

# Female mate choice and the potential for ornament evolution in túngara frogs *Physalaemus pustulosus*

Michael J. RYAN<sup>1, 2\*</sup>, Ximena E. BERNAL<sup>3</sup>, A. Stanley RAND<sup>2, 4</sup>

<sup>1</sup> Section of Integrative Biology C0930, University of Texas, Austin, TX 78712, USA

<sup>2</sup> Smithsonian Tropical Research Institution, P.O. Box 0843-03092 Balboa, Ancón, Republic of Panamá

<sup>3</sup> Department of Biological Science, Box 43131, Texas Tech University, Lubbock, TX 79409, USA

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**Abstract** The potential for ornament evolution in response to sexual selection rests on the interaction between the permissiveness or selectivity of female preferences and the constraints on male development of signaling related traits. We investigate the former by determining how latent female preferences either exaggerate the magnitude of current traits (i.e. elaborations) or favor novel traits (i.e. innovations). In túngara frogs, females prefer complex mating calls (whine-chucks) to simple calls (whine only). The whine is critical for mate recognition while the chuck further enhances the attractiveness of the call. Here we use a combination of synthetic and natural stimuli to examine latent female preferences. Our results show that a diversity of stimuli, including conspecific and heterospecific calls as well as predator-produced and human-made sounds, increase the attractiveness of a call when added to a whine. These stimuli do not make simple calls more attractive than a whine-chuck, however. In rare cases we found stimuli that added to the whine decrease the attractiveness of the call. Overall, females show strong preferences for both elaborations and innovations of the chuck. We argue that the emancipation of these acoustic adornments from mate recognition allows such female permissiveness, and that male constraints on signal evolution are probably more important in explaining why males evolved their specific adornment. Experimentally probing latent female preferences for stimuli out of the species' range is a useful means to gain insights about the potential of female choice to influence signal evolution and thus the astounding diversity in male sexually-selected traits [*Current Zoology* 56 (3): 343–357, 2010].

**Key words** Mate choice, Ornament evolution, Túngara frogs, *Physalaemus pustulosus*

Sexual selection promotes spectacular diversity of male ornaments in animals as heterogeneous as birds, frogs, fish, and insects. Comparative evidence demonstrates that closely related species often tend to be similar in most characteristics but often divergent in sexually selected traits (Sibley, 1957; Kaneshiro, 1980; Dominey, 1984; Ryan and Rand, 1993a). For example, many closely related species of cichlids in the Great Lakes of Africa differ in their hue and coloration pattern, traits used by females in mate choice, but are similar in morphology, genetics and behavior (Coultridge and Alexander, 2002; Stelkens and Seehausen, 2009). The evolutionary lability of sexually selected traits is also reflected in the similarity in plumage of females of related species of birds while males, who are under stronger sexual selective pressure, have distinct colorful plumages (West-Eberhard, 1983; Price, 1998; Price, 2008). Although evolutionary biologists have devoted considerable attention to understanding why females

have evolved strong mating preferences (Darwin, 1859; West-Eberhard, 1983; Kirkpatrick and Ryan, 1991; Andersson, 1994; Endler and Basolo, 1998), there is less known about the potential evolutionary trajectories and the type of changes promoted by female preferences driving ornament evolution (but see Endler et al., 2005).

The extant association between a female preference and a male ornament rests in part on the interaction between the permissiveness or selectivity of female preferences and the constraints on male development to produce the signal. Understanding why specific traits evolve requires knowing what traits were favored by female choice and what types of variation in traits males were more or less likely to exhibit. In this study we attempt to assess the potential power of female choice in promoting trait evolution by examining latent female preferences for various types of ornaments in the túngara frog *Physalaemus* (= *Engystomops*) *pustulosus*. Then, given our knowledge of the underlying mecha-

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\* Corresponding author. E-mail: mryan@mail.utexas.edu

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nisms of trait elaboration we speculate as to how male constraints contribute to the current association between female preference and male ornaments in this species.

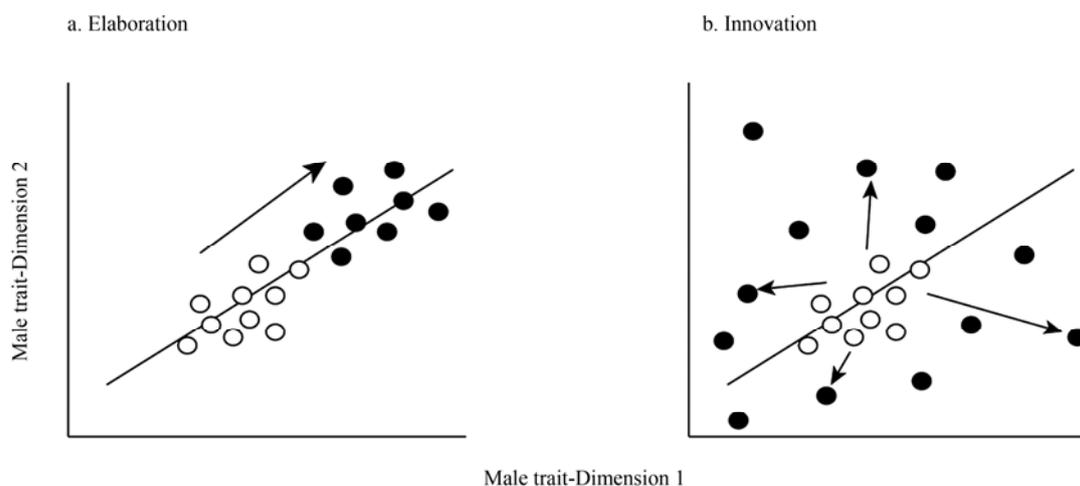
Exaggerated male traits can arise through elaboration or innovation. For example, female choice could favor males with either longer tails (exaggeration) or with novel pigment patterns (innovation). Following Endler et al. (2005), we refer to elaboration as a new form of a trait that accentuates the magnitude of the existing trait along the current axis of variation in the population (Fig. 1a). Sexual selection by female choice often favors trait exaggeration as evidenced by preferences for a greater quantity of a given trait: longer tails in birds (Andersson, 1982, 1994); brighter colors in fish (Houde, 1997) and birds (Andersson, 1994); and longer, louder, and more frequent calls in insects and anurans (Ryan and Keddy-Hector, 1992). Preferences for traits of greater quantity can result from an evolutionary change in female preference. It might be more likely, however, that exaggeration does not require a shift in female preference, as in many cases females have a relatively open-ended preference for a greater quantity of the trait under selection (Ryan and Keddy-Hector, 1992).

Female preferences can also favor the emergence of novel male traits. Again following Endler et al. (2005), we refer to such changes in sexually selected traits as innovations (Fig. 1b). Some examples of an innovation include female preference for males with red leg bands in zebra finches (Burley et al., 1982), and female preferences by two species of mollies for conspicuous orange tumors on the dorsal fins of conspecific males (Schlupp et al., 1999). Receiver biases for novel traits

also have been described in a variety of organisms (Basolo, 1990; Ryan 1990; McClintock and Uetz 1996; Jones and Hunter, 1998). Ornament evolution through innovations can, but need not, require an evolutionary change in female preference. In zebra finches, for example, females have a preference for males with red beaks, and red leg bands might exploit that more general preference for red. Transference of the red trait from one part of the body to another could occur with no change in a general preference for red, or it could result from a change of a specific preference for color in the particular body part.

To date, most examples of preference for trait elaboration or innovation come from studies on single traits (for exceptions see Burley, 1985; Burley and Symanski, 1998; Brooks et al., 2005; Endler et al., 2005; Gerhardt and Brooks, 2009). Here, we seek to investigate the degree to which a series of both exaggerated and innovative ornaments are favored by latent female preference in the túngara frog.

Sexual selection and communication in túngara frogs has been studied extensively (Ryan and Rand, 2003a; Ryan in press). In this species of small Neotropical frogs, as in most anurans, males aggregate in puddles during the breeding season from where they call to attract females and deter rival males (Ryan, 1985). Male túngara frogs produce a frequency modulated whine that is both necessary and sufficient to elicit female response. Males also facultatively add from one to seven secondary call components, called chucks, appended at the end of the whine (Ryan, 1980). Chucks are under opposing selection as female túngara frogs (Rand and



**Fig. 1** Distinction between elaboration and innovation of male ornaments

The x- and y-axis represent different features of the signal (i.e. call duration and energy content) or dimensions from an analysis for variable reduction that accounts for the variability of the trait (i.e. PCA). The open circles indicate the characteristics of the starting traits in the population and the closed ones indicate the ones of new ornaments. Based on Endler et al. (2005).

Ryan, 1981), as well as frog-eating bats (Ryan et al., 1982) and blood-sucking flies (Bernal et al., 2006), are preferentially attracted to whines with chucks (complex calls) than to whines alone (simple calls). Chucks are not produced without a whine, and female túngara frogs are not attracted to chucks only (Ryan, 1985; Farris et al., 2002); thus the chuck acts as an ornament of the whine. The whine is about 350 ms long, and during that time the fundamental frequency sweeps from 900 Hz to 400 Hz. The chuck, in contrast, is about 40ms in duration and has a rich spectrum of 15 harmonics of a fundamental of about 220 Hz, with a dominant frequency of ca. 2500 Hz. The fundamental frequency of the chuck is one-half that of the fundamental of the end of the whine. The tail of the fundamental frequency of the whine and its odd harmonics intervene between the harmonics of the chuck that match those of the whine. Subharmonics are diagnostic of chucks (Ryan, 1985; Gridi-Papp et al., 2006; Bernal et al., 2009a). The chuck is produced by a fibrous mass attached to the vocal folds; males with a surgically removed fibrous mass are unable to produce chucks even though they increase the relative amplitude of the call at the end of the whine (Gridi-Papp et al., 2006).

In túngara frogs, learning is not required for call production in males or for preferences in females (Dawson and Ryan, 2009). The preference for complex calls, in which the chuck simultaneously increases the stimulation of both the amphibian and the basilar papilla of the frog's inner ear, is thought to have evolved through sensory exploitation (Ryan, 1990; Ryan and Rand, 2003a; but see Ron, 2008). Consistent with the sensory exploitation hypothesis is the fact that the chuck can be replaced by a variety of other sounds that are preferred by females. This might be possible because the chuck is not involved in mate recognition, and its function seems to be to increase auditory stimulation of females once they recognize the signaler as an appropriate mate. Thus the acoustic details of the chuck are less constrained to vary and still incite call preferences in females (Ryan and Rand, 1990; Wilczynski et al., 1995). For example, only the upper seven harmonics (above 1.5 kHz) of synthetic chucks are needed for females to show a preference for complex calls. The lower seven harmonics (below 1.5 kHz) do not influence this preference (Ryan and Rand, 1990; Wilczynski et al., 1995). These responses are not unexpected given that about 90% of the energy of the chuck is in the upper harmonics. When the upper and lower harmonics each have the same amplitude as a typical full chuck both types of synthetic

chucks enhance the attractiveness of the whine to females (Ryan and Rand, 1990; Wilczynski et al., 1995). Females are also receptive to variations of the chuck that depart substantially from the natural structure of the chuck. Whines followed by a burst of white noise that lacks the characteristic harmonic structure of the chuck (Ryan and Rand, 1990) and a pure tone (2.1 kHz; Wilczynski et al., 1995) can substitute for a chuck. Similarly, females are flexible regarding the temporal placement of the chuck relative to the whine. Whines with a chuck placed at different times relative to the whine, preceding, overlapping, or following it, are generally more attractive than a single whine, and often as attractive as a normal whine and chuck (Wilczynski et al., 1999; Farris et al., 2005). Females also prefer a whine preceded by the prefix of the call of the closely related species *P. pustulatus* (Ryan and Rand, 1993b).

In this study we examine how variations that include elaborations and innovations of a natural ornament affect female call preferences to determine the potential for latent female preferences to drive call evolution. We include synthetic manipulations of the chuck, calls of closely related species and other anurans, sounds produced by flying frog-eating bats, as well as environmental noises, and man-made sounds, to broadly explore the acoustic space of sounds that can enhance the attractiveness of a whine as does a chuck.

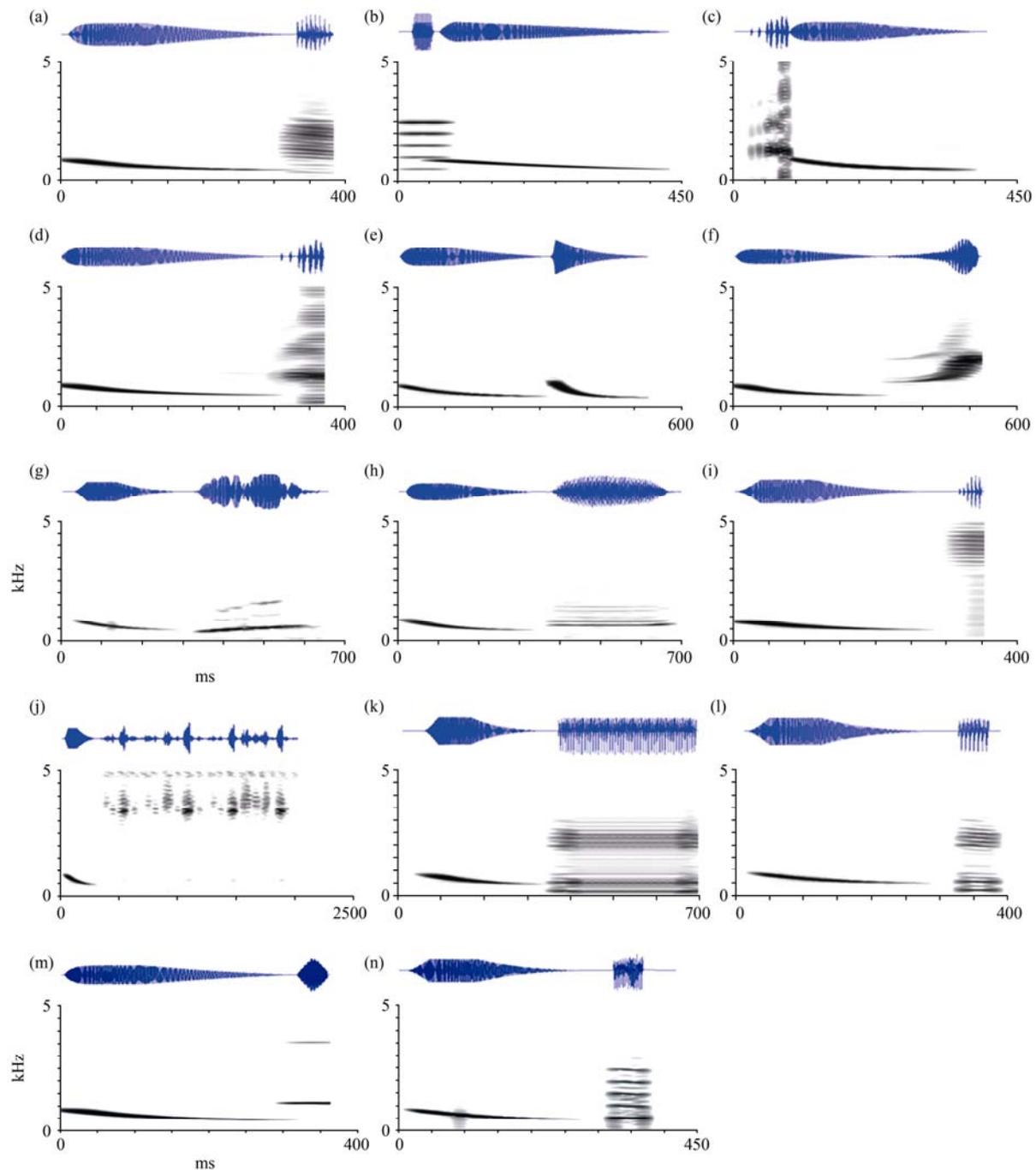
## 1 Materials and Methods

We collected and tested female túngara frogs between 1988 and 2002 at breeding sites near the research facilities of the Smithsonian Tropical Research Institute in Gamboa and its surrounding areas (9°7'N, 79°42'W, Panama). Females visiting choruses to select a mate were captured in amplexus. We took the pairs to the laboratory at the Smithsonian Tropical Research Institute facilities in Gamboa, and performed standard two-choice phonotaxis experiments. Once in the lab, we separated the pair and only used the females for our experiments. We performed the phonotaxis tests in a 1.8 m × 2.7 m acoustic chamber with speakers placed opposite one another in the center of the wall along the longer axis of the arena. The females were released at the center of the chamber where they waited under a funnel for 3 min while calls were broadcast antiphonally from the speakers. Calls were adjusted to a maximum whine amplitude of 82 dB SPL (re. 20  $\mu$  Pascals) at the release point of the females, which mimics the male's call at a distance of approximately 0.50 m (Ryan, 1985). The females were observed remotely using a video

camera and infrared light. A choice was scored if the female approached a speaker within 10 cm without following the walls of the chamber. The stimuli were broadcast until a female chose a speaker, she failed to choose or 15 min passed (see details on the rules used to determine a choice/failure to respond in Ryan and Rand,

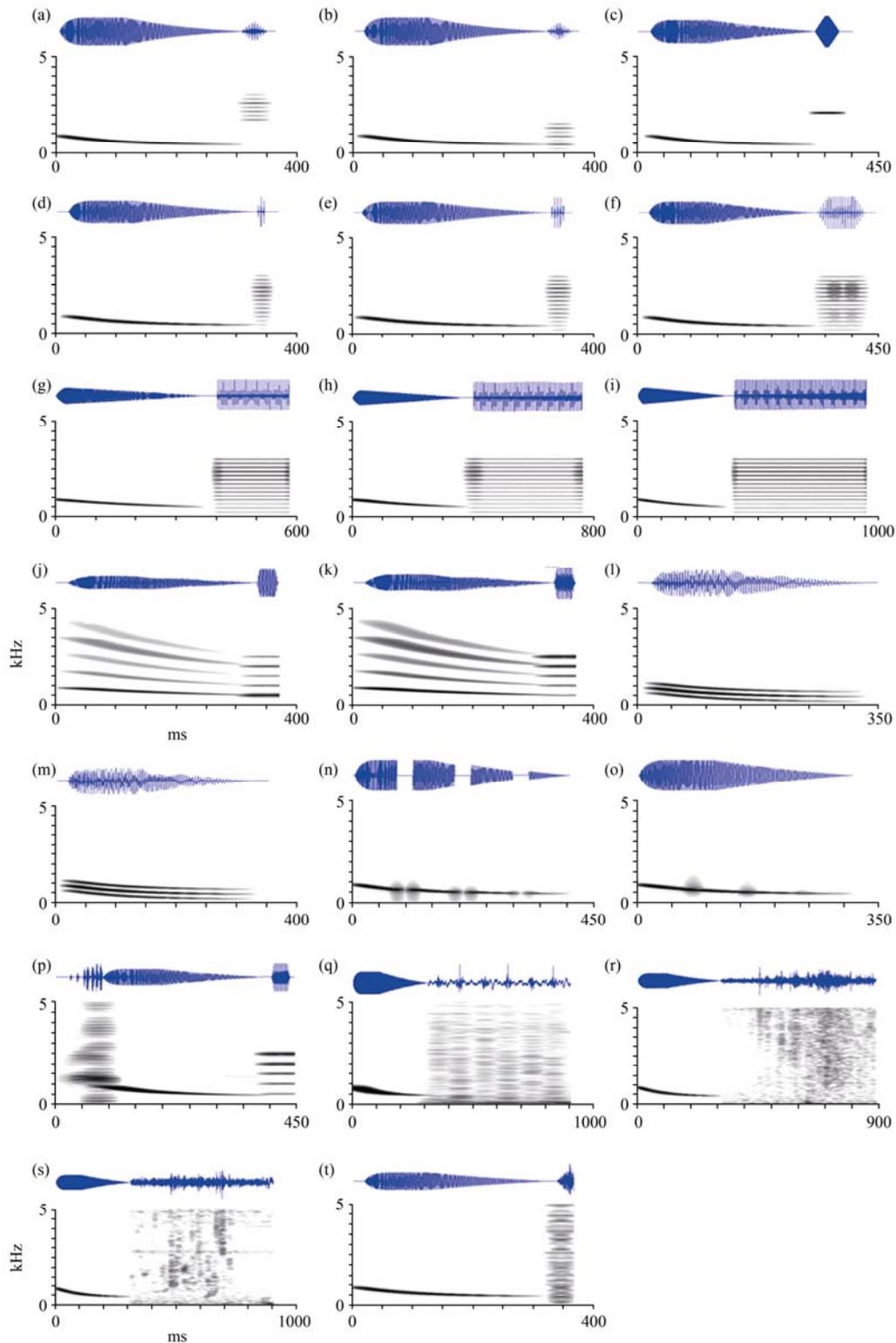
2003b). We released the frogs at the end of the night after marking them using individual toe-clip numbers at the same place where we originally collect them.

We used a combination of synthetic and natural stimuli to attempt to elicit latent female preferences (Figs. 2 and 3). We classify stimuli as elaborations, including



**Fig. 2** Synthetic and natural experimental stimuli used in female preference tests for whines with a variety of sounds as substitutes for a chuck

This figure illustrates 14 stimuli, matching those stimuli used in the tests reported in Table 1. (a) whine plus *P. petersi* squawk; (b) *P. petersi* squawk plus whine; (c) *P. randi* (cf. *pustulatus*) prefix plus whine; (d) whine plus *P. randi* prefix; (e) whine plus *P. petersi*; (f) whine plus *Leptodactylus fragilis*; (g) whine plus *L. pentadactylus*; (h) whine plus *Pleurodema brachyops*; (i) whine plus *Dendrophus phlebodes*; (j) whine plus chorus of hylids; (k) whine plus 321 ms ñññ; (l) whine plus 45 ms ñññ; (m) whine plus bells & whistles; (n) whine plus mew. Note that the frequency, on the Y-axis, is identical for all stimuli and extends from 0-5 kHz, while time, on the X-axis, differs among some stimuli.



**Fig. 3** Synthetic and natural experimental stimuli used in female preference tests for whines with manipulations and substitutions of the chuck in this and previous studies

This figure illustrates 20 stimuli, matching those used in the tests reported in Table 2 but omitting stimuli already depicted in figure 2. (a) whine plus high-half chuck; (b) whine plus low-half chuck; (c) whine plus 2100 Hz tone; (d) whine plus 15 ms chuck; (e) whine plus 23 ms chuck; (f) whine plus 90 ms chuck; (g) whine plus 180 ms chuck; (h) whine plus 360 ms chuck; (i) whine plus 580 ms chuck; (j) whine plus proto-chuck low Hz; (k) whine plus proto-chuck high frequency; (l) whine with 90% AM; (m) whine with 100% AM; (n) fragmented whine with 30 ms gaps; (o) fragmented whine with 2 ms gaps; (p) *P. randi* prefix plus whine plus *P. petersi* squawk; (q) whine plus bat wing-beats; (r) whine plus rustling; (s) whine plus splash; (t) whine plus white noise. Note that the frequency, on the Y-axis, is identical for all stimuli and extends from 0-5 kHz, while time, on the X-axis, differs among some stimuli.

reductions, or as innovations, as defined by Endler et al. (2005; Fig. 1). Although this classification of the stimuli is not definitive, it provides insights into the effect of different types of ornament variations as a chuck. Elaborations we used include variations in the duration, amplitude modulation and continuity of the chuck. Innovations consist of adding the aggressive call, call components of closely related and non-closely related species, and man-made sounds following the whine, as a chuck.

Using synthetic versions of the chuck, we evaluated the responses of females to whines with chucks that were altered in duration and amplitude modulation. We synthesized stimuli based on the mean values of the parameters of the chuck in the population by shaping sine waves using custom software (J. Schwartz, Pace University at Pleasantville, NY; sample rate 20 kHz and 8 bit). The experimental chucks were appended to synthetic whines. Variables for constructing the synthetic whines were based on mean values of a combination of the following eight spectral and temporal call parameters: maximum frequency, final frequency, duration, rise time, fall time, whine shape, rise shape, and fall shape (details in Ryan and Rand, 1995).

We used six synthetic versions of the chuck that vary in duration: two variants were shorter (15 ms, 23 ms), and four were longer (90 ms, 180 ms, 360 ms and 580 ms) than the average chuck in the population (43.8 ms, Ryan and Rand, 2003a). This variation in chuck duration expands beyond the duration of natural chucks, and the longer variants may be perceived as supernormal stimuli (Tinbergen, 1953; Enquist and Arak, 1993). With the exception of the duration of the chuck, all the other properties of this call component were kept constant.

The amplitude of the chuck relative to the whine varies between calls. Often as males start adding chucks in a calling bout, the relative amplitude of the chuck is low and, as the bout progresses the chuck increases in relative amplitude. An increase in the amplitude of the chuck also results in one bout of amplitude modulation. We asked if introducing amplitude modulation (AM) to the whine would make it more attractive, independent of an increase in the peak amplitude of the call as occurs with the addition of a chuck. We used two synthetic whines, the amplitude of both was modulated by a sine wave with a period of 4 ms. In one stimulus the depth of AM was 90% and in the other it was 100%. This manipulation changes the distribution of sound energy within the whine with no effect on the whine's peak amplitude.

The addition of a chuck makes the call discontinuous and sounds fragmented. Males of some species of frogs

partition the end of their call into separate or near-separate components in response to an approaching female. In *Pleurodema brachyops*, for example, males seem to fragment their calls in response to approaching females, and the resulting calls sound similar to a call with a túngara frog chuck (Ryan pers obs). To examine the effects of the discontinuity added by the chuck, we fragmented the whine into four sections of 78 ms each. In one stimulus these sections were separated by 30 ms and in the other stimulus they were separated by only 2 ms gaps. The total energy of the calls was kept intact but the duration of the calls was longer than the standard whine.

We used a variety of natural calls as substitutes for the chuck including aggressive calls of túngara frogs, calls of other species, and man-made sounds. The aggressive call of túngara frogs, the "mew", functions in interactions among males in the chorus and might play a role in maintaining fixed spatial distances among calling males (Ryan, 1985). The aggressive call is clearly different from the whine; it is longer in duration and has higher frequency and amplitude modulation than the advertisement call. We first examined whether mews are attractive to females, and then determined the response of females to whines with a mew in the position of the chuck. To investigate if the mew alone was recognized by females, we paired it with a burst of white noise from a second speaker in the phonotaxis experiment (more details about recognition tests is found in Ryan and Rand, 1995). The mew used in this study was recorded on BCI on May 1<sup>st</sup>, 1965. If this, or any other stimulus, failed to be recognized as a call by the females, we did not test it against the whine chuck. Similarly, if any stimulus was not as attractive as a whine we did not compare it to the whine chuck. We did, however, determine if a mew in the position of a chuck made the whine more attractive.

To investigate the selectivity of females to ornaments added to the whine, we examined the responses of females to whines with chuck-like ornaments from the sister clade to túngara frogs. Complex calls are known in some populations of the sister species of túngara frogs, *P. petersi* and *P. freibergi* (Ryan and Drewes, 1990; Boul and Ryan, 2004; Boul et al., 2007). Cannatella et al. (1998) described the sister clade of *P. pustulosus* as these two species, but given that species boundaries and distributions have not been defined for them, here we follow Funk et al. (2007) and refer to both species as *P. petersi*. In *P. petersi*, males from some populations facultatively add a secondary component to

the main whine-like component of their call. This secondary component, the squawk, seems to be homologous to the chuck produced by túngara frog males. Phonotaxis experiments demonstrated that *P. petersi* females from populations where complex calls are produced strongly preferred the simple call plus a squawk to the simple call alone (Boul et al., 2007). We added a *P. petersi* squawk, in the position of the chuck, to the whine of *P. pustulosus*. The squawk was recorded at Tambopata, Peru. Although other species in the *P. pustulosus* group do not produce complex calls, some species produce a short burst of sound preceding the whine known as the “prefix” (Ryan and Rand, 1993b; Ron et al., 2005). We added the prefix of the call of *P. randi* (the current name for the populations of *P. pustulatus* we studied) to the whine of *P. pustulosus*. We tested the effect of the prefix when added prior to the whine, its natural position, and also in the position of the chuck at the end of the call.

We also added calls of more distantly related species to the túngara frog whine, to further broaden our investigation of how the addition of other sounds in place of a chuck might enhance the attractiveness of the whine. We chose the call of a closely related species (*P. petersi*), the calls of other Leptodactylid species sympatric with túngara frogs (*Leptodactylus fragilis* and *L. pentadactylus*), calls of an allopatric member of the family (*Pleurodema brachyops*), and the calls of a distantly related hyliid (*Dendropsophus phlebodes*). We also offered females whines followed by a recording that contained the calls of several species commonly found at the breeding areas of túngara frogs. This recording of a chorus of mixed species was dominated by *D. ebraccatus*, *D. microcephalus* and *D. phlebodes*. All the calls, with the exception of the call of *P. petersi*, were recorded in central Panama.

We also investigated two arbitrary human-made sounds as substitute for chucks. One of us (ASR) produced a 321ms ‘ñññ’ nasal sound which we appended to a whine. As a variant we also tested a fragment of the ‘ñññ’ that approximated the duration of a chuck, 45 ms long. In addition, the sounds of simultaneous bells and whistles were added to a whine. Both stimuli, the ‘ñññ’ and the bells and whistles, represent novel ornaments that share few features with chucks and had not been previously heard by the frogs. Finally, we compiled the results of previous studies on female phonotaxis in túngara frogs that have explored the effect of variations and replacements of the chuck for different purposes.

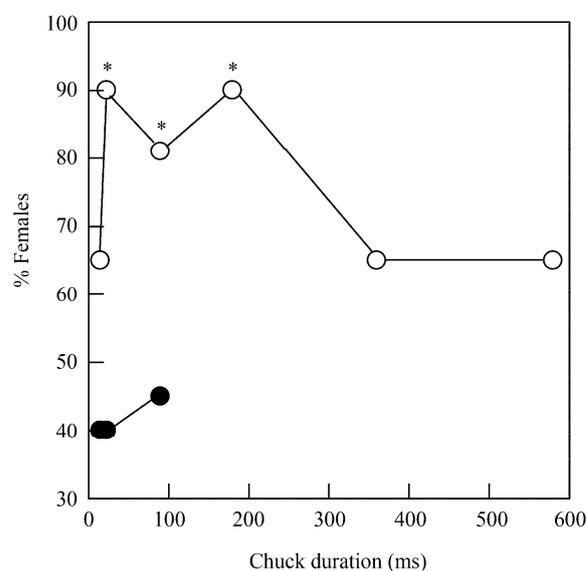
We tested the null hypothesis of no preference (50:50)

by computing the exact binomial probability for each experiment (pair of stimuli presented together). If significantly more females exhibited phonotaxis to one of the stimuli this was considered evidence for female preference. In most experiments we tested a total of 20 females, but for a few only 10 females were tested (i.e. 90% and 100% AM) and for others more females were tested (i.e. 90 ms chuck,  $n=42$ ). Sample sizes for each test are presented in Table 1.

## 2 Results

### 2.1 Elaborations: chuck manipulations

Chuck duration increased the attractiveness of the whine over almost an eight-fold range in duration (23 to 180 ms, Fig. 4). Females were preferentially attracted to whines with chucks that were 23, 90 or 180 ms long compared to whines only. This variation extended from approximately -2 to +12 standard deviations from the mean chuck duration in this population (mean = 43.8,  $SD = 11.0$ ; Ryan and Rand 2003a). Longer chucks were not, however, perceived as supernormal stimuli; that is, they were no more attractive than a normal whine-chuck. Chucks with durations of 15, 360 and 580 ms did not enhance the attractiveness of the call. Whines followed by these extremely short (-3.5  $SD$ ) or long (+28.8 and +48.8  $SD$ ) chucks were similar in attractiveness to a whine-only.



**Fig. 4 Female preferences in phonotaxis test in which the duration of the chuck of a whine-chuck call was varied**

Open circles indicate tests of a whine-chuck against a whine only. Close circles are test versus a whine followed by an average duration chuck (mean = 43.8,  $SD = 11.0$ ; Ryan and Rand, 2003a). In each test  $N=20$  females, with the exception of whines with chucks of 90 ms versus simple whine where  $n=42$ . The asterisk indicates  $P < 0.05$ , exact binomial probability test.

Female preferences were sensitive to amplitude modulation of the whine. When whines were altered with either 90% or 100% amplitude modulation over a 4 ms period, amplitude modulated whines were less attractive than standard whines; when presented with a AM whine versus an average whine, none of the females chose the former (exact binomial  $P < 0.001$ ; 90% AM: 0 out of 10 females; 100%AM: 0 out of 10 females). This lack of preference might result from the AM part of the whine being perceived as a short whine followed by multiple but very short duration chucks reducing the attractiveness of the call.

Fragmenting a whine into four 78 ms sections separated by gaps of either 30 ms or 2 ms did not influence its attractiveness either positively or negatively. The number of females that chose the fragmented whine was about the same to those that chose the normal whine (30

ms gaps, 4/6, exact binomial  $P = 0.754$ ; 2 ms gaps, 7/13, exact binomial  $P = 0.344$ ).

## 2.2 Innovations: chuck substitutions

When a mew was presented in recognition tests (i.e. competed against a speaker producing white noise of equal duration to the whine, (Ryan and Rand, 1995)), only three out of 30 females chose the mew (Table 1). Thus the mew did not signal an appropriate mate to females. Adding the mew to the whine, however, had no effect on the attractiveness of the call.

The squawk of *P. petersi* and the prefix of *P. randi* made the whine more attractive than a simple call (Table 1). Each of these stimuli could be at the end or the beginning of the call and they still increased the attractiveness of a whine. A whine preceded by a prefix and with a squawk appended to it, however, did not act as a supernormal stimulus.

**Table 1** Results of phonotaxis experiments in female túngara frogs for whines with a variety of sounds as substitutes for a chuck

	Stimuli		Female choices	<i>P</i>
	whine+chuck	vs. whine	18:2	<b>&lt;0.001</b>
Aggressive call	mew (only)	vs. noise vs. whine	3:30	0.251
	whine+mew		12:8	0.503
Ornaments from other species	whine+squawk		14:6	0.115
	squawk+whine		18:2	<b>&lt;0.01</b>
	prefix+whine		16:4	<b>0.012</b>
	whine+prefix		18:2	<b>&lt;0.01</b>
		vs. whine+chuck		
	whine+squawk		12:8	0.503
	squawk+whine		11:9	0.823
	prefix+whine		8:12	0.503
	prefix+whine+squawk		12:8	0.503
Calls from other species of frogs		vs. whine		
	whine+ <i>P. petersi</i>		16:4	<b>0.012</b>
	whine+ <i>L. fragilis</i>		11:9	0.823
	whine+ <i>L. pentadactylus</i>		7:13	0.263
	whine+ <i>P. brachyops</i>		10:10	1.000
	whine+ <i>D. phlebodes</i>		6:14	0.078
	whine+ chorus hylids		2:18	<b>&lt;0.01</b>
Man-made sounds	'ñññ' - 321 ms		0:20	<b>&lt;0.01</b>
	'ñññ' - 45 ms		7:13	0.263
	bells & whistles		21:8	<b>0.016</b>

Female choices as the number of females that choose the whine with the chuck substitute: females that choose the whine or whine+chuck depending on the test. *P*-value for a two-tailed exact binomial test.

The call of other species of frogs appended to a whine increased, decreased, or had no effect on the attractiveness of a whine (Table 1). While the call of *P. petersi* in place of a chuck made the call more attractive, the calls of *L. fragilis*, *L. pentadactylus* and *Pleurodema brachyops* did not make the whine more attractive. The call of *D. phlebodes*, or a chorus of frogs dominated by small hylids, made the whine less attractive than a whine only. The man-made sounds we used made the whine more or less attractive. Appending the sounds of bells and whistles enhanced whine attractiveness while the long (341 ms long) nasal sound ‘ñññ’ made it less attractive and the short (45 ms long) ‘ñññ’ made the stimulus as attractive as a whine. The preference for whines with bells and whistles, however, was not significantly different from the preference for whine-chuck (Table 1; Fisher’s exact test,  $P=0.098$ ,  $n=51$ ).

### 3 Discussion

Latent female preferences have the potential to drive the evolution of male ornaments. The results we present here show that such latent potential in túngara frogs is considerable. To evaluate the generality of our findings, we further broadened the stimuli examined by compiling the results of previous studies on female phonotaxis in túngara frogs that explored the effect of variations and replacements of the chuck for different purposes.

Table 2 summarizes the results of this study combined with those of previous ones. The results from previous studies are consistent with those found here. A broad array of stimuli increased attractiveness of the whine to females. Frequency manipulations of the chuck that constrain its frequency range so it would only stimulate one of the two inner ear organs (amphibian papilla or basilar papilla) were, for instance, effective at eliciting a preference as long as each stimulus had the total amount of energy as in the normal chuck. The lower-frequency chuck, however, did not make the whine more attractive if it had the relatively low energy content it typically has in nature, while the higher-frequency chuck did make the whine more attractive with its natural, fairly high, energy content (Ryan and Rand, 1990, 1995). Similarly, a burst of white noise following the whine increased the attractiveness of the whine (Ryan and Rand, 1995).

Some manipulations of the chuck did not enhance the attractiveness of the whine. Males from other species of *Physalaemus* in western Ecuador produce calls in which they increase the amplitude at the end of the whine maintaining the spectral structure of the whine. Those

secondary components mimic the general waveform of a whine-chuck without creating the subharmonics that characterize chucks, which include a fundamental frequency one-half that of the fundamental of the whine (Ryan, 1985; Gridi-Papp et al., 2006; Bernal et al., 2009a). We thus refer to these calls as proto-chucks. Female túngara frogs did not discriminate in favor of whines followed by a proto-chuck. There was, however, a strong tendency to prefer the whine with a proto-chuck when the dominant frequency in that last section of the call was shifted upwards to match that of the natural chuck (unpublished data). Whines with a proto-chuck were less attractive to females than normal whine-chuck calls even when the dominant frequency of the proto-chuck corresponds to that of a chuck (unpublished data).

On a few occasions, sounds appended to a whine reduced the attractiveness of the call. Sounds associated with an increased risk of predation, such as those produced by the wing-beats of a frog-eating bat or approaching predators, made the whine less attractive (Bernal et al., 2007). Interestingly, the call of *D. phlebodes* or a small chorus of small hylids appended to a whine was also discriminated against by female túngara frogs.

This study, combined with results from previous studies, shows that a variety of stimuli increase the attractiveness of the simple whine call to female túngara frogs. These stimuli thus mimic the chuck’s effects but never surpass it; that is, these stimuli when added to the whine do not make that call more attractive than a whine-chuck. Up to this point, we have not found a supernormal stimulus relative to the whine-chuck. Lack of a supernormal stimulus response to the whine is expected given its critical role for species recognition (Márquez and Bosch, 1997), but it is surprising to find that a variety of stimuli can replace a chuck but so far none of the ones tested increases call attractiveness beyond that of a natural whine-chuck. Contrary to our expectations, extra-long chucks (+28.8 and +48.8 SD) did not trigger a supernormal stimulus response but instead had no effect on the attractiveness of the whine. Overall we found that latent female preferences for ornaments can be in the form both of elaborations and innovations.

In terms of elaborations, the basic chuck still maintained its attractiveness to females with changes within certain ranges of duration, including a drastic increase in duration up to 12 standard deviations from the mean, and a drastic reduction in frequency range, including replacing the 15 harmonic frequency spectrum of the

**Table 2 Summary of the responses of female túngara frogs in phonotaxis to whines with manipulations and substitutions of the chuck compiling results from this and previous studies**

Type of ornamentation	Stimulus	energy content <sup>1</sup>	vs. whine			vs. whine+ chuck			References
			ns	+	-	ns	+	-	
<b>Elaborations</b>									
Chuck manipulations	<i>Frequency alterations</i>								Ryan and Rand (1990,1995)
	whine+high Hz chuck	relat			■				
	whine+low Hz chuck	relat	■						
	whine+ high Hz chuck	full			■	■	■		
	whine+ low Hz chuck	full			■	■	■		
	whine+tone (2.1 kHz)	full			■				
	<i>Duration</i>								This study
	whine+15ms	full	■			■	■		
	whine+23ms	full			■	■	■		
	whine+90ms	full			■	■	■		
	whine+180ms	full			■	■	■		
	whine+360ms	full	■						
	whine+580ms	full	■						
	<i>Proto-chucks</i>								unpublished
	whine+protochuck low Hz	manip	■					■	
	whine+protochuck high Hz	manip		■				■	
	<i>Amplitude Modulation</i>								This study
	whine+90% AM	manip.			■				
	whine+100% AM	manip.			■				
	<i>Fragmented whine</i>								This study
	30 ms long gaps	full	■						
	2 ms long gaps	full	■						
<b>Innovations</b>									
<b>Chuck substitutions</b>									
	<i>Aggressive call</i>								This study
	whine+mew	full	■						
	<i>P. petersi</i> squawk	full							This study
	whine+squawk	full	■			■	■		
	squawk+whine	full			■	■	■		
	<i>P. randi</i> prefix	full							This study
	prefix+whine	full			■	■	■		
	whine+ prefix	full			■	■	■		
	prefix+whine+squawk	full				■	■		
	<i>Heterospecific call</i>								This study
	whine+ <i>P. petersi</i>	full			■				
	whine+ <i>L. fragilis</i>	full	■						
	whine+ <i>L. pentadactylus</i>	full	■						
	whine+ <i>P. brachyops</i>	full	■						
	whine+ <i>D. phlebodes</i>	full				■			
	Whine+ chorus hylids	full				■			
	<i>Predator related sounds</i>								Bernal et al. (2007)
	whine+bat wing	full				■	■		
	whine+rustling	full				■	■		
	whine+splash	full				■	■		
	<i>Man-made sounds</i>								
	whine+bells & whistles	full			■				This study
	whine+321ms ‘ñññ’	full				■			This study
	whine+45ms ‘ñññ’	full	■						
	whine+white noise	full			■				Ryan and Rand (1995)

<sup>1</sup> *full*: The chuck variant has the total energy content of a full chuck. Typically the chuck/variant is scaled to have a maximum peak amplitude twice that of the whine. *relat*: the higher and lower half harmonics are in an energy ratio of 9:1, as in the wild (Ryan et al., 1990). *manip*: the chuck variant has an arbitrary amplitude.

Dark grey boxes indicate: ns= no significant preference ( $P > 0.05$ ), + = significant increase on the attractiveness of the whine ( $P < 0.05$ ), - = significant reduction on the attractiveness of the whine ( $P < 0.05$ ). Light grey box indicates a strong tendency ( $P < 0.08$ ).

chuck with a single tone. Disrupting the whine with amplitude modulations, fragmenting it, or appending a proto-chuck to it did not enhance its attractiveness. Although some manipulations defeat the chuck's function of increasing whine attractiveness, substantial changes of this component do not disrupt this function.

The more surprising set of results from this study and some previous ones (Ryan and Rand, 1990, 1995; Bernal et al., 2007), is the range of innovations as attractive as the true chuck. Call prefixes and suffixes from other closely related species, and even a burst of white noise, enhance the attractiveness of the whine to a similar degree as a chuck. This would give the impression that all "bells and whistles" that could be added to the whine would make it more attractive. That assertion is true in a literal sense, as bells and whistles shaped to the waveform of a chuck mimic the chuck's effects on the whine. In the real sense not all "bells and whistles" enhance the whine, as there are a number of stimuli that do not make the whine more attractive, including addition of the túngara frog's aggressive call, a human nasal sound, and sounds associated with predators. A wide range of innovations elicit enhanced attractiveness of the simple mating call, but there are also sounds that do not trigger this response including some that probably behoove the female to avoid, such as sounds that might indicate approaching predators.

### 3.1 Evolution of the chuck

We have shown a wide degree of latent preferences that could have directed the evolution of more attractive male traits along various evolutionary pathways. Why did males evolve chucks and not bursts of noise or bells and whistles? The answer seems to be the starting condition of the male's morphology. This is a constraint in the sense of Maynard Smith et al. (1985) — an aspect of the animal's biology that biases the production of variant phenotypes. As noted previously, túngara frogs have large extensions of the vocal folds, known as fibrous masses, which extend into the region in which the lungs connect to the larynx. It has been suggested that these masses are responsible for the production of the chuck (Drewery et al., 1982). The suggestion was borne out by comparative studies linking laryngeal morphology and bioacoustics among closely related species and between populations of *P. petersi* that did and did not produce complex calls (Ryan and Drewes, 1990; Boul and Ryan, 2004). This hypothesis received experimental verification when Gridi-Papp et al. (2006) surgically ablated the fibrous masses resulting in the inability of males to produce chucks. Although males attempted to produce

chucks by increasing call amplitude at the end of the whine, discrete call components with subharmonics did not appear. Recently, Bernal et al. (2009a) speculated about the biophysics underlying chuck production. They stated that the subharmonics of the chuck provide evidence for a nonlinear phenomenon that appears to be caused by nonlinear mechanical dynamics of the vocal production mechanism. Specifically, fibrous masses attached to the vocal folds might undergo impact oscillation at a sufficiently high excitation level to produce the subharmonics in the chuck. Subharmonic generation by impact oscillation, also known as clapping or impact nonlinearity, has been documented in many dynamic systems (Hindmarsch and Jefferies, 1984; Tournat et al., 2004; Pippard, 2007). Thus to understand why males evolved chucks, we need to understand why males evolved large fibrous masses. Is there something about the male's laryngeal morphology that biases to do so?

Although the large fibrous masses in túngara frogs and some species of *P. petersi* are responsible for chuck production, other species also have fibrous masses, albeit smaller ones (Drewry et al., 1982; Ryan and Drewes, 1990; Boul and Ryan, 2004). One function of these structures is to load mass onto the vocal cords and thus lower the call's frequency (Martin, 1972). There are some general advantages to lower-frequency calls: they transmit for longer distances in the environment (Morton, 1975), and in túngara frogs female prefer lower-frequency chucks (Ryan, 1980; Ryan, 1985) and lower-frequency whines (Bosch et al., 2000). Thus there might be selection favoring large fibrous masses and the resultant lower-frequency calls. An exaggerated response to that selection could lead to the evolution of a mass large enough that when subjected to impact oscillation at sufficiently high excitation levels results in a chuck (Bernal et al., 2009a). Despite the latent preferences for a variety of adornments, the general presence of smaller fibrous masses could have biased males towards eventually evolving a chuck and thus the constraints on male sound production could lead to the association we see today between male chucks and female preferences for them. This is why males did not evolve bells and whistles.

