



Review Paper

Geographic variation in acoustic communication in anurans and its neuroethological implications



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ABSTRACT

Geographic variation of traits may represent the first step for evolutionary divergence potentially leading to speciation. Signals are behavioral traits of particular interest for the study of variation at a geographic scale. The anuran acoustic communication system represents an excellent model for studies of this kind, because their vocalizations play a main role in reproduction and the extant variation in this system may determine the evolution of this group. This review is committed to studies on geographic variation of acoustic communication systems in anurans, focusing on temporal and spectral characteristics of signals, environmental constraints affecting them and sound producing and receiving organs. In addition to the review of the literature on these topics, I highlight the deficit of investigation in some areas and propose alternative directions to overcome these drawbacks. Further, I propose the four-eyed frog, *Pleurodema thaul*, as an excellent model system to study geographic variation using a wide spectrum of approaches.

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1. Introduction

Darwin proposed that species diverge by means of natural selection favoring individuals having fitter traits over other individuals less adapted to compete for limited resources (Darwin, 1859). However, it has been widely recognized that this process is not a generator of variation, but operates on pre-existing trait variation (Jablonka and Lamb, 2002, 2005; Endler, 1986). After Darwin, diverse processes such as mutation, genetic drift and hybridi-

zation have been proposed as sources of variation (Futuyma, 2013). The combined action of these factors produces divergence among populations, causing speciation as a potential final outcome (Butlin et al., 2012; Coyne and Orr, 1998; Coyne and Orr, 2004). Speciation could arise as the consequence of dispersion and colonization phenomena, by which members of a population migrate and settle in new environments (e.g. Bonacum et al., 2005). Also vicariance phenomena have been proposed to produce geographic barriers that isolate and expose populations to different environmental conditions (e.g. Hoskin et al., 2005). Individuals of populations exposed to such conditions may establish distinct behavioral dynamics of interaction between themselves and with their environment, yielding variation of behavioral characters at geographical scales (see Freeman and Herron, 2007 or Turelli

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et al., 2001 for mechanisms of speciation). This kind of variation has provided an excellent model to investigate possible initial stages of speciation and the factors responsible for these divergences. The main factors that have been attributed to generate geographic variation are natural and sexual selection and genetic drift, which have been studied for different kinds of traits (Bush and Schul, 2010; Butlin et al., 2012; Funk et al., 2009).

Animal communication, understood as the transmission of a signal between a sender and a receiver across an environment causing a behavioral change in the second actor (see Bradbury and Vehrencamp, 2011; Hauser, 1997 for a complete discussion on the definition of animal communication), is a widely studied phenomenon that plays a significant role in species divergence (Endler and Basolo, 1998; Ryan and Rand 1990; Boughman, 2002; Wilkins et al., 2013). Because signals travel across an environment as they are transmitted from sender to receiver, the evolution of the communication system may be affected by particular environmental conditions to which different populations are exposed (Narins, 2001; Gridi-Papp and Narins, 2009). Acoustic communication has been an excellent model to study the effects of environmental factors on the structure of signals, the morphology of the sound-producing structures, and auditory sensitivities (Morton 1975; Hermida and Farías, 2009; Schwartz and Gerhardt, 1998).

Anurans possess important features that make them particularly interesting from a bioacoustic standpoint. Anurans spend their first stages in the aquatic environment, depending totally on this resource (Vitt and Caldwell, 2014). The auditory sensitivity of anurans starts developing from premetamorphic stages (Boatright-Horowitz and Simmons, 1997) and some larvae are even capable of emitting acoustic signals in certain contexts (Natale et al., 2011; Salgado Costa et al., 2013). In adult life, anurans become partially independent of the aquatic medium, returning there to complete their reproduction. The change from aquatic to terrestrial life causes modifications at morphological, physiological and behavioral levels in both senders and receivers (Vitt and Caldwell, 2014; Wells and Schwartz, 2007; Vidal and Labra, 2008). Therefore, anurans also represent an excellent model for studies on development, influences of environmental conditions, morphological and physiological changes.

