



The evolution of female mate choice for complex calls in túngara frogs

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Female mate preferences can favour the evolution of complex secondary sex traits in males. A mechanism increasingly explored to explain the origin of such male traits is sensory exploitation. Under sensory exploitation, female mating preferences are by-products of sensory biases that originate previously (often in nonreproductive contexts). Túngara frogs (*Engystomops*) have offered a widely known example for this mechanism. Male *E. pustulosus* make a complex call by adding a chuck to their typical whine call. Previous studies reported that the evolution of complex calls was driven by a female sensory bias that originated in the common ancestor of *Engystomops*, before the origin of complex calls. Here, I carry out mate choice experiments and new phylogenetic and comparative analyses to reevaluate the evolution of female mate choice and courtship calls in túngara frogs. My results indicate that the chuck and its corresponding female preference have coevolved in a process that is compatible with Fisherian or good genes sexual selection. Character reconstructions show that both the chuck and the female preference have originated multiple times among closely related lineages. I found no support for previously proposed scenarios of sensory exploitation in *Engystomops*, and the mate choice experiments indicated that increased stimulation of the sensory system by the male courtship signal does not necessarily translate into increased female preference. This finding highlights the significance of central processing in mate choice decisions.

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Strong female preference for costly, conspicuous or elaborate secondary sexual traits in males explains why such seemingly disadvantageous traits are maintained. How such traits are initially favoured, however, is an ongoing debate. The sensory exploitation hypothesis (also known as 'sensory bias') states that female mating preferences are a by-product of pre-existing sensory biases, which evolved incidentally, under other selective pressures (e.g. prey acquisition). Subsequently, males evolve secondary sexual characters that match those biases (e.g. match the colour of prey) because females prefer them (West-Eberhard 1984; Ryan & Rand 1990, 1993b; Basolo 1995; Fuller et al. 2005). Unlike other sexual selection models (e.g. good genes or Fisherian selection), the sensory

exploitation hypothesis requires the preference to evolve before the trait.

Túngara frogs (genus *Engystomops*, formerly known as the *Physalaemus pustulosus* species group; Ron et al. 2006) provide a well-known example of sensory exploitation (Ryan & Rand 1993a, b, 2003a). For reproduction, túngara males aggregate at night to call in choruses. Females visit these choruses and actively choose a mate based on acoustic features of males' calls (Ryan 1985). The advertisement call is innate and consists of a whine-like sound that is necessary and sufficient for mate recognition. In *E. pustulosus* and some populations of the *E. petersi* species complex (including *E. freibergi*), males can add a facultative 'chuck' to the call (Rand & Ryan 1981; Boul et al. 2007; Fig. 1a). The chuck enhances call attractiveness to females in *E. pustulosus* and *E. petersi* from Yasuní (Ryan & Rand 1990; Boul et al. 2007). Traditionally, calls with chucks and without chucks have been referred to as 'complex' and 'simple', respectively.

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Sensory exploitation was proposed in these species because the origin of the female preference for the chuck seemed to precede the origin of the chuck itself (i.e. the chuck exploits a pre-existing sensory bias; Fig. 1b; Ryan & Rand 1993a). The sequence of origin was inferred from mate choice experiments showing that females of *E. coloradorum* prefer conspecific calls with three chucks even though *E. coloradorum* males do not produce chucks (Fig. 1b). The phylogeny therefore suggested that complex calls had originated more recently than the female preference and therefore exploited a sensory bias of prior origin (Fig. 1b).

There is a complementary, mechanistic hypothesis to explain the preference for complex calls based on neurophysiological characteristics of the frog's inner ear (hereafter referred to as the sensory-matching hypothesis; Wilczynski et al. 2001). The sound frequency sensitivity of the amphibian ear resides in two organs, the amphibian papilla (AP: low frequencies) and the basilar papilla (BP: high frequencies). The sensory-matching hypothesis proposes that females prefer complex calls because they stimulate both organs (whine stimulates the AP; chuck stimulates the BP) and are a better match for the ear's sound frequency sensitivity. Simple calls, in contrast, have been considered as stimulating only the AP (Ryan 1990; Ryan et al. 1990; Wilczynski et al. 2001). Thus, simple and complex calls have been defined by two separate criteria: the presence or absence of the chuck and their capacity to stimulate the BP. The consistency between both criteria, however,

has never been tested statistically. Findings of new call variants suggest that calls without chucks may significantly stimulate the BP (Ron et al. 2005), suggesting that both criteria could be mutually inconsistent.

The sensory exploitation hypothesis in túngara frogs relies on ancestral character reconstruction, which can be biased by taxon exclusion (Ackerly 2000). The reconstructions of ancestral female preferences that support this hypothesis are based on preference data from only two species of *Engystomops* (Ryan & Rand 2003a). Thus, expanding taxon sampling is needed to reliably reconstruct the evolution of female preferences. Recent studies (Boul et al. 2007; Guerra & Ron, in press) have increased female preference sampling and here I present additional preference data for three species. Based on these results, I reevaluate the evolution of advertisement calls and female mate choice in túngara frogs. I also evaluate the covariation between the presence of the chuck and its corresponding female preference and discuss one of the predictions of the sensory-matching hypothesis: that enhanced peripheral stimulation should result in enhanced call attractiveness.

METHODS

Túngara frogs belong to the genus *Engystomops* (Ron et al. 2006). They form a clade of ~10 species of small (16–35 mm) frogs, most closely related to *Physalaemus* (Ron et al. 2006). *Engystomops* contains two basal

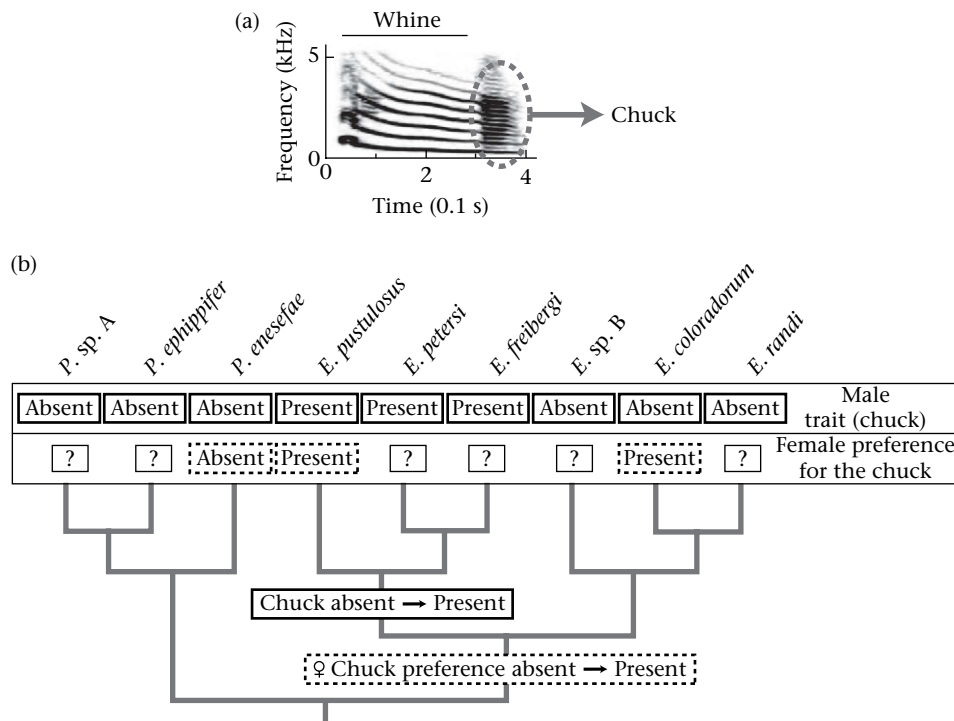


Figure 1. (a) Spectrogram of the complex call of *Engystomops pustulosus* showing the whine and the chuck (simple calls have only the whine). (b) Sensory exploitation hypothesis in túngara frogs as proposed by Ryan & Rand (2003a). In all species, the male call consists of a whine. In *E. pustulosus*, males can increase the attractiveness of the call to females by adding a chuck to the whine. Female mate choice experiments showed that the chuck of *E. pustulosus* also increase attractiveness in *E. coloradorum*. According to ancestral character reconstruction, the preference for the chuck preceded the origin of the chuck. Thus, males that produce the chuck were thought to be exploiting a pre-existing sensory bias in females.

groups: one distributed in western South America (clade Duovox) and the other distributed in Central America, northern South America, and the Amazon Basin (clade Edentulus). Species nomenclature has changed recently (Nascimento et al. 2005), and I follow the most recent review (Ron et al. 2006).

Female Preference and Male Trait Variation

I assessed whether the chuck of *E. pustulosus* enhances the attractiveness of male calls in three species of túngara frogs that do not produce chucks: *E. coloradorum*, *E. randi* and *E. sp. D*. Each phonotaxis experiment had at least 19 replicates. In each replicate, a wild-caught gravid female was given a choice between two male advertisement calls broadcasted antiphonally from speakers at opposite ends of a rectangular arena. One call was the conspecific advertisement call, whereas the other was the same call with one chuck from *E. pustulosus* artificially appended to the end. A choice was scored when the female came within 10 cm of either speaker.

To prepare the stimuli, I used natural calls from different males from the same population: 5 males in *E. randi*, 12 males in *E. coloradorum* and 12 males in *E. sp. D*. In each trial, each female heard two calls from the same male (modified versus unmodified). The specific male call to be used in each trial was selected with a random number generator. Male calls were recorded in the field with a Sennheiser ME-67 directional microphone and an analogue recorder SONY WM-DC6 or digital recorders SONYTCD-D8 or SONYMZ-NH1. I digitally appended a synthetic chuck to the calls with Cool Edit pro 2.0 (Syntrillium Software, Scottsdale, AZ, U.S.A.). Neither of the calls included a facultative high frequency suffix that can be produced by male *E. coloradorum* and *E. sp. D* (S. R. Ron, unpublished data).

I also tested in each of the three species the existence of female phonotaxis towards the conspecific call. Each female was presented with two stimuli broadcasted antiphonally: the conspecific call and white noise.

Experiments were carried out at night between January and February 2005 in two localities in western Ecuador: Tinalandia, Provincia de Pichincha (*E. coloradorum*) and Bosque Protector Puyango, Provincia El Oro (*E. randi* and *E. sp. D*). Females were collected between 1900 and 0100 hours from choruses; most females were in amplexus when caught. The experiments were carried out under darkness in a chamber (180 × 105 × 100 cm) with walls covered with padded foam to reduce reverberation. The distance between the centre of the arena and each speaker was 80 cm.

Before each trial, the female was placed in the centre of the arena under a funnel. Then, the two alternative stimuli were played for 3 min, after which the funnel was lifted. Female movements were monitored in real-time on a TV screen attached to an infrared video camera (Sony DCR-TRV70). The stimuli were broadcast from a computer connected to two speakers (Saul Mineroff Field Speakers SME-AFS, Elmont, NY, U.S.A.). Each stimulus was played from a different channel of a stereo sound file and

they were emitted antiphonally through the speakers. The peak amplitude of the call at the release site was 80 dB SPL (re. 20 µPa). The speakers faced each other from opposite ends of the chamber. Both stimuli were normalized to the same peak amplitude for the white noise. The call rate per channel was 0.5 calls/s for *E. coloradorum* and *E. sp. D* and 1.4 calls/s for *E. randi* (call rates matched natural call rates of each population). To avoid biases, I randomized the side from which the stimuli were broadcast in all trials. The floor of the chamber was always wet and room temperature was maintained between 21° and 23 °C. To score female choices, I followed the methodology of Ryan & Rand (2003b). After the experiment, females were released in the wild at their site of capture.

Female preferences for complex calls were analysed with binomial tests under a maximum likelihood estimator with the software Binomial Test (Engells 1988). For each experiment, I calculated the two-tailed binomial probability of the null hypothesis of no preference (0.5:0.5). Female responses to white noise versus the conspecific call were analysed with a two-tailed Fisher's exact test under a null hypothesis derived from *E. pustulosus* (Rand et al. 1992).

Phylogenetic Analysis

Analyses of character correlations and ancestral character reconstructions are based on phylogenetic trees derived from Bayesian inference. Phylogenetic analyses used the same matrix and alignment of mitochondrial DNA sequence data (genes 12S rRNA valine-tRNA and 16S rRNA; ~2.4 kb) presented by Ron et al. (2006) except that several population-level terminals were removed because they were invariant for the behavioural characters analysed. I also added one *E. freibergi* sample from Tambopata, Perú (GenBank accession EF011551) from Funk et al. (2007) because call data is available for this population. The model of character evolution for the analyses was the same as that used in Ron et al. (2006). Four Markov chains were used in each of two analyses, the prior for the rate matrix was a uniform dirichlet and all topologies were equally probable a priori. Each analysis ran for 2×10^6 generations. For each analysis, the chain was sampled every 200 generations. After 2×10^6 generations, the average standard deviation of split frequencies was ~0.001, indicating that the two analyses converged into a stationary distribution. The first 25% of sampled trees were discarded as the burn-in and the remaining trees were used for estimating Bayesian posterior probabilities. Phylogenetic analyses were carried out in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003).

Ancestral State Reconstruction

Ancestral character states for the preference for chucks in females and the origin of chucks in males were reconstructed with Bayesian inference. Bayesian inference was preferred over maximum likelihood reconstruction because it accounts for the uncertainty in the estimates of model parameters (Ronquist 2004). Preferences and calls were coded as binary characters. Preference data from

phonotaxis experiments with *E. coloradorum*, *E. randi* and *E. sp. D* were combined with results for *P. enesefae* from Tárano & Ryan (2002), *E. pustulosus* from Gridi-Papp & Ryan (2006) and *E. petersi* from Boul et al. (2007) and Guerra & Ron (in press). Experiments with *E. petersi* include three populations from which only one (Yasuni) produces chucks. In the three populations, females had to choose between the local whine versus the local whine with a chuck from Yasuni appended (Boul et al. 2007; Guerra & Ron, in press). I coded female preference as present or absent based on binomial tests on the female phonotaxis data (present if binomial $P < 0.05$).

Calls were classified as complex if they had a chuck. I scored the chuck as present (state 1) or absent (state 0), following Ryan & Rand (2003a) and Boul et al. (2007). Calls from Tampopata were coded as polymorphic (states 0 and 1; Boul et al. 2007). Calls of *E. coloradorum*, *E. pustulosus*, *E. sp. B* and *E. sp. D* can contain suffixes that have some structural resemblance to the chuck of *E. pustulosus* and *E. petersi* (S. R. Ron, unpublished data). The chuck, however, uniquely combines the following features: (1) a fundamental harmonic having half the frequency of the fundamental harmonic in the whine, (2) twice the number of harmonics of the whine, (3) a significant increase in dominant frequency relative to the whine and (4) a sudden increase in call amplitude. Species coded as chuck-absent always lack features (1) and (4) and almost always lack feature (2).

Bayesian character reconstructions were implemented using BayesTraits under the BayesMultistate method (ver. 1.0; Pagel & Meade 2006). To account for phylogenetic uncertainty, posterior probabilities of rate coefficients and ancestral character states were reconstructed using 500 trees (including branch lengths) randomly chosen from the post-burn-in trees from the Bayesian phylogenetic analyses. Posterior probabilities for character states were obtained from the reversible-jump Markov chain Monte Carlo approach described by Pagel & Meade (2006). I used a uniform prior for the models of evolution and an exponential prior for the rate coefficients. I did not specify a mean for the exponential prior but rather seeded this parameter from a uniform hyperprior. The range for the hyperprior was informed by maximum likelihood parameter estimates obtained from BayesTraits 1.0 under the BayesMultistate method. To ensure adequate mixing among chains, the amount of change in rate coefficients among generations in the MCMC (i.e. the *ratedev* parameter) was set to achieve acceptance rates ranging from 20 to 40%.

Support for the probable character states was tested by comparing two alternative models with Bayes factors. Under the first model, the character state of the target node was set to 0; under the second model, it was set to 1. The Bayes factor is the ratio of the marginal likelihoods of both models. Calculating the marginal likelihoods is computationally difficult. However, the harmonic means of the posterior distribution of likelihoods can be used as an alternative to the marginal likelihoods (Pagel & Meade 2006). The test statistic was calculated as twice the difference between the harmonic means of the posterior distributions of log-likelihoods of both models. Values higher

than 2 are considered positive evidence of support for the favoured model. For each test, the Markov chains were run for at least 1×10^8 generations to ensure stability in the values of the harmonic means. Each test was replicated several times to ensure consistent results.

Ancestral female preferences were also reconstructed as a continuous character (proportion of females preferring the whine–chuck call) with squared-change parsimony with weighted branches (Maddison 1991) as implemented in MESQUITE 1.12 (Maddison & Maddison 2005). Bayesian character reconstructions have the advantage over parsimony methods of accounting for mapping and phylogenetic uncertainty and allowing hypothesis testing for alternative character states (Ronquist 2004; Pagel & Meade 2006). However, square-change parsimony allows coding of female preferences as a continuous character, thus avoiding the information loss of discrete coding.

Taxon sampling of male calls includes all known species of *Engystomops* in addition to species from all species groups in the outgroup (*Physalaemus*). Unfortunately, carrying out phonotaxis experiments in regions of difficult access (e.g. *E. freibergeri* in Amazonian Peru) was unfeasible and as a consequence, sampling of female preferences is less exhaustive and includes 50% of the species of *Engystomops* and one species of *Physalaemus*. I tested the possible effects of incomplete taxon sampling in the reconstruction of female preference at the root of *Engystomops*, a crucial node to evaluate the sensory exploitation hypothesis. In this analysis, character states 0 and 1 were assigned randomly, with a probability of 50%, to all terminals lacking preference data in the phylogeny. The 50% probability is conservative because it is higher than the observed probability of preference (25%) and more likely to favour the presence of the preference at the root of *Engystomops*, which would support sensory exploitation. A total of 20 character sets were generated in this manner. Ancestral character states were reconstructed for each set with BayesTraits using the same methodology as that used for the real data set. Support for the state at the root of *Engystomops* was tested with Bayes factors.

Testing the Correlation between Male Trait and Female Preference

Testing for a correlation in the evolution of the sexual trait and its corresponding female preference can help to discriminate among alternative hypotheses of the underlying process of sexual selection. For example, under the Fisherian model of sexual selection, the origin of the trait is predicted to be phylogenetically correlated with the origin of the preference. In sensory exploitation, in contrast, a lack of correlation is expected unless the male trait tracks the sensory bias at a fast rate and between speciation events (Endler & Basolo 1998). I applied Pagel & Meade (2006) Bayesian test for correlated evolution to test the correlation between the chuck presence and its preference in females. The test requires the characters to be coded binarily. Female preference and the male trait were coded as in the ancestral character reconstructions. The test fits a continuous-time Markov model of character

evolution to two traits and tries to find the models that best explain their joint evolution. The search uses a reversible-jump Markov Chain Monte Carlo methodology to explore among dependent and independent models of character evolution. Under the dependent models, the rate of change between character states (e.g. preference present–absent) varies depending on the background state of the other character (e.g. chuck present–absent). Under the independent models, the rates of change are independent between characters. I used Bayes factors to compare the support between dependent and independent models. The test statistic was calculated as twice the difference between the harmonic means of the posterior distributions of log-likelihoods of dependent and independent models. Values higher than 2 are considered positive evidence of support for the favoured model. Markov chains were run for 1×10^8 generations and the test was replicated three times to ensure consistent results. To account for phylogenetic uncertainty, all results were integrated over a sample of 500 randomly chosen trees from the post-burn-in trees from the Bayesian phylogenetic analyses. All procedures were implemented in BayesTraits under the BayesDiscrete method (ver. 1.0; Pagel & Meade 2006).

Frequency Allocation and Sensory Matching

According to the sensory-matching hypothesis, whine–chuck calls are preferred because they stimulate both ear receptors (AP and BP; whine-only calls stimulate the AP only). This hypothesis predicts that complex calls should stimulate more strongly the BP than simple calls. I tested this prediction with two analyses. First, I measured the proportion of energy greater than 1.5 kHz and tested for differences, taking phylogeny into account, between complex calls and simple calls. The proportion of energy greater than 1.5 kHz quantifies the relative amount of energy available for BP stimulation (BP sensitivity is absent below 1.5 kHz in *Engystomops* and *Physalaemus*; Rand et al. 1992; Wilczynski et al. 2001). The test is based on a maximum likelihood ratio from the generalized least squares (GLS) approach proposed by Pagel (1997). A significant result would indicate that energy content differs between complex and simple calls. Analyses were carried out with the software Continuous (Pagel 1997) under the phylogeny presented by Ron et al. (2006).

Second, I compared the strength of the matching between ear frequency sensitivity and call frequency in species with simple and complex calls. Specifically, for each of six species, I compared the upper-frequency peak of male calls (which is closest to the BP best excitatory frequency; Fig. 2) and the best excitatory frequencies of the BP from individuals of the same population. Measurements for best excitatory frequencies were provided by W. Wilczynski and are summarized in Wilczynski et al. (2001). Comparisons were carried out with Kruskal–Wallis tests.

Call energy content and peak frequency were measured using CANARY 1.2.1 (Charif et al. 1995). Voucher and locality information for call recordings is available from the

author upon request. For each taxon, I analysed calls from five individuals. Relative energy content (as energy flux density) was measured over a spectrogram generated with a fast Fourier transformation with a frequency resolution of 87.4 Hz and 2048 points (sampling frequency = 44.1 kHz). In *E. coloradorum*, *E. pustulatus*, *E. sp. B* and *E. sp. D*, I only selected calls containing high frequency suffixes (to be consistent with the choice of chuck-appended calls in *E. pustulosus* and *E. petersi*). All *E. pustulosus* calls had one chuck.

RESULTS

Phylogenetic Analysis

The Bayesian tree topologies for both independent analyses were identical. The consensus phylogeny, including nodal support values, is shown in Fig. 3. Nodal support was strong (posterior probability = 1.0) for all but three nodes. The topology was consistent with the phylogenies reported by Ron et al. (2006) and Funk et al. (2007). The phylogenetic relationships of *Engystomops* are discussed in depth in Ron et al. (2006) and are not further considered here.

Reconstructions of Female Preference and the Origin of Chucks

Females of the three species tested showed the ability to make phonotactic discriminations under my experimental set-up by expressing a strong preference for their natural call versus white noise (Table 1).

Chuck preference tests indicated that addition of the *E. pustulosus* chuck did not increase call attractiveness in any of the three species tested (Table 2). In *E. sp. D* and *E. randi*, only 32% and 26% of the females, respectively, preferred the complex call (Table 2). The lack of preference for complex calls in *E. coloradorum* contrasts with findings by Ryan & Rand (1993a) for the same species (see Discussion).

Both the Bayesian and the parsimony reconstruction of ancestral female preferences showed that the preference for the chuck was absent in *Engystomops*' most recent common ancestor (node B in Fig. 4, Table 3). According to the Bayesian reconstruction, there have been two independent origins of the preference for the chuck: one in *E. petersi* from Yasuní and another in *E. pustulosus* or in the most recent common ancestor of clade Edentulus (node D). The Bayesian relative probability favoured the absence of the preference at node D but its support was nonsignificant (Bayes factor = 0.64; Fig. 4). Therefore, the preference for chucks at node D cannot be ruled out. The reconstruction significantly rejected the preference for the chuck in the most recent common ancestor of clade Duovox (node C). The parsimony reconstruction showed opposite trends in female preference in clades Duovox and Edentulus (Table 3). In the most recent common ancestor of Duovox (node C), only 33% of females were predicted to prefer the chuck. In the ancestor of Edentulus

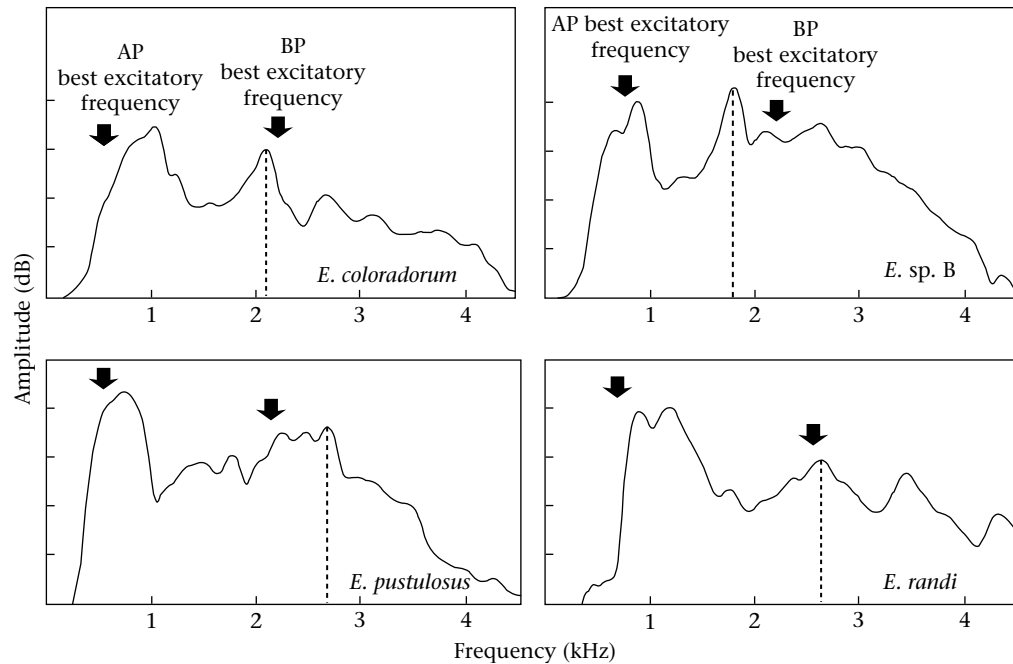


Figure 2. Power spectrum of typical advertisement calls of *Engystomops*. Each graph represents the distribution of sound intensity level across frequencies (from averages along the entire call). Tick marks on the Y axes denote 10 dB intervals. Black arrows indicate the frequencies of highest sensitivity (best excitatory frequency) of the female ear (from Wilczynski et al. 2001): left arrow for the amphibian papilla (AP), right arrow for the basilar papilla (BP). Calls have a low-frequency peak that is close to the AP peak sensitivity and an upper-frequency peak (dashed line) that is close to the BP peak sensitivity. The *E. pustulosus* call is a whine–chuck; the calls for *E. coloradorum*, *E. randi* and *E. sp. B.* are whine-only.

(node D), 81% of females were predicted to prefer the chuck.

The reconstruction of the male trait showed that the presence of the chuck was not the ancestral state in *Engystomops*, suggesting instead that there have been two or three independent origins of the chuck within the clade *Edentulus* (Fig. 4). Under the two-origins scenario, the chuck originated in *E. petersi* from Yasuní and node D; several scenarios are possible for three origins (e.g. in *E. petersi* from Yasuní and nodes I and J). Although the reconstruction favoured the presence of the chuck in the most recent common ancestor of *Edentulus*, support for this state was nonsignificant (Bayes factor = 1.12).

When character states of female preference were randomly assigned to terminals lacking female preference data, ancestral reconstructions became ambiguous for the ancestor of *Engystomops* (node B) in all 20 replicates. The mean \pm SE posterior probability for the presence of the preference was 0.485 ± 0.014 ($N = 20$ replicates).

Correlation between Preference and Trait

Chuck preference tests have been carried out in eight taxa (Table 2). Females have shown a preference for chucks in two of them, precisely in the taxa on which males produce chucks (Fig. 4, Table 2). The test of correlated evolution resulted in a Bayes factor of 3.635, favouring the model of dependent evolution. This result indicates that the chuck and its female preference have evolved in concert.

Frequency Allocation and Sensory Matching

According to the sensory-matching hypothesis, complex calls are preferred because they stimulate both the AP and the BP (simple calls stimulate the AP only). Thus, calls with chucks (complex) should have a higher content of energy available for BP stimulation (above 1.5 kHz). The energy measurements did not support this prediction because calls with and without chucks were not significantly different in energy content above 1.5 kHz (maximum likelihood ratio test $P = 0.546$). Simple calls of most species had more energy available for BP stimulation than did complex calls of *E. pustulosus* (Fig. 3).

Measurements of the strength of sensory matching also suggest that stimulation of the BP is comparable between calls with and without chucks. Contrary to the expectations, the closest signal-receptor match occurred in two species having simple calls (Table 4, Fig. 2). In contrast, the complex call of *E. pustulosus* showed significant differences between call peak frequency and the ear's best excitatory frequency.

DISCUSSION

The Bayesian character reconstructions suggest that both the chuck and the female preference for it originated independently more than once and are highly homoplastic (Fig. 4). Interestingly, the gains of chucks and preferences were clustered on a single clade (*Edentulus*). This pattern clearly suggests that this clade is a phylogenetic hot spot

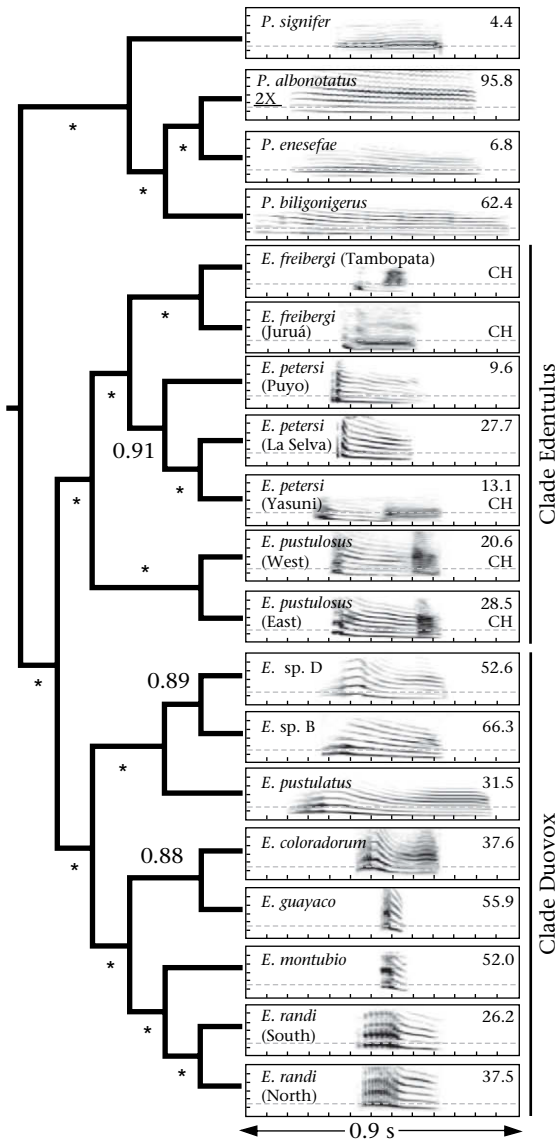


Figure 3. Bayesian consensus tree of *Engystomops* (with *Physalaemus* as outgroup) based on analyses of 2422 base pairs of mitochondrial DNA sequence data. Numbers on branches are Bayesian posterior probabilities from post-burn-in trees; asterisks indicate 1.0 posterior probability. Spectrograms of the advertisement call of all terminals are shown next to the tree. Numbers next to the spectrograms represent the percentage of energy above 1.5 kHz (total energy = 100%; measurements for *E. freibergi* complex calls were not available). The percentage offers an estimate of the amount of energy available for the ear’s basilar papilla (BP) stimulation (> 1.5 kHz) versus amphibian papilla stimulation (< 1.5 kHz); ‘CH’ refers to taxa with whine–chuck (complex) calls. Spectrograms of calls are drawn at the same scale (box = 0.9 × 6 kHz) except for *P. albonotatus* (1.8 × 6 kHz); the horizontal dashed line in each spectrogram shows the 1.5 kHz threshold. See text for details.

for the evolution of novel complex signals and mating preferences.

The phylogenetic clustering of gains of the male trait and the female preference suggests that both traits have coevolved, as indicated by the test of correlated evolution. Endler & Basolo (1998) proposed expected patterns of character correlation on a phylogeny under alternative

Table 1. Female choices between two alternative signals in phonotaxis experiments

	Conspecific call	White noise	Fisher’s exact test <i>P</i>
<i>E. coloradorum</i>	19	2	<0.001
<i>E. randi</i>	18	2	<0.001
<i>E. sp. D</i>	45	1	<0.001

The noise signal had the same amplitude envelope as the conspecific call. Values represent the number of females that chose each signal of the pair. Probabilities are from two-tailed Fisher’s exact tests under a null hypothesis derived for *Engystomops pustulosus* (Rand et al. 1992). A significant value indicates that females recognized the conspecific call under the experimental set-up.

models of sexual selection. Specifically, Fisherian and good genes models of sexual selection are expected to show a phylogenetic correlation of trait and preference (Kirkpatrick & Ryan 1991; Shaw 1995; Endler & Basolo 1998). The correlation found in túngara frogs strongly suggests that Fisherian or good genes sexual selection are plausible models to explain the origin of complex calls.

Revisiting the Sensory Exploitation Hypothesis in Túngara Frogs

Sexual selection by sensory exploitation occurs when the perceptual and cognitive systems of the receiver evolve incidentally as a result of evolutionary forces operating before the origin of the sender’s signal. In túngara frogs, the receiver’s preference for the chuck was considered to have originated in the most recent common ancestor of *Engystomops*, long before the origin of the chuck (Ryan & Rand 1993a). My results, in contrast, show that the preference originated more recently, within the clade Edentulus. Thus, the new results do not support

Table 2. Results for two-choice phonotaxis experiments for females túngara frogs

	No. of females choosing		Proportion choosing wh–ch	Binomial <i>P</i> *
	Wh	Wh–ch		
<i>E. coloradorum</i>	9	11	0.550	0.664
<i>E. petersi</i> Puyo	7	15	0.682	0.093
<i>E. petersi</i> La Selva	7	11	0.611	0.359
<i>E. petersi</i> Yasuní	2	13	0.867	0.004
<i>E. pustulosus</i>	527	3135	0.950	< 0.001
<i>E. randi</i>	14	5	0.263	0.041
<i>E. sp. D</i>	13	6	0.316	0.115
<i>P. enesefae</i>	15	12	0.444	0.572

Stimuli: Wh = conspecific whine (simple call); Wh–ch = conspecific whine followed by one chuck of *E. pustulosus* or *E. petersi* (complex call). Data for *Physalaemus enesefae*, *E. pustulosus* and *E. petersi* are from Tárano & Ryan (2002), Guerra & Ron (in press), Gridi-Papp & Ryan (2006) and Boul et al. (2007).

*Bold values denote a significant preference for the complex call. Note that *Engystomops randi* shows a significant preference for the simple call.

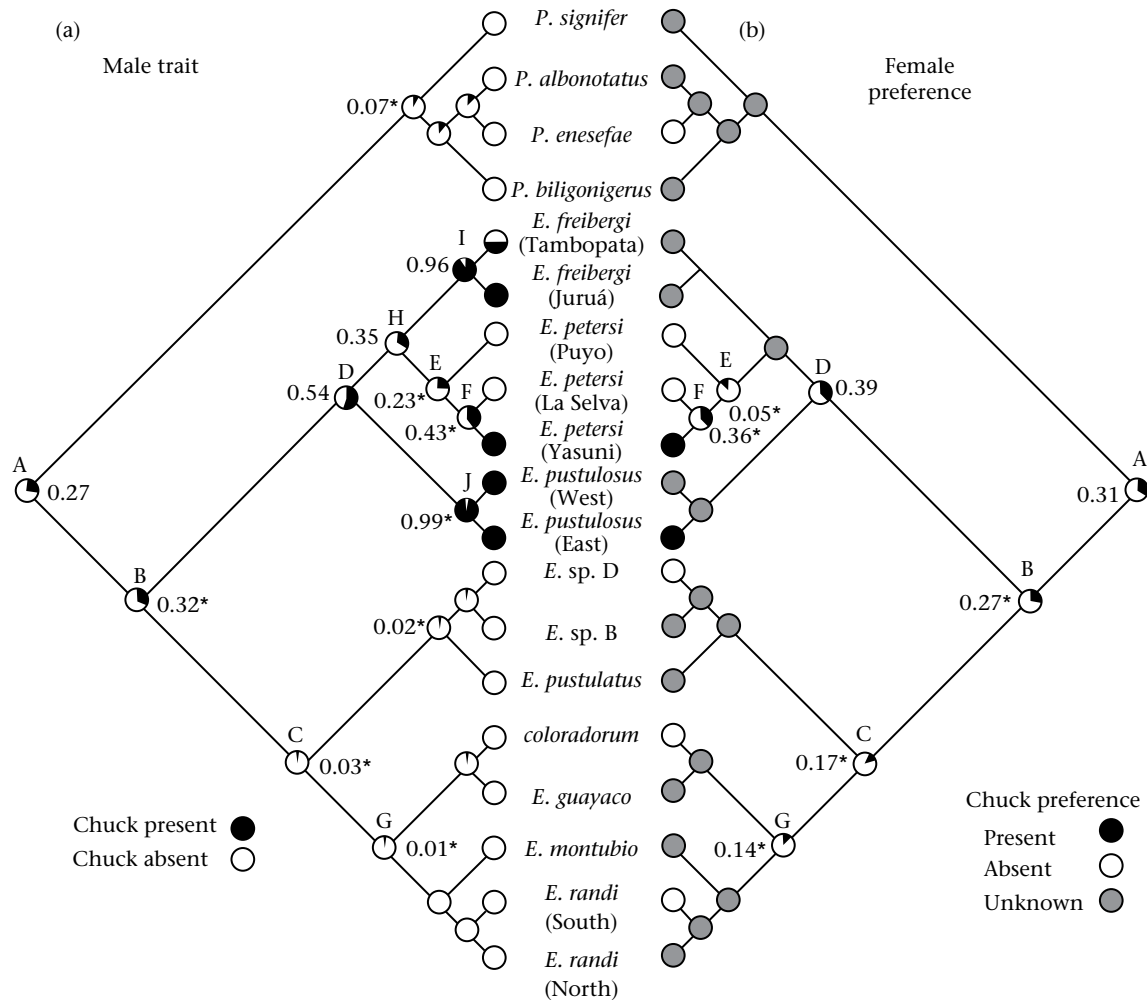


Figure 4. Ancestral character reconstruction for the evolution of the sexual male trait (i.e. chuck) and its corresponding female preference in túngara frogs. Reconstructions were based on Bayesian inference. Pie charts at nodes represent the relative Bayesian support for (a) the presence or absence of chucks in the male calls and (b) the presence or absence of preference by females towards conspecific chuck-appended calls. Numbers next to the nodes are relative Bayesian support values. Asterisks indicate significant support for the favoured character state (as indicated by a Bayes factor >2). Preference data were obtained from two-choice experiments in which females chose between the conspecific call with a chuck from *E. pustulosus* or *E. petersi* appended and the conspecific call without the chuck (Table 2). Preference data for *Physalaemus enesefae*, *E. pustulosus* and *E. petersi* are from Ryan & Rand (1990); Tárano & Ryan (2002); Guerra & Ron (in press) and Boul et al. (2007).

the sensory exploitation hypothesis as proposed by Ryan & Rand (1993a).

The correlation in the evolution of the chuck and its corresponding female preference is also incompatible with Ryan & Rand's (1993a) hypothesis. According to Ryan & Rand (1993a), pre-existing preferences suggest that the male trait evolved after the preference and are inconsistent with models invoking the correlated coevolution of traits and preferences. My results show such correlation and conflict with the sensory exploitation scenario proposed by Ryan & Rand (1993a). Endler & Basolo (1998) noticed that it is possible to recover a phylogenetic correlation under sensory exploitation when character transitions of trait and preference occur in the same tree branch. The sensory exploitation scenario proposed for túngara frogs, however, lacked such a correlation because it reconstructed character transitions of trait and preference occurring in different branches.

According to the Bayesian reconstruction, the character state for the ancestor of clade Edentulus is equivocal (node D in Fig. 4); in the parsimony reconstruction, the presence of the preference was favoured (Table 3). Thus, sensory exploitation (i.e. an earlier origin of the preference relative to the chuck) within this clade is possible, provided that the chuck did not originate in the ancestor of clade Edentulus. It is clear, however, that sensory exploitation lacks support in *E. petersi* from Yasuni because the chuck and its preference originated in the same branch (Fig. 4). Both the Bayesian and the parsimony reconstructions show that the preference for the chuck was absent in nodes B, C and G. Inconsistencies between both reconstructions are evident at nodes F and E and seem to be a consequence of different coding strategies. Under parsimony, preferences were treated as a continuous character; in the Bayesian reconstructions, preferences were coded binarily based on results from binomial tests (which, given the

Table 3. Nodal values of the reconstruction of the proportion of female túngara frogs choosing the whine–chuck call versus the whine in phonotaxis experiments

Node	Proportion of females choosing the whine–chuck
A	0.46
B	0.53
C	0.33
D	0.81
E	0.79
F	0.75
G	0.50

Nodes correspond to those in Fig. 4. Female preferences were coded as a continuous character (proportion of females preferring the whine–chuck call). Ancestral preferences were reconstructed with squared-change parsimony with weighted branches.

experimental sample sizes, could suffer from type II errors). These conflicts, however, are only peripheral and do not compromise the finding of an ancestral lack of preference for complex calls in the most recent ancestor of *Engystomops*. Note, however, that Bayesian inference has advantages over parsimony reconstructions because it accounts for uncertainties in the phylogeny and mapping of characters in its estimates (Ronquist 2004). In addition, the Bayesian approach allows hypothesis testing of alternative character states at each node.

There is a potential caveat with the interpretation of *P. enesefae* preference data. This species has a small area of sympatry with *E. pustulosus* where selection for species recognition could have generated reproductive character displacement, resulting in secondary loss of female preference for the heterospecific call element. Ryan & Rand (2003a) point out, however, that this scenario is unlikely because *E. pustulosus* chucks do not make the calls more or less attractive. Moreover, *P. enesefae* also shows a lack of preference for the acoustically distinctive chucks of *E. petersi* (Tárano & Ryan 2002). Lack of preference for complex calls by *P. enesefae* seems to be independent of whether the chuck belongs to a sympatric or allopatric species.

The occurrence of sensory exploitation has been documented with varying levels of support in diverse taxonomic groups including fish (Basolo 1995; Rodd et al. 2002; Smith et al. 2004), arthropods (Proctor 1992;

McClintock & Uetz 1996) and birds (Pryke & Andersson 2002; Madden & Tanner 2003). The Scenario of sensory exploitation proposed by Ryan & Rand (1993a, b) in túngara frogs, however, was particularly compelling because it traces phylogenetically the precedence of the bias over the trait (only three other examples are known: swordtails (Basolo 1995), water mites (Proctor 1992), and Goodenidae fish (Macías & Ramirez 2005)). Two reasons could explain the incorrect reconstruction of female preference in Ryan & Rand's (1993a) analyses: incomplete taxon sampling and the experimental design of phonotaxis experiments in *E. coloradorum*. I discuss both issues below.

Taxon Sampling and Phonotaxis Experiments in *E. coloradorum*

Simulations have consistently shown that the probability of correctly estimating ancestral character states decreases as taxa are excluded from the analyses (Zhang & