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The mechanism of sound production in túngara frogs and its role in sexual selection and speciation

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Sexual communication can evolve in response to sexual selection, and it can also cause behavioral reproductive isolation between populations and thus drive speciation. Anurans are an excellent system to investigate these links between behavior and evolution because we have detailed knowledge of how neural mechanisms generate behavioral preferences for calls and how these preferences then generate selection on call variation. But we know far less about the physical mechanisms of call production, especially how different laryngeal morphologies generate call variation. Here we review studies of a group of species that differ in the presence of a secondary call component that evolved under sexual selection. We discuss how the larynx produces this call component, and how laryngeal morphology generates sexual selection and can contribute to speciation.

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Introduction: anuran acoustic communication, sexual selection, and speciation

One of the most important decisions an animal makes is choosing a mate, and usually it is the female choosing a male [1,2]. There is strong selection on males to produce signals that indicate its species and on females to be attracted preferentially to its own species' signal. This female call preference leads to species recognition. But there is always variation among conspecific signals and differential female attraction to these signals generates sexual selection, which is the variation in reproductive success due to variation in the ability to acquire mates [3].

Anuran vocal communication is an excellent system to understand the interaction among mating signals, brain, and behavior, as well as the evolutionary consequences of these behaviors on speciation. Capranica studied the evoked vocal responses of bullfrogs to synthetic calls to gain insights into how animals extract information from complex acoustic patterns [4^{*}]. Later studies by Capranica and many others used electrophysiology and gene expression to probe how the auditory system decodes the mating call and passes this information to higher brain centers involved in decision making [5,6^{**}]. Simultaneously, studies of female phonotaxis provided a critical link between the auditory system and behavior, and placed anuran mate recognition firmly within current theories of evolutionary biology [7,8^{**}]. Thus there is a detailed understanding of the neurological and physiological bases of how frogs recognize mating calls and how this leads to mating preferences among and within species. However, we understand much less about how calls are produced. The most detailed studies are on *Xenopus* (Kelley, this issue, and [9,10,11^{**}]). But these under-water calling frogs have a derived vocal production system that differs from most other anurans; for example, *Xenopus* lacks vocal cords [12].

Here we review the case of a more typical frog in order to understand the link between laryngeal morphology and mating calls. We then explore how variation in calls and their underlying morphology can generate sexual selection, behavioral reproductive isolation between populations, and thus potentially drive speciation.

Mechanisms of production

The main organ responsible for sound production in frogs is the larynx. In most frogs, air is expelled by contracting trunk muscles surrounding the lungs, which pushes the air through the larynx [13–15]. The incoming air causes the vibration of the vocal cords and the larynx itself [14]. The air then enters the buccal cavity and passes through the vocal slits to inflate the vocal sac. One of the most conspicuous and near-universal traits of male frogs is the vocal sac. Its main function is to recycle air from the lungs to the vocal sac and back again [16,17]. The vocal sac also radiates sound [18–20], as do the head of some treefrogs [18] and the ears of bullfrogs [21]. Many frogs rely on other cues besides the call [22], and the inflating vocal sac can provide a visual cue to the receiver [23–25] and can generate surface-water disturbances that are then detected by receivers [26,27]. It is important to note that the individual components that contribute to the

production of the anuran vocalization do not act in isolation; for example, Kime *et al.* [28**] modeled how the vibration patterns of the vocal folds are influenced by pressure in both the lungs and the buccal cavity, and may also be modulated by the oscillatory behavior of other vocal system components such as the arytenoid cartilages.

Complex calls, sexual selection, and call production in túngara frogs

The túngara frog, *Physalaemus* (= *Engystomops*) *pustulosus*, produces a call with two components, a frequency-modulated whine which can be produced by itself (simple call) or followed by 1–7 harmonic bursts or chucks (complex calls). The whine temporally overlaps the first chuck, thus the two components can be produced simultaneously (Figure 1). The whine is necessary and sufficient to elicit female phonotaxis and is critical for species recognition. The chuck by itself is not a salient signal, but when a single chuck is added to the whine it increases the whine's attractiveness fivefold. Males can facultatively add to up to seven chucks and females also prefer calls with more versus fewer chucks [29–31]. Thus these complex calls, and the structures that produce it, are favored by sexual selection. Chucks also impose a cost by attracting the frog eating bat (*Trachops cirrhosis*) and other acoustically orienting predators [29,31]. The preference for chucks might precede the evolution of the chucks themselves. *Physalaemus coloradorum* lack chucks but females are attracted to their own call followed by three *P. pustulosus* chucks, but they do not prefer their

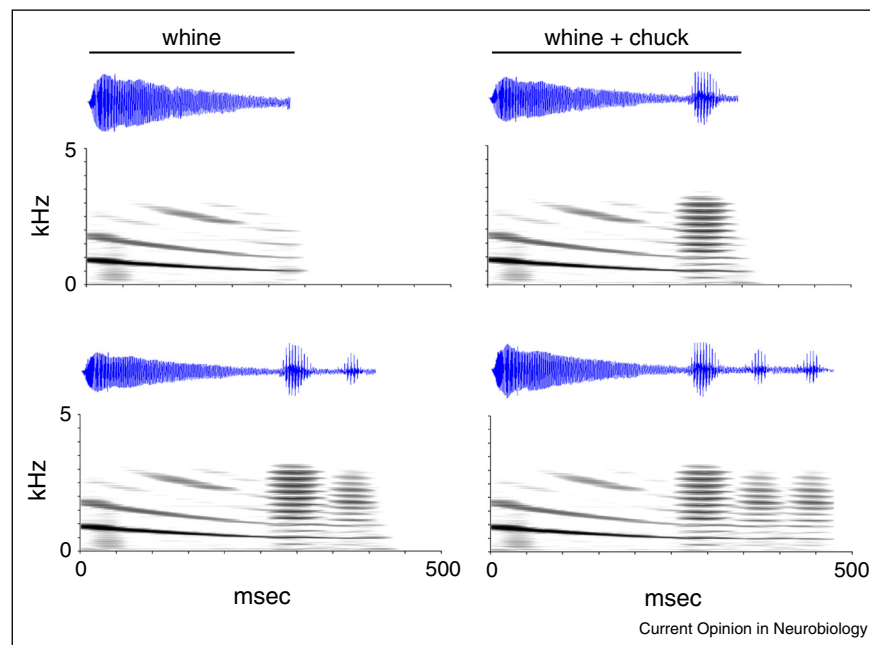
own call plus one chuck over their normal chuck-less call (reviewed in [31]).

How can the túngara frog produce two call components that overlap in time? In 1976, Charles Greenwalt, the pioneer of oscine song production [32], speculated: 'There is no very good evidence that frogs have the two acoustical systems which birds possess. There is a hint in *Engystomops* [= *Physalaemus*] *pustulosus* that something of this sort might exist, but frankly I doubt it.' (p. 72 in [29]).

Nothing then known about anuran call production could account for the whine-chuck. Drewry *et al.* [33] sought the mechanism of the 'two-voiced frog call' by comparing the laryngeal morphology of three congeners. They showed that the túngara frog differed from the others by possessing a larger fibrous mass (FM) that was connected to the vocal cords and wall of the larynx and suspended into the bronchial passage (Figure 2). This configuration, they speculated, allowed the FM to vibrate independently of the vocal cords and produce the chuck. Another species that produced only a whine had a small FM, while a third species with a call that resembled a long, continuous chuck had a large FM whose anchoring prohibited it from vibrating independently of the vocal cords. Thus, despite Greenwalt's skepticism there seemed to be two acoustical systems within the larynx of the túngara frog that enabled production of its complex call.

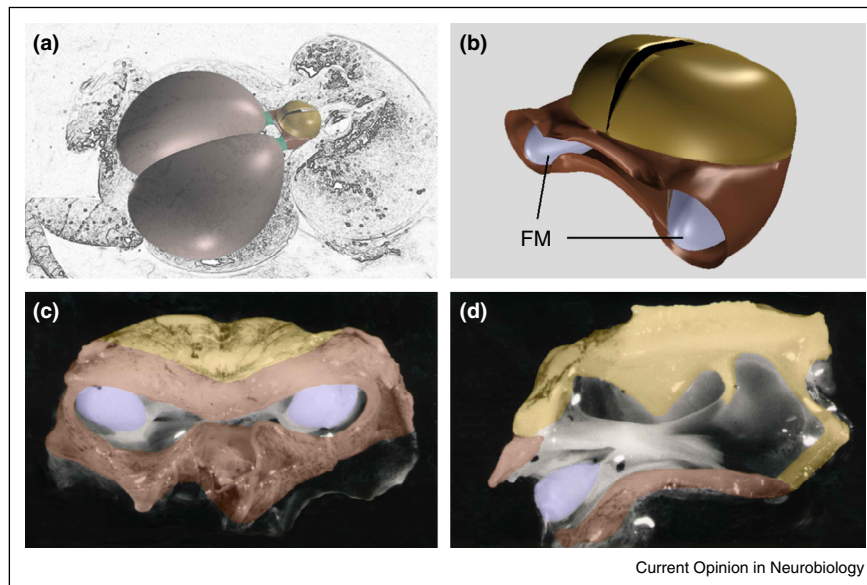
The key support for the 'two-voiced' complex call is experimental. When Gridi-Papp *et al.* [34] surgically

Figure 1



The complex advertisement call of the túngara frog, *Physalaemus pustulosus*. Top, blue illustrations are waveforms and bottom gray-scale illustrations are spectrograms. The figure illustrates calls of varying complexity of the same male: (top, left) whine, (top, right) whine plus chuck, (bottom, left) whine plus two chucks, and (bottom, right) whine plus three chucks.

Figure 2



Laryngeal morphology of the túngara frog. The arytenoid cartilages are in yellow, the cricoid cartilage in red, the fibrous masses in blue, the vocal folds in white, the bronchi in green and the lungs in pink. **(a)** Approximate position of the larynx and lungs in the calling frog. **(b)** Simplified illustration of the larynx without bronchi or lungs. **(c)** A view of the larynx from the lungs showing the expansion of the fibrous masses toward the bronchi. **(d)** A medial section of the larynx showing the attachment of the fibrous mass to the vocal fold. From Gridi-Papp *et al.* [34].

ablated the FM, male túngara frogs were unable to produce chucks. This is an unusual situation in which a specific morphological structure in the larynx is associated with a specific signal component.

Ryan and Drewes [35] conducted phylogenetic comparisons that offered insights into the pattern of complex call evolution and the underlying morphology that determines it. They compared species from two clades of the *P. pustulosus* species group. One clade contains *P. coloradorum* and *P. pustulatus*, which are restricted to regions west of the Andes. These two species lack chucks and have smaller larynges and FMs.

The other clade contains *P. pustulosus* and the Amazonian *Physalaemus petersi*. Ryan and Drewes examined individuals from two populations of *P. petersi* (both are now called *Physalaemus freibergi* [36,37]), one in southern Peru and one in western Brazil. Males in the former population only infrequently added chucks (sometimes called ‘squawks’) and had a smaller mass. The Brazilian *P. petersi* had a large FM similar to the homologous mass in *P. pustulosus*. Larynx and FM size covaried, presumably because a larger larynx is needed to house a larger FM [38]. But larynx and FM size are not inextricably coupled in their development and evolution. As we note below, some populations of *P. petersi* produce low-frequency whines typical for males with larger larynges but lack chucks and presumably the large FM.

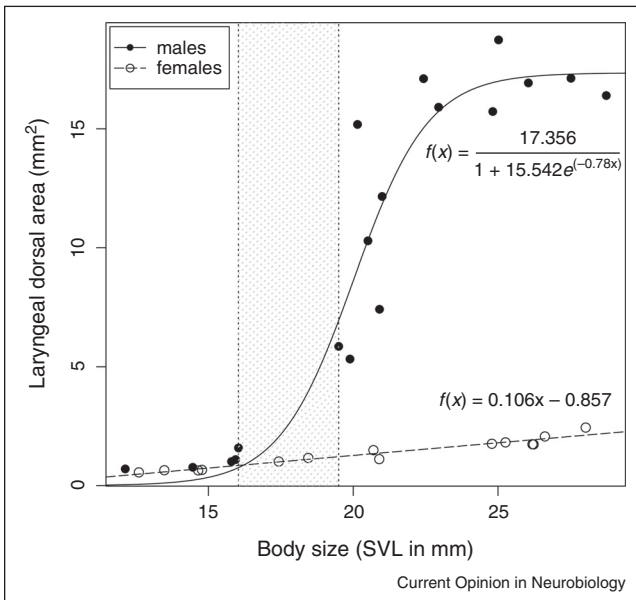
These species comparisons suggest that the chuck evolved in the common ancestor of *P. pustulosus* and *P. petersi*-*P. freibergi*, and was subsequently lost or independently evolved in some populations of the *P. petersi* clade (Figure 4).

The patterns of development that result in the large larynx and large FM of túngara frogs has also been documented. As only the male frog produces advertisement calls, it is not surprising that there is a strong sexual dimorphism in larynx size (Figure 3). Túngara frogs are less than 13 mm snout-to-vent length (SVL) when they metamorphose and the larynges of the sexes are indistinguishable until about 16 mm when males show strong positive allometric growth in overall larynx size (Figure 3) as well as the size of the vocal cords and the FM [39]. Interestingly, larynx growth plateaus when males first call in the field, at about 24 mm SVL [29]. Thus male reproductive behavior is triggered when the larynx is fully developed.

Speciation and the larynx

In *P. petersi* and *P. freibergi* the simple calls consist of a whine preceded by a short prefix. As far as we know, all *P. pustulosus* and *P. freibergi* males are able to produce chucks. But the presence of complex calls varies among populations of *P. petersi* [39–41]. Larynx morphology maps onto call variation among populations as it does among species. Both the larynx and the FM are larger in

Figure 3



Larynx area growth relative to body size in *Physalaemus pustulosus*. Shaded area corresponds to the time window in which strong positive allometry occurs in males. From Guerra *et al.* [39].

populations where males produce complex calls compared with populations in which males produce only simple calls (prefix plus whine) [38]. Moreover, FM size is correlated with larynx size, which in turn influences the frequency of the call (larger larynx → lower frequency) as it does in many vertebrates [42,43]. Thus *P. petersi* that produce chucks also have lower-frequency whines.

Females from *P. petersi* populations where males produce chucks prefer calls with chucks, while females from populations where males do not produce chucks have no such preference [40,44]. Boul *et al.* [40] compared preferences for simple calls from populations with and without chucks. The population with no chucks has high-frequency simple calls and the populations with chucks has low-frequency complex calls. Females exhibited a near unanimous preference for the local simple call versus the foreign simple call. The most obvious difference between the two calls was the whine frequency. Males from populations that produced chucks had larger larynges and FM and lower-frequency whines, thus it seems that the population-based preference was based on whine frequency. Boul *et al.* suggested that in *P. petersi* sexual selection favors the evolution of the chuck, and when the chuck and the larger larynx that produces it evolve, so do low-frequency whines. Thus the low-frequency whines and the population-based preferences for them are incidental consequences of sexual selection favoring chucks, they result in behavioral

Figure 4

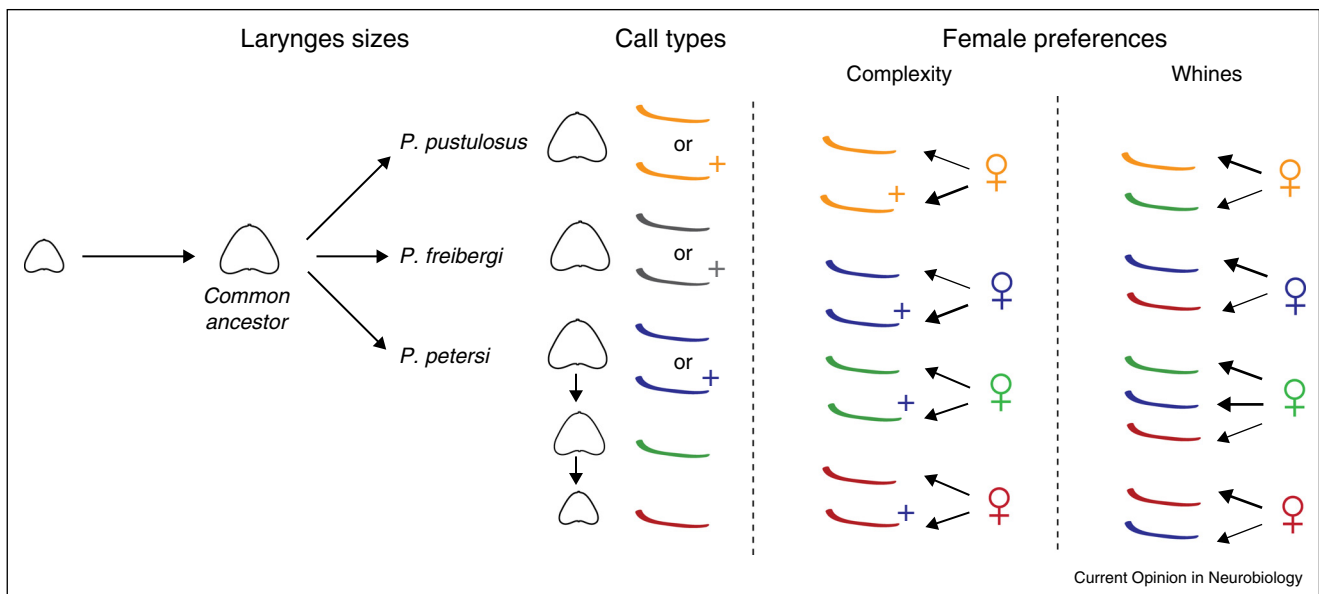


Diagram of the suggested evolution of laryngeal size with the call type each larynx produces (on the left). Call types: orange: *P. pustulosus*, gray: *P. freibergeri*, blue: *P. petersi* with low-frequency complex calls, green: *P. petersi* with low-frequency simple calls; and red: *P. petersi* with high-frequency simple calls. Plus sign denotes the presence of chucks. The third column shows female discrimination between simple vs. complex calls. Arrows weight denotes the frequency of female responses to each call type. The fourth column shows female discrimination between simple calls. Again, arrows weight denotes the frequency of female responses to each call type. From Boul and Ryan [38], Boul *et al.* [40] and Guerra and Ron [34].

reproductive isolation between populations, and thus could potentially contribute to speciation.

A later study by Guerra and Ron [44] added more complexity to the situation and also demonstrates the importance of whine-frequency for population-based preferences. They tested females from a population where males did not produce chucks but had low-frequency whines (low-frequency simple). These females preferred low-frequency whines to high-frequency whines whether the low-frequency whines were from populations that did or did not produce chucks (Figure 4). These phonotaxis results are consistent with Boul *et al.*, in that low-frequency versus high-frequency whines generate strong preferences for local calls. Their results, however, also showed that low-frequency whines can exist in the absence of chucks. Thus low-frequency whines can evolve independently of complex calls. If females show preferences for whines from their own populations, then the divergence of larynx morphologies and the calls they produce could contribute to behavioral isolation among populations and thus potentially drive speciation.

Conclusions and future directions

The studies reviewed here have revealed the links between laryngeal morphology, mating call variation, and both sexual selection and speciation. Female preference for lower-frequency whines produced by larger larynges can act as a pre-mating isolating mechanism. In some cases the preference for chucks drives the evolution of larger larynges and lower-frequency calls, while in other cases larger larynges evolve independently of chucks. These studies have some similarities to the well-known system of Darwin's finches in which variation in beak morphology is correlated to variation in diet, which then drives ecological speciation [45] and also has incidental effects on song production that contribute to species divergence [46]. In addition, there is a good understanding of the genetic and developmental mechanisms that underlie variation in beak morphology [47,48]. A remaining challenge in the túngara frog system is to dig one layer deeper and identify the patterns of gene expression that account for the laryngeal variation that is subject to sexual selection and contributes to behavioral reproductive isolation and speciation.

Conflict of interest statement

Nothing declared.

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