In most anuran species males aggregate in chorusing ensembles, producing advertisement calls which attract females (Gerhardt and Huber, 2002; Ryan, 2001), in a reproductive system called lek, widely used by birds and mammals (Bradbury and Vehrencamp, 2011; Alcock, 2005). Furthermore, males of some species set territories by means of their calls (Gerhardt and Huber, 2002; Ryan, 2001). Various structures including lungs, larynx, mouth and vocal sac participate in the production of these calls (Martin, 1971; Martin, 1972; Martin and Gans, 1972; see Walkowiak, 2007 for a review of vocal production in anurans). By contracting the flank muscles air is forced to flow through the larynx, which contains a series of muscles (e.g. dilator, constrictor), cartilage (e.g. arytenoid, cricoid) and folds (vocal cords). As the air flows the vocal cords vibrate, determining the fundamental frequency of frog vocalizations. After its passage through the larynx, the different frequencies of the sound produced are amplified or attenuated in the mouth cavity and vocal sac, determining the final spectral content of the vocalizations produced. The temporal structure of the vocalizations may be modulated in active or passive modes (Martin, 1971; Gerhardt and Huber, 2002). In the active mode, the contraction of the flanks determines the number of pulses, the duration of each pulse and the duration of the inter-pulse interval. In contrast, in passive control the amplitude modulations exerted by both laryngeal muscles and arytenoid valves are responsible for the fine temporal properties of vocalizations such as the number and depth of intra-pulse modulations (i.e. ratio of

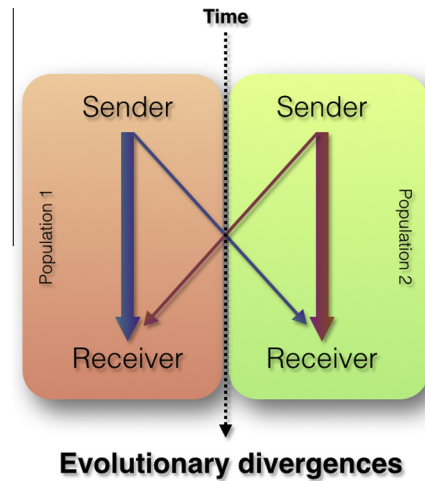


Fig. 1. Schematic diagram of geographic variation in the anuran acoustic communication system. In each population, a signal travels from a sender to a receiver, but the signal emitted by a sender from population 1 is perceived or triggers behavioral responses mainly from receivers of the population 1 compared to receivers of population 2 and vice versa. Over time and by means of different evolutionary mechanisms (e.g. genetic drift, natural and sexual selection), the signals emitted and the sound producing-receptor structures diverge between the populations.

the difference between the maximum and minimum envelope amplitude within a pulse, expressed as a percentage). Sound reception in anurans has peculiar characteristics because at the auditory periphery the frequency sensitivity is segregated in two different organs. The low and mid frequencies are processed by the amphibian papilla and the high frequencies by the basilar papilla (Feng et al., 1975). These two structures represent the first filter in the auditory system, in contrast with temporal filters that are located upstream in the anuran auditory system (Narins et al., 2007).

Geographic variation of the acoustic communication system in anurans may generate evolutionary divergences, representing the initial stages of speciation. If the signals emitted by senders differ between populations, and if the receivers from different populations perceive them as distinct, different behavioral responses may be triggered. In addition, if the environments across which these signals travel are different between populations, different conditions for propagation and degradation may be generated. Thus the main evolutionary forces (e.g. genetic drift, natural and sexual selection) may act with different intensities in each population, producing their divergence (Fig. 1).

Signal variation at a geographic scale represents an excellent model to investigate the factors that influence signal divergence between populations at both sender and receiver levels. Several studies in anurans have undertaken efforts in this direction. In addition, since behavior is the expression of structure and organization of the nervous system in a particular environment (Maturana-Romesin and Varela, 2003), the neurobiological mechanisms underlying this behavioral variation at a geographic scale are also relevant to be understood. The receptor responses are so important that these may induce drastic changes in the configuration of certain signal parameters, which could in turn generate important evolutionary changes. For this reason it is crucial to consider the geographic variation of both morphology and physiology of the sound receptor structures. However, few investigators have carried out studies on the neurobiological implications of the geographic variation of the communication system and these efforts have received considerably less attention than behavioral studies. Thus the aim of this review is to comment on studies regarding geographic variation in the acoustic communication systems of anurans from behavioral and neurobiological approaches, and to

propose possible lines of investigation for researchers potentially interested in these issues.

2. Geographic variation of acoustic signals

2.1. Spectral and temporal parameters

The spectral contents of acoustic signals are fundamental for species recognition and therefore variation of these features at a geographic level would generate divergence between populations of the same species, leading to biases in female preferences or changes in males' responses to acoustic competition (Burmeister et al., 2002). It is well known that spectral parameters are closely related to body size. In general, the dominant frequency of advertisement calls emitted by males is inversely related to body size (Castellano and Giacoma, 2000; Gerhardt and Huber, 2002) and it is also related to auditory sensitivity (Gerhardt and Schwartz, 2001; see also 2.4). It is expected that at localities with higher temperatures, experiencing faster desiccation and shorter metamorphosis times, smaller individuals will develop (Marquez-Garcia et al., 2009), and therefore calls emitted by adult males would have higher dominant frequencies. In addition to temperature and desiccation, plant cover imposes diverse constraints on propagation, refraction and reflection of sound, affecting the spectral contents of signals of different populations. One of the best known cases of relationships between signal structure and environment occurs in the North American cricket frog, *Acris crepitans*. This frog has a wide geographic distribution in the United States with two subspecies within this range, *A. c. crepitans* in the east and *A. c. blanchardi* in the west (Wilczynski and Ryan, 1999). The first taxon always inhabits pine forest zones while the second inhabits open savanna zones, with isolated populations inhabiting pine forests. These subspecies differ in various parameters of their calls such as call rate, call duration and dominant frequency; the last character presents the most notable differences associated with habitat. *A. c. crepitans* also emits shorter calls with slower pulse rate and higher dominant frequency than *A. c. blanchardi*. Interestingly, populations of *A. c. blanchardi* present call parameter values more similar to populations of *A. c. crepitans* inhabiting pine forests (Nevo and Capranica, 1985; Ryan, 2001; Wilczynski and Ryan, 1999). Another classic case of geographic variation is the Puerto Rican coqui, *Eleutheroedactylus coqui*, which shows a highly negative correlation between spectral parameters and population altitude. Males from altitudinal extremes give weaker responses to calls displayed by males from the opposite population (Narins and Smith, 1986).

Variation in temporal parameters at a geographic scale, in addition to being associated with variation in conspecific recognition as occurs for spectral parameters, may denote differences in the motivational and energetic conditions between males (Gerhardt 1991; Castellano and Giacoma, 1998; Zimmitti, 1999; Castellano et al., 2000, 2002). Relationships between some temporal parameters and the genetic condition of taxa have been shown to occur. The North American tree frog *Hyla* group is a genus comprising diploid, tetraploid and triploid hybrid species. These frogs emit a pulsed advertisement call with a pulse rate inversely related to the ploidy level (Foster and Endler, 1999; Gerhardt and Huber, 2002). Similar to *Hyla*, the *Bufo viridis* complex is a group of uncertain phylogenetic relationships that includes populations with different ploidy levels. However, independent of the ploidy, different populations of this group present a remarkable variation in the call pulse rate, exhibiting a high positive correlation with body temperature (Giacoma and Castellano, 2001; Castellano et al., 2002). In addition, studies on *Hyla labialis* have also demonstrated that temporal parameters of their advertisement calls show variation related to the altitude of the populations of the recorded subjects: subjects

from lower altitude localities emit calls with higher pulse repetition rate and lower call duration because of the inverse relationship between altitude and temperature (Luddeke and Sánchez, 2002).

Variation in temporal parameters in anurans has also been associated with physiological constraints. Calls having longer duration or higher number of pulses imply larger energy expenditure, but provide higher reproductive success and fitness (Wells and Taigen, 1989). A number of studies have measured oxygen consumption during calling behavior (for a review see Wells, 2001), but to my knowledge none has compared these traits among populations from different geographic locations. In some cases the geographic variation of acoustic parameters has been linked to genetic variation among populations (Funk et al., 2009), and not others (Pröhl et al., 2006, 2007). In the former study, the divergence of the acoustic signals was attributed to genetic drift but in most other cases, the geographic variation of anuran acoustic signals has been associated with other evolutionary forces (Boul et al., 2007; Velásquez et al., 2014; Bernal et al., 2005). Gerhardt (Gerhardt, 1991; Gerhardt, 1994) proposed that anuran call variables having intra-individual variation below 5% are static variables subjected to stabilizing selection, and those above that value correspond to dynamic variables that are subject to directional selection (Gerhardt, 1991; Gerhardt, 1994; Castellano et al., 2002; Castellano and Giacoma, 1998; Castellano et al., 2000). However, various authors have considered that this nomenclature applies only to the extremes of a continuum and different combinations within an extended range can be found in nature (Castellano and Giacoma, 1998; Pröhl, 2003; Velásquez et al., 2013).

Considering the antecedents outlined above, the four-eyed frog *Pleurodema thaul* arises as an excellent model to study the geographic variation of acoustic signals and factors that influence this variation. This species has an extensive geographic distribution in Chile, ranging from the Atacama Desert (27°06'S, 69°53'W, Correa et al., 2007) to Patagonia (45°24'S, 72°42'W, Veloso, 2006), and has a prolonged reproductive season from July to December, depending on the population. During this season, males aggregate in chorusing ensembles where they emit advertisement calls with their vocal sacs inflated, floating on the water or perched on the surface vegetation (Penna and Veloso, 1990; Solís, 1994; Penna et al., 2008). By means of their calling activity during the reproductive season, males establish territories and attract females. A recent study showed that advertisement calls present important geographic variation in their temporal and spectral parameters, congruent with the genetic variation among the populations studied (Velásquez et al., 2013; Fig. 2). Furthermore, this divergence of the acoustic signals emitted by the males of *P. thaul* is not likely to have been the outcome of inter-sexual selection processes, because females of different populations of this species do not show preferences for local or foreign calls (N.A. Velásquez, personal observation). I postulate that the extant signal divergence is the product of the intense vocal interaction between males within each population, because playback experiments show remarkable geographic variation in the evoked vocal responses of males from different populations (Velásquez et al., 2014), in contrast to the stability of female phonotactic responses.

2.2. Influence of environmental factors

Along the extensive geographic distribution of anuran species, various factors associated with the different environments to which these taxa are exposed influence the variation of acoustic signals. Differences in temperature, humidity, and plant cover result in different conditions for sound propagation and degradation, and in diverse biotic and abiotic background noise. Due to

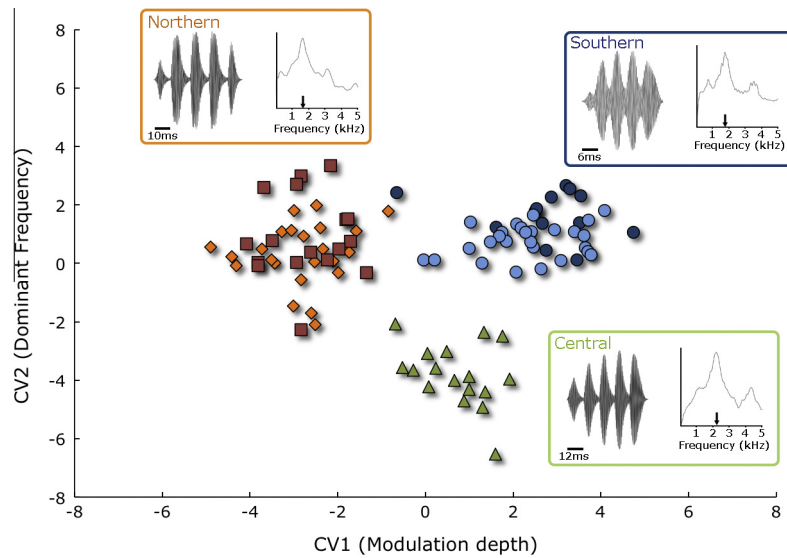


Fig. 2. Geographic variation of acoustic parameters in the advertisement calls of *P. thaul*. Graph of discriminant analysis where each point represents one subject. The two first discrimination axes were mostly correlated with the modulation depth and dominant frequency, respectively. Insets show both pulses and power spectra of representative calls from each zone within the latitudinal distribution of *P. thaul*. Pulses highlight the differences in the depths of the intra-pulse modulations while power spectra show the differences in the dominant frequency. The arrows in the power spectra indicate the dominant frequencies. Symbols: Blue circles: Carrera Pinto; Light blue circles: Totoral; Green triangles: Los Maitenes; Orange diamonds: Osorno; Brown squares: La Picada.

the ectothermic condition of anurans, studies on effects of environmental conditions on geographic variation of their acoustic signals have focused on understanding the relationships between temperature and signal parameters. It has been found that temperature is positively associated with temporal parameters such as pulse rate, call duration and number of pulses. In contrast, a relationship of this kind with spectral parameters has not been found (Ryan, 2001; Gerhardt and Huber, 2002).

A number of studies have investigated the consequences of pond desiccation for diverse phenotypic characteristics (Marquez-García et al., 2009; Semlitsch, 1987; Wilbur, 1987; Semlitsch and Reyer, 1992; Rowe and Dunson, 1995; Denver, 1997). These studies have shown that in ponds subjected to higher temperatures and experiencing faster drying, both postmetamorphic and adult individuals are smaller than individuals from ponds exposed to lower solar radiation. This size difference is due to the shorter metamorphosis time in ponds with fast desiccation (Crump, 1989; Ryan and Winne, 2001; Leips et al., 2000; Loman, 2002). However, to my knowledge no studies focused on the relationship between pond drying time and call spectral characteristics have been conducted. If temperature varies between populations, metamorphosis time and body size are also expected to vary. Because body size changes, the fundamental and/or the dominant frequency of adult calls will also change. Furthermore, desiccation increases larval density, so different populations are expected to have distinct larval dynamics. Larval vocalizations have been reported when they are manipulated or in instances of cannibalism (Natale et al., 2011; Salgado Costa et al., 2013). Different types of interactions among larvae and also vocalizations with distinct temporal and spectral contents probably occur among different populations.

Plant cover is another important factor affecting the variation of signals at a geographic scale, because this factor alters the spectral contents of propagating signals. This issue has been widely studied in birds, finding that animals living in distinct environments differ in the quality of their vocalizations (Gish and Morton, 1981; Shy, 1983; Ryan and Brenowitz, 1985; Sorjonen, 1986; Slabbekoorn and Smith, 2002). Closed environments cause higher signal degradation and attenuation than open environments, and calls of species from closed environments have shorter duration, fewer

pulses and lower dominant frequencies (Ryan et al., 1990; Wiley, 2009; Velásquez et al., 2013). According to this evidence, local signals are emitted in an optimum way in native localities, a condition systematized as the “acoustic adaptation hypothesis” as proposed by Morton (1975). This hypothesis has not been demonstrated to operate in anurans, since contrasting results have been reported, finding that signals propagate similarly well in local and foreign environments (Penna and Solís, 1998; Kime et al., 2000; Wilczynski and Ryan, 1999; Penna et al., 2006).

Calls of anurans having wide geographic distributions are also affected by the biotic and abiotic noise present in different localities. Acoustically active accompanying fauna may cause variation in the dominant frequency and in the timing of emissions, depending on the call characteristics of the accompanying species (Penna and Velásquez, 2011; Penna and Meier, 2011; Penna and Zúñiga, 2014). In addition, abiotic noises such as wind, rain, creeks and also synthetic band-pass noise may modify both the emission rate and the duration of calls. For instance, males of *Eusophus calcaratus* increase their vocal activity, measured as increases in both rate and duration of their calls, when they are confronted to abiotic noises such as rain, creeks and a band-pass noise encompassing the frequency spectrum with more energy in the natural call of this species. In contrast, males of *E. emiliopugini*, a congeneric and sympatric species, do not show significant changes in vocal activity when confronted with the same noises (Penna et al., 2005; Penna and Hamilton-West, 2007). Even anurans may have extensive frequency ranges surpassing 20 kHz in environments with high noise levels, as has been shown to occur in the Chinese frog *Odorrana tormota* (Feng et al., 2006). According to this evidence, variation in both types and levels of noises in different environments could be related to variation in the vocal activity of calling males from different populations.

Preliminary results suggest that the sound environments where *P. thaul* communicates are not related to geographic variation in its advertisement calls. Propagation experiments of a white noise along a 16 m transect in eight populations along the latitudinal distribution of this frog in Chile showed that degradation of this sound with distance in each population is not related to the pattern of variation of advertisement calls. Thus populations within the same biogeographic region may exhibit contrasting degradation

characteristics not related to the characteristics of calls emitted by local frogs (N. Velásquez, personal observation). Because of the important geographic variation of the evoked vocal responses of males of this species, it is probable that the acoustic environments in which they communicate have a secondary role relative to intra-sexual selection processes (Velásquez et al., 2014). The interactions between males during the reproductive season may be determinant for divergence of the signals and for the evoked vocal response selectivity (ten Cate et al., 2002; West-Eberhard, 1983).

3. Geographic variation of the organs involved in the production and reception of sounds

Because of the close relationship of behavioral patterns to the structure and organization of the nervous system, geographic variation at the level of the organs involved in the production of spectrally and temporally organized signals is expected to occur, as well as in organs involved in the reception and processing of these sounds. Studies on the relationship between the acoustic characteristics of frog calls and sound producing structures and receptors are certainly less numerous than those focused exclusively on behavior. One of the few studies considering sound-producing organs was conducted in six populations of *Acris crepitans*, in which significant relationships between laryngeal morphology and temporal and spectral call characteristics were found (Mc Clelland et al., 1996). However, this relationship disappears when body size is removed as a variable. This dependence on body size is interesting from an evolutionary point of view because different environments will give rise to individuals of different sizes (see point 2.3), and thus the size of laryngeal structures and their relationship to call parameters will be different in each population (Mc Clelland et al., 1998). Because of the significant geographic variation observed in the advertisement calls of *P. thaul*, mainly in the depth of the intra-pulse modulations and the dominant frequency, it is expected that the morphology of the larynges of males from these populations will vary correspondingly. In particular, it would be expected that the volume of the vocal cords will be larger in populations having lower dominant frequency. In addition, the volume of laryngeal muscles and arytenoid valves should vary among male from populations that differ in their modulation depths. Morphometric characteristics of sound-receiving structures in *A. crepitans*; i.e., outer ear (diameter of the tympanic membrane), middle ear (both extracolumella and middle ear total volume), and inner ear (inner ear volume) are also positively related with body size (Mc Clelland et al., 1996). Interestingly, this evidence is in agreement with reports on geographic variation of auditory sensitivity. Studies using multi-unit recordings on the cranial eighth nerve fibers which innervate the inner ear (Ryan and Wilczynski, 1988; Keddy-Hector et al., 1992; Wilczynski et al., 1992), and single-unit recordings of neurons of the dorsal medullary nucleus (Capranica et al., 1973) of *Acris crepitans* have shown that the best excitatory frequency is negatively correlated with body size, and that this variation is congruent with the variation of the dominant frequency of the advertisement calls emitted by males in each population. In addition, studies have related temperature to auditory reception in anurans (Narins, 2001). These studies, using single fiber recordings from the eighth nerve, whole-cell patch-clamp recordings from isolated hair cells and recordings of spontaneous otoacoustic emissions (SOAEs), demonstrated that the tuning and thresholds of the amphibian papilla, the oscillation frequency of the membrane potential of hair cells from amphibian papilla and sacculus and the emission frequency of SOAEs are strongly dependent on temperature. Therefore it is likely that individuals from populations which are subjected to different temperatures will have distinct

auditory sensitivities and consequently different behavioral responses.

The analysis of the variation in dominant frequency, its relationship to body size and the concomitant variation of frequency sensitivity are relevant for divergence of the sound communication system, but there are species for which the main differences at a geographical scale reside in variations in temporal rather than in spectral parameters (Gerhardt and Huber, 2002; Castellano et al., 2002; Velásquez et al., 2013). Electrophysiological studies have demonstrated that midbrain neurons of males of *P. thaul* respond selectively to temporal parameters contained in the advertisement calls of this species (Penna et al., 1997) and that both evoked vocal responses and auditory thresholds are closely matched (Penna et al., 2008). Among the anurans studied, *P. thaul* is the species presenting both the highest selectivity in single-unit responses to temporal characteristics of conspecific calls at the midbrain level and the highest correspondence between the thresholds of evoked vocal responses measured in the field and auditory thresholds measured from multi-unit recordings in the same brain area. However, these studies have been carried out with individuals from a single population. It would be interesting to replicate these experiments with subjects from locations encompassing the full range of geographic distribution to establish correlates between the variation at the genetic and bioacoustic levels with studies at the neurobiological level. Because advertisement calls have remarkable latitudinal variation it is likely that both single and multi-unit responses vary accordingly.

To my knowledge, no studies have examined neural responses from different populations to stimuli differing in temporal parameters such as the number of pulses, call duration, pulse rate or depth of amplitude modulations. A limitation for this type of study is that gross techniques such as multi-unit recordings and auditory threshold determinations do not provide appropriate resolution at a fine temporal scale, and single-unit approaches are needed to classify the types of responses into tonic or phasic and to measure response latencies and post-stimulus histograms or raster plots (Elliott et al., 2011). Unfortunately, in small animals such as frogs this kind of recording does not generate neuron sample sizes appropriate for a thorough statistical analysis and to draw robust conclusions unless a large number of animals is used (Wilczynski and Ryan, 2010). Rather, other techniques are currently in use as markers of neural activity, such as immediate early gene expression (Burmeister et al., 2008; Hoke et al., 2004; Hoke et al., 2008; Chakraborty et al., 2010; Mangiamele and Burmeister, 2011), or intra-cellular recordings (Kostarakos and Hedwig, 2012) that might succeed in addressing the issue of geographic variation in the activation of auditory responses.

4. Conclusions

Geographic variation of acoustic communication systems is a topic that has received increasing attention, but researchers have been primarily focused on studies of signal variation among different populations. Efforts to establish the neurobiological mechanisms underlying signal variation have been undertaken to a much lesser extent. Some studies showing relationships between the dominant frequencies of the signals emitted by males, laryngeal morphology, auditory sensitivity and body size of different populations of a single species have been conducted. In some cases, these relationships have accounted for population differences between male signals and female preferences. However, there is a total lack of knowledge about the neurobiological mechanisms involved in processing the temporal variation of parameters of acoustic signals at a geographic scale. From the accumulated knowledge, the four-eyed frog *P. thaul* arises as an excellent model

to study geographic variation with a wide spectrum of approaches at the bioacoustic, genetic, morphological, energetic and neurobiological levels, using immunohistochemistry techniques for early neural activation.

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