### 3.2 Evolution of preference for chucks

The above argument assumes the existence of latent female preferences and asks why the chuck but not other sounds evolved. We can also ask why females have this particular set of latent preferences. One possibility is that the preference for this set of adornments results from the additional neural stimulation added to

the whine. This argument suggests that the whine is critical for species recognition, as has been aptly demonstrated (Wilczynski et al., 1995), and once the female recognizes the male as conspecific added acoustic energy functions to further stimulate her, even if this energy is not very specific. This latter point has been demonstrated in this study.

Frogs have two inner ear-organs that are sensitive to air-born sound: the amphibian papilla (AP), which is most sensitive to frequencies below 1200 Hz, and the basilar papilla (BP), which is most sensitive to frequencies above 1200 Hz (Capranica, 1977; Gerhardt and Schwartz, 2001). The low-frequency of the whine primarily excites the AP. The chuck's dominant frequency is fairly close to the most sensitive frequency of the BP. Thus the chuck might be especially efficient at increasing neural stimulation (Ryan et al, 1990). But adornments that do not stimulate the BP, such as a synthetic chuck with only the lower harmonics, also make the whine more attractive. Thus both inner ear organs can serve as conduits for increased stimulation favored by sexual selection. By this argument, the preference for call adornments in general, and the chuck specifically, is a by-product of the general design and function of the frog's auditory system.

An alternative, and not mutually exclusive, view is that selection has favored females to prefer calls with adornments. In one scenario, calls with adornments might be easier to localize by females, and thus reduce search time, or might be more efficient at stimulating the female's reproductive state. If so, selection could favor preferences for adornments added to the whine. It is also possible that there is an association between male quality and the ability to produce complex calls, whether the call ends in a chuck or in some other adornment. If so, selection would favor females to prefer complex calls to simple calls regardless of the acoustic details of the complex call. It might then follow that either the chuck was the most likely adornment to be produced given the male's calling morphology, or for some reason chucks were better indicators of male quality than other adornments. The latter seems less likely since we have shown that other adornments can be as attractive as chucks.

There are two types of quality that could be indicated by the call. One is genetic. Males that produce more complex calls might advertise heritable variation for survivorship. This has never been directly tested and thus remains a possibility. Another quality relates to the direct benefits that females obtain from males, and there

is evidence for this sort of benefit (Ryan 1985). In túngara frogs, males only provide sperm and assist in nest-building. Females mating with larger males enjoy lower numbers of unfertilized eggs (Ryan, 1985). Male size correlates with the dominant frequency of both the chuck and the whine, and the female's preference for larger males results from their preference for lower-frequency chucks (Ryan, 1980, 1985) and, to a lesser extent, for lower-frequency whines (Bosch et al., 2000). Thus females might gain a benefit from preferring complex calls because it gives them added information about male size. As males tend to escalate call complexity in response to calls of other males (Ryan, 1985; Bernal et al. 2009b; Goutte et al. 2010), the number of chucks being produced is a good indication of male density and thus a larger pool of males from which females can sample, which might also be advantageous to females (Bernal et al. 2007).

Earlier comparative analysis suggested the preferences for chucks existed before the chucks evolved (Ryan and Rand, 2003a), deeming it not parsimonious to invoke an adaptive advantage for the origin of the preference for chucks. Recent studies by Ron (2008), however, have shown that some other species of *Phyllotriton* are repelled by their own mating calls to which chucks of a túngara frog call are added. In addition, Boul et al. (2007) have shown that although there are not statistically significant differences in the strength of preference for complex calls between females from populations with and without complex calls, the trend is for a stronger preference in populations with complex calls. Thus the presence of preference for complex calls in species lacking complex calls themselves is more labile than we had previously suspected, and at this point it is equivocal as to whether the behavioral preference for the chuck arose prior to and coincident with the chuck.

### 3.3 Latent preferences in other taxa

Responses to novel stimuli, such as sticklebacks attacking red postal vans and geese retrieving large balls instead of eggs into their nests, are a hallmark of early ethological studies (Tinbergen, 1953). A number of studies have used artificial traits to test the responses of females to novel stimuli in a variety of taxa. Adding sexually selected traits from heterospecific males to conspecific males has shown that females often prefer males with foreign and novel traits. For example, females from an unsworded relative of swordtail fish, *Xiphophorus maculatus*, prefer males with a sword (Basolo, 1990, 1995). Similarly, in wolf spiders of the

species *Schizocosa rovnieri*, females are attracted to males with tufts of bristles on their forelegs, as the ones present in males of the closely related species *S. ocreata* even though conspecific males lack tufts (McClintock and Uetz, 1996). Manipulating males to evaluate female preferences has gone a step further adding novel traits, not present in closely related species and often chosen arbitrarily. In a series of elegant studies, Burley and her co-workers (1982, 1986) added leg bands and feather ornaments to zebra finches, *Taeniopygia guttata*. Female zebra finches are more attracted to males wearing red leg bands, white crests or flashy anklets of red, yellow, lavender or power blue than to standard conspecific males. Similarly, in two species of grassfinches (Burley and Simanski, 1998) and in Javanese mannikins (Witte and Curio, 1999) females prefer artificially added crests, and female American goldfinches are more attracted to males wearing orange bands (Johnson et al., 1993). Female mating preferences for novel traits in birds can arise through sexual imprinting (Witte et al., 2000; Burley, 2006). This probably is not the case, however, for other taxa in which imprinting has not been as clearly demonstrated. Besides the examples of platyfishes and wolf spiders noted above, this would include female preferences for novel traits in sailfin mollies using visual signals (Ptacek and Travis, 1997; Schlupp et al., 1999), jumping spiders producing seismic signals (Elias et al., 2006) and flat lizards using chemical cues (Lewis et al., 2007). As this short summary illustrates, latent female preferences are apparent in a broad diversity of taxa and across sensory modalities. Although perhaps more intensely investigated in túngara frogs, these frogs do not appear to be an exception to the rule.

In conclusion, many studies have examined in great detail how and why females exert preferences for extant male traits to gain some understanding about the process of sexual selection. Here we employed a more expansive view of female preferences, and we exploited the female potential to generate selection for traits that do not exist. We have uncovered in female túngara frogs an amazing range of latent preferences for traits that do not exist in túngara frogs nor have any history of existing in their antecedents. Studies of this latent potential of females, we suggest, might provide some additional insights into how sexual selection by female choice is responsible for the evolution of the incredible diversity of morphologies and behaviors that so impressed Darwin and most of his intellectual descendants.

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