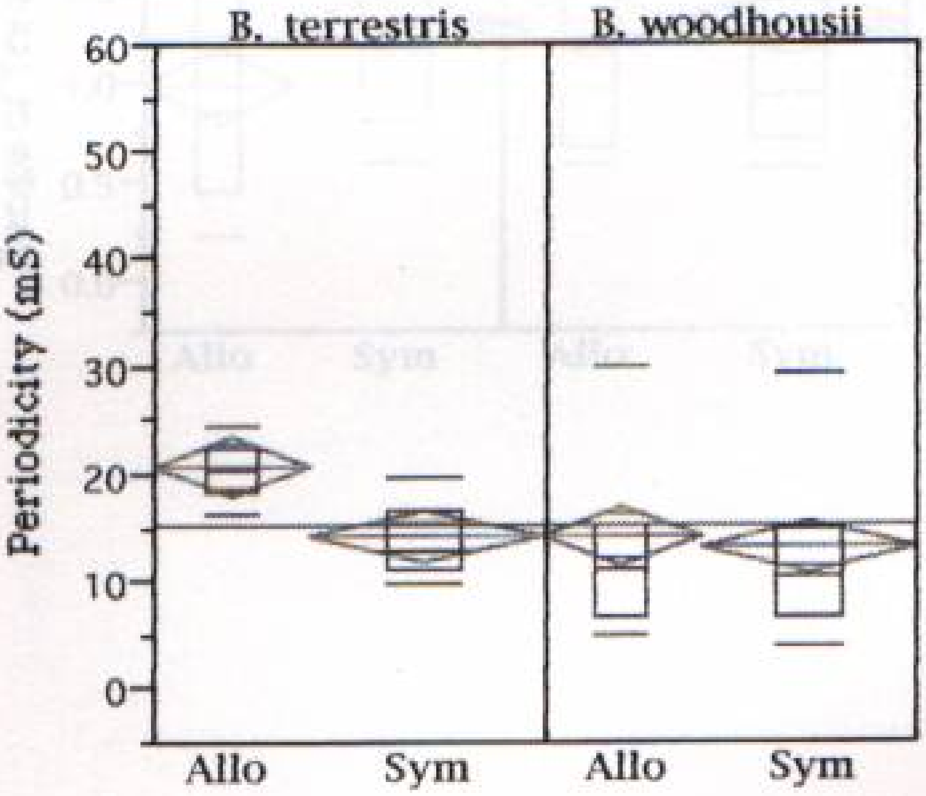
B

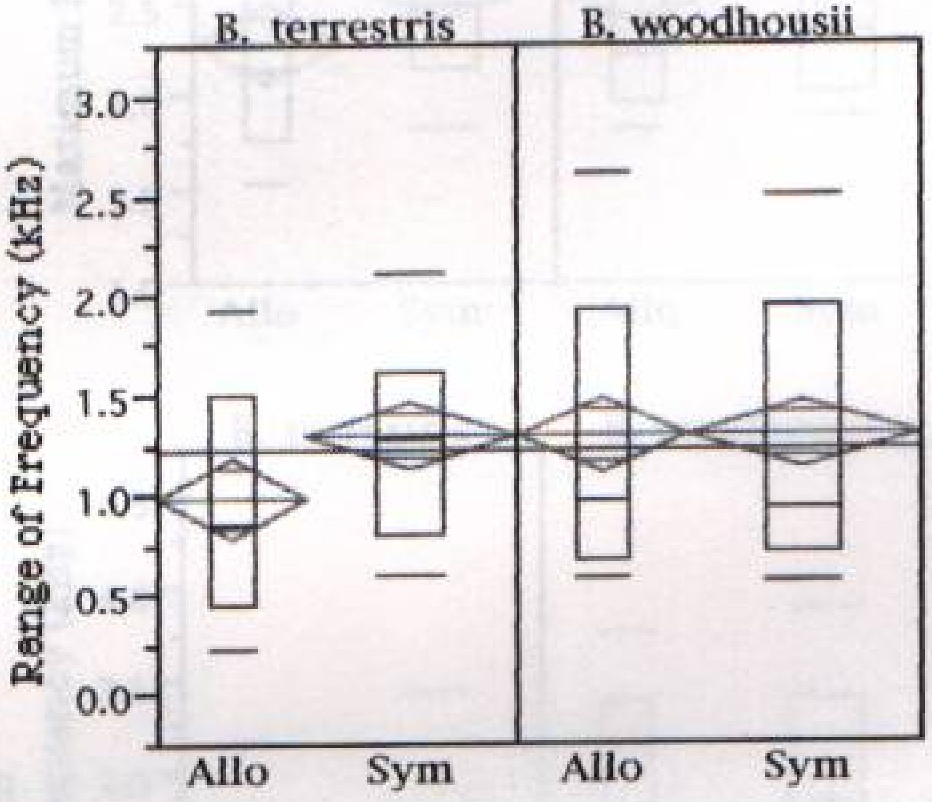

B. terrestris
B. woodhousii
Number of Pulses
10
0
Allo
Sym
Allo
Sym

A

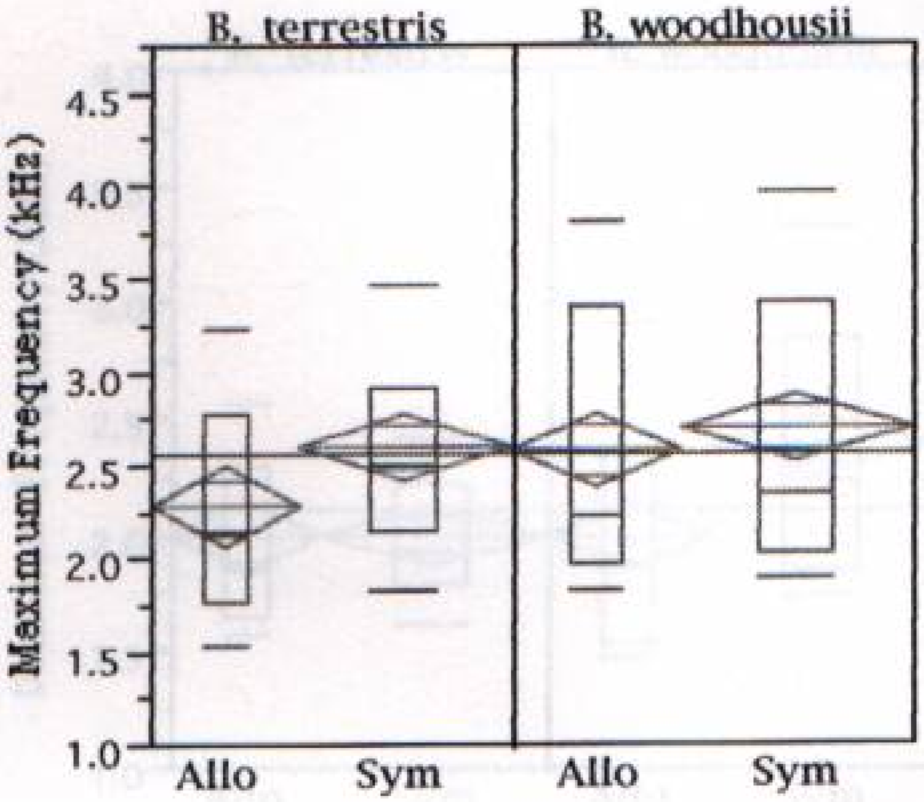

B. terrestris
B. woodhousii
Periodicity (mS)
60
50
40
30
20
10
0
Allo
Sym
Allo
Sym

B

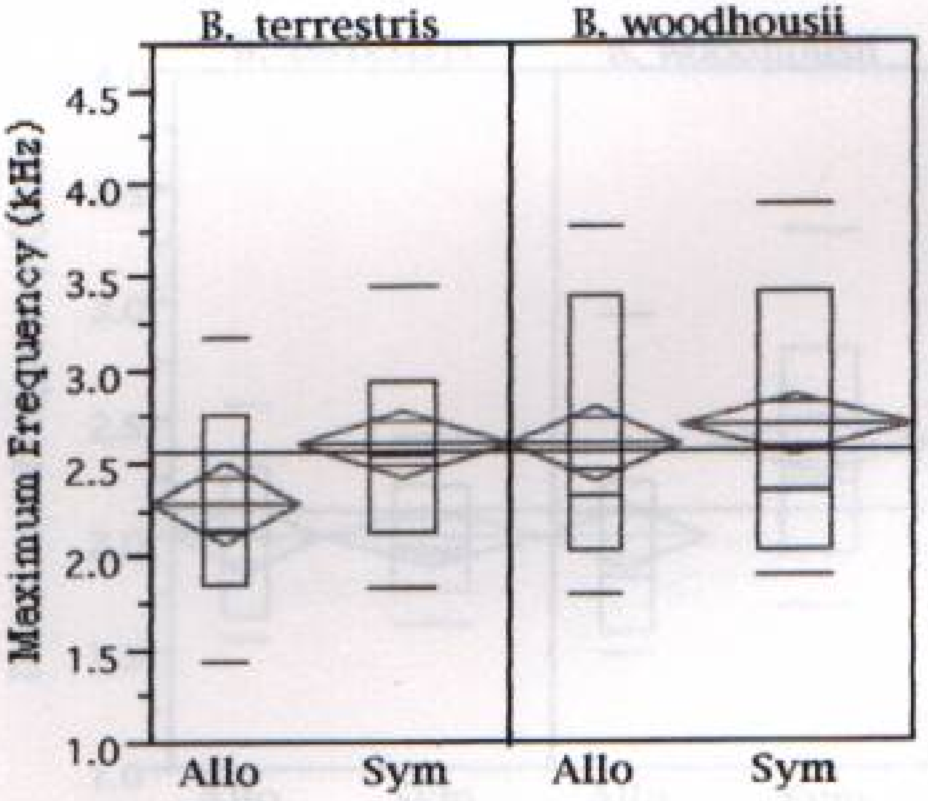

B. terrestris
B. woodhousii
Periodicity (mS)
60
50
40
30
20
10
0
Allo
Sym
Allo
Sym

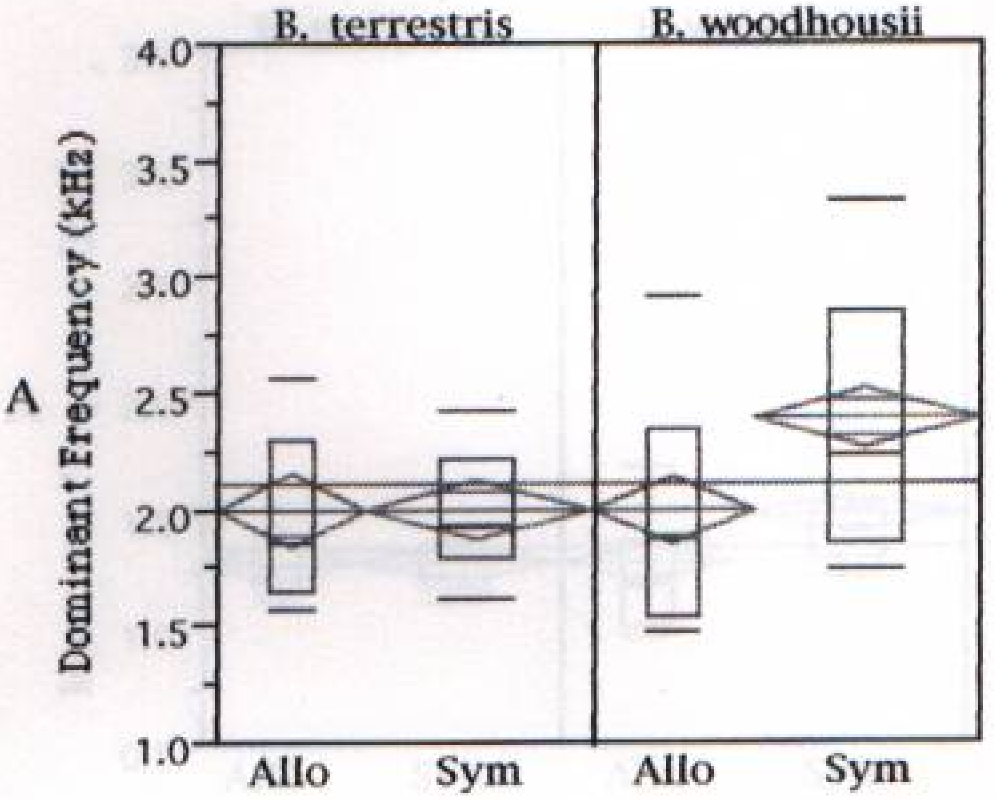

B. terrestris
B. woodhousii
Range of Frequency (kHz)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Allo
Sym
Allo
Sym

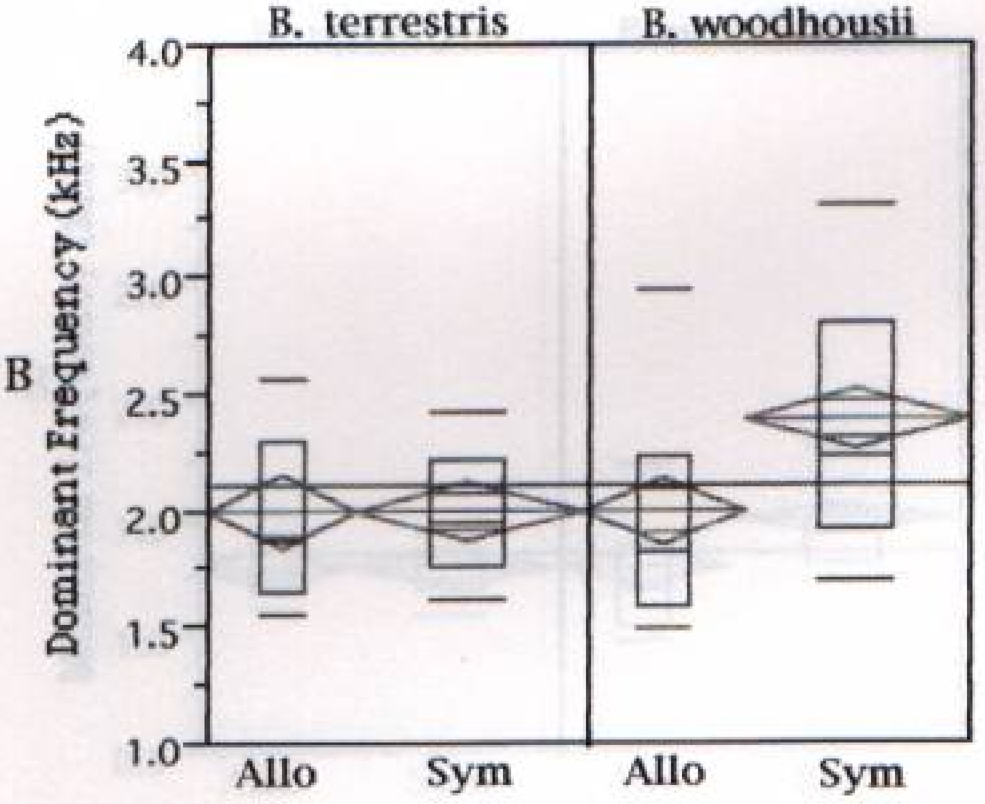
A


B. terrestris
B. woodhousii
Maximum Frequency (kHz)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym

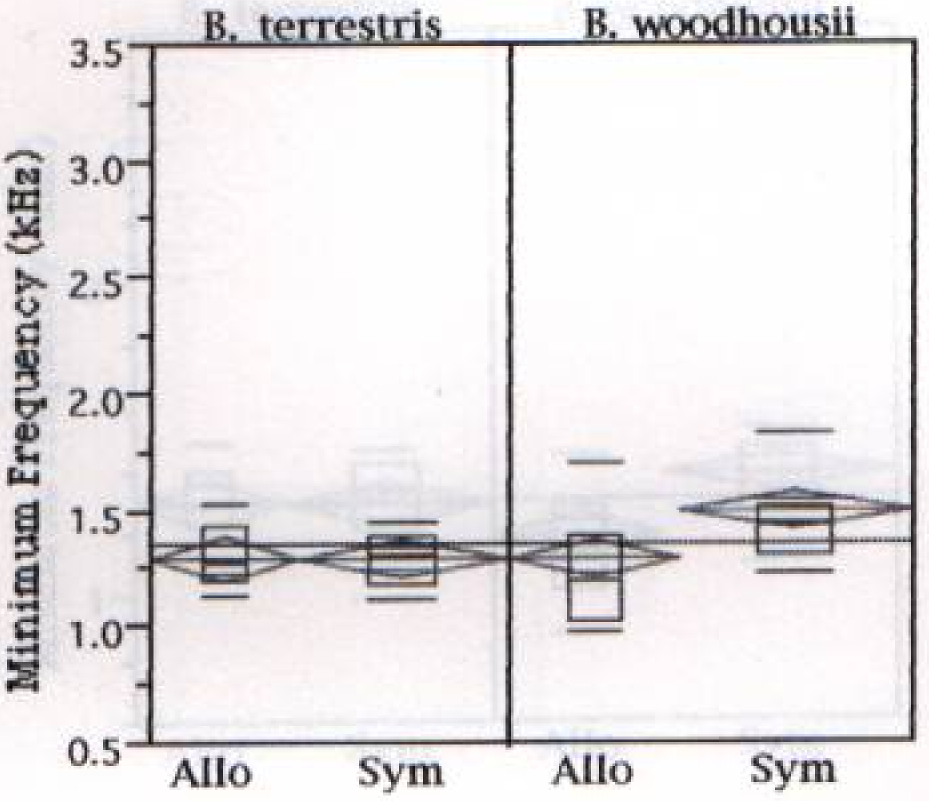
B


B. terrestris
B. woodhousii
Maximum Frequency (kHz)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym

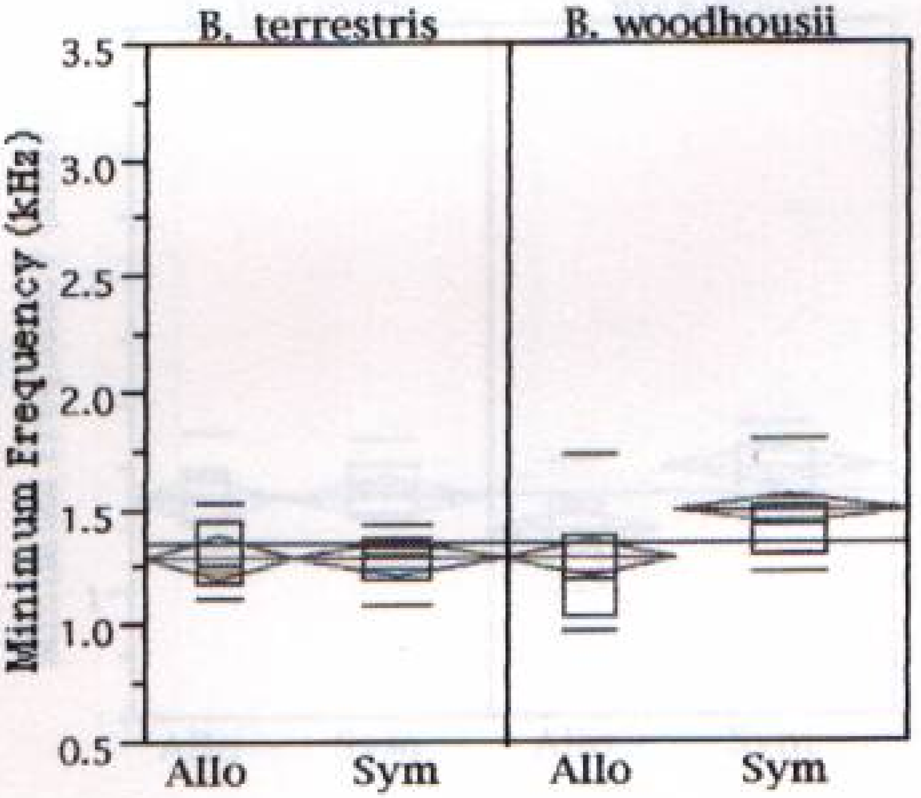

A
B. terrestris
B. woodhousii
Dominant Frequency (kHz)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym


B
B. terrestris
B. woodhousii
Dominant Frequency (kHz)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Allo
Sym
Allo
Sym

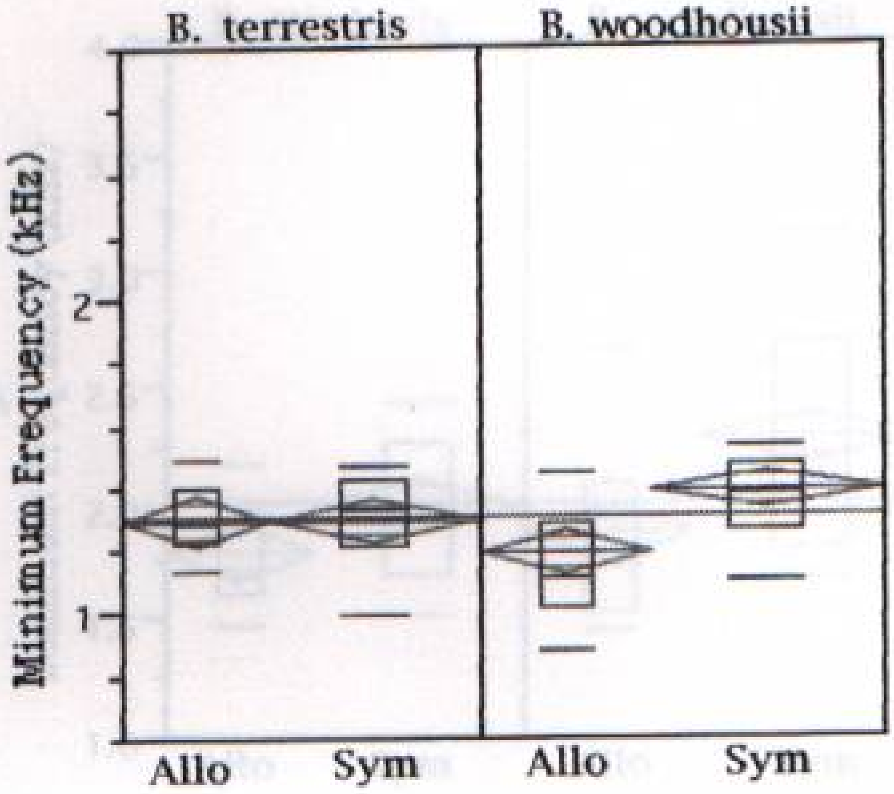
A


B. terrestris
B. woodhousii
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Minimum Frequency (kHz)
Allo
Sym
Allo
Sym

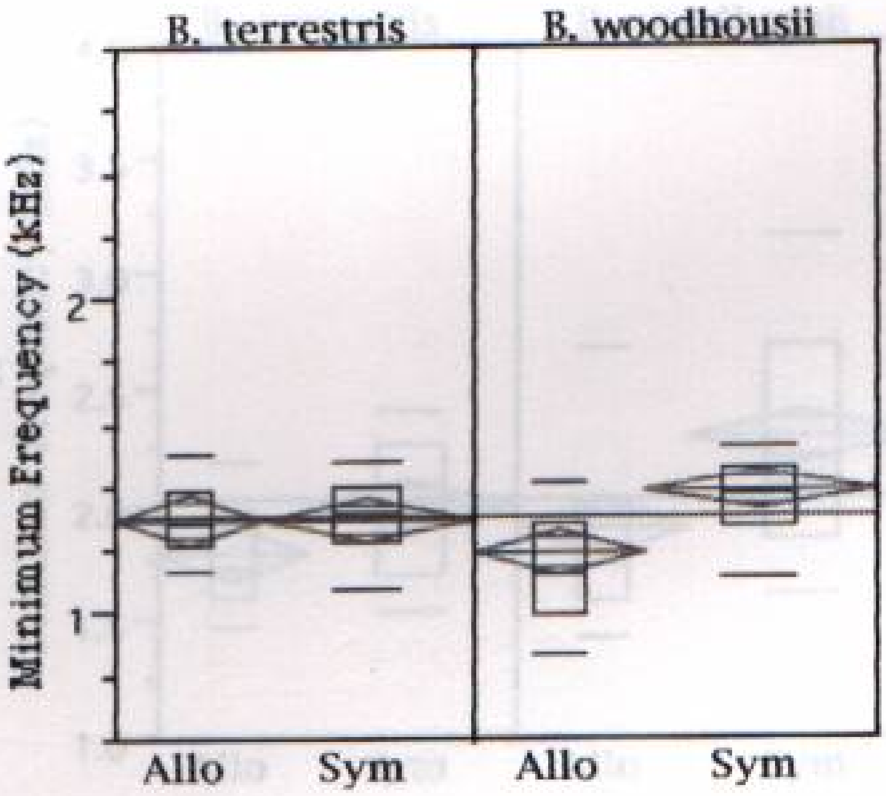
B


B. terrestris
B. woodhousii
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Minimum Frequency (kHz)
Allo
Sym
Allo
Sym

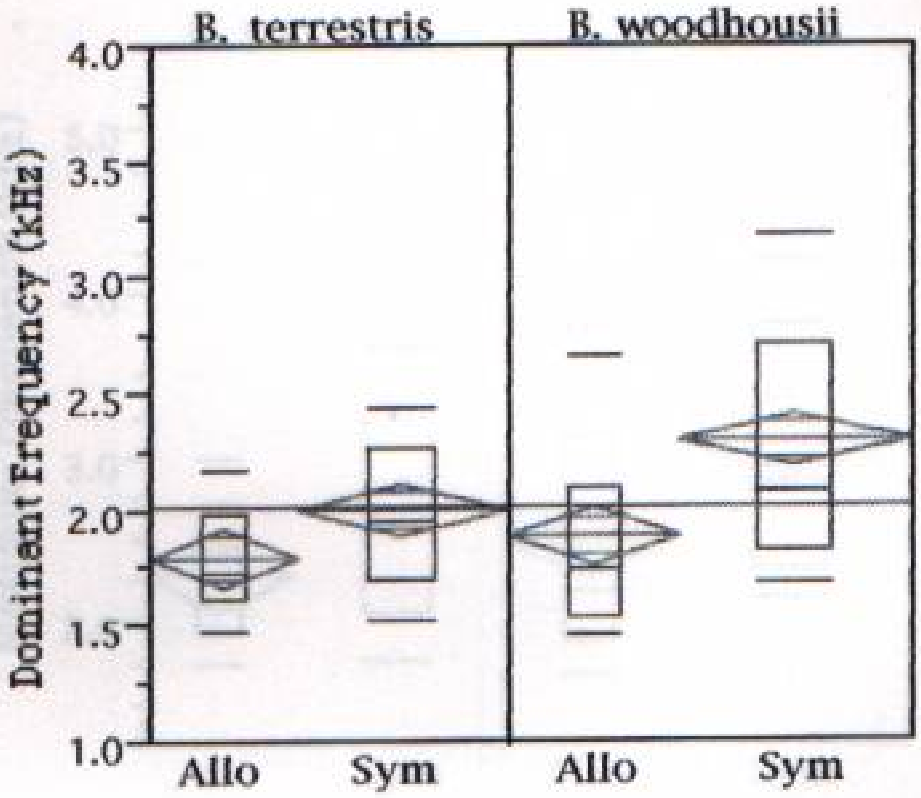
A


B. terrestris
B. woodhousii
Minimum Frequency (kHz)
2
1
Allo
Sym
Allo
Sym

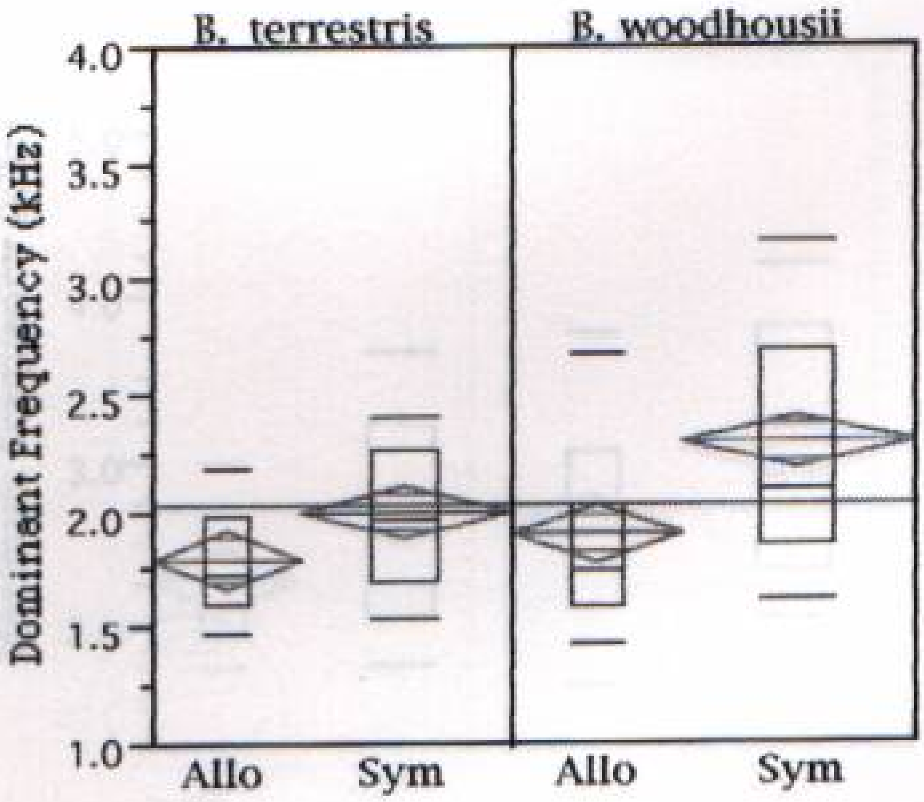
B


B. terrestris
B. woodhousii
Minimum Frequency (kHz)
2
1
Allo
Sym
Allo
Sym

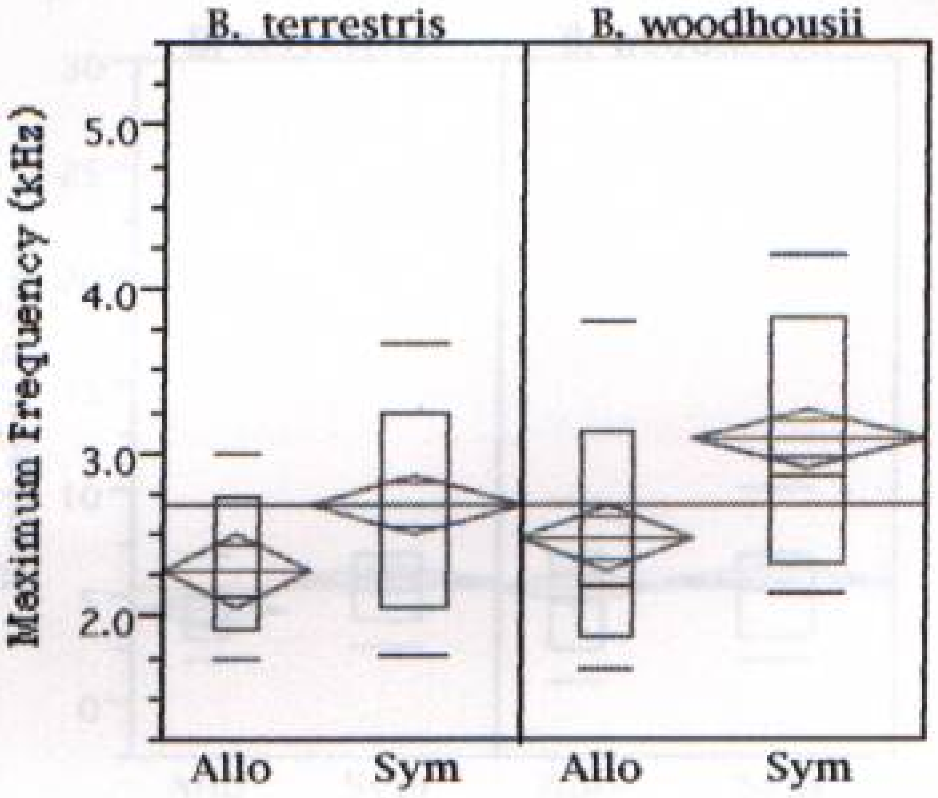
A


B. terrestris
B. woodhousii
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Dominant Frequency (kHz)
Allo
Sym
Allo
Sym

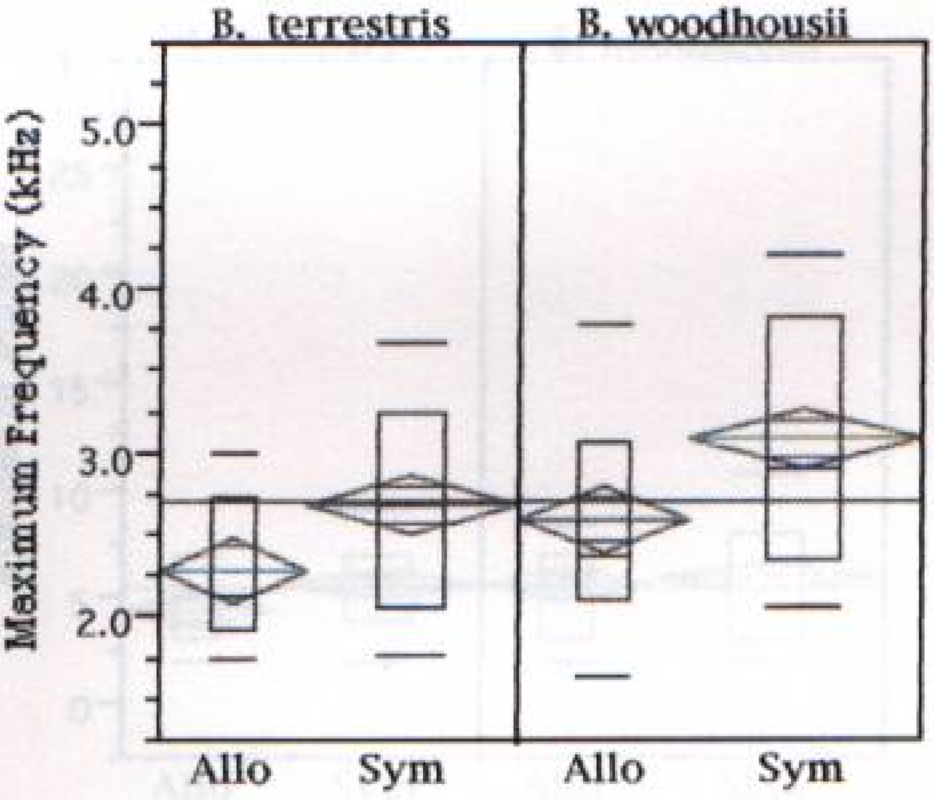
B

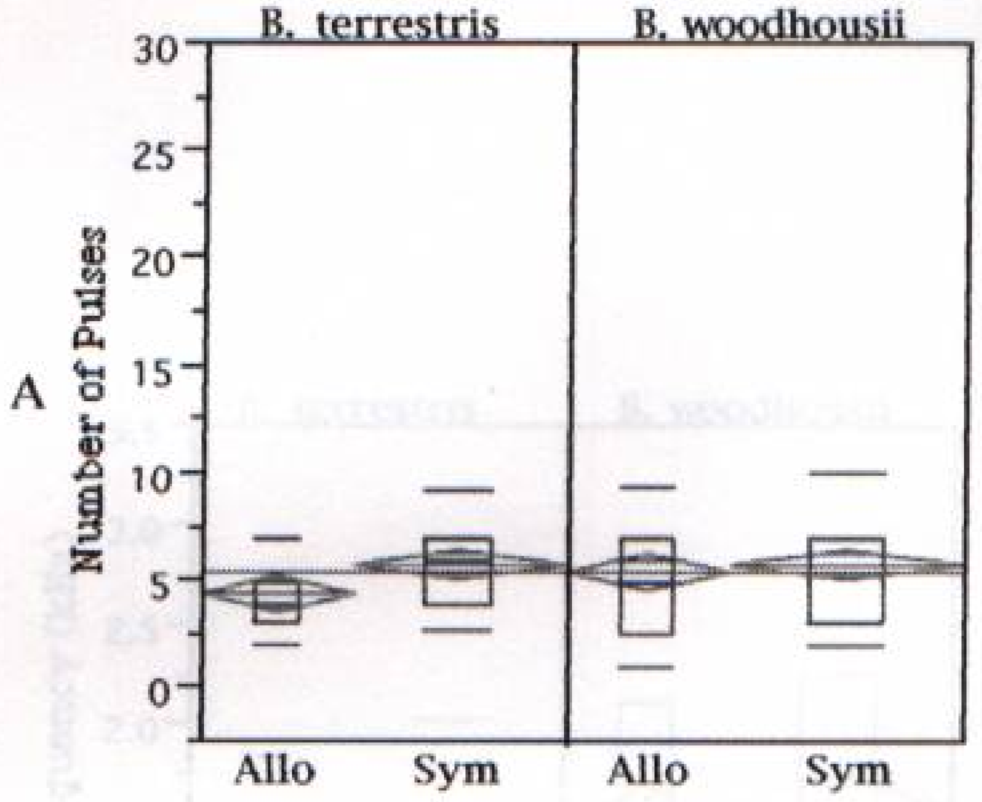

B. terrestris
B. woodhousii
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Dominant Frequency (kHz)
Allo
Sym
Allo
Sym

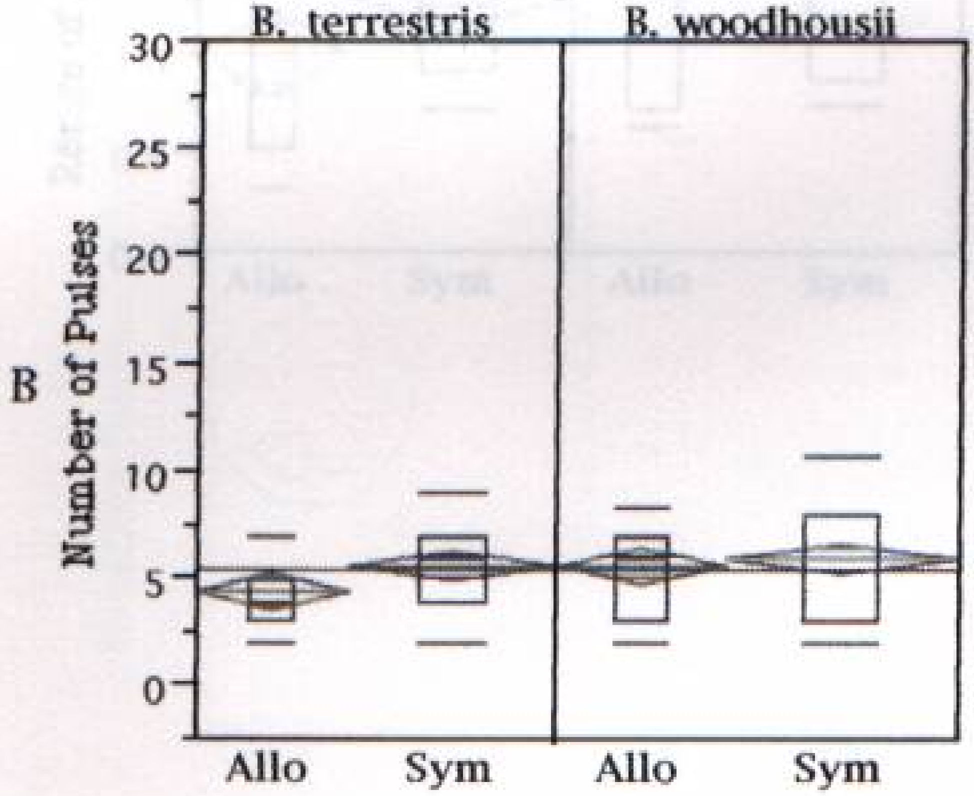
A

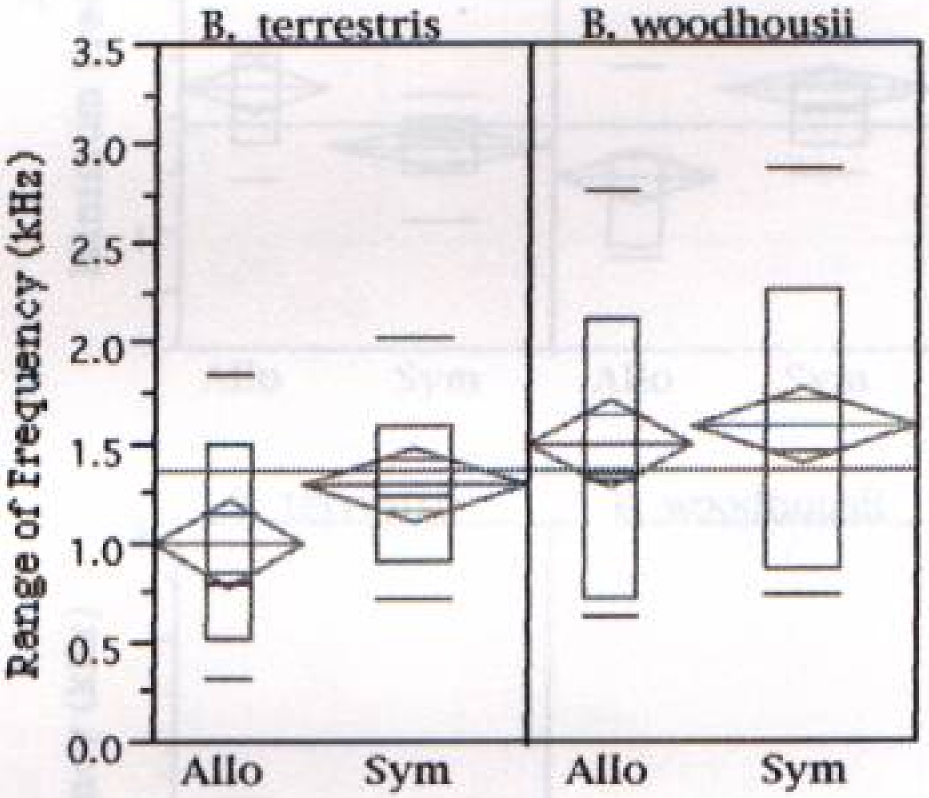

B. terrestris
B. woodhousii
Maximum Frequency (kHz)
5.0
4.0
3.0
2.0
Allo
Sym
Allo
Sym

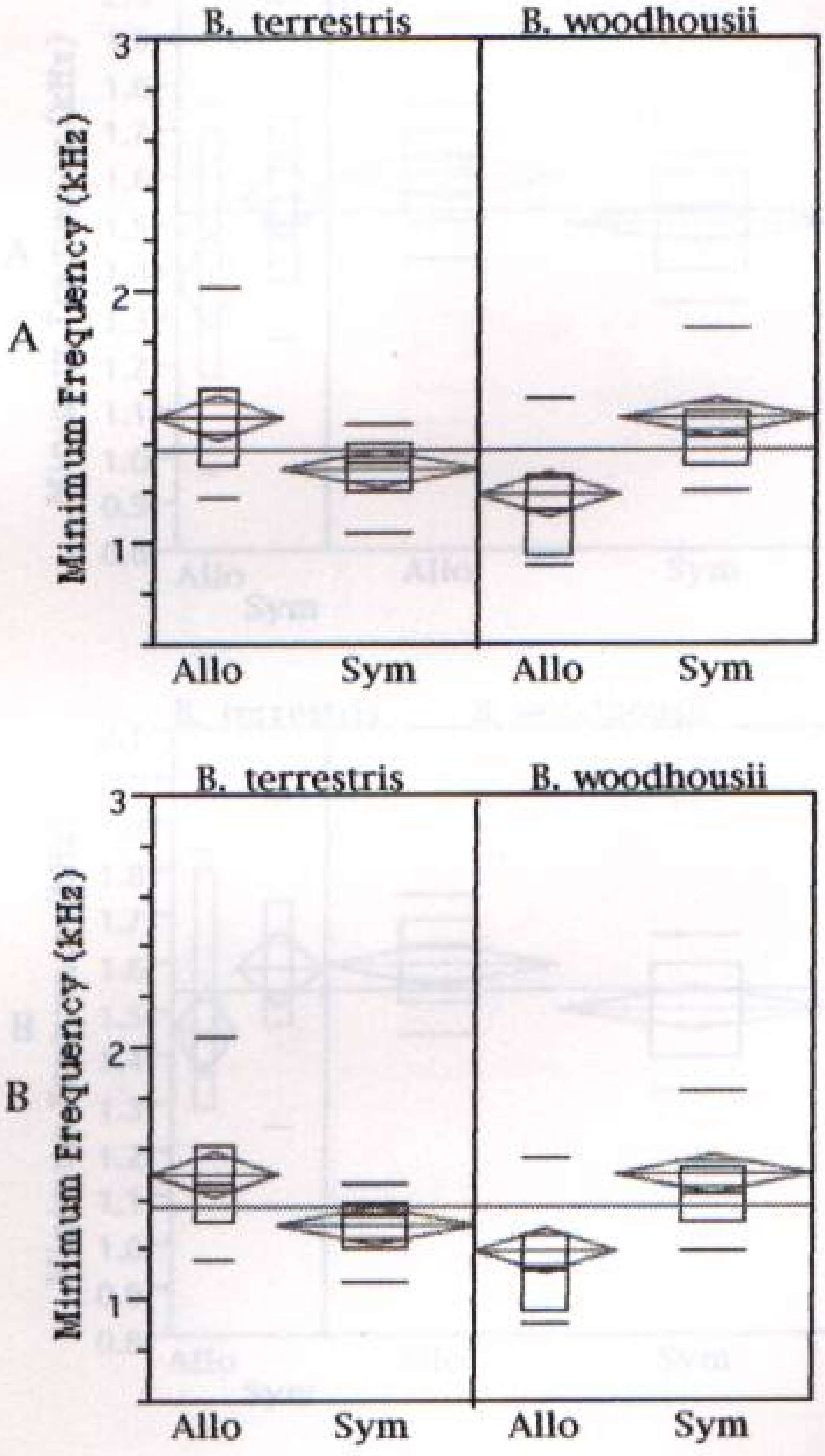
B


B. terrestris
B. woodhousii
Maximum Frequency (kHz)
5.0
4.0
3.0
2.0
Allo
Sym
Allo
Sym

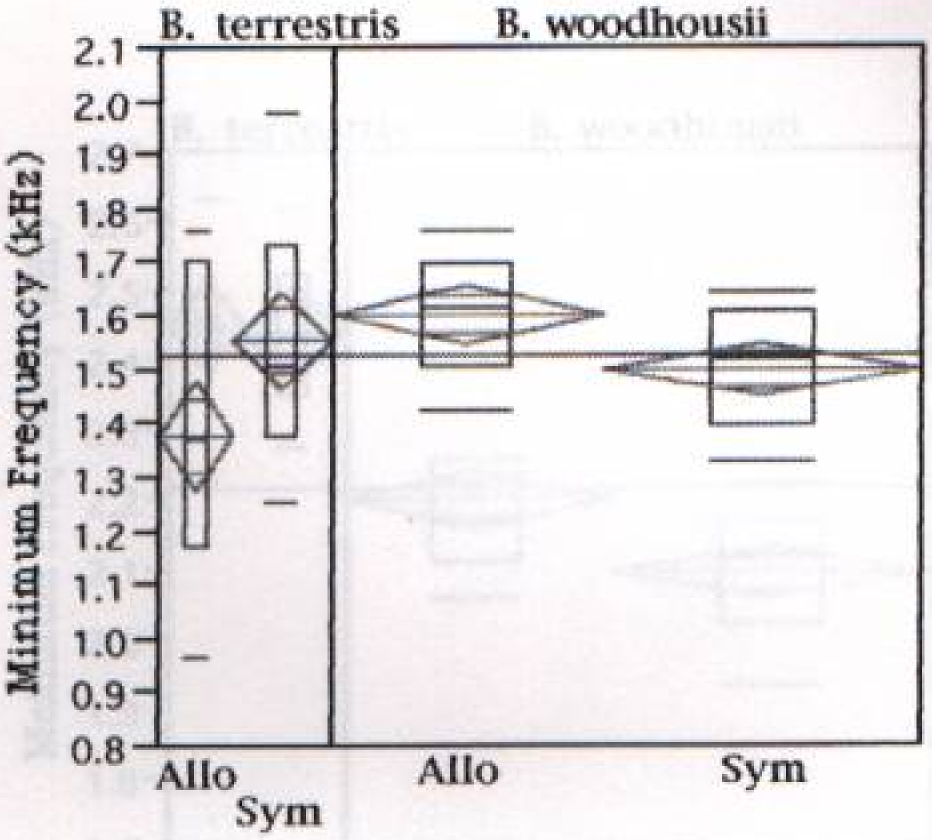

A
B. terrestris
B. woodhousii
Number of Pulses
30
25
20
15
10
5
0
Allo
Sym
Allo
Sym


B
B. terrestris
B. woodhousii
Number of Pulses
30
25
20
15
10
5
0
Allo
Sym
Allo
Sym

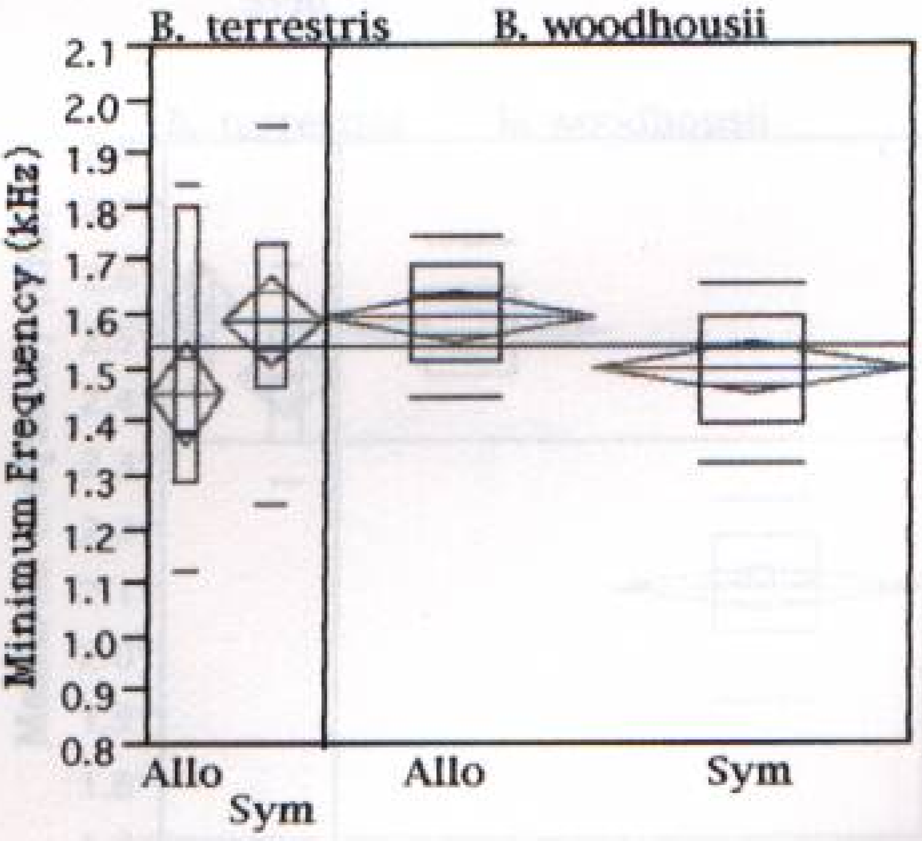

B. terrestris
B. woodhousii
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Range of Frequency (kHz)
Allo
Sym
Allo
Sym


A
B. terrestris
B. woodhousii
Minimum Frequency (kHz)
3
2
1
Allo
Sym
Allo
Sym
B
B. terrestris
B. woodhousii
Minimum Frequency (kHz)
3
2
1
Allo
Sym
Allo
Sym

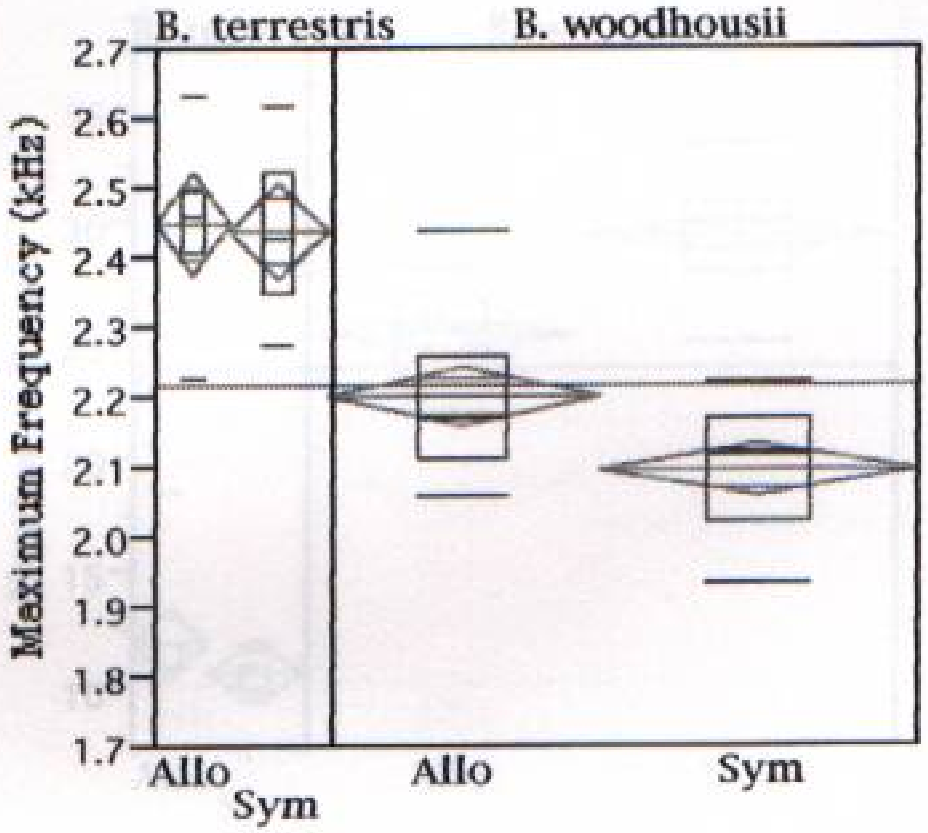
A


B. terrestris
B. woodhousii
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
Minimum Frequency (kHz)
Allo
Sym
Allo
Sym

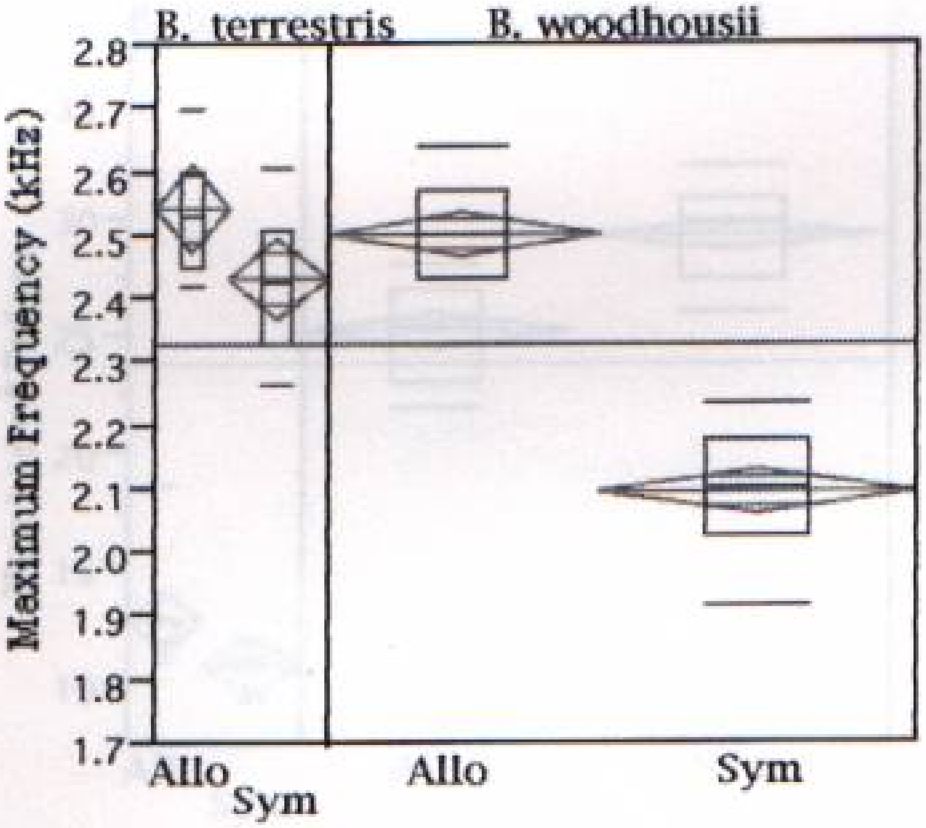
B

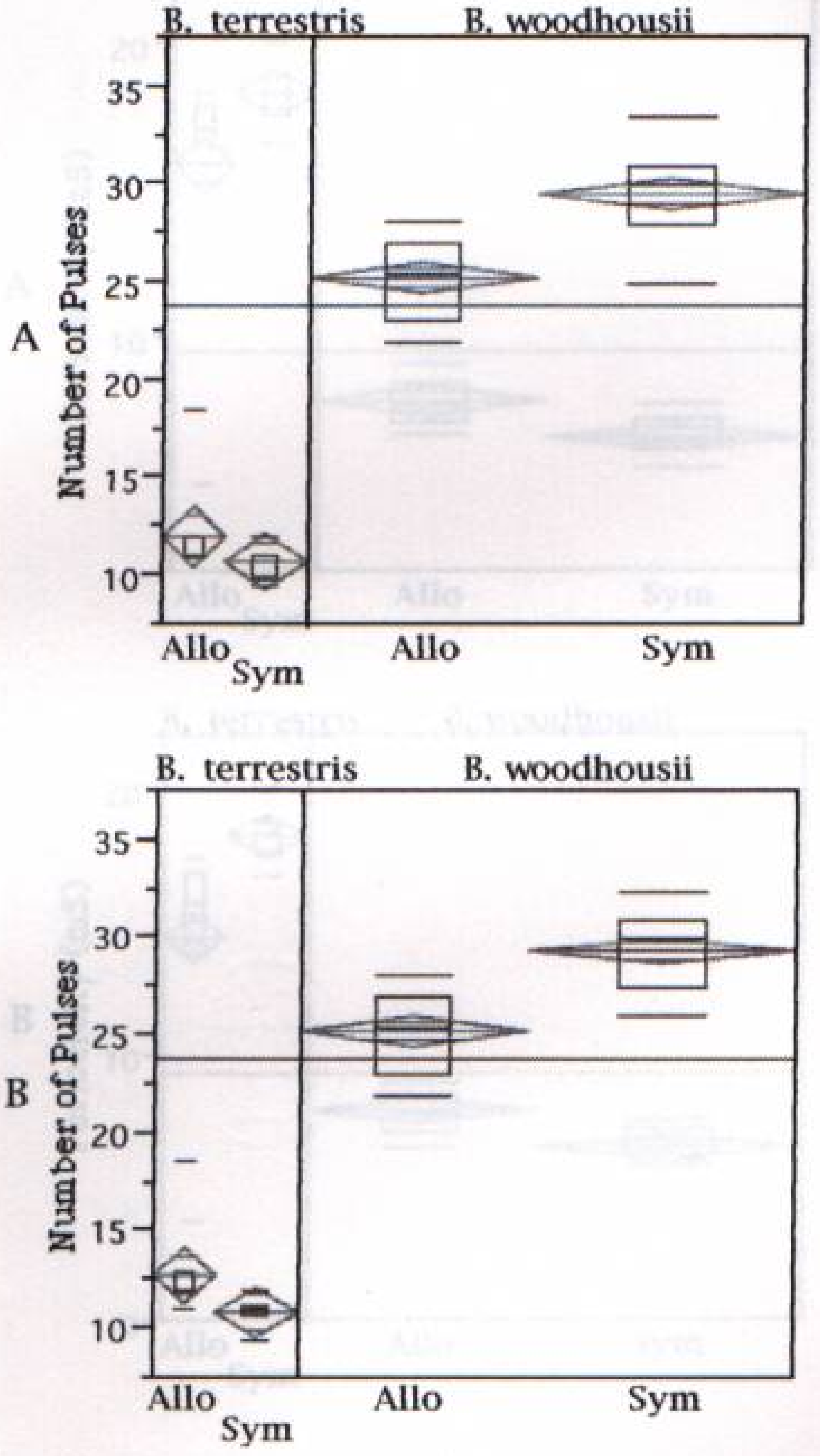

B. terrestris
B. woodhousii
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
Minimum Frequency (kHz)
Allo
Sym
Allo
Sym

A

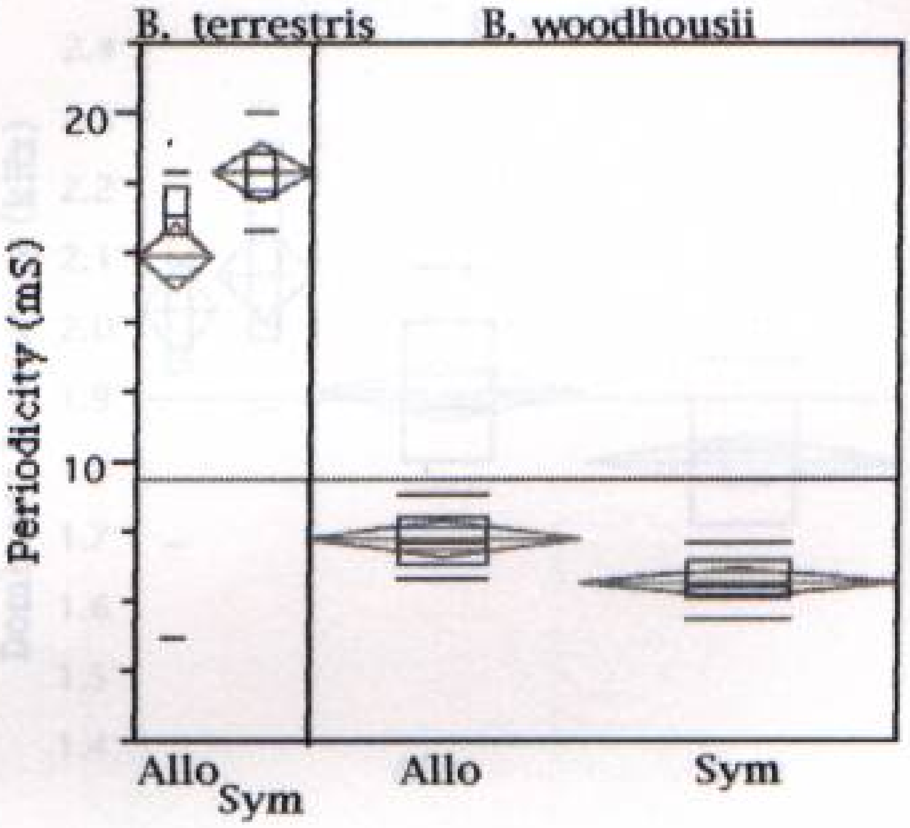

B. terrestris
B. woodhousii
Maximum Frequency (kHz)
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
Allo
Sym
Allo
Sym

B

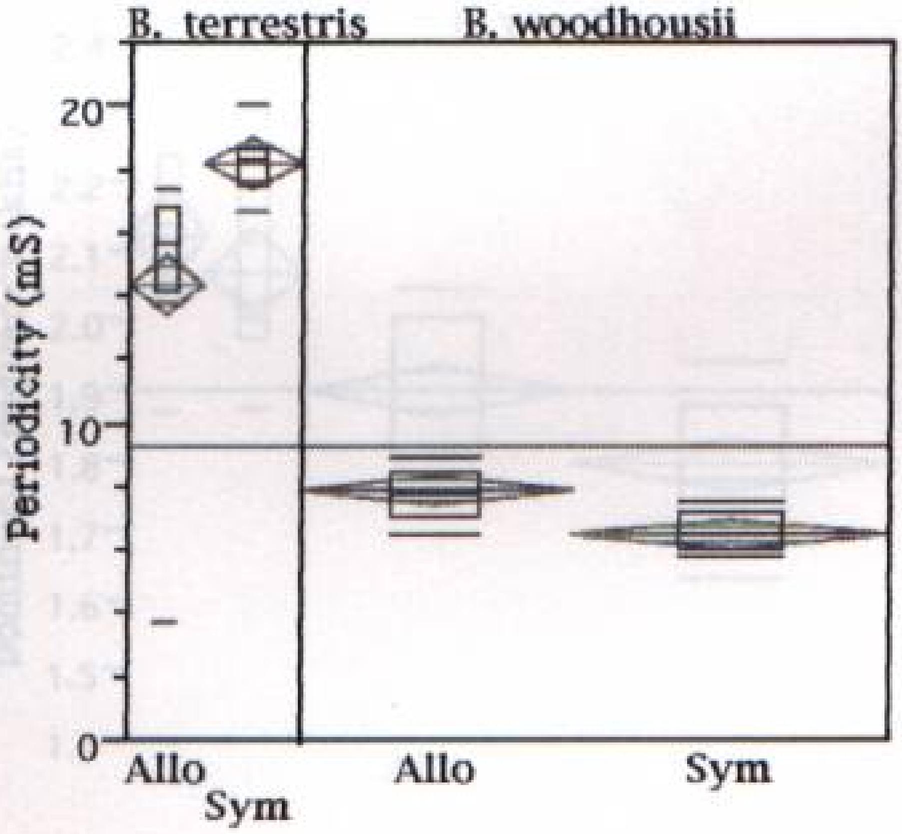

B. terrestris
B. woodhousii
Maximum Frequency (kHz)
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
Allo
Sym
Allo
Sym


B. terrestris
B. woodhousii
35
30
25
A
20
15
10
Number of Pulses
Allo
Sym
Allo
Sym
B. terrestris
B. woodhousii
35
30
25
B
20
15
10
Number of Pulses
Allo
Sym
Allo
Sym

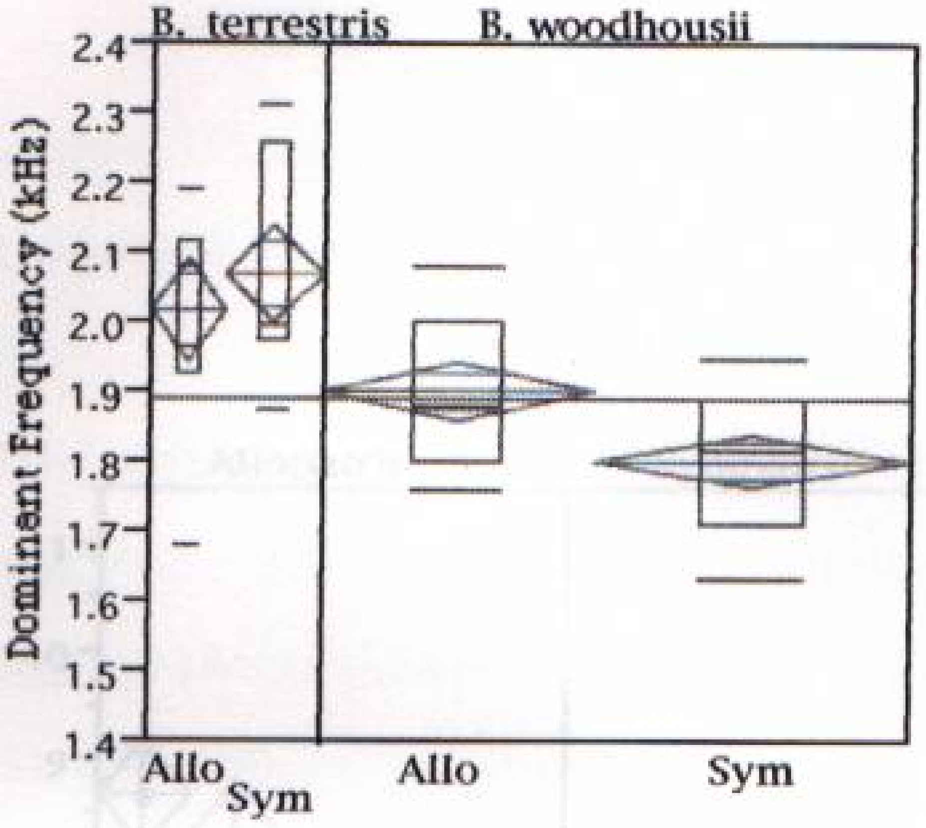
A


B. terrestris
B. woodhousii
Periodicity (mS)
20
10
Allo
Sym
Allo
Sym

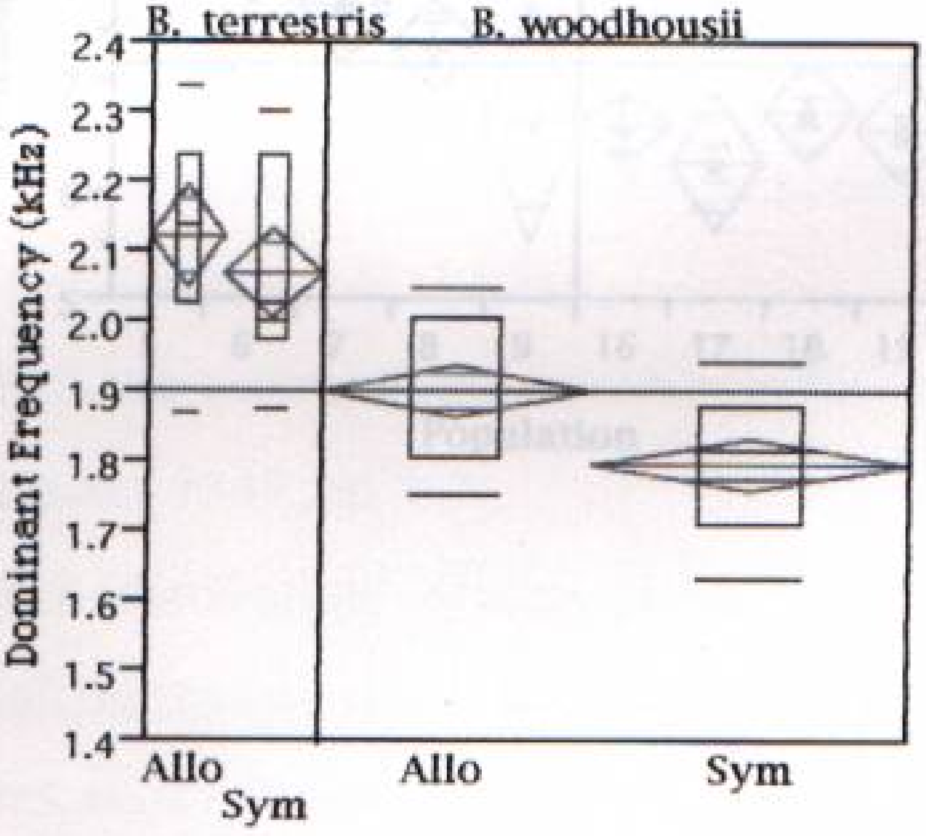
B

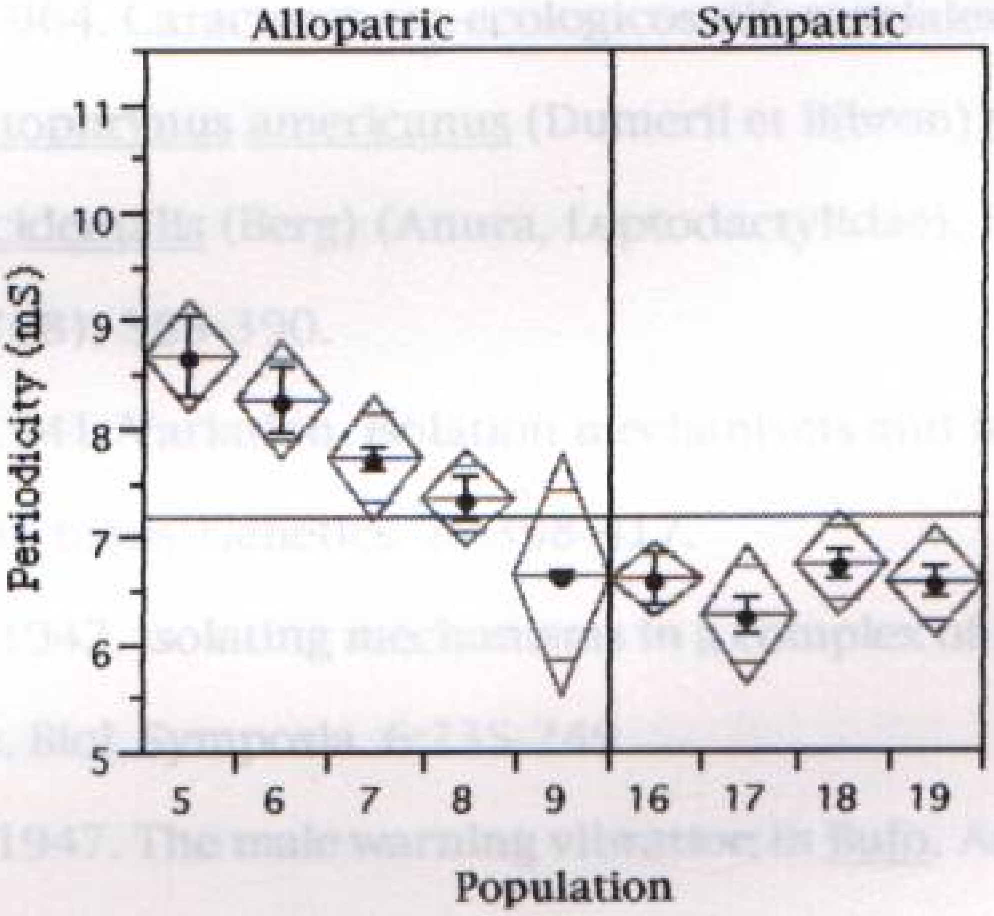

B. terrestris
B. woodhousii
Periodicity (mS)
20
10
0
Allo
Sym
Allo
Sym

A


B. terrestris
B. woodhousii
Dominant Frequency (kHz)
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
Allo
Sym
Allo
Sym

B


B. terrestris
B. woodhousii
Dominant Frequency (kHz)
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
Allo
Sym
Allo
Sym


Allopatric
Sympatric
Periodicity (mS)
11
10
9
8
7
6
5
5
6
7
8
9
16
17
18
19
Population

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APPENDIX I. Mean, standard deviation (SD), variance (SS) and minimum (min) and maximum (max) values for all parameters of the release vocalizations of B. americanus and B. woodhousii in allopatry (Allo) and in sympatry (Sym) adjusted for the effects of temperature (T)(21°C) and both temperature and SVL (T + SVL). Values are blank for those parameters where both temperature and SVL adjustments were not necessary (see text). Freq. = frequency.

	B. americanus				B. woodhousii			
	Allo		Sym		Allo		Sym	
Parameter	T	T + SVL	T	T + SVL	T	T + SVL	T	T + SVL
Interchirp Duration (mS)								
mean	125.0		129		155.9		154.5	
SD	45.8		59.5		74.8		57.2	
SS	2095.4		3546.2		5594.1		3274.6	
min	37.9		20.2		19.9		89.3	
max	267.0		421.4		427.5		436.9	
Chirp Duration (mS)								
mean	134.8		95.8		72.9		68.7	
SD	42.2		464.0		44.9		26.3	
SS	1778.4		1717.3		2016.5		691.3	
min	68.2		8.1		13.5		26.1	
max	245.7		274.4		291.8		142.8	
Pulses First Chirp								
mean	4	4	3	3	4	5	5	5
SD	2	2	2	2	4	4	3	3
SS	4	4	3	3	16	15	11	11
min	1	1	1	1	1	-1	1	1
max	11	11	7	7	15	16	13	13
Pulses Mid Chirp								
mean	6	5	4	4	7	7	6	6
SD	2	2	2	2	5	5	3	3
SS	4	4	2	2	21	20	11	11
min	3	2	1	1	0	0	1	1
max	15	14	8	8	18	18	14	14
Pulses Last Chirp								
mean	5	4	4	4	5	6	5	5
SD	2	2	2	2	4	4	3	3
SS	3	3	2	2	19	18	9	9
min	1	0	1	1	1	-1	1	1
max	8	7	6	6	28	28	12	12
Periodicity First Chirp (mS)[*]								
mean	30.9	19.6	28.9	30.7	17.3	17.4	13.4	13.4
SD	19.4	18.7	16.9	16.5	11.0	11.0	8.6	8.6
SS	374.6	349	281.8	270.9	120.2	120.1	73.6	73.2
min	1.1	-7.8	4.8	4.2	3.9	4.2	4.7	5.5
max	78.2	65.5	100.8	103.2	45.7	46.0	45.6	45.8

Periodicity Mid Chirp (mS)								
mean	25.2	37.9	28.2	29.9	15.2	14.2	15.6	15.6
SD	15.5	13.8	16.8	16.3	12.5	12.2	13.5	13.5
SS	240.8	191.1	281.6	265.2	156.8	149.5	181.1	180.9
min	13.1	19.2	1.5	6.5	3.7	1.1	3.4	4.1
max	100.5	102.8	141.9	135.7	57.8	58.4	83.7	83.8
Periodicity Last Chirp (mS)								
mean	30.6	32.7	35.2	36.1	15.6	15.4	13.5	13.3
SD	17.5	17.5	17.8	17.7	9.2	9.1	7.6	7.6
SS	307.1	305.8	316.4	311.6	84.1	82.9	58.1	57.1
min	9.0	11.4	7.5	10.1	8.3	7.4	5.8	5.9
max	94.5	96.9	101.7	101.4	61.0	61.3	51.9	51.5
Dominant Freq. First Chirp (kHz)								
mean	1.40	1.50	1.50	1.50	1.90	1.90	2.00	2.00
SD	0.18	0.17	0.22	0.21	0.51	0.49	0.61	0.60
SS	0.03	0.03	0.05	0.05	0.26	0.24	0.37	0.36
min	1.05	1.16	1.15	1.15	1.21	1.16	1.23	1.21
max	2.05	2.16	2.30	2.26	3.50	3.56	3.61	3.59
Dominant Freq. Mid Chirp (kHz)								
mean	1.40	1.50	1.50	1.50	2.00	2.00	1.43	1.43
SD	0.17	0.16	0.20	0.19	0.54	0.53	0.88	0.90
SS	0.03	0.03	0.04	0.04	0.29	0.28	0.77	0.81
min	1.05	1.15	1.20	1.21	1.25	1.19	0.13	0.02
max	2.05	2.12	2.21	2.19	3.48	3.54	3.74	3.61
Dominant Freq. Last Chirp (kHz)								
mean	1.30	1.40	1.40	1.40	1.90	1.90	1.88	1.88
SD	0.12	0.11	0.21	0.21	0.50	0.48	0.59	0.59
SS	0.01	0.01	0.05	0.04	0.25	0.23	0.35	0.34
min	1.01	1.12	1.10	1.12	1.23	1.17	1.01	0.98
max	1.61	1.63	2.14	2.12	3.07	3.06	3.14	3.24
Range First Chirp (kHz)								
mean	0.90		0.80		1.20		1.20	
SD	0.19		0.33		0.76		0.88	
SS	0.03		0.11		0.58		0.77	
min	0.56		0.42		0.37		0.15	
max	1.65		2.37		3.02		3.25	

Range Mid Chirp (kHz)								
mean	0.90	2.0	0.90	1.90	1.50	2.30	1.30	[illegible]
SD	0.23	0.24	0.34	0.36	0.79	0.79	0.83	[illegible]
SS	0.05	0.06	0.12	0.13	0.63	0.62	0.69	[illegible]
min	0.59	1.59	0.44	1.16	0.54	1.48	0.27	[illegible]
max	2.03	3.10	2.41	3.48	3.02	4.26	3.13	[illegible]
Range Last Chirp (kHz)								
mean	0.82	1.50	0.80	1.90	1.30	2.50	1.30	2.60
SD	0.14	0.15	0.34	0.36	0.77	0.74	0.88	0.91
SS	0.02	0.02	0.12	0.13	0.59	0.55	0.77	0.83
min	0.59	1.41	0.40	1.41	0.46	1.54	0.14	1.15
max	2.03	2.43	2.35	3.48	2.85	4.00	3.34	4.63
Minimum Freq. First Chirp (kHz)								
mean	0.90	0.90	1.00	1.00	1.30	1.30	1.40	1.40
SD	0.14	0.13	0.14	0.14	0.43	0.43	0.32	0.32
SS	0.02	0.02	0.02	0.02	0.19	0.19	0.10	0.10
min	0.67	0.68	0.70	0.69	0.82	0.84	1.00	0.99
max	1.48	1.49	1.46	1.42	3.00	3.02	3.11	3.10
Minimum Freq. Mid Chirp (kHz)								
mean	0.90	1.00	1.00	1.00	1.20	1.20	1.30	1.30
SD	0.13	0.12	0.13	0.13	0.38	0.38	0.25	0.25
SS	0.02	0.02	0.02	0.02	0.14	0.14	0.06	0.06
min	0.69	0.82	0.72	0.71	0.78	0.80	0.89	0.88
max	1.32	1.43	1.48	1.44	2.81	2.80	2.25	2.25
Minimum Freq. Last Chirp (kHz)								
mean	0.90	1.00	1.00	1.00	1.20	1.20	1.30	1.30
SD	0.12	0.11	0.14	0.13	0.38	0.37	0.23	0.22
SS	0.01	0.01	0.02	0.02	0.14	0.14	0.05	0.05
min	0.71	0.82	0.72	0.71	0.80	0.72	0.96	0.91
max	1.19	1.30	1.48	1.44	2.71	2.69	2.14	2.13
Maximum Freq. First Chirp (kHz)								
mean	1.80	1.90	1.90	1.90	2.50	2.60	2.60	2.60
SD	0.22	0.22	0.36	0.36	0.78	0.75	0.97	0.94
SS	0.05	0.05	0.13	0.13	0.60	0.56	0.94	0.89
min	1.43	1.55	1.48	1.41	1.46	1.39	1.26	1.17
max	2.61	2.72	3.52	3.47	4.21	4.31	4.63	4.59

Maximum Freq. Mid Chirp (kHz)								
mean	1.90	2.0	1.90	1.90	2.80	2.80	2.70	2.70
SD	0.25	0.24	0.36	0.36	0.81	0.79	0.90	0.89
SS	0.06	0.06	0.13	0.13	0.66	0.62	0.80	0.79
min	1.48	1.59	1.17	1.16	1.65	1.48	1.47	1.30
max	2.89	3.00	3.51	3.48	4.15	4.26	4.72	4.61
Maximum Freq. Last Chirp (kHz)								
mean	1.70	1.80	1.90	1.90	2.60	2.60	2.60	2.60
SD	0.15	0.15	0.37	0.36	0.75	0.74	0.92	0.91
SS	0.02	0.02	0.13	0.13	0.57	0.55	0.84	0.83
min	1.40	1.51	1.41	1.41	1.61	1.54	1.32	1.15
max	2.32	2.43	3.53	3.48	3.94	4.00	4.74	4.63

APPENDIX II. Mean, standard deviation (SD), variance (SS) and minimum (min) and maximum (max) values for all parameters of the advertisement vocalizations of B. americanus and B. woodhousii in allopatry (Allo) and in sympatry (Sym) adjusted for the effects of temperature (T)(21°C) and both temperature and SVL (T + SVL). Values are blank for those parameters where both temperature and SVL adjustments were not necessary (see text). Dom. Freq., max. freq. and min. freq. = dominant, maximum and minimum frequencies.

	B. americanus				B. woodhousii			
	Allo		Sym		Allo		Sym	
Parameter	T	T + SVL	T	T + SVL	T	T + SVL	T	T + SVL
Pulses								
mean	8	8	8	8	25	25	26	26
SD	0.3	0.3	1	1	3	3	2	0.4
SS	0.08	0.08	2	2	8	8	6	5
min	7	7	7	7	20	20	21	20
max	9	9	13	13	33	33	30	30
Periodicity (mS)								
mean	23.2	23.4	21.2	21.9	7.9	7.9	7.7	7.8
SD	1.4	1.3	6.1	5.9	1.0	1.0	0.7	0.7
SS	1.8	1.8	37.2	35.3	1.0	1.0	0.5	0.5
min	20.0	20.0	2.1	2.0	6.2	6.0	6.2	6.5
max	25.7	25.8	31.5	31.3	11.2	11.3	9.2	9.3
Dom. Freq. (kHz)								
mean	1.52	1.49	1.79	1.77	1.90	1.90	1.90	1.90
SD	0.13	0.13	0.12	0.11	0.13	0.11	0.1	0.12
SS	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.01
min	1.25	1.27	1.59	1.53	1.69	1.63	1.70	1.69
max	1.73	1.72	2.03	2.03	2.21	2.09	2.11	2.11
Range (kHz)								
mean	0.83		0.61		0.54		0.55	
SD	0.17		0.17		0.09		0.11	
SS	0.03		0.03		0.01		0.01	
min	0.58		0.24		0.40		0.35	
max	1.20		0.96		0.76		0.82	
Min. Freq. (kHz)								
mean	1.03	1.01	1.40	1.39	1.60	1.60	1.70	1.70
SD	0.17	0.17	0.16	0.15	0.12	0.12	0.14	0.14
SS	0.03	0.03	0.03	0.02	0.02	0.01	0.02	0.02
min	0.77	0.79	0.89	0.97	1.38	1.33	1.45	1.44
max	1.36	1.30	1.69	1.68	1.86	1.81	1.97	1.97
Max. Freq.								
mean	1.86	1.87	2.03	2.04	2.20	2.50	2.20	2.20
SD	0.12	0.12	0.14	0.13	0.13	0.12	0.11	0.11
SS	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01
min	1.65	1.66	1.70	1.68	2.04	2.28	1.99	1.99
max	2.12	2.12	2.34	2.37	2.60	2.76	2.38	2.39

APPENDIX III. Mean, standard deviation (SD), variance (SS) and minimum (min) and maximum (max) values for all parameters of the release vocalizations of B. terrestris and B. woodhousii in allopatry (Allo) and in sympatry (Sym) adjusted for the effects of temperature (T)(21°C) and both temperature and SVL (T + SVL). Values are blank for those parameters where both temperature and SVL adjustments were not necessary (see text). Freq. = frequency.

Parameter	B. terrestris				B. woodhousii			
	Allo		Sym		Allo		Sym	
	T	T + SVL	T	T + SVL	T	T + SVL	T	T + SVL
Interchirp Duration (mS)								
mean	177.4		147.1		155.9		159.6	
SD	51.4		101.4		74.8		71.4	
SS	2646.9		10274		5594.1		5097.2	
min	93.5		72.7		19.9		86.2	
max	64.9		812.7		427.5		544.6	
Chirp Duration (mS)								
mean	80.5		90.7		72.9		82.9	
SD	30.4		32.2		44.9		29.9	
SS	921.9		1036.3		2016.5		893.5	
min	28.4		39.9		13.5		40.3	
max	161.8		161.5		291.8		198.6	
Pulses First Chirp								
mean	4	4	5	5	4	5	5	5
SD	2	2	4	4	4	4	3	3
SS	6	6	14	14	16	14	10	10
min	1	0	0	0	1	-1	1	1
max	11	10	12	12	15	16	13	13
Pulses Mid Chirp								
mean	4	4	7	7	7	7	7	7
SD	2	2	3	3	5	5	4	4
SS	5	5	8	8	20	20	17	17
min	0	0	1	1	0	0	1	1
max	9	9	12	12	18	18	18	18
Pulses Last Chirp								
mean	4	4	6	6	5	6	6	6
SD	2	2	3	3	4	4	3	3
SS	4	4	8	8	19	17	10	10
min	1	1	0	0	1	-1	1	1
max	10	10	13	13	28	28	16	16
Periodicity First Chirp (mS)								
mean	17.5	17.6	17.3	17.2	17.3	17.4	17.5	16.1
SD	4.5	4.5	9.2	9.2	11.0	11.0	13.2	12.7
SS	20.5	20.3	85.2	84.6	120.2	120.1	174.5	160.9
min	8.3	9.3	7.4	6.5	3.9	4.2	4.6	0.5
max	37.7	37.4	50.9	49.3	45.7	46.0	74.5	66.6

Periodicity Mid Chirp (mS)								
mean	21.1	21.0	14.4	14.3	15.2	14.2	13.9	13.4
SD	5.0	5.0	5.1	5.1	12.5	12.2	10.8	10.7
SS	25.1	24.6	26.1	25.9	156.8	149.5	117.4	115.2
min	11.1	9.5	5.1	5.1	3.7	1.1	-0.5	-1.1
max	42.5	40.9	36.0	36.0	57.8	58.4	51.4	50.8
Periodicity Last Chirp (mS)								
mean	18.5	18.5	17.4	16.9	15.6	15.4	16.0	15.1
SD	3.4	3.3	4.5	4.3	9.2	9.1	13.8	13.5
SS	11.6	11.2	20.4	18.5	84.1	82.9	190.5	183.0
min	10.5	11.0	9.9	8.2	8.3	7.4	4.9	1.9
max	26.6	27.1	32.7	30.3	61.0	61.3	67.8	66.8
Dominant Freq. First Chirp (kHz)								
mean	1.80	1.80	2.00	2.00	1.90	1.90	2.30	2.30
SD	0.26	0.26	0.34	0.34	0.51	0.49	0.59	0.59
SS	0.07	0.07	0.12	0.12	0.26	0.24	0.35	0.35
min	1.34	1.35	1.39	1.40	1.21	1.16	1.48	1.44
max	2.39	2.39	2.91	2.92	3.50	3.56	3.97	3.97
Dominant Freq. Mid Chirp (kHz)								
mean	2.00	2.00	2.00	2.00	2.00	2.00	2.40	2.40
SD	0.42	0.42	0.32	0.32	0.54	0.53	0.61	0.60
SS	0.18	0.18	0.11	0.10	0.29	0.28	0.37	0.36
min	1.42	1.46	1.31	1.41	1.25	1.19	1.62	1.49
max	1.74	3.17	2.82	2.85	3.48	3.54	3.81	3.80
Dominant Freq. Last Chirp (kHz)								
mean	1.80	1.80	2.00	2.00	1.90	1.90	2.10	2.10
SD	0.37	0.37	0.36	0.36	0.50	0.48	0.55	0.55
SS	0.14	0.13	0.13	0.13	0.25	0.23	0.30	0.30
min	1.05	1.09	1.31	1.30	1.23	1.17	1.53	1.50
max	2.55	2.54	2.72	2.73	3.07	3.06	3.65	3.65
Range First Chirp (kHz)								
mean	0.90	2.30	1.40	2.70	1.20	2.60	1.60	3.10
SD	0.52	0.52	0.67	0.68	0.76	0.75	0.75	0.84
SS	0.27	0.27	0.45	0.46	0.58	0.56	0.56	0.71
min	0.21	1.56	0.22	1.49	0.37	1.39	0.49	1.85
max	2.27	3.57	2.74	3.92	3.02	4.31	3.69	5.26

Range Mid Chirp (kHz)								
mean	1.00		1.30		1.50		1.60	
SD	0.58		0.50		0.79		0.82	
SS	0.34		0.25		0.63		0.67	
min	0.11		0.46		0.54		0.66	
max	2.34		2.59		3.02		3.30	
Range Last Chirp (kHz)								
mean	1.00		1.30		1.30		1.30	
SD	0.65		0.57		0.77		0.74	
SS	0.43		0.33		0.59		0.55	
min	-0.14		0.13		0.46		0.39	
max	2.74		2.63		2.85		3.10	
Minimum Freq. First Chirp (kHz)								
mean	1.30	1.30	1.30	1.30	1.30	1.30	1.50	1.50
SD	0.18	0.18	0.15	0.15	0.43	0.43	0.39	0.39
SS	0.03	0.03	0.02	0.02	0.19	0.19	0.15	0.15
min	0.70	0.73	0.95	0.94	0.82	0.84	1.01	1.00
max	1.60	1.60	1.88	1.87	3.00	3.02	3.26	3.26
Minimum Freq. Mid Chirp (kHz)								
mean	1.50	1.50	1.30	1.30	1.20	1.20	1.50	1.50
SD	0.30	0.30	0.15	0.15	0.38	0.38	0.34	0.34
SS	0.09	0.09	0.02	0.02	0.14	0.14	0.12	0.12
min	0.88	0.90	0.88	0.95	0.78	0.80	1.01	1.00
max	2.26	2.72	1.62	1.69	2.81	2.80	2.73	2.73
Minimum Freq. Last Chirp (kHz)								
mean	1.30	1.30	1.30	1.30	1.20	1.20	1.40	1.40
SD	0.16	0.16	0.16	0.14	0.38	0.37	0.31	0.31
SS	0.02	0.02	0.03	0.02	0.14	0.14	0.10	0.10
min	0.75	0.76	0.87	0.95	0.80	0.72	0.78	0.78
max	1.55	1.56	1.56	1.53	2.71	2.69	2.75	2.75
Maximum Freq. First Chirp (kHz)								
mean	2.30	2.30	2.70	2.70	2.50	2.60	3.10	3.10
SD	0.52	0.52	0.68	0.68	0.78	0.75	0.85	0.84
SS	0.27	0.27	0.46	0.46	0.60	0.56	0.72	0.71
min	1.57	1.56	1.49	1.49	1.46	1.39	1.96	1.85
max	3.57	3.57	3.92	3.92	4.21	4.31	5.76	5.26

Maximum Freq. Mid Chirp (kHz)								
mean	2.50	2.50	2.70	2.70	2.80	2.80	3.10	3.20
SD	0.62	0.62	0.54	0.53	0.81	0.79	0.88	0.88
SS	0.39	0.39	0.29	0.28	0.66	0.62	0.78	0.77
min	1.68	1.61	1.70	1.85	1.65	1.48	2.03	1.94
max	4.30	4.27	3.96	3.98	4.15	4.26	4.98	5.07
Maximum Freq. Last Chirp (kHz)								
mean	2.30	2.30	2.60	2.60	2.60	2.60	2.70	2.70
SD	0.67	0.67	0.59	0.59	0.75	0.74	0.80	0.80
SS	0.45	0.44	0.35	0.35	0.57	0.55	0.64	0.63
min	1.07	1.13	1.36	1.34	1.61	1.54	1.68	1.68
max	4.07	3.99	3.94	3.88	3.94	4.00	4.54	4.54

APPENDIX IV. Mean, standard deviation (SD), variance (SS) and minimum (min) and maximum (max) values for all parameters of the advertisement vocalizations of B. terrestris and B. woodhousii in allopatry (Allo) and in sympatry (Sym) adjusted for the effects of temperature (T)(21°C; raw data for B. terrestris) and both temperature and SVL (T + SVL). Values are blank for those parameters where both temperature and SVL adjustments were not necessary (see text). Dom. Freq., max. freq. and min. freq. = dominant, maximum and minimum frequencies.

	B. terrestris				B. woodhousii			
	Allo		Sym		Allo		Sym	
Parameter	T	T + SVL	T	T + SVL	T	T + SVL	T	T + SVL
Pulses								
mean	12	13	11	11	25	25	30	29
SD	3	2	1	1	3	3	3	3
SS	6	6	1	1	8	8	9	7
min	11	11	9	9	20	20	24	25
max	20	20	12	12	33	33	35	36
Periodicity (mS)								
mean	16.0	14.4	18.3	18.3	7.9	7.9	6.6	6.6
SD	4.7	4.5	1.1	1.1	1.0	1.0	0.8	0.7
SS	21.7	20.4	1.2	1.2	1.0	1.0	0.6	0.5
min	2.1	1.3	16.4	16.4	6.2	6.0	5.2	5.1
max	18.5	17.5	20.8	20.8	11.2	11.3	8.0	8.2
Dom. Freq. (kHz)								
mean	2.02	2.12	2.07	2.07	1.90	1.90	1.80	1.80
SD	0.16	0.15	0.16	0.15	0.13	0.11	0.12	0.12
SS	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01
min	1.63	1.85	1.83	1.82	1.69	1.63	1.48	1.47
max	2.20	2.36	2.35	2.34	2.21	2.09	2.02	2.01
Range (kHz)								
mean	1.08		0.88		0.54		0.59	
SD	0.25		0.20		0.09		0.18	
SS	0.06		0.04		0.01		0.03	
min	0.58		0.57		0.40		0.34	
max	1.30		1.26		0.76		1.31	
Min. Freq. (kHz)								
mean	1.38	1.45	1.56	1.59	1.60	1.60	1.50	1.50
SD	0.27	0.26	0.24	0.22	0.12	0.12	0.15	0.15
SS	0.07	0.07	0.06	0.05	0.02	0.01	0.02	0.02
min	0.92	1.12	1.17	1.16	1.38	1.33	0.87	0.86
max	1.77	1.85	2.04	2.03	1.86	1.81	1.73	1.70
Max. Freq.								
mean	2.45	2.54	2.44	2.43	2.20	2.50	2.10	2.10
SD	0.11	0.09	0.12	0.11	0.13	0.12	0.14	0.14
SS	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02
min	2.20	2.42	2.22	2.22	2.04	2.28	1.79	1.79
max	2.65	2.71	2.65	2.63	2.60	2.76	2.64	2.62