GEOGRAPHIC VARIATION IN *ACRIS CREPITANS* A MORPHOLOGICAL AND BEHAVIORAL ASSESSMENT ACROSS THREE CENTRAL TEXAS RIVER BASINS,WITH COMMENTS ON SYSTEMATICS

THESIS

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CHAPTER I

INTRODUCTION

Recent publications have brought to light several questions regarding the systematics of cricket frogs (*Acris*; Hylidae), a small anuran with a large geographic distribution in eastern North America (Gray, 1983; Gorman, 1986; Conant and Collins, 1998; McCallum and Trauth, 2004). The genus *Acris* is divided into two species: *Acris crepitans*, the northern cricket frog, and *Acris gryllus*, the southern cricket frog. Both species are further divided into subspecies (*A c crepitans*, *A c blanchardi*, and *A c paludicola*; *A g gryllus* and *A g dorsalis*) (Conant and Collins, 1998). Of particular interest and uncertainty is the organization of the subspecies of *A crepitans*.

McCallum and Trauth (2006) published an argument for collapsing *A c blanchardi* into *A. c. crepitans*, based on ambiguities in the morphological characters originally used to describe the former. However, these subspecies are known to differ in characteristics of the acoustic communication system (Ryan and Wilczynski, 1991; McClelland et al., 1996, 1998), which is the primary means of mate recognition in most anuran species (e.g., Blair, 1958; Duellman and Trueb, 1986; Giacoma and Castellano, 2001). Sexual and environmental selection pressures on mate recognition systems in many taxa (Ptacek, 2000; Pannhuis et al., 2001; Schwartz and Hendry, 2006), and particularly on the acoustic communication system of anurans (Ryan and Wilczynski,

1988; Wilczynski and Ryan, 1999; Lougheed et al., 2006), are thought to contribute to evolutionary lineage divergence at the genetic level (Pannhuis et al., 2001; Lougheed et al., 2006, Schwartz and Hendry, 2006) It is suggested that this genetic divergence may occur prior to the evolution of significant observable morphological differences (Camargo et al , 2006). While references to morphological differences among *A crepitans* subspecies exist in the literature (Harper, 1947; Cagle, 1954; McLelland et al , 1996, 1998) and in anecdotal accounts, the work of McCallum and Trauth (2006) is the only recent attempt at quantifying morphological differences, and tautologically dismisses known behavioral differences as having been documented based on a flawed subspecific definition. In failing to account for differences in acoustic communication behavior, these authors effectively dismiss an evolutionary mechanism that is thought to significantly contribute to the diversification of anuran lineages.

This thesis represents a combined morphological/behavioral evaluation of Central Texas *Acris*, conducted to assess differences at a small geographic scale, and to shed light on the systematic conundrum represented by this group. The specific goals of this project are to 1) characterize the vocal qualities of the advertisement call of populations of *A crepitans* from three adjacent river drainages: the Colorado river drainage, including the sample locality from Lost Pines in Bastrop County identified as exhibiting significant behavioral differences from other nearby populations; one sample locality from the Guadalupe river drainage in Hays County; and one sample locality from the Brazos river drainage in Milam County; 2) generate a detailed description of morphological characters for each of these sample localities; 3) assess variation in behavioral and morphological characters within and between these sample localities; and 4) document any geographic

patterns found to exist in covariation among suites of these characters. It is unclear at this time the degree of genetic connectivity of these localities, thus the use of the term 'population(s)' refers to the discrete geographic sampling units for the purpose of this study. Results of this work will prove informative in the ongoing process of reevaluating the systematic organization of *Acris* subspecies and should provide a framework for future molecular systematics work.

The work contained in this document focuses on the subspecies *A c blanchardi* and *A c crepitans*. The following introductory subsections contain information that frame the research questions and that provide background on the evolutionary importance of the anuran acoustic communication system

TAXONOMIC HISTORY OF *ACRIS*

The genus *Acris*, originally described by Dumeril and Bibron (1841, in Frost, 2007), is composed of two species, *A crepitans* (Northern Cricket Frog; Baird, 1854, in Frost, 2007) and *A gryllus* (Southern Cricket Frog; Le Conte, 1825, in Frost, 2007, as *Rana gryllus*). Two subspecies of *A gryllus* were described in 1827 (*A g gryllus* and *A g dorsalis*; Harlan, 1827, in Frost, 2007, as members of the genus *Rana*), with another (*A. g crepitans*; Cope, 1875, in Frost, 2007) described later in the 19th century. Two additional subspecies were described by Burger et al. (*A. g paludicola*; 1947, in Frost, 2007) and Harper (*A. g blanchardi*; 1947) in the mid 20th century. The latter three *A. gryllus* subspecies were subsequently placed in the species *A. crepitans* (*A. c crepitans, A c blanchardi, A. c paudicola*; Conant, 1958, in Frost, 2007; Duellman, 1977, in Frost, 2007; Rose et al., 2006). Most recently, McCallum and Trauth (2006) suggest

synonomizing *A c blanchardi* with *A c crepitans*, a suggestion that has not been incorporated in the most recent online version (updated 10 April 2007) of the Standard Common and Current Scientific Names (Collins and Taggart, 2002). Thus at the time of this writing, the genus *Acris* contains two species and five subspecies: *A c crepitans*, *A c blanchardi*, *A c paludicola*, *A g gryllus*, and *A g dorsalis* The assumption for the purpose of this thesis is that this organization is correct at the current state of knowledge about this genus.

The above-documented additions and revisions to the taxonomic organization, particularly at the subspecific level, have largely resulted from distinctions in morphological characters that are somewhat subjective. These characters include overall bulkiness, overall wartiness, overall coloration, presence and characteristics of one or more dark markings on the posterior of the thigh, presence and characteristics of anal warts, and degree of toe webbing, among others (Harper, 1947; Neill, 1950; Conant and Collins, 1998; McCallum and Trauth, 2006). While Neill (1950) was able to discern between *A crepitans* and *A gryllus* using series of both species obtained from an area of sympatry in Georgia, determining the identity of individual specimens can be challenging, particularly in regions where both species occur. This difficulty is magnified at the subspecific level within *A crepitans*, as noted in the recent literature regarding these frogs (McCallum and Trauth, 2006; Rose et al., 2006), particularly given that both of these publications suggest that geographic boundaries as currently understood are poorly defined.

Evolutionary Position and Molecular Phylogenetics

The genus *Acris* is a member of the order Anura, comprised of 5227 species (Frost et al., 2006) of frogs and toads. Recent efforts to bring the taxonomy of anurans into concordance with their phylogeny have confirmed the position of Hylidae (which includes *Acris*) within the Neobatrachian clade (Frost et al., 2006). Specifically, *Acris* is a member of the tribe Hylini, nested in subfamily Hylinae, family Hylidae. The large molecular phylogeny of hylid frogs recently published by Faivovich et al. (2005) confirms that *Acris* is evolutionarily closer to *Pseudacris* (Chorus Frogs) than either genus is to any other. This relationship agrees with the traditional morphology-based taxonomy. However, the scope of the tree-of-life studies mentioned above (Faivovich et al., 2005; Frost et al., 2006) precluded any investigation into the intrageneric or intraspecific diversity in *Acris*. The most recent published molecular work at this level is that of Rose et al. (2006), which confirmed the position of *A c paludicola* as a subspecies of *A crepitans,* and not, as originally defined, of *A gryllus*.

The works cited above acknowledge the need for denser taxon sampling from Anura generally (Faivovich et al., 2005; Frost et al., 2006), and within *Acris* in particular (Rose et al., 2006) in the ongoing effort to sort out evolutionary relationships and systematics at both the large and small scale. While population genetic analyses are beyond the scope of this thesis, the information and analyses contained herein should prove valuable as the state of knowledge of anuran biodiversity continues to increase, and as evolutionary hypotheses are updated in light of combined distributional, morphological, and molecular datasets.

DISTRIBUTION AND MORPHOLOGY

The historical range of *Acris* extends from the eastern seaboard of the United States westward to Nebraska, Kansas, Oklahoma, and Texas, and extending into easternmost Colorado and New Mexico. At the northern extent of their range, cricket frogs are documented from southern Michigan and New York State (Conant and Collins, 1998) The range extends southward to the Rio Grande and into northeast Mexico (Gray, 1983). The majority of this range is occupied by the subspecies of *A crepitans*, with *A gryllus* occurring only in the southern states (Viginia, the Carolinas, Georgia, Florida, Alabama, Mississippi, and southeastern Louisiana; Nevo, 1973a and 1973b), typically below the fall line that separates the coastal plains from upland areas in the southern Atlantic and Gulf Coast states (Neill, 1950).

A c blanchardi is the northern- and westernmost subspecies, occupying roughly half of the overall range of the genus. The geographic delineation between *A c blanchardi* and *A c crepitans* as it is presently understood runs through Tennessee, Arkansas, and Texas (Conant and Collins, 1998). The position of this line of contact does not correspond to any natural geographic barrier, and is potentially innacurate (McCallum and Trauth, 2006). In Texas, the accepted delineation follows the boundary of the Trinity and Neches river drainage basins (Conant and Collins, 1998) with *A c crepitans* occurring in the eastern, mesic pine forests, and *A c. blanchardi* occupying more xeric and open habitats westward and southward to the Pecos and Rio Grande rivers. *A. c paludicola* (Coastal Cricket Frog) occurs only in the easternmost Gulf Coast counties in Texas, and its range and conservation status are unclear at the present time (Rose et al., 2006).

All of the members of *Acris* are small (<40 mm; Conant and Collins, 1998), and typically have a posterior-pointing triangular marking between the eyes (Harper, 1947; Conant and Collins, 1998) and one or more dark markings on the posterior of the thigh (Conant and Collins, 1998). *A c blanchardi* is considered the largest member of the genus (16-38 mm), though there is considerable overlap in size among the species and subspecies (Conant and Collins, 1998). As noted above, the subspecies of *A crepitans* lack consistent and definitive morphological distinctions. Generally speaking, subspecies of *A. crepitans* may have smooth to somewhat warty skin (Conant and Collins, 1998); a dorsal ground color that ranges from pale grey to tan to olive and occasionally bright green (Gray, 1995); a vertebral stripe that is reddish-brown, green, or gray (Gray, 1983; Gorman, 1986); a ragged-edged dark or dusky stripe on the dorsoposterior surface of the thigh (Harper, 1947; Neill, 1950; McCallum and Trauth, 2006); shorter legs than *A. gryllus* (Conant and Collins, 1998); and more extensive toe webbing than *A gryllus* (Conant and Collins, 1998). A pectoral fold may be present, as may dark areolae on the otherwise pale venter (Harper, 1947; McCallum and Trauth, 2006). Males of all *Acris* have a single vocal pouch (Conant and Collins, 1998), that during the breeding season is much darker than the rest of the ventral surface.

Harper (1947), in his initial description of *A. c. blanchardi*, states that the most reliable character difference between this subspecies and *A. c. crepitans* lies in the characteristics of the dark markings found on the posterior surface of the thighs. In both subspecies, a single dark stripe is present on the dorsoposterior surface. Ventral to this marking and flanking the anus are dark or dusky areas, overlaid with white papillae. In *A. c. crepitans*, the stripes are bordered above and below by distinct pale areas, and the more

ventral dark patches are disjunct from this stripe. The thigh stripe of *A. c. blanchardi* is contiguous with the more ventral dark patches, which are also larger in area in this subspecies. Harper (1947) also states that the mass of *A. c. blanchardi* is twice that of *A. c. crepitans*, leading to a bulkier appearance overall.

Mate Recognition and Acoustic Communication

Given that there is nearly always a cost associated with heterospecific matings, it was long thought that stabilizing selection should act in the maintenance of species-recognition properties of mating signals (Blair, 1958). Empirical evidence has not borne out this prediction (Ryan and Wilczynski, 1991), and it has become clear that while recognition of conspecifics is an important component of the mate recognition system, mating signals do exhibit a high degree of intraspecific variation. Sexual selection in the form of female mate choice, coupled with localized environmental selection and genetic drift, can result in interpopulation divergence in mating signals, and may be a potent driver of prezygotic reproductive isolation and speciation (reviewed in Ptacek, 2000 and Panhuis et al., 2002).

The anuran acoustic communication system was recognized early on as a powerful evolutionary mechanism, as it plays a crucial role in directing mate choice (Blair, 1958). Local differences in female preference, habitat acoustics and climate may affect detectable differences in call characteristics among subpopulations, and broadly distributed species may display a high degree of intraspecific variation across their ranges (Ryan et al., 1996; Wilczynski and Ryan, 1999; Lougheed et al., 2006). Since properties of acoustic signals can be unambiguously quantified, the mating system of anurans has

served as an excellent model for testing various evolutionary hypotheses, particularly those that involve sexual and environmental selection and their role in the evolution of reproductive isolation mechanisms (Gergus et al., 1997; Smith et al., 2003a; Smith et al., 2003b) In this acoustic mate recognition system, male anurans broadcast a species-specific advertisement call from appropriate breeding sites; females are attracted to the breeding site by these calls, where they approach calling males (Wells, 1977; Duellman and Trueb, 1987). Thus the communication system of anurans is coupled, in that it involves both the signal-producing structures of the sender (males) and the sensory structures of the receiver (females). Evolutionary changes to one aspect of the system must be accompanied by compatible changes to (or be compatible with) the other in order for the mate recognition system to continue to function (Ryan and Wilczynski, 1988; McClelland et al., 1996).

Two factors are particularly important in directing the evolution of anuran acoustic mate recognition systems: 1) tuning of the female auditory system, which directs mate choice (Wilczynski and Ryan, 1999) and 2) environmental effects, which can be multiple (Ryan et al., 1990; Wilczynski and Ryan, 1999). Anurans have two inner ear structures, the basilar and amphibian papillae, which detect sounds in different ranges of the frequency spectrum (Duellman and Trueb, 1986). Dominant frequency (the highest-amplitude frequency band) of advertisement calls usually closely corresponds to the range of greatest sensitivity, or tuning, of one of these papillae in the female (Ryan et al., 1992). Both dominant frequency and papillae tuning are influenced by the size of the structures involved in producing or perceiving the sound (McClelland et al., 1996). Lower frequencies are associated with larger structures, and size of both sound

production and sensory structures are positively correlated with body size (Ryan et al., 1992; McClelland et al , 1998; Wilczynski and Ryan, 1999). Thus tuning of the female papillae and frequency-based mating preferences are negatively correlated with body size (Ryan et al., 1992).

Environment can affect the evolution of calls in several ways. Larger individuals are frequently found in more xeric environments (Nevo, 1973a), as greater resistance to desiccation is associated with smaller surface area to volume ratio (Ralin and Rogers, 1972). Since dominant frequency (both in sender production and receiver sensitivity) is inversely related to body size (Ryan et al., 1992; McClelland et al., 1998; Wilczynski and Ryan, 1999), climatic characteristics that affect body size can result in pleiotropy on both dominant frequency of calls and female preference. Environmental complexity, chiefly in the form of vegetation structure, is also an important factor, as dense vegetation tends to increase degradation of auditory signals (Ryan et al., 1990). Both spectral and temporal aspects of calls can be influenced, with dominant frequency tending to decrease and temporal elements such as pulse rate tending to increase as the habitat becomes more complex (Wilczynski and Ryan, 1999). Selection favors call characteristics that increase transmission efficiency based on habitat acoustics.

ACRIS CREPITANS ACOUSTIC COMMUNICATION

Both *A. c blanchardi* and *A. c. crepitans* produce short, click-like calls, similar in quality to the noise produced by tapping together two small rocks or marbles. Calls vary in length from very short (<20 ms) to considerably longer (>100 ms), and may consist of amplitude-modulated pulses, which are often grouped into two or more subsets (pulse

groups) within a call. Calls are usually repeated from 10 to more than 30 times, forming call groups. Call rate, duration, number of pulses per call, and pulse groups per call vary dependent on position in the call group, with more slowly-repeated and shorter calls broadcast at the beginning of call groups These temporal characteristics of *Acris* vocalizations represent several sources of overall variation that can be quantified in acoustic behavioral analysis. Spectral analysis is typically confined to measurement of dominant frequency, as the calls of *Acris* are not frequency-modulated. Further, dominant frequency changes little through the call group

As noted above, Texas *A c blanchardi* occupy more open, western habitats, while *A c crepitans* occur in the more eastern pine forests. Ryan and Wilczynski (1988; 1991) documented significant differences in both spectral and temporal call parameters among populations of both subspecies from Texas. Calls of *A c. blanchardi* are lower in dominant frequency, longer in duration and consisting of more pulses, and repeated at a slower rate. Call groups also contain fewer calls than the more eastern subspecies (Ryan et al., 1992). Variation in both spectral and temporal call characters was determined to be clinal across the transect sampled in the studies mentioned above, a result that remained strong after removing the effects of body size. The authors attribute this observation to tuning of the basilar papilla in females and its effect on dominant frequency based mate choice preferences, which has been determined through neurophysiology and phonotaxis experiments to be lower than the average dominant frequency of male advertisement calls (Ryan and Wilczynski, 1991). These authors suggest that sexual selection may generate a tendency for gene flow from east to west, due to female preference for lower frequency calls (Ryan and Wilczynski, 1991; Wilczynski and Ryan, 1999).

In their work on Texas *Acris*, Ryan and Wilczynski (1988; 1991; Wilczynski and Ryan, 1999) described a population of *A c blanchardi* in eastern Bastrop County, Texas, that has call characteristics that group with calls of *A c crepitans* when analyzed in a principle component analysis. This population lies well within the range of *A c blanchardi* (Conant and Collins, 1998), though evidence regarding the genetic affinities of this population is lacking at the present time. The area, known as the Lost Pines, does differ from the typically more open western habitats, in that the vegetation community consists primarily of Loblolly pine (*Pinus taeda*) and understory, and is structurally similar to the habitat of East Texas (Ryan and Wiczynski, 1988; Al-Rabab'ah and Williams, 2004), where *A c crepitans* occurs. Preliminary collection of behavioral data (male advertisement call) was undertaken to investigate the interaction between sexual and environmental selection in these frogs at a small geographic scale, and to examine the variation in call characteristics among geographically proximal populations. The results of this initial investigation suggest that significant behavioral differences may exist among populations of *A. crepitans* in Central Texas, indicating that further research of the kind documented in this thesis is warranted. While detailed morphometric data were not collected in this preliminary investigation, *Acris* individuals from the Lost Pines were smaller in size than the more western populations in the pilot study. Further, extensive research conducted in Central and South American *Eleutherodactylus* frogs has described an enormous diversity of species based on subtle morphological differences (e.g., Kwet and Solé, 2005). These studies provide a wealth of potentially diagnostic morphological characters that have yet to be examined in *Acris*.

The research presented here represents a multi-modal investigation of intraspecific variation at a small geographic scale. Further, this work permits a reevaluation of the currently accepted geographic delineations between the subspecies of *A crepitans*, and should prove informative in future exploration of the taxonomic status and evolutionary history of these subspecies

CHAPTER II

METHODS

At the most general, this thesis seeks to illuminate differences that may exist among Central Texas populations of cricket frogs. Specifically, the goals of this research involve the population of *Acris* inhabiting the Lost Pines, which Ryan and Wilczynski (1988; 1991) identified as having significantly different advertisement calls from other Texas populations. The sampling strategy makes use of the natural boundaries represented by three adjacent river drainage basins: the Brazos drainage to the north and east, the Colorado drainage at the central portion of the distribution of interest, which includes the Lost Pines population studied by Ryan and Wilczynski (1988; 1991; Wilczynski and Ryan, 1999); and the Guadalupe drainage to the south and west. It is not clear whether the topographic boundaries of these drainage basins represent a significant barrier to gene flow between groups of *Acris* residing within. However, the populations sampled span a geologic and climatologic gradient, thus local environmental selection may favor different morphological and behavioral characters across the three sites. Given that *Acris* exhibit sexual dimorphism in overall size, and female frogs do not vocalize, all analyses are confined to male individuals. This sampling strategy facilitates statistical evaluation of variation among all three basins, and patterns of geographic variation in coordinated suites of characters.

Drainage basins sampled including specific locality, abbreviation, and geographic coordinates are listed in Table 1. Behavioral variables measured including variable type, abbreviations, and units (where appropriate) are listed in Table 2a. Morphological measurements including variable type, abbreviations, and units are listed in Table 2b.

Field Sampling Localities

From the Brazos basin, a population of *Acris* inhabiting a small tributary in Milam County was sampled twice during July 2007. This tributary runs adjacent to Milam County Road 264 (N30.80661°, W-096.74443°; Mil CR264), and flows into the Brazos River approximately 10 km from the sampling location. Additional specimens were collected for morphometric analysis from a small roadside pond approximately 8 km away on Milam County Road 342 (N30.71334°, W-096.78821°; Mil CR342). The vegetation community in this portion of the basin is predominantly hardwood forest, some of which has been cleared for farmland; substrate at the two localities sampled is primarily silt and sand. At both locations, male *Acris* were calling from clumps of riparian vegetation within 1 m of the water. While both sites lie adjacent to roads, the woody vegetation structure (trees up to 10 m in height and understory) was intact to within 10 m of the roadway.

One location was sampled from the Lost Pines in Bastrop County within the Colorado river basin. This sample site lies within a ~2000 ha ranch, owned by the Capitol Area Boy Scouts, and managed as a refuge for the endangered Houston toad (*Bufo houstonenesis*) since 1999. While some areas of the property are cleared pasture, much of the original Loblolly pine (*Pinus taeda*) forest remains undisturbed. Two ponds were sampled from this ranch: one lies in forested habitat, with numerous trees (primarily *P.*

taeda) within 10 m of the pond edge (N30 21623°, W-097.24188°; GLR Pond 2); the second pond sits at the edge of a cleared area (N30 20938°, W-097.24309°; GLR Pond 5a) All individuals recorded at GLR Pond 2 were found calling at the pond edge, within 1m of the water. It should be noted that there is little emergent or riparian plant growth at this location, thus all frogs were recorded calling from open sites. The substrate is sand, overlaid with pine duff. GLR Pond 5a is surrounded by emergent and riparian vegetation, and while the substrate does consist primarily of sand and some gravel, cobbles represent a large proportion of the substrate composition. Given the proximity of these two ponds (<2 km), data collected from both sites are treated as a single population for analysis.

Sampling from the Guadalupe drainage was conducted on the Blanco River in Hays County, at a low water crossing near San Marcos, Texas (N29.93674°, W-097.89530°; Post Road river crossing). The riverbed is composed of limestone gravel with larger cobbles, boulders, and exposed limestone bedrock. Frogs were recorded and collected calling from pools of water, which were cut off from the main river channel subsequent to high water events earlier in the season. The substrate in these pools is gravel and bedrock, with little to no emergent or riparian vegetation.

Collection of Field Data

Field data collection was conducted from March through August 2007. On all field-sampling excursions, efforts were made to retain every individual recorded. However, in some cases males escaped during recording and were not captured. Recordings of these individuals were only used in acoustic analyses of characters that are known to be independent of body size. Further, from most sampling localities additional individuals were retained for morphometric analysis and were not recorded. Table 3

summarizes sampling dates, specific locations, and data type collected for all individuals used in this study.

Acoustic data were recorded on a Marantz PMD 670 Professional digital recording unit with a Sennheiser ME66 microphone with windscreen. Vocalizations were recorded at a sampling rate of 48 kHz, which exceeds the Nyquist frequency of approximately 10 kHz for this species. All recordings were stored as uncompressed .wav files using the pulse code modulation (PCM) algorithm option of the Marantz PMD 670. Each male was recorded for 3-5 minutes at a distance of approximately 5 m. Input levels were set at 20% or lower, to reduce clipping of calls and to minimize acoustic capture of males calling close to the individual of interest. Appropriate input levels were gauged using the level meter of the Marantz PMD 670. Air temperature, relative humidity, wind speed, and substrate type were documented for each individual recorded using a Kestrel 3000 Pocket Weather Meter. Individuals were captured by hand and maintained in 16 oz. plastic containers, with sphagnum moss moistened with water from the sampling location as substrate. Permission to retain specimens was granted under the authority of IACUC permit #0714_0482_07.

Acoustic Measurements

Measurements of temporal and spectral characters of calls were made using Raven Pro 1.3 (Charif et al., 2004). Each recording was first edited to a length of 30-90 seconds, in order to reduce processor time. The best call groups were retained for measurement, with priority placed on segments of recordings containing the least amount of abiotic noise (e.g., wind or vehicles) and the fewest vocal competitors. Much of the previous research on advertisement calls of *Acris* has utilized only the longest call

groups. These call groups are characterized by short, simple (single pulse-group) calls, repeated at an increasing rate at the beginning of the group, followed by a series of longer and more rapidly repeated calls, and ending with a series of much longer and more complex calls, repeated at a slower rate (see Fig. 1 for a waveform of this type of call group). Not all calling bouts follow this pattern, however, and some males did not produce any call groups that matched the description above during the recording period. Given that the character of the call group is one component of the overall variation in acoustic communication behavior, these shorter call groups were retained in this analysis.

After editing, most recordings contained over 100 individual calls. Calls were randomly selected from each recording using a table of random numbers that ranged from 1 to 10. Spectral and temporal characters were then recorded for each selected call. As previously mentioned, dominant frequency (DF) is the only spectral datum included in this analysis. This is due to the fact that the calls of male *Acris* are not frequency-modulated. The spectrogram shown in Fig. 2 illustrates this quality with the frequency-modulated call of a canyon wren shown for comparison.

Dominant frequency (also referred to as max or peak frequency by some authors) was calculated using the Discrete Fourier Transform (DFT) function available in Raven Pro 1.3 (Charif et al., 2004). While the actual calculation is a complex, three dimensional integration involving the spectral, amplitude and time axes of a sound, the output is an easily read graph of amplitude as a function of frequency (Fig. 3). The software implements this calculation using the Selection Spectrum View, which permits users to define values for settings used to make the calculations in the DFT. Each call chosen for analysis was selected and analyzed in the following manner. All DF calculations were made using the Blackman window type, which uses a 3 dB filter bandwidth of 158 Hz

and reduces the magnitude of side peaks in the frequency graph. A window size of 500 samples was used, which corresponds to the minimum length of calls in the recordings (about 5 ms), with the exception of all but the shortest calls (>1% of the total dataset). DF was calculated for these very short calls by expanding the selection to 500 samples and recording DF for the enlarged area of the waveform. Amplitude of background noise was minimal for all of these cases, and thus does not affect the accuracy of DF measurements. Window size of 500 samples represents the best possible trade-off between accuracy of frequency calculation (achieved through larger window sizes) and the temporal constraints of the data (i.e., call length). Time grid settings determine the distance traveled by the window along the waveform in calculating the DFT. 'Hop size' is one method of setting the time grid, and refers to the number of samples the center of the window moves between each iteration of the DFT. Percent overlap, or the amount of data resampled each time the window is moved, depends on hop size. Hop size was set to 10 samples, leading to an overlap of 98%. Frequency grid spacing determines resolution in the spectrogram view, but has no direct effect on the value calculated for DF. Grid spacing was set to 23.4 Hz for DF calculations in this analysis. The DF values of all windows sampled are averaged to compute the overall DF for a call. These DF values were then averaged for all calls sampled for a given individual, and this mean DF value used in all statistical analyses

Temporal characters were assessed by visual inspection of the waveforms in Raven Pro 1.3 (Charif et al., 2004). The amplitude-modulated pulses are clearly visible in the waveform when the time grid (x axis) is expanded. A 'pulse' was delimited by an amplitude modulation of at least a 20% decrease in the relative amplitude (y axis) of the preceding peak in the waveform. Pulse groups were defined by gaps of at least 10 ms

between consecutive pulses. For the purpose of this study, calls consisting of a single pulse group are termed 'simple' calls (s), and those consisting of two or more pulse groups are termed 'complex' calls (cc). Number of pulse groups per call (PG/C) and number of pulses per pulse group (P/PG) were recorded using this set of definitions. Call duration (CD) and call group duration (CG) were calculated in Raven Pro 1.3 (Charif et al., 2004) by including the 'Delta Time' parameter in selection tables. CD is measured in miliseconds (ms or s^{-3}) and CG is measured in seconds (s). Pulse rate (PR, in pulses/ms) was calculated by dividing the total number of pulses present in a call by CD. Call rate (CR) was determined by dividing the total number of calls in a call group by CG. Figure 4a and b depict simple and complex calls for comparison, and illustrate pulses and pulse groups.

Additional non-numeric data were recorded regarding the overall calling style (weak vs. strong pulses; simple vs. complex calls, simple vs. complex call groups) and presence of vocal competitors within 2 m during the recording period. Frogs characterized as being 'weak pulsers' had a majority of pulses that were modulated by less than 40% (Fig. 5). Complex call groups were defined as having the characteristic pattern described above, with a fluctuation in call rate and complexity from beginning to end. Simple call groups were defined as consisting of predominantly simple calls, with little fluctuation in rate through the duration of the group (Fig. 6).

MORPHOLOGIC MEASUREMENTS

Most previous work on morphological variation within and among subspecies of *Acris* has focused on either markings or overall size, with some comments on relative length of the hind legs To date the largely anecdotal observations that hind legs of *A c crepitans* are relatively longer than those of *A c blanchardi* have yet to be put to a statistical test. Roy et al. (1998) suggest that correlation of variation in calling structure with variation in certain ratios of body structures may be useful in identifying new species of frogs in India. Numerous new species of tropical anurans have been described using detailed morphological measurements, particularly in the genus *Eleutherodactylus* (e.g., Campbell and Savage, 2000; Kwet and Solé, 2005) Taking previous systematics research in other anuran taxa as a guide, this work utilizes strict linear measurements, proportions of overall body length, and some relative proportions to more deeply investigate variation among populations of *Acris*. Features that were quantified in previous work, some of which are purported to be of little utility in discerning subspecies (McCallum and Trauth, 2006) were documented as well.

Prior to collection of linear measurements, specimens were euthanized by application of Benzocaine (Oragel®) to the ventral body surface, as described by McDiarmid (1994). Specimens were fixed in 95% ethanol for ~24 hrs and stored in 70% ethanol. All measurements were made to the nearest .1 mm using Mitutoyo digital calipers. In order to investigate the role of geographic distance between populations in morphological variation within the genus, fourteen *A. crepitans* from Eddy Co, New Mexico were included, as were six from Louisiana, five *A. c. paludicola* from Jefferson Co, Texas, and five *A. gryllus* from Louisiana, Alabama, and Mississippi.

Linear measurements of the following anatomical structures were collected: snout-urostyle length (SUL) from the tip of the snout to the tip of the urostyle, with spine and pelvic girdle held flat; femur length (FL) as measured from the tip of the urostyle to the distal end of the femur; tibia length (TL) as measured from knee to heel; hind foot length (HF) as measured from heel to tip of 4th toe; distance from the heel of the foot to the distal margin of the medial tubercle (HT); distance from the proximal margin of the medial tubercle to the tip of the fourth toe (Toe) (Fig. 7), head width measured at the point of the mandible (HW); head length from the point of the mandible to the tip of the snout (HL); interorbital distance at the narrowest point (IO); internareal distance (IN) (Fig. 8). longitudinal eye diameter (ED), and distance from the anterior margin of the eye orbit to the nares (EN) (Fig. 9). Ratios were calculated for the following relationships: hind leg length relative to SUL [(FL+TL)/SUL]; head length to head width (HL/HW); length of hind foot relative to leg length [HF/(FL+TL)]; length of toes relative to overall foot length (Toe/HF); eye diameter relative to head length (ED/HL). These relationships were chosen for investigation based on general observations of specimens and anecdotal accounts of differences among populations of *Acris*.

Additional non-numeric morphological data were collected regarding characteristics of various markings that have been analyzed in the past, or that were noted by Harper (1947) to be of use in discriminating *A. c. blanchardi* from *A c crepitans*. In cases involving binary characters, the number 1 was used to code for *crepitans*-like characters, and 2 used for *blanchardi*-like characters. Harper (1947) remarked that the dark or dusky markings on the posterior face of the thighs (markings adjacent to the anus, Fig. 10, and characteristics of the postfemoral stripe, Fig. 11) were the best diagnostic characters in distinguishing *A. c blanchardi* from *A c crepitans*. McCallum and Trauth

(2006) appear to have misinterpreted this description as referring only to the more dorsally located dark marking on the thighs, and took absence of a light mark superior to this dark area to be a *blanchardi*-like character (Fig. 11). Characteristics of the postfemoral stripe (Fig. 11) and presence and extent of the more ventrally located dusky areas (Fig. 10) were included in this analysis. McCallum and Trauth (2006) took some features from the type description of Harper (1947) to be blanchardi-like characters, though the author did not explicitly state a comparative use. These include presence of warts on the snout, presence of a pectoral fold (Fig. 12), and areolae on the venter. The former two characters were encoded using the above-described method and included in the current analysis. None of the frogs examined had ventral areolae, thus this character was not analyzed. Extent of toe webbing was noted by both Harper (1947) and analyzed by McCallum and Trauth (2006) as a potential subspecific diagnostic feature, and thus was coded for the current analysis when possible. Given the small size and delicate nature of this feature, in cases where damage to toe webbing was suspected, the animal was not included in analysis. Relative length of the fingers has proven useful in systematics and taxonomy in tropical Leptodactylid frogs, and was codified and analyzed for *Acris* in this study.

Data Analyses

All statistical analyses were performed using JMP 7.0 (SAS Institute, 2007).

Descriptive Statistics – Means, standard deviations, and variances were calculated and normality assessment was conducted for each quantitative variable measured and for all ratios. Descriptive statistics were calculated for all individuals

together and for discrete sampling locations/populations. Normality was assessed by inspection of frequency histograms, outlier boxplots, and normal quantile plots

CORRELATIONS WITH TEMPERATURE AND SUL – Correlation of DF with SUL and temperature has been demonstrated in previously published behavioral research on *Acris* (Ryan and Wilczynski, 1988, Wagner, 1989; Ryan et al., 1990; Ryan and Wilczynski, 1991; Ryan et al., 1992; Wilczynski and Ryan, 1999). Temporal features of calls are also known to vary with temperature (Wagner, 1989). Effects of body size and temperature on spectral and temporal characters were assessed using correlation and regression analysis and by model fitting using the Generalized Linear Model (GLM) personality. These methods were used to assess the need for scaling of the data to remove spurious effects on call properties.

The chosen model was used to remove the effect of body size and longitude on DF by standardizing SUL at 20.6 mm, the grand mean of all populations sampled. Subsequent tests involving DF were performed on both unscaled and scaled values.

ANOVA – Variation among populations in each quantitative variable was assessed using a *k*-sample Van Der Waerden test (k=3 populations). This is a non-parametric test of equality of means among different categorical levels and uses the quantile position of observations to assign a 'normal score' which functions as the response variable in the test. This test returns similar results as parametric analysis of variance for normally distributed data, but is also applicable to data that violate assumptions of normality and homoscedasticity upon which parametric hypothesis testing

is not valid. This test was chosen as the best option given that some variables have non-normal distributions.

Levene's test of equal variance was used to assess homoscedasticity. In cases where variances were unequal, the Welch ANOVA was used in lieu of the Van Der Waerden test Welch ANOVA assesses differences among groups or levels having unequal standard deviations For cases where the probability of variances being unequal was between .05 and .1, results of both Welch ANOVA and Van der Waerden tests are reported

For variables having significant results for the Van der Waerden test, a Tukey-Kramer HSD test was performed. Tukey-Kramer HSD tests all possible level pairs (populations in this analysis) and is similar to sequential Student's t-tests with Bonferroni correction but is more conservative Given the small sample sizes, this test was chosen to reduce type-1 error.

CATEGORICAL DATA ANALYSES – Geographic variation in binomial categorical data was assessed using logisitc regression analysis with both latitude and longitude as regressors. Small sample sizes, resulting in low expected values for many cells, prevented the use of χ^2 analysis. Of the behavioral variables, pulse style, call complexity, and call group complexity were analyzed by this method. Morphological characters analyzed by logistic regression included relative finger length, presence of pectoral fold, presence of warts on the snout, extent of toe webbing, presence of a light dorsal border adjacent to the dark marking on the dorsoposterior surface of the thigh, and presence of an extensive dusky marking on the posteriolateral thigh surface adjacent to the vent.

MULTIVARIATE ANALYSES – Given the large number of variables underconsideration and lack of prior knowledge about variation in these characters at the scale examined in this study, principal component analysis (PCA) was employed to examine covariation among a large number of characters without *a priori* assumptions regarding geographic groupings. Only quantitative variables having significant geographic variation identified in the univariate or bivariate analyses described above were used in the PCA.

Given uncertainties regarding the validity and distribution of subspecies through the area sampled, separate PCAs were run using scaled and unscaled DF. This allows consideration of two competing hypotheses: 1) that variation in DF can be attributed to variation in body size, temperature, or local effects of habitat selection, or 2) that variation in DF represents divergence of mating signals among populations that may be experiencing some degree of reproductive isolation. Agreement of score plots between the two PCAs should be taken to indicate that body size fails to entirely account for observed variation in DF, and that biologically meaningful geographic variation may exist among these populations. In order to examine the usefulness of marginally significant characters (those having significant results of Van der Waerden tests but for which Tukey-Kramer failed to identify contrasts), separate PCAs were run using all significantly variable characters (liberal: DF, CD, CR, SUL, HF, Toe, IO, EN, Leg:SUL, HL:HW, HF:Leg) and only those with clear contrasts (conservative: DF, CD, SUL, IO, EN, Leg:SUL, HL:HW, HF:Leg). Comparison of the PCA plots should help inform potential behavioral, systematic, and taxonomic interpretations.

PCA was also performed on morphological characters alone, with the inclusion of the set of individuals measured from the Texas Cooperative Wildlife Collection (TCWC)

and individuals collected from the Black River, NM. Grouping of individuals measured from any of the three populations in this study with known *A c paludicola*, *A c crepitans* (Louisiana), or *A gryllus* may aid interpretation of the results from a taxonomic perspective. As in the combined morphological and behavioral PCA, only characters exhibiting significant geographic variation were included in the analysis. The exception was toe length, which was not measured on museum specimens and thus was not included in the morphology PCA

CHAPTER III

RESULTS

DESCRIPTIVE STATISTICS

Results of measurements of all variables for each individual are presented in Table 4. A sample of the frequency histograms, boxplots, normal quantile plots, and summary statistics used to assess normality is shown in Fig. 13 Data presented are for all populations considered together The following variables deviated from normality. DF (bimodal), PG/C (left-skewed), CG (left-skewed), SUL (right-skewed), Toe (bimodal), HL (bimodal), IN (bimodal), HF·Leg (right-skewed). Normality was judged primarily on the basis of linearity of normal quantile plots and boxplots. A summary of means, variances, standard error means, and coefficients of variation are shown in Table 5.

CORRELATIONS WITH TEMPERATURE AND SUL

Conflicting results were obtained in correlation and regression analysis of dominant frequency as related to both temperature and SUL. Results of these analyses including response variable, regressor, grouping, function, number of observations, response means, r^2, r^2_{adj}, root mean square error, test statistic, probability of obtaining an equal or greater test statistic by chance, and model statement of fit for significant linear or polynomial relationships are summarized in Table 6. For all populations together, simple

linearegression using DF as the response variable and temperature as the regressor revealed a weakly positive relationship of DF to temperature for this dataset (r^2 = 0 1623, r^2_{adj} = 0.1312, F ratio = 5.2294, prob >F = 0.0303). Addition of a 2nd degree polynomial term to the equation yielded a significant increase in the value of r^2 (r^2 = 0.4988, r^2_{adj} = 0.4602, F ratio = 12.9376, prob >F = 0 0001). However, analysis of this relationship at the population level revealed that the relationship of DF to temperature was not consistent among populations. DF of only one population (LP) had a significant relationship to temperature (r^2 = 0.5853, r^2_{adj} = 0.5853, F ratio = 12.7029, prob >F = 0 0061), and the slope of the regression was negative, in contrast with the positive relationship determined for all populations together. Adding a 2nd degree polynomial term increased r^2 and r^2_{adj}, but the t ratio of the parameter estimate for the 2nd degree polynomial term was low and the probability of obtaining a similar absolute value of the t ratio was not significant at α=.05 (t ratio = 1.78, prob >|t| = 0.1134). Neither Bln (r^2 = 0.0850, r^2_{adj} = -0.0675, F ratio = 0.5571, prob >F = 0.4836) nor Brz (r^2 = 0.2760, r^2_{adj} = 0.1855, F ratio = 3.0499, prob >F = 0.1189) had significant linear relationships with temperature. Addition of a 2nd degree polynomial term did not improve the predictive relationship for either population (Bln: r^2 = 0.1254, r^2_{adj} = -0.2244, F ratio = 0.3584, prob >F = 0.7154; Brz: r^2 = 0.3473, r^2_{adj} = 0.1608, F ratio = 1.8622, prob >F = 0.2247).

Correlation of DF to SUL was similarly confounded by differences among populations. A significant, negative linear correlation of DF to SUL was determined to exist (r^2 = 0.5516, r^2_{adj} = 0.5312, F ratio = 27.0611, prob >F = <.0001) for all populations considered together. Correlation analyses at the individual population level were not significant for any of the populations, and addition of 2nd degree polynomial

terms did not improve the predictive ability of any of the models (see Table 6 for relevant statistics and models considered).

Given the ambiguity of relationships between DF and both temperature and SUL, models were fit using the Akaike information criterion (AIC) to choose a scaling equation. Previous work in this genus demonstrated a longitudinal gradient in call DF in Texas (Ryan and Wilczynski, 1988; Ryan and Wilczynski, 1991; Wilczynski and Ryan, 1999), with western populations having lower DF and eastern populations having higher DF. In consideration of this potential source of variation, longitude was included as a parameter in fitting the model. The stepwise model fitting personality was used, which allows evaluation of increasingly complex models in the fitting process. A summary of models tested, AIC values, r^2, and r^2_{adj} values is shown in Table 6. The model that best fit the data included SUL and longitude as parameters (AIC= -92.3062, r^2= 0.6548, r^2_{adj}= 0.6219). Maximal absolute value of AIC, r^2, and r^2_{adj} values were taken to indicate optimal predictive ability in the model selection process. These values with parameters and models considered are shown in Table 7.

All temporal characters were evaluated for relationship to SUL and to temperature using correlation and regression analysis. Characters having no significant relationship with SUL or temperature for all data considered together were not analyzed at the population level. Likewise, if 2nd degree polynomial relationships were not significant for all data together, this relationship was not assessed at the population level. Results of these analyses including response variable, regressor, grouping, function, number of observations, response means, r^2, r^2_{adj}, root mean square error, test statistic, probability of obtaining an equal or greater test statistic by chance, and model statement of fit for significant linear or polynomial relationships are summarized in Table 8.

A significant, positive linear relationship was found between SUL and pulse groups per call for all populations together (r^2 = 0.2684, r^2_{adj} = 0.2351, F ratio = 8.0695, prob >F = 0.0095) and for the Brazos population (r^2 = 0.7187, r^2_{adj} = 0.6784, F ratio = 8 0695, prob >F = 0.0039). SUL was also significantly correlated with call duration (CD) for all populations together (r^2 = 0 2443, r^2_{adj} = 0.2100, F ratio = 7.1122, prob >F = 0.0141). None of these relationships had a significant 2nd degree polynomial component. Given high within-individual variation for these characters and a lack of a consistent pattern to this relationship among populations, scaling for the correlated temporal variables was not performed.

ANOVA AND PAIRWISE CONTRASTS

Results of Van der Waerden analyses of variance and Levene's test for each behavioral and morphological character are shown in Table 9. DF (unscaled and scaled to SUL=20.6 mm.), CD, CR, SUL, HF, IO, EN, Leg:SUL, HL:HW, and HF:Leg all exhibited significant variation among populations. HF (Levene's F = 2.9559, P > F = 0.0793) and $DF_{unscaled}$ (Levene's F = 3.0825, P > F = .0629) were the only variables exhibiting heteroscedasticity. Welch ANOVA allowing unequal standard deviations was significant for both of these variables.

Tukey-Kramer HSD results for each pairwise comparison of variables exhibiting significant geographic variation are shown in Table 10. The Brazos population was different from both Colorado (LP) and Guadalupe (Bln) populations for $DF_{unscaled}$, CD, EN and HL:HW. This population differed from the Guadalupe population but not the Colorado in DF_{scaled}, SUL, and IO. For Leg:SUL and HF:Leg, the Brazos population differed from the Colorado population but not the Guadalupe. The Colorado population

did not differ significantly from the Guadalupe population for any of the variables tested Tukey-Kramer HSD failed to identify the pairwise contrast for call rate and hind foot length

Categorical Variables

Logistic regression results for categorical variables is summarized in Table 11. Negative log likelihood values are reported for the reduced model (intercept only), the full model (with a term for each observation), and the difference model, which includes a term for the categorical variable of interest. Also reported are degrees of freedom, the χ^2 test statistic, the probability of obtaining a similar test statistic by chance ($P>\chi^2$), and the U value (analogous to r^2).

Pulse style exhibited significant variation in the longitudinal dimension only, with the probability of observing weakly pulsed calls increasing as sampling moves eastward. Call complexity exhibited significant variation in both dimensions, with probability of observing simple calls increasing as sampling moves north and east. Variation in character of the thigh stripe was also tied to both latitude and longitude, with probability of observing a light dorsal border adjacent to the darker dorsoposterior stripe increasing as sampling moves north and east. Character of markings adjacent to the vent varied by longitude only, with probability of observing extensive markings on the posterior surface of the thigh increasing as sampling moves eastward. None of the other categorical measurements exhibited significant geographic patterns.

MULTIVARIATE ANALYSES

Principal component analyses using the 'conservative' character set was nearly identical for both DF_{scaled} and $DF_{unscaled}$ (Fig. 14 and 15). A similar pattern was observed for 'liberal' PCAs (Fig 16 and 17). Though the arrangements of individual points shifted slightly depending on the character set (liberal or conservative) and the value of DF (scaled or unscaled) used, the overall pattern observed remained the same for each iteration of the analysis. In all four PCAs, the Brazos population forms a distinct cluster in the left half of component space. While use of the liberal character set resulted in somewhat tighter groupings of points in the Colorado and Guadalupe populations, a clear delineation of these populations was not evident.

PCA of morphological variables, which included individuals from TCWC, individuals collected but not recorded from sampled sites, and individuals collected from the Black river, NM, is shown in Fig. 18. Raw data for these individuals are tabulated in Table 12. While some grouping is evident, particularly for the five *A c paludicola* individuals measured, no distinct geographic or taxonomic pattern exists with respect to coordinated variation of morphological characters.

Graphs of PCAs depict the first two principal components only. Cumulative percent of variation explained by these two components for each PCA is shown in Table 13. Vector loadings for each variable for each analysis are shown in Tables 14-16.

CHAPTER IV

DISCUSSION

Recent revisions to long-standing taxonomies resultant from large-scale phylogenetic analyses (e.g., Faivovich et al., 2005; Frost et al., 2006) represent a merging of traditional methods of classifying organisms with evolutionary theory. While overall morphological similarity offered the best method for naming and grouping organisms for much of the history of biological inquiry, modern methods demonstrate that no single dataset is definitive in elucidating ancestral lineages and relationships among extant species. Within species, population genetic studies illuminate ancient radiation patterns and can detect lineage divergence within species prior to the evolution of unique morphologies. Whether broad or narrow in taxonomic scope, investigations of evolutionary history benefit from a multi-modal approach and ideally should include morphological, behavioral, ecological, and molecular data. The ultimate goal is to better understand the evolutionary dynamics involved in lineage divergence and speciation, whether the actual events are ancient or incipient with respect to the present point in time. The research presented here opens new lines of inquiry in regard to the evolutionary history and modern interactions of a widely distributed nearctic anuran.

The genus *Acris* is distributed through much of the continental United States east of the Rocky Mountains (Conant and Collins, 1998). The distribution is similar in extent to that of the closely related genus *Pseudacris*. These two genera form a reciprocally monophyletic group, which holds a basal position in the Middle American/Holarctic clade of Hylid frogs published by Faivovich et al. (2005). Implicit in this phylogenetic hypothesis and the current distributions of these two groups is that both experienced similar climatologic and geologic conditions in their evolution to the present day. Recent molecular studies demonstrate that traditional morphology based taxonomy in *Pseudacris* does not reflect patterns of lineage divergence (Moriarty and Cannatella, 2004) and that cryptic lineages exist in at least one *Pseudacris* subspecies (*P crucifer crucifer*; Austin et al., 2002). Although the potential exists for similar, evolutionarily distinct lineages within *Acris* beyond the current taxonomy (two species), the only population genetic analysis to date that investigates relationships within the genus is that of Rose et al. (2006), which confirmed the genetic affinity of *A c paludicola* to *A crepitans*, but not to *A gryllus*. The very limited distribution of this distinct subgroup suggests the potential existence of similarly distinct groups within the greater range of the genus.

McCallum and Trauth (2006) reviewed a large number of specimens of both *A. c crepitans* and *A. c. blanchardi* from allopatry and from sympatry and could not differentiate these subspecies based on the characters they measured. However, many of their characters did not appear in Harper's (1947) original description of *A c blanchardi* as being useful in discriminating the subspecies, and one character listed as most diagnostic (character of markings adjacent to the vent) was not quantified. Both Rose et al. (2006) and McCallum and Trauth (2006) suggest that subspecific boundaries in *A.*

crepitans may be misplaced. In Texas, the accepted zone of contact between *A c blanchardi* and *A c crepitans* roughly follows the boundary of the Trinity and Neches river drainage basins (Conant and Collins, 1998). It is not clear in the literature how or when this line was established, and Harper (1947) in his original description of *A c blanchardi* makes reference to a zone of sympatry with *A. c. crepitans* that extends to the Rio Grande in Texas Thus the boundary between these subspecies as originally envisioned must lie several hundred miles to the west of the currently accepted line. Given uncertainties regarding the distribution and validity of the subspecies occupying the area sampled in this research, no explicit assumptions are made regarding potential subspecific affinity of the animals included in the analysis, and taxonomic implications are discussed in terms of future research directions.

BEHAVIORAL VARIATION

While the role of sexual selection in the speciation process is difficult to measure (Pannhuis et al., 2001), speculation about the potential participation of selection on characters involved in mate choice in lineage divergence can be traced back to the writings of Darwin (1859, 1871). Female preference for conspecific signals has been demonstrated in a diverse range of taxa (reviewed in Ptacek, 2000). Intraspecific variation in advertisement call has been demonstrated for many anurans (e.g., Ryan et al., 1996; Lougheed et al., 2006), including *Acris* (Ryan and Wilczynski, 1991; Wilczynski and Ryan, 1999), and studies of intraspecific variation in female preference for variable male signals show that females often prefer the signals characteristic of males from the same population (Ryan and Wilczynski, 1988; Ptacek, 2000). Thus variation among

populations in the coupled sender-receiver communication system can lead to divergence of those populations in mating signal properties, and ultimately may lead to prezygotic reproductive isolation.

Ryan and Wilczyniski and collaborators (1988; with Cocroft, 1990; 1991; with Perril, 1992; 1999) have demonstrated among-population variation in male advertisement call and in female preference in Texas subspecies of *Acris*. Specifically, these researchers found that *A c crepitans* produce calls with significantly higher DF, shorter duration, fewer pulses per call, faster call rate, and more calls per call group (Ryan et al., 1990). The Bastrop county population sampled in these works had call characteristics similar to that of *A c. crepitans*, though it lies within the range of *A. c blanchardi*. McClelland et al (1996, 1998) found that laryngeal structures involved in producing calls and the auditory structures that perceive them can vary independent of body size. Thus pleiotropic effects of larger, more desiccation resistant body size in western populations cannot fully explain clinal variation in call characters or female preference (Ryan and Wilczynski, 1991; Wilczynski and Ryan, 1999).

While Ryan and Wilczynski (1988) report that DF and best excitatory frequency (BEF) of the VIIIth cranial nerve are matched within but differ between populations of *Acris* sampled in Travis and Bastrop counties, most reports that employ female phonotaxis to measure call preferences find that females prefer calls with lower DF, regardless of mean call DF for their home population (Ryan et al., 1992; Perril and Lower, 1994). It is important to note that most of the experimental work of this nature employs artificial calls, which alter only one call component for each two-choice test. Thus these experiments cannot capture variation in female preference for coordinated

suites of characters (i.e. variation through multivariate call space). Higgins and Waugaman (2004) found that univariate call measures were insufficient to discriminate between species of field cricket, and that multivariate measures performed better at separating the calls of allopatric species.

Results of the current analysis found significant variation among populations in three of the call variables (DF, CR, CD) known to differ between *A c blanchardi* and *A c crepitans*. Further, unscaled DF for all populations considered together is distinctly bimodal (Fig. 13), with no individual recorded having a mean DF between 3.9 and 4.0 kHz. This distribution suggests the presence of two distinct groups within the region sampled. The Brazos population differed from the other two sample locations in unscaled DF and CD, both of which are known to vary between subspecies and to function in female preference. While the Van der Waerden test revealed significant difference in CR among populations, the Tukey-Kramer HSD did not identify a significant contrast in any of the population pairs. Two of the categorical call characters (pulse style and call complexity), showed significant geographic variation. The probability of observing weakly pulsed, multiple pulse group calls decreased as observations moved eastward. Call complexity exhibited significant longitudinal variation as well, with a probability of observing complex calls decreasing as observations moved northward. Previous studies have not examined the function of either call character in female mate choice.

Considering only univariate differences among the sampled populations, it appears that the Brazos group contains individuals more similar to *A c crepitans* than either of the other two groups. This analysis was unable to repeat the findings of Ryan and Wilczynski and collaborators (1988; with Cocroft, 1990; 1991; with Perril, 1992; 1999),

in that the Bastrop County group was more similar to *A c blanchardi* in univariate call characters It should be noted that, given the apparent presence of two distinctly different groups through the range sampled, it is possible that the earlier investigation sampled a breeding locality containing more *A c crepitans*-like individuals, while the population sampled for this analysis contained more *A c blanchardi*-like individuals. One individual sampled in Bastrop County did have a mean DF over 4 0 kHz (MAG084), placing it well above the normal range for *A c blanchardi*, and suggesting a need for more robust sample sizes and denser geographic sampling.

Significant differences in advertisement call observed in this analysis suggest the potential for assortative mating among the populations sampled. While female preference was not measured for this study, these results indicate a need for further investigations of the reproductive communication system within the range sampled. Should call preferences of females match the vocal qualities of males from their home population as reported by Ryan and Wilczynski (1998), some degree of lineage sorting is likely. These investigations should be accompanied by population genetic analyses in order to better understand the greater role of sexual selection in lineage divergence.

MORPHOLOGICAL VARIATION

Unlike many of the genera in Hylidae, members of the genus *Acris* lack distinctive and dramatic differences in morphology among species. *A crepitans* is distinguished from *A gryllus* by its greater length and bulk, by having a more pointed snout, and by differences in femoral striping (Conant and Collins, 1998). Neill (1950) was able to distinguish these species in an analysis of a series of specimens that spanned an area of sympatry in Georgia, indicating that although morphological differences are not pronounced, they are sufficient for positive species identification.

Within *A crepitans*, in the original description of *A c blanchardi,* Harper (1947) stated that the best identifying mark to distinguish this subspecies was "the more extensive dusky area on the posterior face of the femora in the vicinity of the vent" Harper also listed "slightly greater linear measurements", "decidedly greater bulk", and "somewhat more extensive webbing of the toes" as distinguishing characters. The argument for elimination of *A c blanchardi* by McCallum and Trauth (2006) used a suite of characters of dubious utility, resulting from what appears to be a flawed interpretation of Harper's original description. The characters they used included warts on the snout, areolae on the ventral body surface, presence of a pectoral fold, web of fourth toe extending to last joint, presence of a light dorsal border to the dorsoposterior leg stripe, SUL, and body mass of preserved specimens. Harper (1947) mentions the first three characters in his type description, but does not offer that any are unique to *A c blanchardi*. Toe webbing has diagnostic potential, but can be variable and is easily damaged in nature and in examination of specimens. Harper does mention the light dorsal border of the dorsoposetrior leg stripe in *A c crepitans* in the context of describing the

diagnostic characteristics of the more posterior dusky femoral marking, which was not included or even mentioned as a *blanchardi*-like character by McCallum and Trauth (2006). SUL has potential in discerning these subspecies, though this is a highly variable character and lack of knowledge of the age, gender, or maturity of a specimen when collected could introduce bias. Body mass is similarly confounded, a situation that could be exacerbated by differences in preservation method and handling of preserved specimens. Thus, of the seven *blanchardi*-like characters examined in McCallum and Trauth (2006), only one objectively measurable character was employed (light dorsal border of leg stripe) that followed the original subspecific description by Harper (1947), and the most diagnostic character (postfemoral dusky area) was ignored completely.

The present study included all of the characters reviewed by McCallum and Trauth (2006) except body mass, for reasons noted above. A large number of additional morphological measurements were taken, and ratios calculated for some relative body proportions. SUL was the only quantitative character measured by McCallum and Trauth (2006), who found no significant difference in the male specimens they measured between South Dakota-Nebraska samples and those from Georgia-Florida. SUL did vary significantly in the present study: frogs sampled from the Brazos basin were significantly smaller in SUL than the frogs sampled from the Guadalupe basin. Colorado basin frogs did not differ significantly from either population. This observation is consistent with the possibility of having a mixed or intergrade population in the Lost Pines habitat.

Of the other *blanchardi*-like characters measured by McCallum and Trauth (2006), pectoral fold and snout warts showed no pattern of geographic variation. This finding is consistent with their assertion that these characters are of no use in determining geographic origin, and with the fact that Harper (1947) did not describe either as unique to either subspecies. Presence of a light dorsal border to the dorsoposterior leg stripe did exhibit geographic variation in both latitude and longitude in the present analysis, with the probability of having a light dorsal border (an *A c crepitans*-like character) increasing as sampling moved north and east. McCallum and Trauth (2006) found that this was the most reliable character in their analysis. Toe webbing exhibited roughly equal probabilities of observation across latitude and longitude of the region sampled, indicating that this character is not useful for delimiting populations at this geographic scale.

Additional morphometrics exhibiting significant differences between the Brazos group and both of the other sampling locations included eye-nares distance and the ratio of head length to head width. Both of these characters suggest that the Brazos frogs have smaller heads, an observation that may be the result of their overall smaller size. Alternatively, these observations could mirror differences in the auditory production and perception structures. Additional work is necessary to ascertain potential correlation of these external morphological measures with internal components of the acoustic communication system. Should relationships exist between these external and internal characters, these external measurements could reflect evolutionary processes at work in the auditory system of *Acris*, and could prove useful for field identification.

The Brazos population differed from the Guadalupe population in toe length and

interorbital distance, and from the Colorado population in leg length to SUL ratio and in proportion of hind foot to leg. For hind foot length, Tukey-Kramer HSD did not identify the contrasting pair. These results are difficult to interpret: while the Brazos population differs from one other population in each significant result, the pairwise contrasts are inconsistent. This could indicate the presence of conflicting clinal variation among characters, or a greater pattern of geographic variation that could become apparent with denser geographic sampling.

Results obtained in univariate morphological analyses highlight the difficulties associated with using morphological traits to infer evolutionary relatedness. Nearly every species exhibits some degree of morphological variation, and diagnostic characters may not be absolute. Clinal variation or geographic mosaic in distribution of morphological traits may go undetected if sampling of specimens is patchy or discontinuous. Again, population genetic analysis is needed to identify the degree to which any of the morphological characters are associated with evolutionary history.

Multivariate Analyses

Previous studies in anurans (Ryan et al., 1996; Gergus et al., 1997; Wycherley et al., 2002; Smith et al., 2003; Lougheed et al., 2006) and crickets (Higgins and Waugaman, 2004) have demonstrated the importance of multivariate analyses in detecting both interspecific and intraspecific differences in communication systems. The degree to which observed geographic variation in calling behavior reflects population history (i.e., lineage divergence) remains to be thoroughly investigated in the literature (Ryan et al., 1996; Lougheed et al., 2006; Robillard et al., 2006). Given the importance of

the anuran communication system in directing mate choice, the potential exists for local variations in the mating system to drive speciation (Blair, 1958; Ptacek, 2000; Panhuis et al., 2001). Multivariate approaches offer a better tool for detecting this variation, and can provide a means of generating hypotheses regarding population history that can be tested using molecular methods.

In the current analysis, PCAs of morphological and call variables – whether using scaled or unscaled DF, liberal or conservative datasets (Fig. 14-17) shared similar patterns of geographic variation: Brazos individuals form a distinct cluster on the left side of component space, while Colorado and Guadalupe individuals are somewhat randomly distributed on the right side of component space. This finding supports univariate results that suggested the Brazos population was distinct, but that were ambiguous in the direction of pairwise differences among populations. The larger morphological analysis, which included specimens of *A. gryllus*, *A c crepitans*, and *A c. paludicola*, does not distinctly separate any of the species or subspecies (Fig. 18), with the possible exception of *A c paludicola*. Comparison of plots of behavioral and morphological data vs. morphology-only clearly indicates the utility of using combined datasets in describing geographic variation at the scale considered in this analysis. Further, as noted in Higgins and Waugaman (2004), univariate analyses may fail to identify parental populations when intermediate forms exist, where multivariate methods often capture covariance among suites of characters that may be biologically relevant.

The findings of Ryan, Wilczynski and collaborators (1988; with Cocroft, 1990; 1991; with Perril, 1992; 1999) were not duplicated in the multivariate analyses: the Lost Pines/Colorado drainage population was not distinct from the Guadalupe group. Instead it

appears that the Brazos males, with the exception of one individual, (MAG095), were distinct in analyses of combined morphological and behavioral datasets. Ryan, Wilczynski and collaborators (1988; with Cocroft, 1990; 1991; with Perril, 1992; 1999) suggest environmental or habitat selection on the calls of Lost Pines *Acris* that favors signal transmission in a structurally complicated environment. Should this be the primary factor affecting the differences in call structure and frequency they describe, the expected result in the present analysis would be clustering of Colorado/Lost Pines individuals with Brazos individuals, since both sample sites were within forested habitat. This is clearly not the case, however. Thus an alternative explanation could be misplacement of a subspecific boundary. Again, only increased density of geographic sampling can begin to address this possibility, preferably coupled with population genetic analyses.

CONCLUSIONS

The taxonomic organization within the genus *Acris* remained stable from the middle 20th century until 2006 (McCallum and Trauth, 2006, Rose et al., 2006). While large-scale phylogenetic analyses have rearranged many of the relationships in Amphibia in recent years (Faivovich et al., 2005; Frost et al., 2006), the broad scope of these projects precluded intrageneric and intraspecific analyses within *Acris*. Thus in spite of an explosion in molecular population and systematic analyses, the only analysis of this type in *Acris* that exists in the literature to date is that of Rose et al. (2006) which focused on the specific affinity of *A. c. paludicola*. The results of the research presented here provide directions for future research, and call into question the contentions of McCallum and Trauth (2006) that *A. c. blanchardi* is not a valid subspecies.

The original intent of this research was to examine interpopulation variation in behavior and morphology of *A crepitans*, without concern for potential subspecific interactions. Considering the results of multivariate analyses, it becomes necessary to consider the possibility that a line of contact between two distinct forms exists within the region sampled. Alternatively, *A crepitans* may represent a species complex that is poorly differentiated morphologically but in which prezygotic reproductive isolation has occurred. Cagle (1954) reported a population of *A crepitans* near Doss, Texas (N30.44, W-99.13), that he identified as *A c crepitans* based on the characters used by Harper (1947) in the original description This locality is far west of the currently accepted zone of contact of these subspecies, thus it appears possible that a mosaic of finely distinguished forms exists within Texas. Lacking broad-scale population genetic analyses, it is impossible to say if the variation observed in this study and in those that preceded it represents the result of local differences in sexual or environmental selection, genetic drift, or a combination of these factors. None of the analyses conducted thus far can address the possibility of reproductive isolation, whether nonexistent, incipient, or well established.

Considering the state of knowledge about this genus, some conclusions are clear, and some require further investigation. First, the results of this work suggest that multiple taxonomic units within *A. crepitans* beyond the two proposed by McCallum and Trauth (2006, *A c crepitans* and *A c paludicola*) must be considered until population genetic analyses can be completed. Second, the geographic delineation between *A c blanchardi* and *A c. crepitans* is either misplaced or a mosaic of intermediate forms, and increased density of sampling within and beyond the area surveyed in this study is necessary. Third,

the morphological distinctiveness of *A c paludicola* merits further investigation, as this group may represent a unique species Given the very limited range and anecdotal evidence of sharp declines in this subspecies, immediate conservation efforts may be needed

In the broader evolutionary context of the role of reproductive communication in driving speciation, studies are needed that address the plasticity of mating signals and preferences in anurans. It is unknown whether a frog from a forested area will continue to produce forest-adapted calls if placed in an open habitat. While knowledge of the degree of within-individual behavioral plasticity is necessary for biologically relevant interpretations of results, there are conservation implications as well. Should it be found that individual animals are narrowly constrained in their reproductive signals, alteration of habitat could generate negative selection on formerly well-adapted signal properties, and reduce the reproductive output of populations. In short, while the role of sexual signals in reproductive isolation and speciation is relatively clear, the potential contribution to extinction must also be addressed.

Finally, the results of this analysis highlight the need for sound molecular hypotheses in evaluating the evolutionary importance of behavioral and morphological variation. If observed variation in these characters coincides with the geographic distributions of genotypes, their utility as indicators of population history is enhanced. Alternatively, if no or only weak correlation exists between the observed characters and population history, questions arise about the degree of divergence in mating signals necessary to result in lineage divergence. Knowledge of the real importance of mating signals in predicting underlying evolutionary dynamics is lacking at the present time,

though many assumptions are made in the behavioral literature. Combined behavioral, morphological, and molecular analyses offer the potential to clarify these relationships.

TABLE 1: Drainage basins sampled, including specific locations, abbreviations used in text, and geographic coordinates.

Drainage	Location	Abbreviation	Latitude	Longitude
Brazos		Brz		
	Milam County Road 264	Mil CR264	30 80661	-97 74443
	Milam County Road 342	Mil CR342	30 71334	-96 78821
Colorado		Col		
	Lost Pines/Griffith League Ranch	LP		
	Pond 2	GLR P2	30.21623	-97 24188
	Pond 5a	GLR P5	30 20938	-97 24309
Guadalupe	Blanco River	Bln	29 93674	-97 8953

TABLE 2A: Acoustic variables measured, including type of measurement (spectral, temporal, or categorical), abbreviations used and units. Categorical variables are unitless.

Type	Variable	Abbreviation	Units
Spectral	Dominant Frequency	DF	kHz
Temporal	Pulse Groups per Call	PG/C	#/call
	Pulses per Pulse Group	P/PG	#/pulse grp
	Call Duration	CD	ms
	Call Group Duration	CG	s
	Pulse Rate	PR	pulses/ms
	Call Rate	CR	calls/s
Categorical	Pulse Style		
	Weak	w	
	Strong	s	
	Call Complexity	Ratio cs cc	
	Simple	cs	
	Complex	cc	
	Call Group Complexity		
	Simple	gs	
	Complex	gc	
	Competitors	comp	
	yes/no		

TABLE 2B: Morphological variables measured, including type of measurement (linear, ratio, or categorical), abbreviations used and units. Categorical variables are unitless.

Variable Type	Variable	Abbreviation	Units
Linear	Snout-Urostyle Length	SUL	mm
	Femur Length	FL	mm
	Tibia Length	TL	mm
	Hind Foot Length	HF	mm
	Heel-Tubercle Distance	HT	mm
	Tubercle-Tip of 4th Toe	Toe	mm
	Head Width	HW	mm
	Head Length	HL	mm
	Interorbital Distance	IO	mm
	Eye Diameter	ED	mm
	Eye-Nares Distance	EN	mm
	Internareal Distance	IN	mm
Ratios	Hind Leg SUL	Leg SUL	
	Head Length Head Width	HL HW	
	Hind Foot Hind Leg	HF Leg	
	Toe Length Foot Length	TL FL	
	Eye Diameter Head Length	ED HL	
Categorical	Relative Finger Length	Fin Len	
	2>1>3>4	1	
	2>1=3>4	2	
	Pectoral Fold	PF	
	yes/no		
	Warts on Snout	Wart	
	yes/no		
	Toe Web	Web	
	Proximal to last phalangeal joint	1	
	Distal to last phlangeal joint	2	
	Dorsoposterior thigh stripe	TS	
	Bordered by light dorsal stripe	1	
	Blended with dorsal pigment	2	
	Anal marking	AM	
	Absent or reduced in size	1	
	Present, extensive	2	

TABLE 3: Individuals sampled by location, date, and data type collected.

Individual	Location	Date Collected	Acoustic Data	Morphologic Data
MAG033	Brz, Mil CR342	29-Apr-07	no	yes
MAG034	Brz, Mil CR342	29-Apr-07	no	yes
MAG035	Brz, Mil CR342	29-Apr-07	no	yes
MAG036	Brz, Mil CR342	29-Apr-07	no	yes
MAG093	Brz, Mil CR264	9-Jul-07	yes	yes
MAG094	Brz, Mil CR264	9-Jul-07	yes	yes
MAG095	Brz, Mil CR264	9-Jul-07	yes	yes
MAG096	Brz, Mil CR264	9-Jul-07	yes	yes
MAG097	Brz, Mil CR264	9-Jul-07	yes	yes
MAG098	Brz, Mil CR264	13-Jul-07	yes	yes
MAG099	Brz, Mil CR264	13-Jul-07	yes	yes
MAG100	Brz, Mil CR264	13-Jul-07	yes	yes
MAG101	Brz, Mil CR264	13-Jul-07	yes	yes
MAG102	Brz, Mil CR264	13-Jul-07	yes	yes
MAG068	Col, LP, GLR P2	2-Jun-07	no	yes
MAG069	Col, LP, GLR P5	2-Jun-07	yes	yes
MAG080	Col, LP, GLR P2	16-Jun-07	yes	no
MAG081	Col, LP, GLR P2	16-Jun-07	yes	yes
MAG082	Col, LP, GLR P2	16-Jun-07	yes	yes
MAG083	Col, LP, GLR P2	16-Jun-07	yes	no
MAG084	Col, LP, GLR P2	16-Jun-07	yes	yes
MAG085	Col, LP, GLR P2	16-Jun-07	yes	yes
MAG086	Col, LP, GLR P2	16-Jun-07	yes	yes
MAG087	Col, LP, GLR P2	16-Jun-07	yes	yes
MAG088	Col, LP; GLR P2	16-Jun-07	yes	no
MAG089	Col, LP; GLR P2	16-Jun-07	yes	yes
MAG070	Bln	6-Jun-07	yes	yes
MAG071	Bln	6-Jun-07	yes	yes
MAG072	Bln	6-Jun-07	yes	yes
MAG073	Bln	6-Jun-07	yes	no
MAG074	Bln	6-Jun-07	yes	yes
MAG075	Bln	6-Jun-07	yes	no
MAG076	Bln	6-Jun-07	yes	no
MAG077	Bln	6-Jun-07	yes	yes
MAG078	Bln	6-Jun-07	no	yes

TABLE 4: Raw data for all individuals from primary sampling localities Means and standard deviations (SD) are reported for behavioral characters with multiple observations per individual. ND indicates missing data (see text)

Individual	Location	Latitude	Longitude	Date	Temperature	Relative Humidity	Avg Wind (kmh)	DF	SD(DF)	PG/C
MAG033	Brz	30 71334	-96 78821	29-Apr-07	20 0	78 4	0 0	nd	nd	nd
MAG034	Brz	30 71334	-96 78821	29-Apr-07	20.0	78 4	0 0	nd	nd	nd
MAG035	Brz	30 71334	-96 78821	29-Apr-07	20 0	78 4	0 0	nd	nd	nd
MAG036	Brz	30 71334	-96.78821	29-Apr-07	20 0	78 4	0 0	nd	nd	nd
MAG068	LP	30 21623	-97 24188	2-Jun-07	25 5	87 7	0 0	nd	nd	nd
MAG069	LP	30 20938	-97 24309	2-Jun-07	25 5	87 8	0 0	3 542	0 082	2 167
MAG070	Bln	29 93674	-97.89530	6-Jun-07	26.7	87 6	2 8	3 623	0 161	1 579
MAG071	Bln	29 93674	-97.89530	6-Jun-07	26 7	87 6	2.8	3 775	0 059	1 579
MAG072	Bln	29 93674	-97 89530	6-Jun-07	26 7	87 6	4 0	3 608	0 079	1 000
MAG073	Bln	29 93674	-97 89530	6-Jun-07	26 7	87 6	4 0	3 615	0 199	2 346
MAG074	Bln	29 93674	-97 89530	6-Jun-07	25 8	86 0	7 1	3 571	0 191	2 024
MAG075	Bln	29 93674	-97 89530	6-Jun-07	26 4	89 3	7 2	3 575	0 207	1 600
MAG076	Bln	29 93674	-97 89530	6-Jun-07	26 4	89 3	7 2	3 615	0 128	1 238
MAG077	Bln	29.93674	-97 89530	6-Jun-07	26 4	89 3	7 1	3 796	0 065	1 900
MAG078	Bln	29 93674	-97 89530	6-Jun-07	26 4	89 3	7 1	nd	nd	nd
MAG079	Bln	29 93674	-97 89531	6-Jun-07	26 4	89 3	7 1	nd	nd	nd
MAG080	LP	30 21623	-97 24188	16-Jun-07	23 9	84 5	0 0	3 749	0 132	1 857
MAG081	LP	30 21623	-97 24189	16-Jun-07	24 8	84 6	0 0	3 695	0 081	1 565
MAG082	LP	30 21623	-97 24190	16-Jun-07	24 3	84 6	0 0	3 724	0 165	2 200
MAG083	LP	30 21623	-97 24191	16-Jun-07	24 0	86 8	0 0	3 771	0 094	1 029
MAG084	LP	30 21623	-97 24192	16-Jun-07	23 4	88 3	0 0	4 133	0 094	1 619
MAG085	LP	30 21623	-97 24193	16-Jun-07	24 1	86 5	0 0	3 823	0 086	1 714
MAG086	LP	30 21623	-97 24194	16-Jun-07	23 9	94 6	1 1	3 865	0 094	1 362
MAG087	LP	30 21623	-97.24195	16-Jun-07	23 9	94 6	1 1	3 877	0 060	1 594
MAG088	LP	30 21623	-97 24196	16-Jun-07	23 9	94 6	1 1	3 725	0 081	1 273
MAG089	LP	30 21623	-97 24197	16-Jun-07	23 9	94 6	1 1	3 670	0 051	1 138
MAG093	Brz	30 80661	-97 74443	9-Jul-07	29 0	78 7	1 0	4 099	0 086	1 696
MAG094	Brz	30 80661	-97 74443	9-Jul-07	27.0	83 2	0 0	4 118	0 084	1 077
MAG095	Brz	30 80661	-97 74443	9-Jul-07	27 1	85 9	0 0	3 711	0 101	2 138
MAG096	Brz	30 80832	-97 74462	9-Jul-07	27 0	85 0	1 9	3 785	0 100	1 000
MAG097	Brz	30 80832	-97 74463	9-Jul-07	27 2	84 6	1 9	4 144	0 108	1 000
MAG098	Brz	30 80661	-97.74443	13-Jul-07	27 0	91 9	0 0	4 227	0 306	1 225
MAG099	Brz	30 80661	-97 74443	13-Jul-07	26 7	94 3	0 0	3 781	0 350	1 250
MAG100	Brz	30.80661	-97 74443	13-Jul-07	29 0	83 9	0 0	4 138	0 065	1 067
MAG101	Brz	30 80661	-97 74443	13-Jul-07	28 8	85 3	0 0	4 228	0 064	1 154
MAG102	Brz	30 80661	-97 74443	13-Jul-07	28 5	84 7	0 0	4 153	0 066	1 024

TABLE 4 CONTINUED

Individual	Location	SD(PG/C)	CD	SD(CD)	PR	SD(PR)	P/PG	SD(P/PG)	n calls	CG	SD(CG)
MAG033	Brz	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG034	Brz	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG035	Brz	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG036	Brz	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG068	LP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG069	LP	0 707	67 690	32 070	0 189	0 078	5 870	3 480	18	20 760	0 000
MAG070	Bln	0 507	37 577	23 533	0 360	0 170	7 263	2 054	19	4 329	0 969
MAG071	Bln	0 507	53 263	25 627	0 192	0 084	6 289	1 008	19	10 323	2 759
MAG072	Bln	0 000	29 269	12 667	0 416	0 072	12 231	5 523	26	7 703	3 018
MAG073	Bln	0 892	72 731	35 634	0 110	0 052	3 019	1 277	26	4 730	1 463
MAG074	Bln	1 275	56 024	45 835	0 175	0 097	3 931	1 956	41	6 345	2 084
MAG075	Bln	0 621	47 300	26 366	0 149	0 060	3 944	1 605	30	5 563	1 091
MAG076	Bln	0 539	34 810	21 956	0 264	0 075	7 524	4 794	21	10 232	5 901
MAG077	Bln	0 852	60 300	31 493	0 213	0 091	6 217	2 443	20	5 156	1 091
MAG078	Bln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG079	Bln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG080	LP	0 727	45 381	28 949	0 207	0 089	4 611	3 922	21	8 834	3 200
MAG081	LP	0 662	46 452	29 846	0 326	0 092	9 449	5 115	23	11 060	3 030
MAG082	LP	0 707	77 320	37 945	0 279	0 478	7 757	6 134	25	17 723	5 298
MAG083	LP	0 171	24 382	13 616	0 323	0 065	1 029	0 171	34	15 947	7 065
MAG084	LP	0 669	48 286	34 421	0 279	0 108	7 508	3 935	21	9 351	0 762
MAG085	LP	0 717	58 000	47 433	0 290	0 180	7 429	5 146	21	13 209	5 298
MAG086	LP	0 613	36 793	28 732	0 246	0 091	5 411	1 546	58	10 080	4 418
MAG087	LP	0 615	45 750	28 170	0 165	0 085	4 151	1 881	32	5 920	3.896
MAG088	LP	0 703	33 818	27 412	0 244	0 067	5 852	2 470	22	7 485	3 453
MAG089	LP	0 351	33 138	14 394	0 254	0 056	7 241	2 007	29	8 415	4 557
MAG093	Brz	0 695	48 239	30 329	0 244	0 117	5 540	1 619	46	9 099	2 954
MAG094	Brz	0 272	25 269	11 567	0 393	0 114	8 846	3 130	28	11 362	1 689
MAG095	Brz	0 743	45 828	22 504	0 183	0 103	3 471	1 501	29	6 581	4 895
MAG096	Brz	0 000	22 405	6 525	0 283	0 058	6 135	1 494	37	11 432	6 228
MAG097	Brz	0.000	17 440	4 583	0 378	0 100	6 320	1 215	25	19 442	11 999
MAG098	Brz	0 423	21 975	14 513	0 462	0 160	8 013	5 874	40	8 086	3 358
MAG099	Brz	0 585	24 964	21 221	0 363	0 130	6 488	3 501	28	9 637	7 966
MAG100	Brz	0 254	29 433	9 587	0 319	0 099	9 150	4 289	30	3 428	0 944
MAG101	Brz	0 368	32 346	14 937	0 296	0 052	8 115	2 197	26	4 021	0 769
MAG102	Brz	0 156	19 073	7 986	0 252	0 076	4 427	1 302	41	5 944	5 961

TABLE 4 CONTINUED

Individual	Location	CR	SD(CR)	n CG	Pulse style	Call Complexity	CG Complexity	Competitors	SUL	FL
MAG033	Brz	nd	nd	nd	nd	nd	nd	nd	23 0	12 5
MAG034	Brz	nd	nd	nd	nd	nd	nd	nd	20 3	11 1
MAG035	Brz	nd	nd	nd	nd	nd	nd	nd	21 7	11 7
MAG036	Brz	nd	nd	nd	nd	nd	nd	nd	20 9	11 6
MAG068	LP	nd	nd	nd	nd	nd	nd	nd	23 0	12 5
MAG069	LP	3 613	0 000	1	s	cc	gc	n	22 9	11 4
MAG070	Bln	7 004	0 697	4	s	cc	gc	y	21 8	11 9
MAG071	Bln	4.434	0 527	2	w	cc	gc	n	21 1	11 5
MAG072	Bln	5 724	1 269	4	s	s	gs	n	23 4	12 6
MAG073	Bln	4.992	0 709	5	w	cc	gs	y	nd	nd
MAG074	Bln	4 501	0 613	5	w	cc	gc	y	22 5	11 4
MAG075	Bln	5 778	0 570	5	w	cc	gc	n	nd	nd
MAG076	Bln	4 735	1 312	3	s	cc	gs	n	21 4	10 6
MAG077	Bln	4 507	0 500	5	s	cc	gs	n	20 3	12 3
MAG078	Bln	nd	nd	nd	nd	nd	nd	nd	22 8	11 6
MAG079	Bln	nd	nd	nd	nd	nd	nd	nd	20 7	13 2
MAG080	LP	4 267	1 340	3	s	cc	gs	n	19 3	9 3
MAG081	LP	3 824	1 084	3	s	cc	gc	n	19 2	10 1
MAG082	LP	3 555	0 373	2	s	cc	gc	n	22 8	11 6
MAG083	LP	3 769	0 273	3	s	s	gc	n	nd	nd
MAG084	LP	4 308	0 474	3	s	cc	gc	y	19 4	10 2
MAG085	LP	2 741	0 876	3	s	cc	gc	n	20 9	10 4
MAG086	LP	5 427	0 902	6	s	cc	gc	n	22 1	10 9
MAG087	LP	5 002	0 857	6	w	cc	gc	n	21 2	9 8
MAG088	LP	4 739	0 764	4	w	v	gc	n	nd	nd
MAG089	LP	5 493	1 493	4	s	s	gc	n	22 3	11 1
MAG093	Brz	5 343	0 560	5	s	cc	gc	n	nd	nd
MAG094	Brz	3 965	0 361	3	s	s	gc	y	16 7	9 9
MAG095	Brz	5 698	0 548	4	s	cc	gc	y	23 6	12 1
MAG096	Brz	4 363	1 576	3	s	s	gc	n	18 4	10 3
MAG097	Brz	2 214	0 906	4	w	s	gs	n	19 1	12 1
MAG098	Brz	5 657	1 112	5	s	cc	gc	n	18 9	10 1
MAG099	Brz	6 291	2 508	3	s	cc	gc	y	21 3	13 5
MAG100	Brz	8 415	0 808	6	s	s	gc	y	18 7	11 1
MAG101	Brz	6 129	0 780	7	s	s	gc	n	18 7	11 1
MAG102	Brz	7 073	1 970	7	s	s	gs	y	19 2	9 7

TABLE 4 CONTINUED

Individual	Location	TL	LEG	HF	HT	Toe	HW	HL	IO	ED	EN
MAG033	Brz	14.8	27 3	18 8	7 5	12 9	7 3	8 0	2 0	2 8	2 0
MAG034	Brz	13 2	24 3	18 5	7 9	12 3	6 5	7 7	1 8	3 1	1 6
MAG035	Brz	13 8	25 5	19 3	7 7	12 6	6 9	7 8	2 0	2 8	2 2
MAG036	Brz	13 0	24 6	17 5	7 4	12 1	6 4	8 2	1 6	3 0	2 0
MAG068	LP	14 8	27 3	18 8	7 5	12 9	7 3	8 0	2 0	2 8	2 0
MAG069	LP	14 7	26 1	20 8	8 1	14 0	7 3	8 3	2 0	3 0	1 8
MAG070	Bln	14 4	26 3	18 5	6 8	11 7	7 4	8 0	2 1	3 2	1 7
MAG071	Bln	13 1	24 6	18 3	6 8	12 8	6 8	7 3	2 1	2 8	1 7
MAG072	Bln	14 1	26 7	20 4	7 8	13 0	7 9	8 2	1 8	3 1	1 6
MAG073	Bln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG074	Bln	14 1	25 5	19 1	7 1	12 5	7 3	8 1	2 2	2 7	2 0
MAG075	Bln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG076	Bln	13 3	23 9	18 6	7 6	12 4	6 6	7 7	2 0	3 4	1 7
MAG077	Bln	13 8	26 1	18 9	7 3	12 7	6 9	7 3	1 7	2 5	2 0
MAG078	Bln	14 2	25 8	19 8	7 1	12 4	8 2	8 2	1 8	3 2	2 0
MAG079	Bln	13 2	26 4	18 8	7 9	12 5	8 0	7 9	2 0	2 3	1 6
MAG080	LP	12 0	21 3	16 5	7 4	11 2	6 4	7 1	1 5	2 6	1 7
MAG081	LP	11 3	21 4	16 3	6 6	10 5	6 3	7 1	1 7	2 8	1 5
MAG082	LP	14 3	25 9	19 3	8 0	12 6	8 0	8 1	2 0	3 1	1 7
MAG083	LP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG084	LP	11 8	22 0	16 5	7 1	10 9	6 5	6 8	1 9	2 2	2 0
MAG085	LP	12 0	22 4	17 5	6 8	11 6	7 4	7 1	1 5	2 5	1 6
MAG086	LP	12 4	23 3	18 1	7 4	11 7	6 5	7 4	1 5	3 2	1 4
MAG087	LP	12 4	22 2	17 2	7 0	10 9	7 1	7 9	1 9	3 4	1 8
MAG088	LP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG089	LP	13 9	25 0	19 9	7 6	13 2	7 0	8 3	2 1	3 2	1 6
MAG093	Brz	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG094	Brz	11 5	21 4	16 3	6 3	10 6	7 0	6 7	1 6	2 4	1 3
MAG095	Brz	13 9	26.0	19 0	8 2	12 6	8 3	7 8	2 1	3 2	1 7
MAG096	Brz	12 6	22 9	17 5	7 5	11 2	7 2	6 8	1 7	3 1	1 5
MAG097	Brz	12 6	24 7	16 5	6 4	10 8	6 8	6 7	1 7	2 5	1 3
MAG098	Brz	13 8	23.9	18 2	7.7	10 9	6 9	6 8	1 7	2 3	12 0
MAG099	Brz	14.2	27 7	19 5	7 7	12 5	7 7	7 8	1 5	2 7	1 4
MAG100	Brz	12 0	23 1	17 1	7 1	11 3	7 9	7 1	1 4	2 7	1 5
MAG101	Brz	12 7	23 8	17 6	7.3	11 9	7 7	7 6	1 4	2 8	1 7
MAG102	Brz	12 7	22 4	17 3	6 9	11 2	7.2	7 1	1.8	2 7	1 3

TABLE 4 CONTINUED

Individual	Location	IN	Leg·SUL	HL HW	HF·Leg	TL FL	ED HL	Relative Fin Len	Fold?	Warts?
MAG033	Brz	1 6	1 187	1 785	0 689	0 686	0 350	2>1>3>4	n	y
MAG034	Brz	1 4	1 197	1 946	0 761	0 665	0 403	2>1=3>4	n	n
MAG035	Brz	1 2	1 175	1 887	0 757	0 653	0 359	2>1>3>4	n	n
MAG036	Brz	1 6	1 177	1 993	0 711	0 691	0 366	2>1>3>4	y	n
MAG068	LP	1.6	1 187	1 785	0 689	1 184	0 350	2>1>3>4	n	y
MAG069	LP	1 6	1 140	1 137	0 797	1 289	0 361	2>1>3>4	y	n
MAG070	Bln	1 7	1 206	1 081	0 703	1 210	0 400	2>1=3>4	y	n
MAG071	Bln	1 2	1 166	1 074	0 744	1 139	0 384	2>1=3>4	y	n
MAG072	Bln	1.8	1 141	1 038	0 764	1 119	0 378	2>1>3>4	n	n
MAG073	Bln	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG074	Bln	1 6	1 133	1 110	0 749	1 237	0 333	2>1=3>4	n	n
MAG075	Bln	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG076	Bln	1 7	1 117	1 167	0 778	1 255	0 442	2>1>3>4	n	n
MAG077	Bln	1 5	1 286	1 058	0 724	1 122	0 342	2>1>3>4	y	n
MAG078	Bln	1 6	1 132	1 000	0 767	1 224	0 390	2>1>3>4	n	n
MAG079	Bln	1 3	1 275	0 988	0 712	1 000	0 291	2>1>3>4	n	N
MAG080	LP	1 2	1 104	1 109	0 775	1 290	0 366	2>1>3>4	n	n
MAG081	LP	1 4	1 115	1 127	0 762	1 119	0 394	2>1>3>4	n	n
MAG082	LP	1 3	1 136	1 013	0 745	1 233	0 383	2>1=3>4	y	n
MAG083	LP	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG084	LP	1 4	1 134	1 046	0 750	1 157	0 324	2>1>3>4	n	y
MAG085	LP	1 2	1 072	0 959	0 781	1 154	0 352	2>1>3>4	y	n
MAG086	LP	1 3	1 054	1 138	0 777	1 138	0 432	2>1>3>4	n	n
MAG087	LP	1 4	1 047	1 113	0 775	1 265	0 430	2>1>3>4	y	n
MAG088	LP	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG089	LP	1 9	1 121	1 186	0 796	1 252	0 386	2>1=3>4	y	n
MAG093	Brz	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAG094	Brz	1.7	1 281	0 957	0 762	1 162	0 358	2>1=3>4	n	n
MAG095	Brz	1 7	1 102	0 940	0 731	1 149	0 410	2>1>3>4	n	n
MAG096	Brz	1 6	1 245	0 944	0 764	1 223	0 456	2>1>3>4	n	n
MAG097	Brz	1 3	1 293	0 985	0 668	1 041	0 373	2>1>3>4	y	n
MAG098	Brz	1 2	1 265	0 986	0 762	1 366	0 338	2>1>3>4	n	n
MAG099	Brz	1 8	1 300	1 013	0 704	1 052	0 346	2>1>3>4	n	n
MAG100	Brz	1 2	1 235	0.899	0 740	1 081	0 380	2>1>3>4	n	n
MAG101	Brz	1 6	1 273	0 987	0 739	1 144	0 368	2>1>3>4	n	n
MAG102	Brz	1 4	1 167	0 986	0 772	1 309	0 380	2>1>3>4	n	n

TABLE 4 CONTINUED

Individual	Location	Toe Web Code	Leg Stripe Code	Anal Marking Code
MAG033	Brz	2	1	1
MAG034	Brz	2	1	1
MAG035	Brz	2	1	2
MAG036	Brz	2	1	1
MAG068	LP	2	1	1
MAG069	LP	1	1	2
MAG070	Bln	2	2	1
MAG071	Bln	2	2	1
MAG072	Bln	2	2	1
MAG073	Bln	nd	nd	nd
MAG074	Bln	2	2	1
MAG075	Bln	nd	nd	nd
MAG076	Bln	2	1	1
MAG077	Bln	2	2	1
MAG078	Bln	2	1	1
MAG079	Bln	damaged	2	2
MAG080	LP	2	1	2
MAG081	LP	1	2	2
MAG082	LP	2	2	2
MAG083	LP	nd	nd	nd
MAG084	LP	1	2	2
MAG085	LP	1		
MAG086	LP	2	1	2
MAG087	LP	2	1	1
MAG088	LP	nd	nd	nd
MAG089	LP	2	1	2
MAG093	Brz	nd	nd	nd
MAG094	Brz	1	2	2
MAG095	Brz	2	1	1
MAG096	Brz	1	1	2
MAG097	Brz	nd	nd	nd
MAG098	Brz	1	1	2
MAG099	Brz	1	1	2
MAG100	Brz	1	1	2
MAG101	Brz	1	1	2
MAG102	Brz	1	1	2

TABLE 5. Summary statistics for all quantitative variables CV = Coefficient of Variation

Variable	Location	Mean	Std Dev	Std Err Mean	Variance	CV
DF	All	3 832	0 221	0 041	0 049	5 756
	Bln	3 647	0 087	0 031	0 008	2 396
	Brz	4 038	0 198	0 063	0 039	4 903
	LP	3 779	0 150	0 045	0 023	3 980
PG/C	All	1 497	0 416	0 077	0 173	27 791
	Bln	1 658	0 430	0 152	0 185	25 902
	Brz	1 263	0 370	0 117	0 137	29 325
	LP	1 593	0 383	0 115	0 146	24 029
CD	All	41 216	16 220	3 012	263 082	39 353
	Bln	48 909	14 552	5 145	211 764	29 753
	Brz	28 697	10 641	3 365	113 222	37 079
	LP	47 001	15 672	4 725	245 617	33 344
PR	All	0 271	0 085	0 016	0 007	31 353
	Bln	0 235	0 106	0 037	0 011	44 969
	Brz	0 317	0 083	0 026	0 007	26 120
	LP	0 255	0 052	0 016	0 003	20 440
P/PG	All	6 318	2 272	0 422	5 164	35 966
	Bln	6 302	2 916	1 031	8 504	46 273
	Brz	6 651	1 878	0 594	3 528	28 245
	LP	6 028	2 264	0 683	5 127	37 563
CG	All	9 386	4 493	0.834	20 184	47 866
	Bln	6 798	2 385	0 843	5 691	35 093
	Brz	8 903	4 627	1 463	21 413	51 975
	LP	11 708	4 663	1 406	21 740	39 825
CR	All	4 950	1 318	0 245	1 738	26 630
	Bln	5.209	0 901	0 318	0 811	17 291
	Brz	5 515	1 717	0 543	2 947	31 129
	LP	4 249	0 855	0 258	0.732	20 135
SUL	All	20 633	1 848	0 377	3 414	8.954
	Bln	21 750	1 078	0 381	1 163	4 958
	Brz	20 038	1 977	0 548	3 909	9 867
	LP	21.310	1 547	0 489	2.392	7 258
FL	All	11 265	1.058	0 190	1 120	9 394
	Bln	11 888	0 804	0 284	0 647	6 766
	Brz	11.292	1 114	0 309	1 241	9 864
	LP	10 730	0 955	0 302	0 302	8 896
TL	All	13.245	1 016	0 183	1 033	7 672
	Bln	13 775	0 506	0 179	0.256	3 676
	Brz	13 138	0 927	0.257	0 859	7 055
	LP	12 960	1 319	0 417	1 740	10 179
Leg	All	24 510	1 897	0.341	3 599	7 740
	Bln	25.663	0 964	0 341	0 928	3 755
	Brz	24 431	1 843	0 511	3 396	7.543
	LP	23 690	2 193	0 694	4 810	9 258

TABLE 5: Summary statistics continued

Variable	Location	Mean	Std Dev	Std Err Mean	Variance	CV
HF	All	18 271	1 226	0 220	1.504	6 712
	Bln	19 050	0 711	0 251	0 506	3 733
	Brz	17 931	1 037	0 288	1 076	5 784
	LP	18 090	1 563	0 494	2 443	8 641
HT	All	7.339	0 488	0 088	0 238	6 645
	Bln	7 300	0 428	0 151	0 183	5 858
	Brz	7 354	0 556	0 154	0 309	7 563
	LP	7 350	0 486	0 154	0 236	6 611
Toe	All	12 013	0 885	0 159	0 782	7 364
	Bln	12 500	0 385	0 136	0 149	3 084
	Brz	11 762	0 792	0 220	0 628	6 735
	LP	11 950	1 162	0 367	1 349	9 721
HW	All	7 184	0 568	0 102	0 323	7 908
	Bln	7 388	0 599	0 212	0 358	8 104
	Brz	7 215	0 557	0 154	0 310	7 713
	LP	6 980	0 547	0 173	0 300	7 841
HL	All	7 577	0 528	0 095	0 278	6 964
	Bln	7 838	0 370	0 131	0 137	4 722
	Brz	7 392	0 539	0 150	0 291	7 294
	LP	7 610	0 569	0 180	0 323	7 471
IO	All	1 810	0 233	0 042	0 054	12 869
	Bln	1 963	0 177	0 063	0 031	9 008
	Brz	1 715	0 223	0 062	0 050	13 002
	LP	1 810	0 238	0 075	0 057	13 139
ED	All	2 842	0 330	0 059	0 109	11 627
	Bln	2 900	0 385	0 136	0 149	13 291
	Brz	2 777	0 274	0 076	0.075	9 879
	LP	2 880	0 371	0 117	0 137	12 868
EN	All	1 681	0 256	0 046	0 066	15 241
	Bln	1 788	0 181	0 064	0 033	10 113
	Brz	1 592	0.315	0 087	0 099	19 770
	LP	1 710	0 197	0 062	0 039	11 516
IN	All	1 484	0 210	0 038	0 044	14 146
	Bln	1 550	0 207	0 073	0 043	13 356
	Brz	1 485	0 212	0 059	0 045	14 248
	LP	1 430	0 216	0.068	0 047	15 125
Leg SUL	All	1 176	0 074	0 013	0 005	6 257
	Bln	1 182	0 067	0.024	0 004	5 645
	Brz	1 223	0 060	0 017	0 004	4 919
	LP	1 111	0 043	0 014	0 002	3 883
HL HW	All	1 059	0 088	0.016	0 008	8 347
	Bln	1 064	0 058	0 021	0 003	5 463
	Brz	1 030	0 111	0 031	0 012	10 762
	LP	1 092	0 067	0.021	0.005	6.173

TABLE 5: Summary statistics continued.

Variable	Location	Mean	Std Dev	Std Err Mean	Variance	CV
HF Leg	All	0 747	0 033	0 006	0 001	4 391
	Bln	0 743	0 027	0 010	0 001	3 670
	Brz	0 735	0.033	0.009	0 001	4 480
	LP	0 765	0 032	0 010	0 001	4 134
Toe HF	All	0 747	0 033	0 006	0 001	4 391
	Bln	0 743	0.027	0 010	0 001	3 670
	Brz	0 735	0 033	0 009	0 001	4 480
	LP	0 765	0.032	0 010	0 001	4 134
Toe FL	All	0 657	0 022	0 003	0 000	3 323
	Bln	0 657	0 024	0 009	0 001	3 707
	Brz	0 656	0 023	0 006	0 001	3 558
	LP	0 660	0 016	0 005	0 000	2 474
ED HL	All	0.375	0.036	0 006	0 001	9 520
	Bln	0 370	0 046	0 016	0 002	12 510
	Brz	0 376	0 032	0 009	0 001	8 433
	LP	0 378	0 035	0 011	0 001	9 205

TABLE 6: Results of regression and correlation analyses for dominant frequency.

Regressor	Grouping	Function	n	Resp. Mean	r^2	r^2_{adj}	RMS_e	TS	P > TS	Model Statement
Temp	All	Linear	29	3.832	0 162	0 131	0 206	F=5 23	0 0303	DF = 2 479 + 0 052(T)+ ε
Temp	All	Polynomial (2°)	29	0 114	0 499	0 460	0 162	t=4 18	0 0003	DF = 2 468 + 0 048(T) + 0 046(T -26 024)^2 + ε
Temp	LP	Linear	11	3.779	0 585	0 539	0 102	F=12 70	0 0061	DF = 8 716 - 0 205(T)+ ε
Temp	LP	Polynomial (2°)	11	3 779	0 703	0 628	0 092	t=1 78	0 1134	DF = 11 131 - 0 306(T) + 0 141(T-24 146)^2+ ε
Temp	Bln	Linear	8	3 647	0 085	0 068	0 090	F=0 56	0 4836	DF = 1 475+ 0 082(T)+ ε
Temp	Bln	Polynomial (2°)	8	3 647	0 125	0 224	0 097	t=-0 48	0 651	DF = 3 480 + 0 007(T)- 0 193(T)-26 475)^2+ ε
Temp	Brz	Linear	10	4 038	0 276	0 186	0 179	F=3 05	0 1189	DF = 1 035+ 0 108(T)+ ε
Temp	Brz	Polynomial (2°)	10	4 038	0 347	0 161	0 181	t=-0 87	0 411	DF = -0 474 + 0 168(T) - 0 1573(T-27 73)^2+ ε
SUL	All	Linear	24	3 848	0 552	0 531	0 154	F=27.06	< 0001	DF = 5 7078408 - 0 0901423(SUL)+ ε
SUL	All	Polynomial (2°)	24	3 848	0 552	0 509	0 157	t=-0 03	0 9768	DF = 5 710 - 0 090(SUL) - 0 0002 (SUL-20 63)^2+ ε
SUL	LP	Linear	9	3 786	0 222	0 111	0 157	F=2 00	0 2002	DF = 4 883 - 0.052(SUL)+ ε
SUL	LP	Polynomial (2°)	9	3 786	0 386	0 181	0 151	t=-1 26	0 253	DF = 5 285 - 0 066(SUL) - 0 049(SUL-21 122)^2+ ε
SUL	Bln	Linear	6	3 665	0 622	0 527	0 066	F=6 57	0 0624	DF = 5 164 - 0 069(SUL)+ ε
SUL	Bln	Polynomial (2°)	6	3 665	0 792	0 653	0 056	t=1 57	0 2148	DF = 5 331 - 0 078(SUL) + 0 0357(SUL-21 75)^2+ ε
SUL	Brz	Linear	9	4 032	0 439	0 359	0 167	F=5 49	0 0517	DF = 5 398 - 0 070(SUL)+ ε
SUL	Brz	Polynomial (2°)	9	4 032	0 497	0 329	0 171	t=-0 83	0 44	DF = 5 018 - 0 049(SUL) - 0 011(SUL-19 4)^2+ ε

TABLE 7: Results of model fitting for scaling of dominant frequency. * indicates the model chosen for scaling of DF

Response	Parameters	Estimate	AIC	p	Cp	r^2	r^2_{adj}	Model
DF	Intercept	3 8322	-70.7811	1	37.699	0	0	DF=3 832+ ε
DF	Intercept	2 4786	-72 7449	2	30 61	0.1522	0 1137	DF=2 479+0 053(T)+ ε
	Temperature	0 0526						
DF	Intercept	35 2253	-82 2778	2	14 02	0 4301	0 4042	DF=35 225+0 323(Lon)+ ε
	Longitude	0 3228						
DF	Intercept	5 7079	-88 02967	2	6 7701	0 5516	0 5312	DF=5 708-0 090(SUL)+ ε
	SUL	-0.0901						
DF	Intercept	31 431	-82 007	3	13 654	0 4698	0 4193	DF=31 431+0 028(T)+0 291(Lon)+ ε
	Temperature	0 0282						
	Longitude	0 2913						
DF	Intercept	5.0007	-87.3343	3	7 354	0 5753	0 5349	DF=5 001+0 022(T)-0 084(SUL)+ ε
	Temperature	0 022						
	SUL	-0.0836						
DF	Intercept	23.1131	-92 3062*	3	2 62	0 6548	0 6219	DF=23 113-0 067(SUL)+0 184(Lon)+ ε
	SUL	-0 067						
	Longitude	0 184						
DF	Intercept	21.7009	-91 0269	4	4	0 665	0 6147	DF=21 701-0 064(SUL)+0 174(Lon)+0 015(T)+ ε
	SUL	-0 0639						
	Longitude	0 174						
	Temperature	0 0146						

TABLE 8: Results of regression and correlation analyses for temporal call characters. TS=test statistic (t ratio used for linear models; F ratio used for polynomial models). Model statement is given for significant relationships only

Response	Regressor	Grouping	Function	n	Mean	r^2	r^2_{adj}	RMSe	TS	P > TS	Model Statement
PG/C	Temperature	All	Linear	24	1 497	0 053	0 018	0 412	F=1 5204	0 2282	
PG/C	Temperature	All	Polynomial (2°)	24	1 497	0 101	0 031	0 409	t=-1 17	0 2521	
PG/C	SUL	All	Linear	24	1 478	0 268	0 235	0 355	F=8 0695	0 0095	PG/C = -0 873 + 0 114(SUL)+ ε
PG/C	SUL	All	Polynomial (2°)	24	1 478	0 269	0 200	0 363	t=-0 18	0 8599	
PG/C	SUL	LP	Linear	9	1 691	0 034	0 104	0 364	F=0 2491	0 633	
PG/C	SUL	Bln	Linear	6	1 553	0 211	0 013	0 384	F=1 0668	0 36	
PG/C	SUL	Brz	Linear	9	1 215	0 719	0 678	0 203	F=8 0695	0 0039	PG/C = -1.783 + 0 155(SUL)+ ε
CD	Temperature	All	Linear	29	41 216	0 090	0 057	15 754	F=2 6813	0 1131	
CD	Temperature	All	Polynomial (2°)	29	41 216	0 110	0 042	15 878	t=-0 76	0 4536	
CD	SUL	All	Linear	24	40 366	0 244	0 210	14 274	F=7 1122	0 0141	CD = -48 276 + 4 296(SUL)+ ε
CD	SUL	All	Polynomial (2°)	24	40.366	0 255	0 184	14 504	t=-0 55	0 5853	
CD	SUL	LP	Linear	9	50 979	0 123	0 002	14 285	F=0 9803	0 3551	
CD	SUL	Bln	Linear	6	45 207	0 359	0 199	11 534	F=2 2392	0 2089	
CD	SUL	Brz	Linear	9	26 526	0 402	0 317	7 125	F=4 7144	0 0665	
PR	Temperature	All	Linear	29	0 271	0 034	0 002	0 085	F=0 9553	0 3371	
PR	Temperature	All	Polynomial (2°)	29	0 271	0 034	0 040	0 087	t=-0 07	0 6349	
PR	SUL	All	Linear	24	0 283	0 142	0 103	0 077	F=3 6403	0 0695	
PR	SUL	All	Polynomial (2°)	24	0 283	0 189	0 112	0 077	t=1 10	0 111	
P/PG	Temperature	All	Linear	29	6 318	0 029	0 007	2 281	F=0 7976	0 3797	
P/PG	Temperature	All	Polynomial (2°)	29	6 318	0 029	0 046	2 324	t=0 01	0 6848	
P/PG	SUL	All	Linear	24	6 827	0 032	0 012	2 023	F=0 7377	0 3997	
P/PG	SUL	All	Polynomial (2°)	24	6 827	0 085	0 002	2 013	t=1 10	0 2822	
CR	Temperature	All	Linear	29	4 950	0 255	0 228	1 159	F=9 2506	0 0052	CR = -5 196 + 0 390(T)+ ε
CR	Temperature	All	Polynomial (2°)	29	4 950	0 329	0 277	1 121	t=1 69	0 1025	
CR	Temperature	LP	Linear	11	4 249	0 182	0 092	0 815	F=2 0083	0 1901	
CR	Temperature	Bln	Linear	8	5 209	0 175	0 038	0 883	F=0 9553	0 3016	
CR	Temperature	Brz	Linear	10	5 515	0 292	0 204	1 532	F=3 3060	0 1065	

TABLE 9: Variance testing and ANOVA for each quantitative variable by population. Analyses having significant or marginal (.05<P<.1) results for Levene's test of equal variance were analyzed using the Welch ANOVA.

Variable	Levene's				Welch				Van Der Waerden		
	F ratio	DF	DF Density	P > F	F ratio	DF	DF Density	P > F	ChiSquare	DF	Prob>ChiSq
DF(unscaled)	3.0825	2	26	0 0629	15 6151	2	16 746	0 0001	13 3867	2	0 0012
DF(scaled@20.6°C)	2.4998	2	21	0 1062					5 7722	2	0 0558
PG/C	0 146	2	26	0 8649					5 2158	2	0 0737
CD	0 5561	2	26	0 5801					10 2932	2	0 0058
PR	2 3374	2	26	0 1165					4 8433	2	0 0888
P/PG	0.2879	2	26	0 7522					0 622	2	0 7327
CR	1 6451	2	26	0 2125					6 1839	2	0 0454
SUL	0 5866	2	21	0 5651					6 6888	2	0 0353
FL	1 4369	2	21	0 2601					4 8117	2	0.0902
TL	3 3292	2	21	0.0555	4 3617	2	13 874	0 0339	3.4436	2	0 1787
LEG	1 1549	2	21	0 3343					5.7087	2	0 0576
HF	2 9559	2	21	0 0739	3 8316	2	13 77	0.0475	3 1986	2	0 2020
HT	0.6043	2	21	0.5557					0 0982	2	0 9521
Toe	3 4583	2	21	0 0503	6 1082	2	13.8	0 0126	4 2975	2	0.1166
HW	0 1787	2	21	0 8376					2 8589	2	0 2394
HL	1 8477	2	21	0.1823					5 8807	2	0 0528
IO	0 6704	2	21	0 5221					6 2525	2	0 0439
ED	0 8413	2	21	0 4452					2.1837	2	0 3356
EN	0 0428	2	21	0 9582					10.3447	2	0 0057
IN	0 4077	2	21	0 6703					1 9169	2	0.3835
Leg·SUL	0 8751	2	21	0.4315					11 3307	2	0 0035
HL:HW	2 5748	2	21	0 1					12.583	2	0 0019
HF.Leg	0 9666	2	21	0 3967					7 9714	2	0 0186
Toe:HF	0.6778	2	21	0.5185					0 8845	2	0 6426
ED:HL	0.054	2	21	0 9476					0 8845	2	0 6426

TABLE 10: Tukey-Kramer HSD matrices for variables having significant ANOVA results.

Variable	Tukey/Kramer HSD matrix			
DF(unscaled)	Abs(Dif)-LSD	Brz	LP	Bln
	Brz	-0 17334	0 08969	0 20732
	LP	0 08969	-0 16527	-0 04797
	Bln	0 20732	-0 04797	-0 1938
DF(scaled@20 6 mm C)	Abs(Dif)-LSD	Brz	LP	Bln
	Brz	-0 16205	-0 03189	0 02856
	LP	-0 03189	-0 16205	-0 10161
	Bln	0 02856	-0 10161	-0 19847
CD	Abs(Dif)-LSD	Bln	LP	Brz
	Bln	-17 156	-14 035	3 936
	LP	-14 035	-14 631	3.311
	Brz	3 936	3 311	-15 345
CR	Abs(Dif)-LSD	Brz	Bln	LP
	Brz	-1 3701	-1 1477	-0 0725
	Bln	-1 1477	-1 5318	-0 463
	LP	-0 0725	-0 463	-1 3063
SUL	Abs(Dif)-LSD	Bln	LP	Brz
	Bln	-2 3592	-1 5258	0 1964
	LP	-1 5258	-1 9263	-0 204
	Brz	0 1964	-0 204	-1 9263
HF	Abs(Dif)-LSD	Bln	LP	Brz
	Bln	-1 8364	-0 7209	-0 3764
	LP	-0 7209	-1 4994	-1 155
	Brz	-0 3764	-1 155	-1 4994
IO	Abs(Dif)-LSD	Bln	LP	Brz
	Bln	-0 32376	-0 10111	0 03222
	LP	-0 10111	-0 26435	-0 13102
	Brz	0 03222	-0 13102	-0 26435
EN	Abs(Dif)-LSD	Bln	LP	Brz
	Bln	-0 25875	-0 13065	0 1138
	LP	-0 13065	-0 21127	0.03318
	Brz	0 1138	0 03318	-0 21127
Leg.SUL	Abs(Dif)-LSD	Brz	Bln	LP
	Brz	-0 06577	-0 00834	0 07182
	Bln	-0 00834	-0 08056	-0 00114
	LP	0 07182	-0 00114	-0.06577
HL HW	Abs(Dif)-LSD	LP	Bln	Brz
	LP	-0 06392	-0 0673	0 06178
	Bln	-0.0673	-0 07829	0 05007
	Brz	0 06178	0 05007	-0 06392
HF.Leg	Abs(Dif)-LSD	LP	Bln	Brz
	LP	-0.03216	-0 00651	0 00295
	Bln	-0.00651	-0 03939	-0 03029
	Brz	0 00295	-0.03029	-0 03216

TABLE 11: Results of logistic regression of categorical data.

Variable	Dimension	Model	-LogLikelihood	DF	ChiSquare	P > ChiSq	U
Pulse style	latitude	Difference	1 657803	1	3 315605	0 1034	0 1034
		Full	14 369471				
		Reduced	16 027274				
	Longitude	Difference	1 984315	1	3 968629	0 0464	0 1238
		Full	14 042959				
		Reduced	16 027274				
Call complexity	latitude	Difference	2 968928	1	5 937856	0 0148	0 1653
		Full	14 992984				
		Reduced	17 961912				
	longitude	Difference	2 573459	1	5 146918	0 0233	0 1433
		Full	15 388453				
		Reduced	17 961912				
Call group complexity	latitude	Difference	0 525541	1	1 051082	0.3053	0 0328
		Full	15 501733				
		Reduced	16 027274				
	longitude	Difference	1 123289	1	2 246578	0 1339	0 0701
		Full	14 903985				
		Reduced	16 027274				
Pectoral fold	latitude	Difference	1 157865	1	2 31573	0 1281	0 0594
		Full	18 334916				
		Reduced	19 492781				
	longitude	Difference	0 645327	1	1 290655	0 2559	0 0331
		Full	18 847454				
		Reduced	19 492781				
Snout warts	latitude	Difference	0 0000753	1	0 000151	0 9902	0
		Full	9 8559649				
		Reduced	9 8560402				
	longitude	Difference	0 114846	1	0 229692	0.6318	0 0117
		Full	9 7411942				
		Reduced	9 8560402				
Thigh stripe character	latitude	Difference	4 641459	1	9 282917	0 0023	0.2485
		Full	14 039929				
		Reduced	18 681388				
	longitude	Difference	4 940424	1	9.880849	0 0017	0 2645
		Full	13 740963				
		Reduced	18 681388				
Anal marking	latitude	Difference	1 142608	1	2 285216	0 1306	0 0569
		Full	18 941416				
		Reduced	20 084023				
	longitude	Difference	2 077425	1	4 154849	0.0415	0 1034
		Full	18 006599				
		Reduced	20 084023				

TABLE 12: Morphological data for museum specimens and individuals collected from Eddy County, NM.

Individual	taxon	State	County/Parish	SUL	FL	TL	LEG	HF	HT	Toe	HW	HL
TCWC9178	*A gryllus*	LA	St Tammany	21.2	10 7	13 4	24 1	18 4	nd	nd	5 9	7.8
TCWC9179	*A gryllus*	LA	St. Tammany	22.1	12 1	12 8	24 9	18 3	nd	nd	6 3	8 1
TCWC15310	*A gryllus*	MS	Coahoma	17 2	10 7	11 6	22 3	15 2	nd	nd	4 7	5 8
TCWC15311	*A gryllus*	MS	Coahoma	23	12 9	14 4	27 3	19 7	nd	nd	5 9	7
TCWC01670	*A gryllus*	AL	Mobile	20 8	10 4	13	23 4	18 5	nd	nd	5 2	7
TCWC72712	*A c paludicola*	TX	Jefferson	17 1	9 8	10 6	20 4	15.2	nd	nd	4 5	7 3
TCWC72713	*A c paludicola*	TX	Jefferson	17	9 2	11	20 2	14 6	nd	nd	4 6	8 1
TCWC72714	*A c paludicola*	TX	Jefferson	17 9	9 9	11 3	21 2	16 1	nd	nd	4.9	6 8
TCWC72715	*A c paludicola*	TX	Jefferson	17 9	9 5	11 1	20 6	13.8	nd	nd	4 5	6 8
TCWC72716	*A c paludicola*	TX	Jefferson	17 3	9 4	10.4	19 8	14 5	nd	nd	4 3	7 2
TCWC78860	*A crepitans*	LA	St Tammany	17 2	10 2	11 6	21 8	16 5	nd	nd	4 5	7 5
TCWC23193	*A crepitans*	LA	St Landry	21	11 8	12 5	24 3	18 8	nd	nd	5	7 5
TCWC17750	*A crepitans*	LA	Iberville	21 1	12 2	13 2	25 4	18 5	nd	nd	5 8	7 5
TCWC23187	*A crepitans*	LA	St Landry	21 4	12 3	13 2	25 5	18 4	nd	nd	5 7	7 7
TCWC17757	*A crepitans*	LA	W Baton Rouge	23 3	13 4	14 3	27 7	20 2	nd	nd	4 8	7 5
TCWC23185	*A crepitans*	LA	St Landry	21	11 8	12 6	24 4	17 3	nd	nd	5 8	6 9
MAG048	*A c blanchardi*	NM	Eddy	22 8	11 8	14 9	26 7	21 5	8 1	13 9	7 8	7 9
MAG049	*A c blanchardi*	NM	Eddy	24 8	12 6	14 7	27 3	21 5	7 9	13 7	8 3	8 0
MAG050	*A c blanchardi*	NM	Eddy	22 5	11 8	14 0	25 8	20 1	7 6	13 0	7 8	8 2
MAG051	*A c blanchardi*	NM	Eddy	23 8	12 8	14 4	27 2	21 2	8 5	14 0	7 9	8 1
MAG052	*A c blanchardi*	NM	Eddy	24 2	12 3	15 1	27 4	22 1	8 0	14 0	7 9	9 2
MAG053	*A c blanchardi*	NM	Eddy	23 6	12 1	13 5	25 6	20 7	7 9	13 5	7 1	8 3
MAG054	*A c blanchardi*	NM	Eddy	22 5	12 5	13 9	26 4	20 8	7 8	13 9	7 5	7 9
MAG055	*A c blanchardi*	NM	Eddy	22 5	12 2	14 2	26 4	20 5	7.4	13 5	6 9	8 0
MAG056	*A c blanchardi*	NM	Eddy	22 8	11 5	14 1	25 6	20 6	8 1	12 8	7 8	8 4
MAG057	*A c blanchardi*	NM	Eddy	23 2	12 1	13 9	26 0	20.6	7 9	13 3	7 5	7 8
MAG058	*A c blanchardi*	NM	Eddy	23 7	12 5	14 2	26 7	20 4	8 1	13 3	7 2	7 8
MAG059	*A c blanchardi*	NM	Eddy	21 9	11.5	14 2	25 7	20 1	7 6	14 5	6 8	7 7
MAG060	*A c blanchardi*	NM	Eddy	21 8	11 7	13 6	25 3	19 6	7 8	13 3	7 4	8 0
MAG061	*A c blanchardi*	NM	Eddy	22 2	11 4	13 4	24 8	20 2	8 4	13 7	7 4	8 4

TABLE 12 CONTINUED: Morphological data for museum specimens and individuals collected from Eddy County, NM.

Individual	taxon	State	IO	ED	EN	IN	Leg SUL	HL HW	HF Leg	TL FL	ED HL
TCWC9178	*A gryllus*	LA	3 5	3 1	2 4	1 3	1 137	1 322	0 763	1 252	0 397
TCWC9179	*A gryllus*	LA	2 8	2 8	2 3	1 9	1 127	1 286	0.735	1 058	0 346
TCWC15310	*A gryllus*	MS	2 4	2 2	1 4	1 7	1 297	1 234	0 682	1 084	0 379
TCWC15311	*A gryllus*	MS	3 1	2 9	1 8	2	1 187	1 186	0 722	1 116	0 414
TCWC01670	*A gryllus*	AL	2 3	2 6	2 3	1 7	1 125	1 346	0 791	1 250	0 371
TCWC72712	*A c paludicola*	TX	3 4	2 4	1.6	1 4	1 193	1 622	0 745	1 082	0 329
TCWC72713	*A. c paludicola*	TX	1 8	2 9	1.4	1	1.188	1 761	0 723	1 196	0 358
TCWC72714	*A c paludicola*	TX	2 7	2 1	2	1 6	1 184	1 388	0 759	1 141	0 309
TCWC72715	*A c paludicola*	TX	2 6	2 5	2	1 7	1 151	1 511	0.670	1 168	0 368
TCWC72716	*A c paludicola*	TX	2 1	2.2	1 4	1 2	1 145	1 674	0 732	1 106	0 306
TCWC78860	*A crepitans*	LA	2 4	2 9	1 9	1 5	1 267	1 667	0 757	1 137	0 387
TCWC23193	*A crepitans*	LA	3 1	2.3	1 7	2 2	1 157	1 500	0 774	1 059	0 307
TCWC17750	*A crepitans*	LA	2 5	2 7	1 9	1.8	1 204	1 293	0 728	1 082	0 360
TCWC23187	*A crepitans*	LA	2 7	2 9	1 8	1 7	1 192	1 351	0 722	1 073	0 377
TCWC17757	*A crepitans*	LA	2	2 3	2 2	1 9	1 189	1 563	0 729	1 067	0 307
TCWC23185	*A crepitans*	LA	2	2.9	1 9	1 7	1 162	1 190	0 709	1 068	0 420
MAG048	*A c blanchardi*	NM	2 3	3 1	1 8	1 7	1 171	1 818	0 805	0 647	0 392
MAG049	*A c blanchardi*	NM	2 0	2 8	1 8	2 0	1 101	1 751	0 788	0 637	0 350
MAG050	*A. c blanchardi*	NM	2 0	3 0	1 7	1 8	1 147	1 830	0 779	0 647	0 366
MAG051	*A c blanchardi*	NM	1 7	2 7	1 8	1 7	1 143	1 805	0 779	0 660	0 333
MAG052	*A c blanchardi*	NM	2 7	2 9	2 1	2 0	1 132	1 971	0 807	0 633	0 315
MAG053	*A c blanchardi*	NM	2 2	3 2	1 6	1 9	1 085	1 978	0 809	0 652	0 386
MAG054	*A c blanchardi*	NM	2 2	2.9	1 6	1 6	1 173	1 841	0 788	0 668	0 367
MAG055	*A c blanchardi*	NM	2 0	2 6	3 0	1 8	1.173	1 936	0 777	0 659	0 325
MAG056	*A c blanchardi*	NM	2 1	3 2	2 3	1 8	1.123	1 882	0 805	0 621	0 381
MAG057	*A c blanchardi*	NM	1 8	2 6	2 3	1 6	1 121	1 832	0 792	0.646	0 333
MAG058	*A c. blanchardi*	NM	1 8	3 2	1.7	1.2	1 127	1 847	0 764	0 652	0 410
MAG059	*A c blanchardi*	NM	1 7	2 9	2 0	1 2	1 174	1 914	0 782	0 721	0 377
MAG060	*A c blanchardi*	NM	1 7	3 2	1 9	1 7	1 161	1 856	0 775	0 679	0 400
MAG061	*A c blanchardi*	NM	2 2	3 4	1 7	2 1	1 117	1 950	0 815	1 175	0 405

TABLE 13: Percent variation explained by first two principal components in PCA.

DF used	Character Set	Cumulative Variation Explained
Scaled to 20 6 mm	Liberal	58 03
	Conservative	62 411
Unscaled	Liberal	61 646
	Conservative	67 024
Morphology	Conservative	61 263

TABLE 14: Vector loadings for PCA on conservative character set.

	Scaled DF		Unscaled DF	
Variable	PC 1	PC 2	PC 1	PC 2
DF	-0 2967	0 0469	-0 41117	-0 1022
CD	0 38617	0 22768	0 3638	0 17281
SUL	0 38669	0 19784	0 39762	0.18081
IO	0 35444	0 30184	0 34534	0 27964
EN	0 36929	0 36475	0 3446	0 31107
Leg SUL	-0 38971	0 37575	-0 36784	0 42014
HL HW	0 38102	-0 21475	0 3545	-0 22035
HF Leg	0 23293	-0 70382	0·20373	-0 72593

TABLE 15: Vector loadings for PCA on liberal character set.

	Scaled DF		Unscaled DF	
Variable	PC 1	PC 2	PC 1	PC 2
DF	-0 25291	0 19929	-0 38166	-0 02757
CD	0 33637	-0 18673	0 31328	-0 22719
CR	-0 09356	0 41895	-0 07824	0 40915
SUL	0 39616	0 24753	0 39136	0 20322
HF	0 33315	0 4587	0 32984	0 44052
Toe	0.36295	0.38003	0 35351	0 35923
IO	0 32656	0 09008	0 31182	0 06568
EN	0 31522	-0 08604	0.29192	-0 11692
Leg SUL	-0 28404	0 3769	-0 26876	0 43767
HL HW	0 31368	-0 22653	0 29199	-0 23528
HF Leg	0 17144	-0 35343	0 15235	-0 38849

TABLE 16: Vector loadings for PCA on morphological data for multiple taxa.

Variable	PC 1	PC 2
SUL	0 53443	0 08282
HF	0 54172	-0 02549
IO	-0 09590	0 64144
EN	0 00623	-0 22383
Leg SUL	-0 36038	-0 40630
HL HW	-0 32347	0 57920
HF Leg	0 42093	0 17434
SUL	0 53443	0 08282
HF	0 54172	-0 02549
IO	-0 09590	0 64144
EN	0 00623	-0 22383

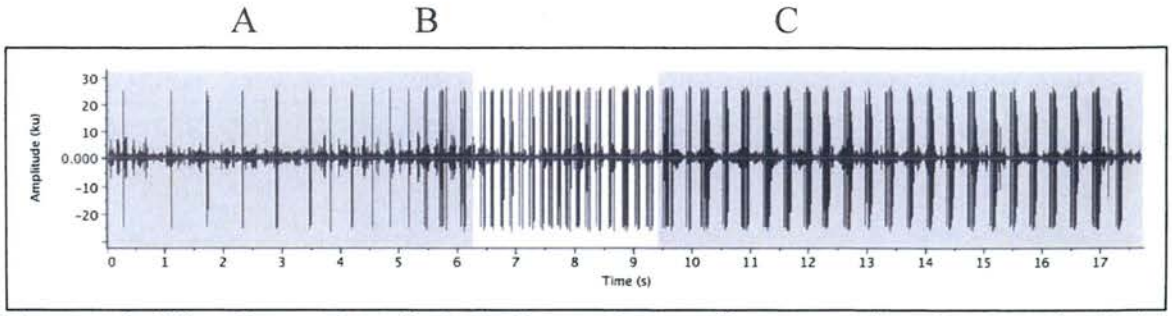

FIGURE 1: Waveform of typical *Acris crepitans* call group. Time in seconds (s) is shown on the x-axis and relative sound pressure (ku, kilounits, a measure of relative amplitude ascribed by Raven Pro 1.3 [Cornell Labs, 2008] when actual broadcast sound power is unknown) is shown on the y-axis. Section A is characterized by short, simple calls consisting of one or two pulse groups, repeated at a slow rate. Section B consists of rapidly repeated short calls with multiple pulse groups. Section C consists of long calls with multiple pulse groups, repeated at a slow rate. Example was taken from recording of individual MAG085, recorded at GLR P2.

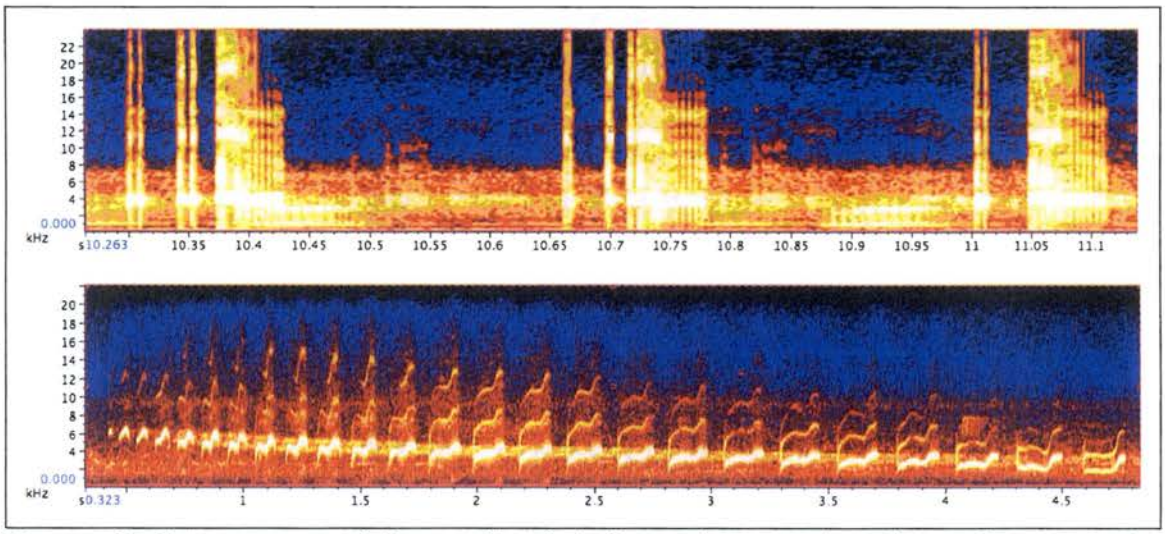

FIGURE 2: Spectrogram of three calls of *Acris crepitans* (top) and complete song of a canyon wren (bottom). Time in seconds (s) is represented on the x-axis and frequency in kHz is represented on the y-axis. In both images, bright white or yellow shades represent frequencies containing the greatest sound pressure (dominant frequency). Note that dominant frequency in *Acris* remains consistent at 3.5-4kHz through all calls, thus are not frequency-modulated. Two types of frequency modulation are evident in the canyon wren spectrogram: a frequency sweep from lower to higher frequency in each note, and overall decrease in frequency from approximately 6 to 2 kHz through the duration of the song. *A. crepitans* example was taken from recording of individual MAG085, recorded at GLR P2. Canyon wren recording provided as a sample file in Raven Pro 1.3 (Cornell Labs, 2008).

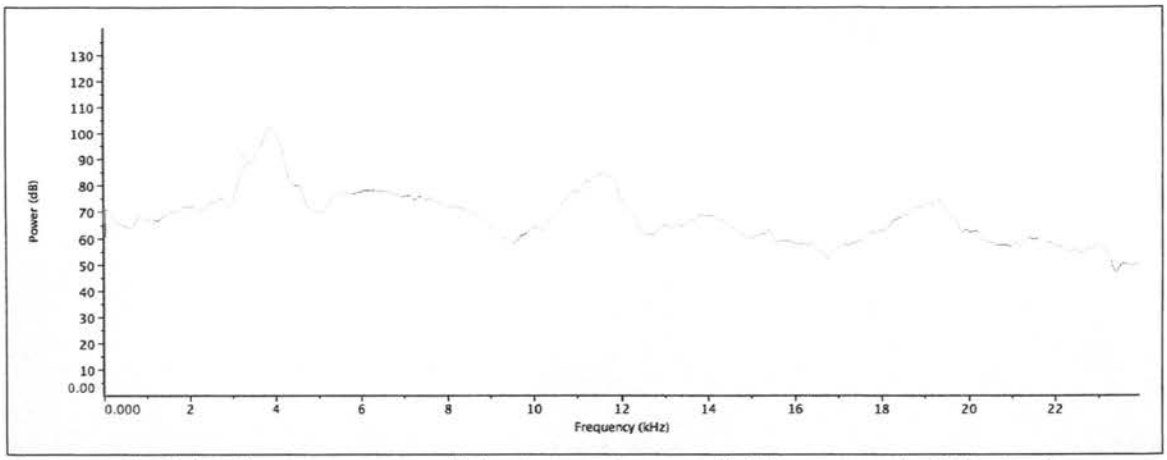

FIGURE 3: Selection spectrum of *Acris crepitans* call. Frequency in kHz is shown on the x-axis and relative sound pressure (dB) is shown on the y-axis. Applying the Discrete Fourier Transform (DFT) to a selection from a waveform or spectrogram in Raven Pro 1.3 (Cornell Labs, 2008) produces this graph. The peak with the greatest power represents the Dominant Frequency (DF). The precise value for DF is obtained from the selection table (not shown) that is displayed with this graph in Raven Pro 1.3. Example was taken from recording of individual MAG085, recorded at GLR P2.

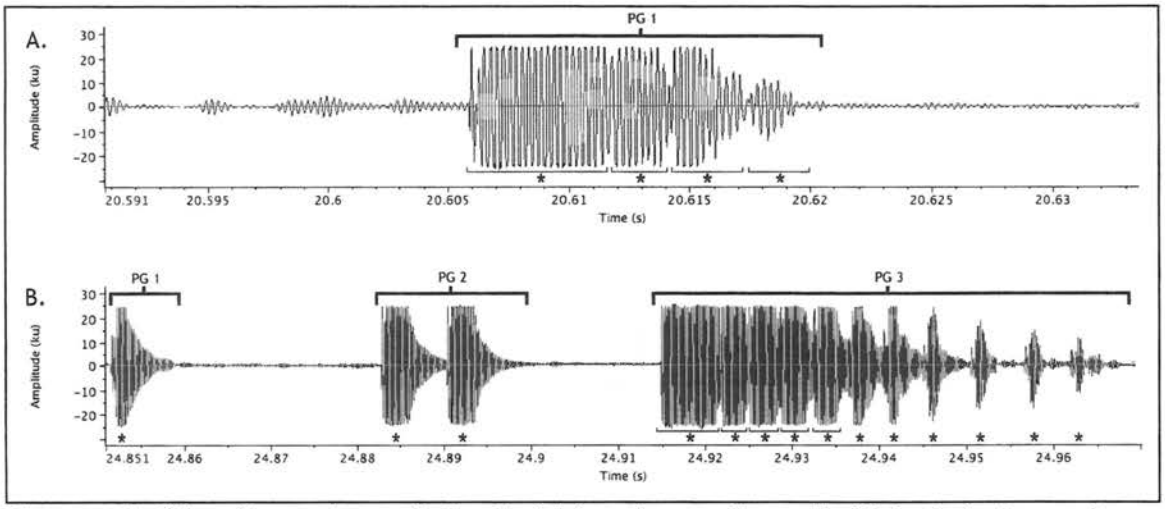

FIGURE 4: Waveform views of simple (A.) and complex calls (B.) of *Acris crepitans*. Simple calls with single pulse groups, as shown in A, are typical of calls that fall at the beginning of calls groups (segment A from Fig. 1). Calls become more complex, having multiple pulse groups (PG) later in the call group (segments B and C in Fig. 1). Pulse groups are denoted with brackets, and pulses are marked with an asterisk (*). Note the difference in duration of the two call types: the simple call (A.) is approximately 15 ms, while the complex call (B.) is 120 ms in duration. Both calls were taken from recording of individual MAG085, recorded at GLR P2.

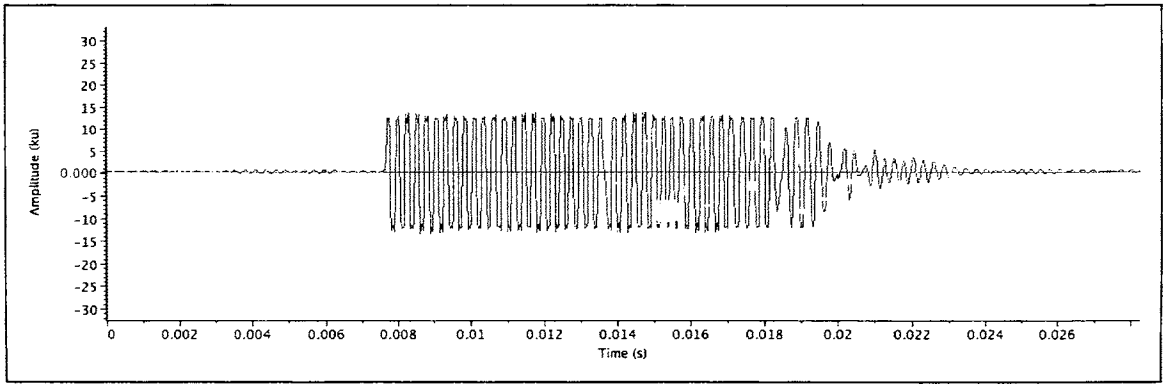

FIGURE 5: Waveform view of weakly pulsed, simple call of *Acris crepitans*. Frogs were classified as weak pulsers if calls contained few or no distinct pulses, or if most pulses were modulated by <40% of the maximum call amplitude. Strongly pulsed calls are shown in Fig. 4A and B. Call was taken from recording of individual MAG087, recorded at GLR P2.

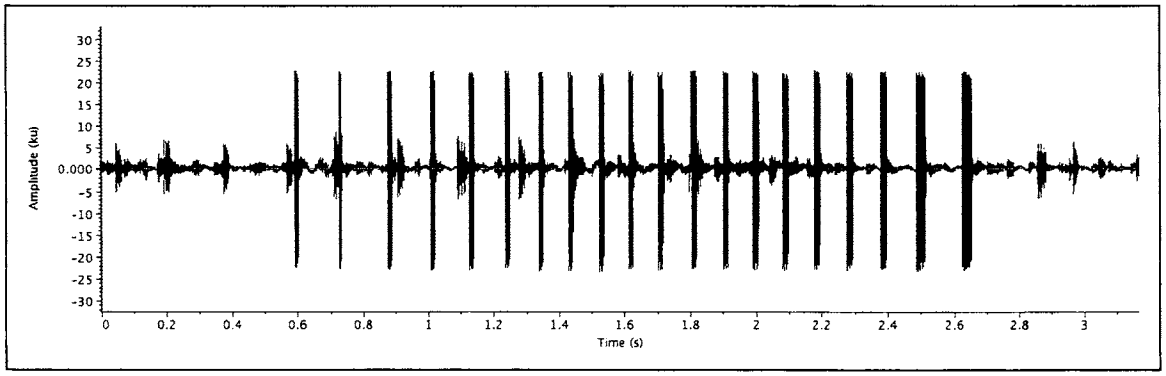

FIGURE 6: Waveform view of simple call group of *Acris crepitans*. Call groups categorized as simple consisted of primarily simple calls (cs) and were shorter in duration than complex call groups (cc, depicted in Fig. 1). Call was taken from recording of individual MAG102, recorded at Mil CR264.

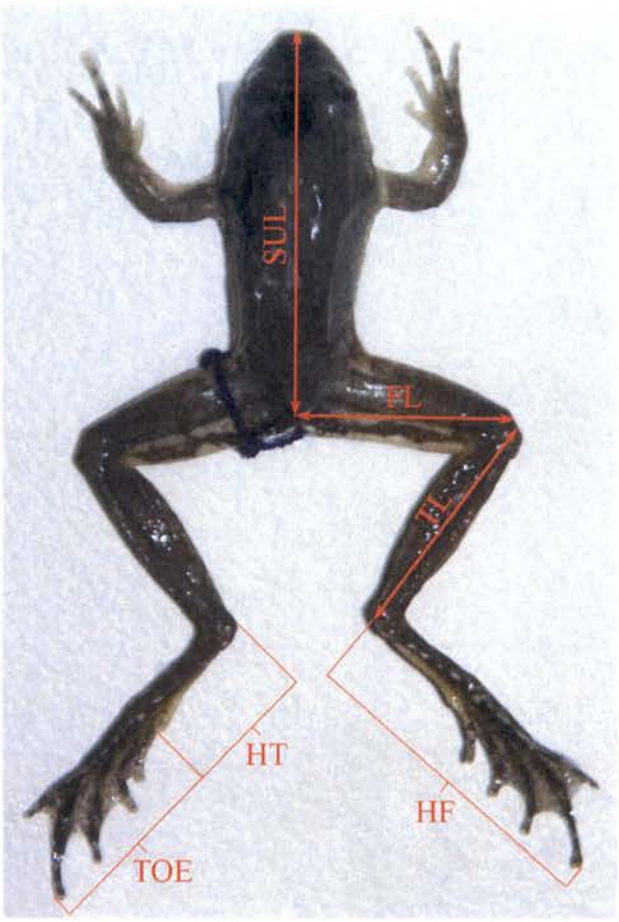

FIGURE 7: Linear morphological measurements. SUL = snout urostyle length; FL = femur length; TL = tibia length; HF = hind foot; HT = heel - tubercle distance; TOE = distance from tubercle to tip of the fourth toe.

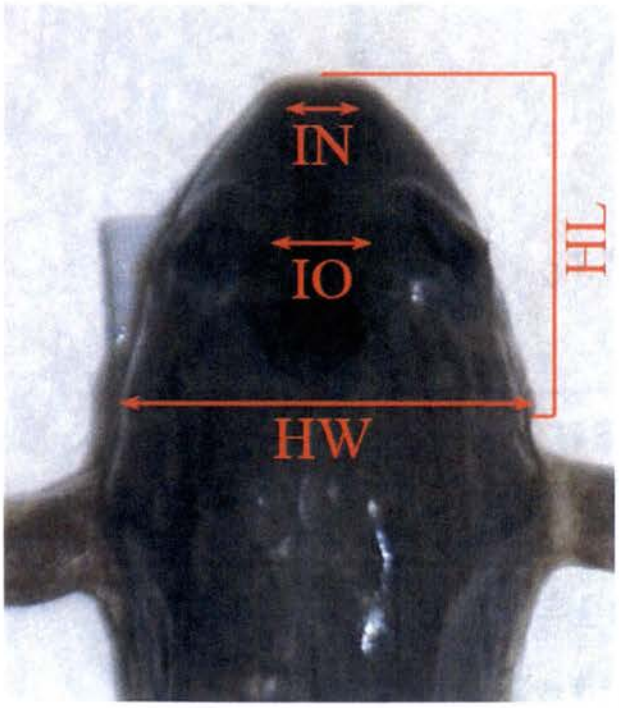

FIGURE 8: Linear morphological measurements: detail of head measurements (dorsal view). HW = head width; HL = head length; IO = interorbital distance; IN = internareal distance.

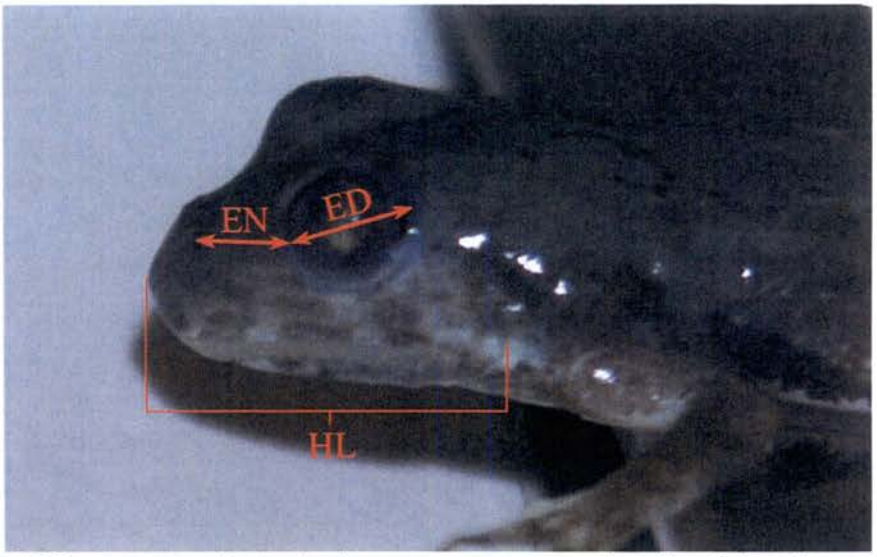

FIGURE 9: Linear morphological measurements: detail of head measurements (lateral view). HL = head length; ED = eye diameter; EN = eye - nares distance.

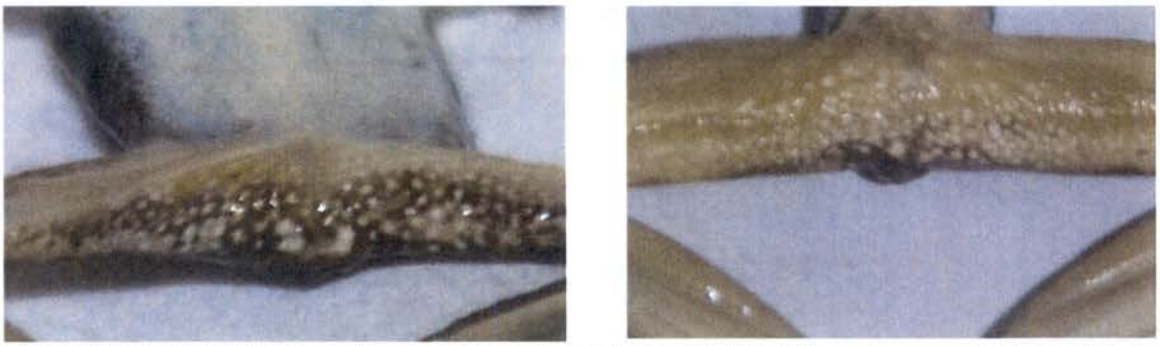

FIGURE 10: Detail of markings adjacent to the vent. On left, extensive dark markings characteristic of *Acris creptians blanchardi*. Or right, sparse markings characteristic of *A. c. crepitans*.

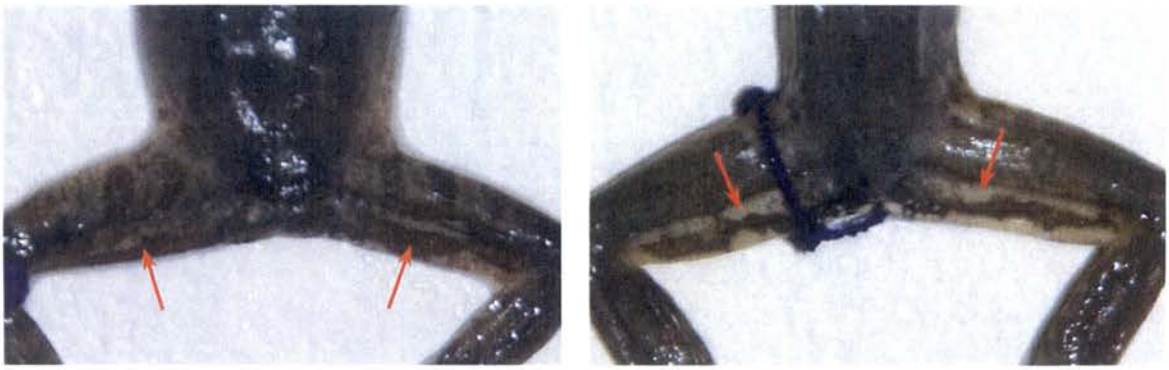

FIGURE 11: Detail of dorsoposterior thigh markings. McCallum and Trauth (2006) took absence of a light border to the dorsoposterior thigh stripe, indicated by arrows in the left photo, as a blanchardi-like character. On the right, arrows indicate the light borders of thigh striping considered crepitans-like by these authors.

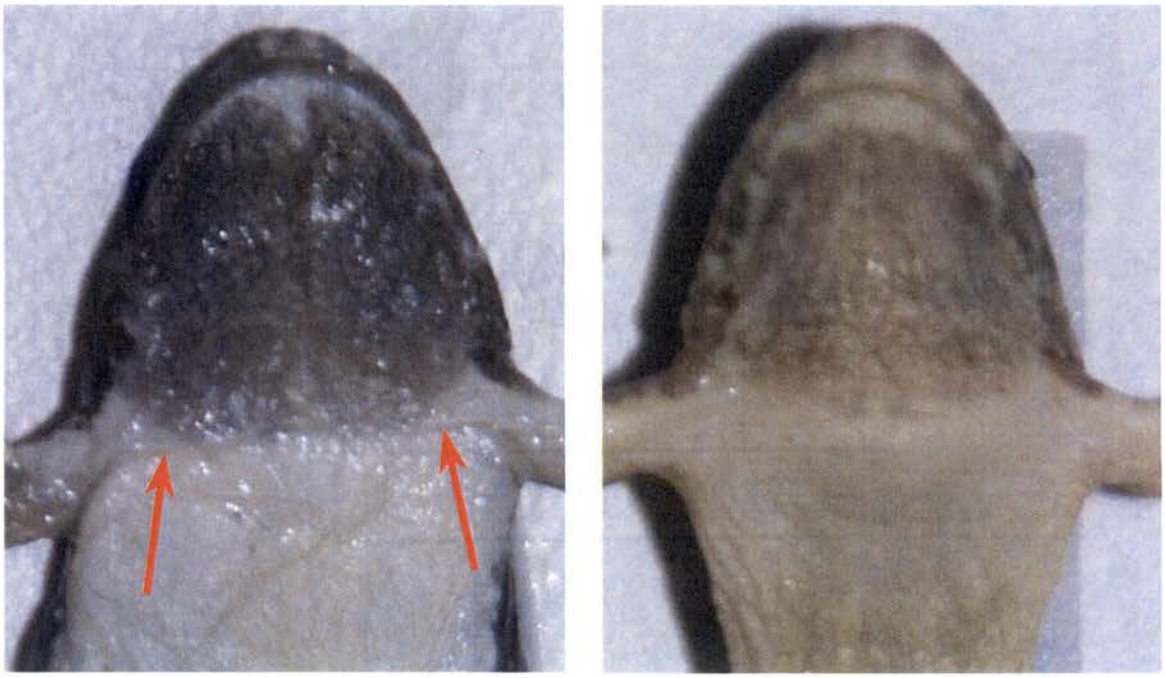

FIGURE 12: Detail of pectoral fold. McCallum and Trauth (2006) took presence of a pectoral fold, indicated by arrows in the left photo, as a blanchardi-like character. On the right, a crepitans-like individual, lacking a pectoral fold.

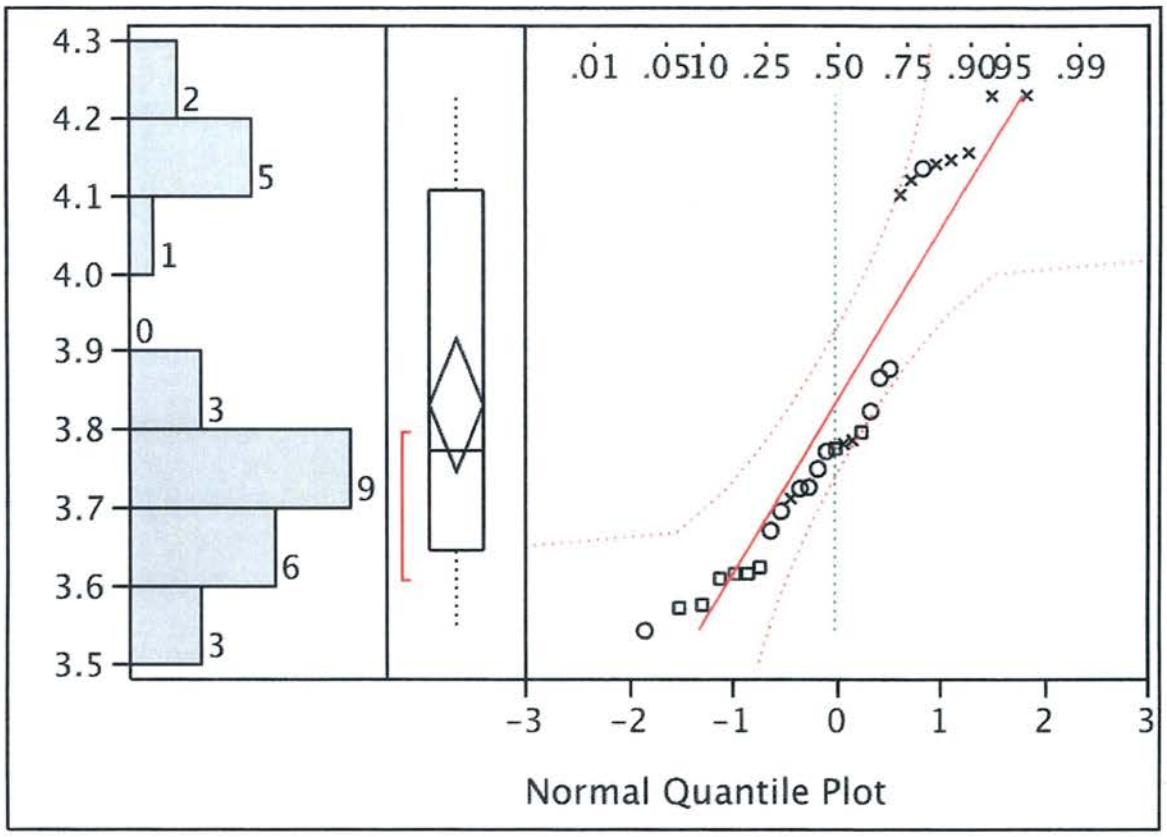

FIGURE 13: Frequency histogram, boxplot, and normal quantile plot of DF for all populations. Figure is provided as an example of output examined for each variable to assess normailty. Note that this distribution is bimodal.

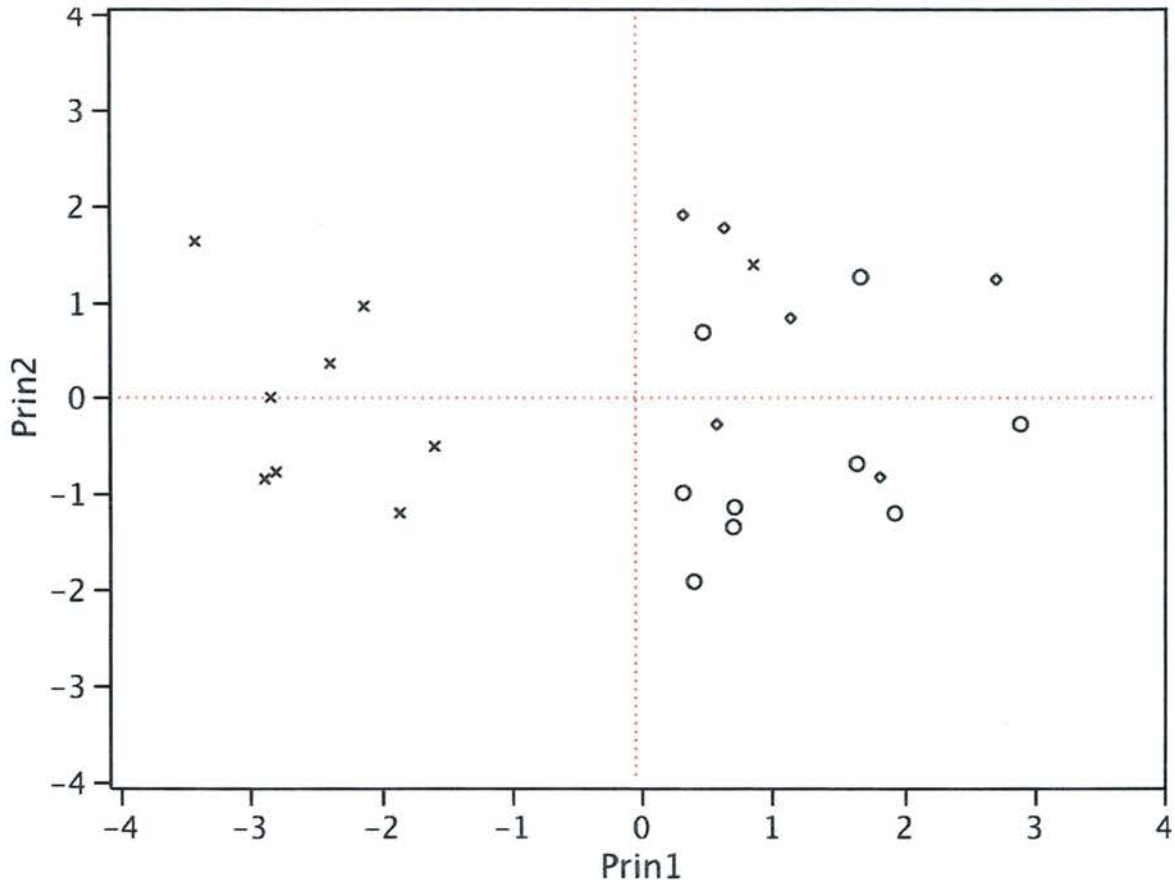

FIGURE 14: PCA using conservative character set, with DF scaled to 20.6 mm. Diamonds represent Guadalupe population; circles represent Colorado population; Xs represent Brazos population.

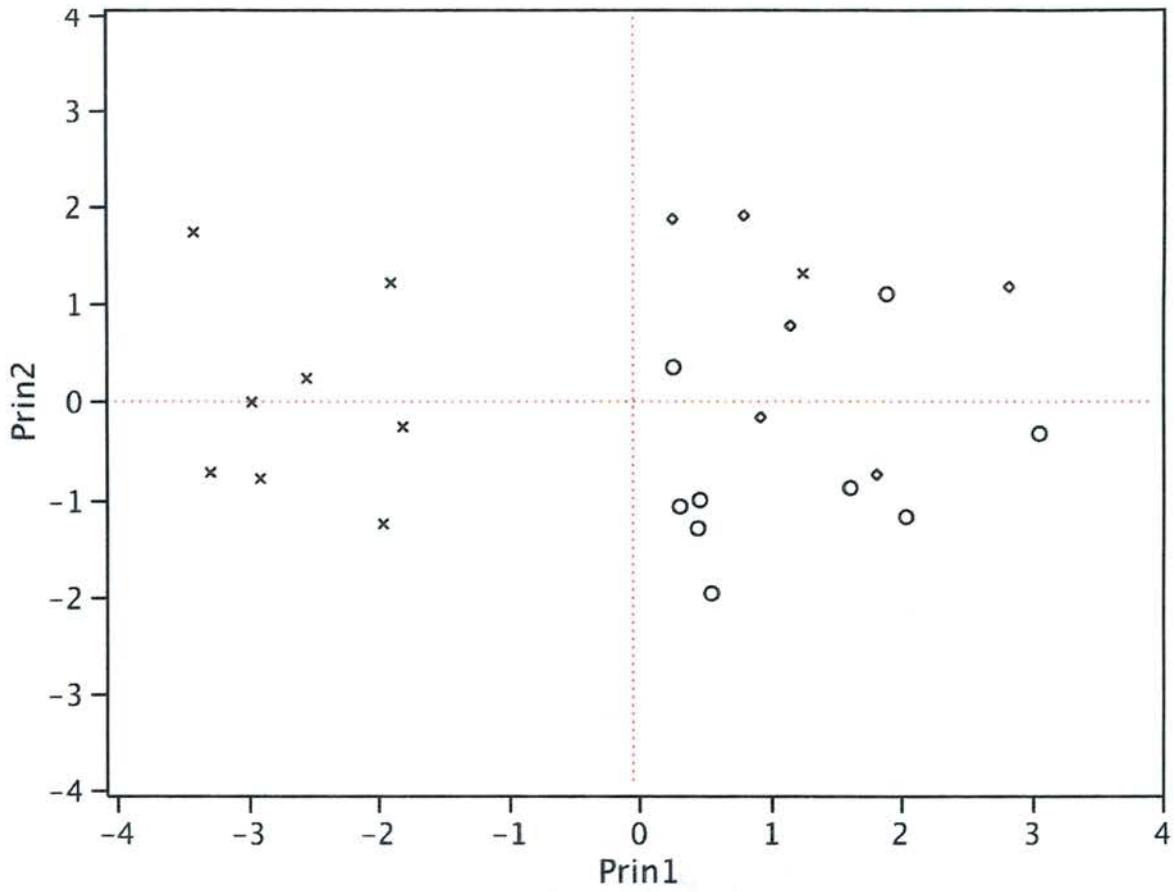

FIGURE 15: PCA using conservative character set, with unscaled DF. Diamonds represent Guadalupe population; circles represent Colorado population; Xs represent Brazos population.

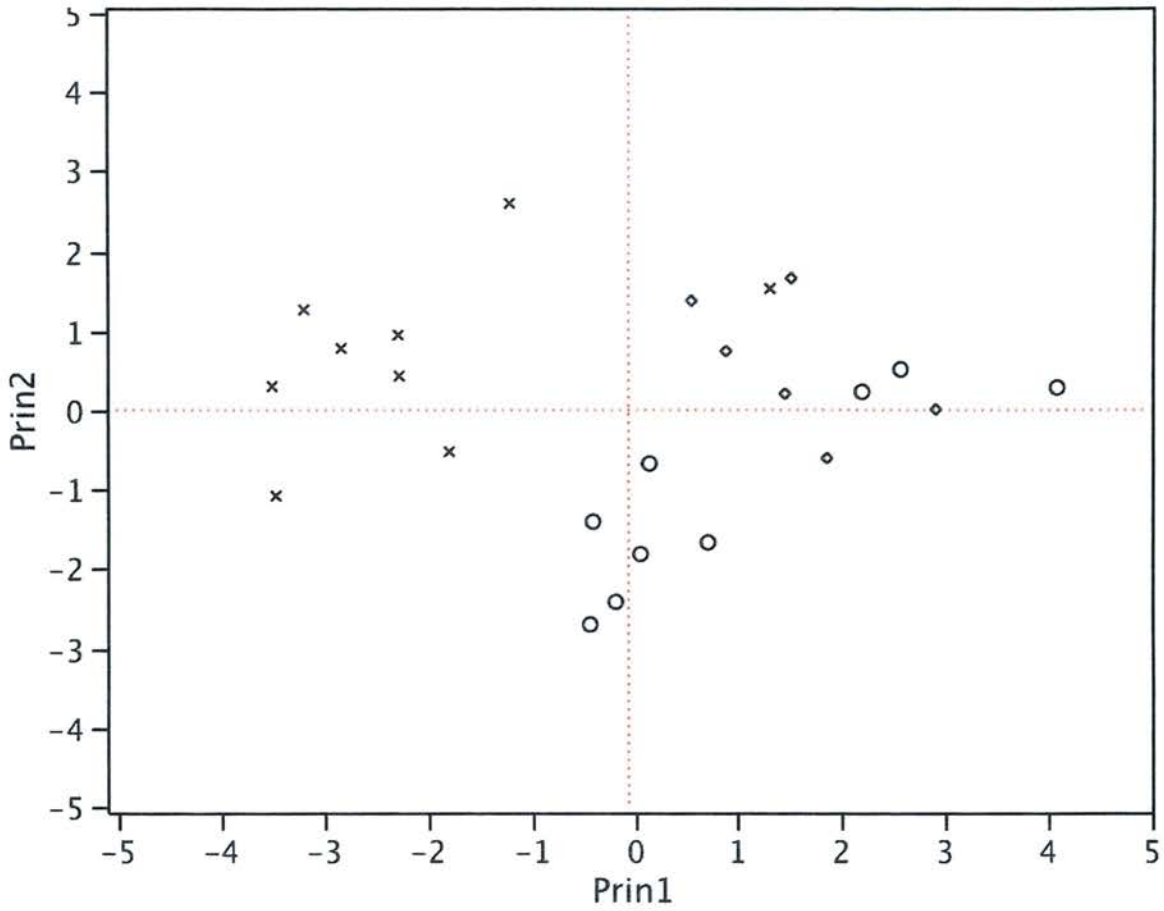

FIGURE 16: PCA using liberal character set, with DF scaled to 20.6 mm. Diamonds represent Guadalupe population; circles represent Colorado population; Xs represent Brazos population.

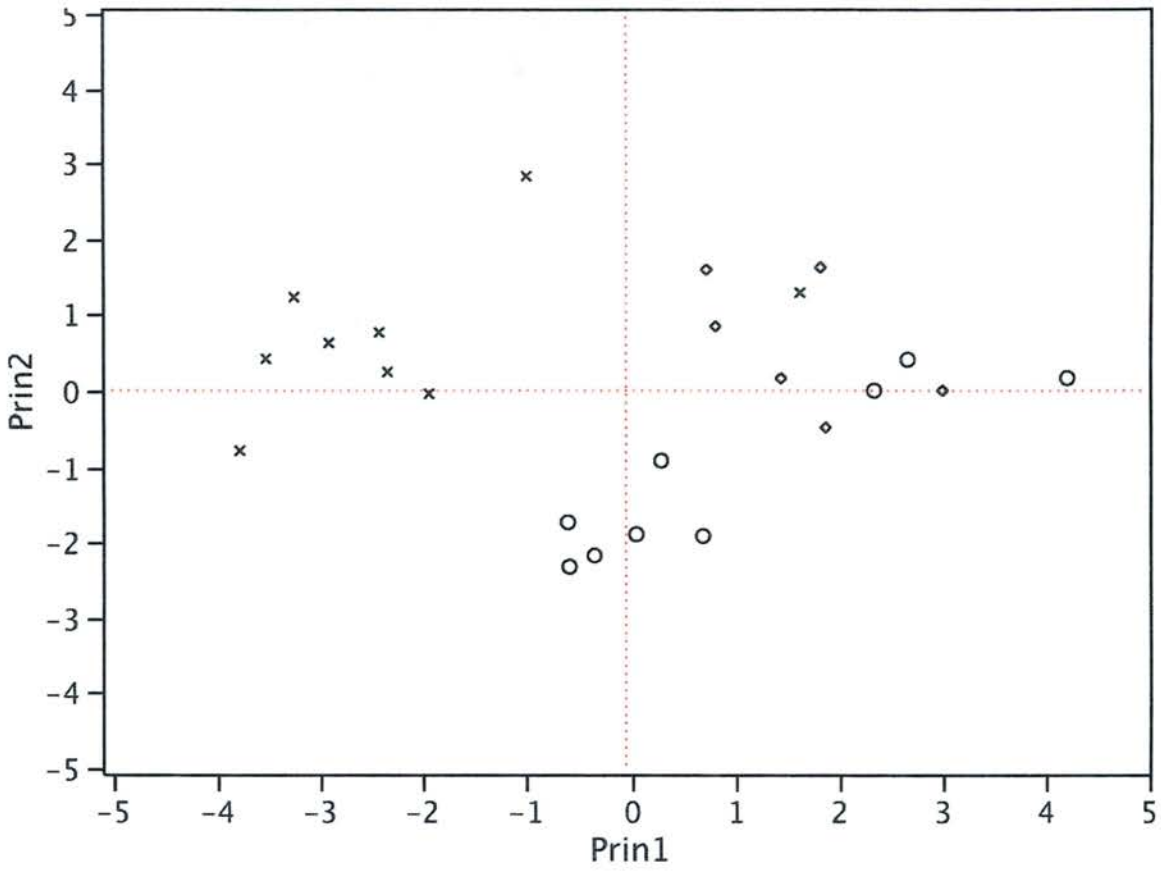

FIGURE 17: PCA using liberal character set, with unscaled DF. Diamonds represent Guadalupe population; circles represent Colorado population; Xs represent Brazos population.

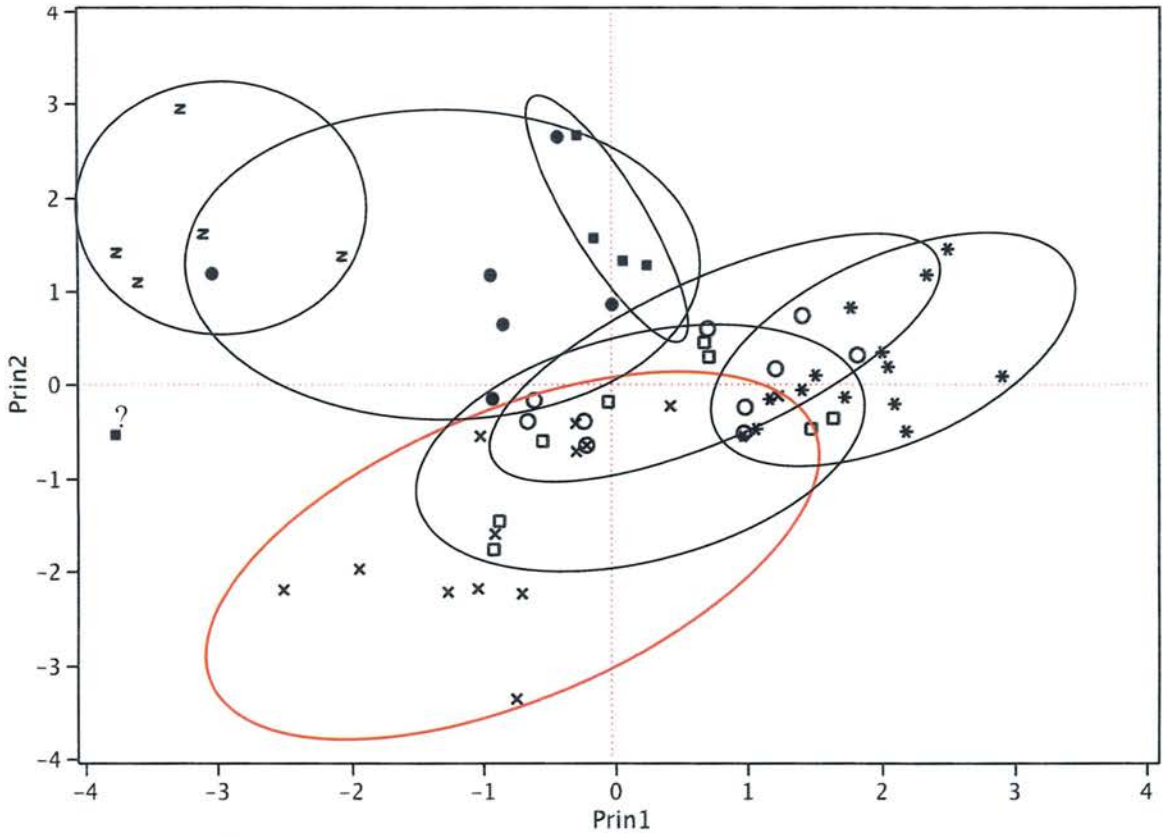

FIGURE 18: PCA on morphological data using conservative dataset and multiple taxa. Open squares represent Guadalupe population; open circles represent Colorado population; Xs represent Brazos population; Zs represent *Acris crepitans paludicola* from Jefferson Co., TX (TCWC72712-16); closed rectangles represent *A. gryllus* from LA, MS, and AL (TCWC 1670, 9178, 9179, 15310, 15311); closed circles represent *A. crepitans* from LA (assumed to be *A. c. crepitans*; TCWC 17750, 17757, 23185, 23187, 23193, 84315); asterisks represent *A. crepitans* collected from the Black river, NM.

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VITA

Born on December 24th, 1970, the only child of Dr. Audley D and Gayle Gaston, Michele Anne Gaston developed a deep appreciation for the natural world at an early age. After graduating from McCallum High School in Austin, TX. in 1988, she worked at a variety of jobs and traveled extensively in the U.S. and Europe prior to completing a Bachelor of Science degree in Geography (Resource and Environmental studies) at then Southwest Texas State University in 2000. An increasing desire to pursue a career as a research biologist led her to obtain a second Bachelor of Science degree from the University of Texas-Austin, which she completed in the fall of 2006 while concurrently enrolled as a Master's student in Wildlife Ecology program of the Biology department at Texas State University-San Marcos. While completing her Master's studies, she worked as an Instructional Assistant for Anatomy and Physiology labs, participated in grant-funded Houston toad research, and worked as a biological consultant for Houston toad management through Environmental Defense Fund. She begins her Doctoral studies in Aquatic Resources at Texas State in the fall of 2008.

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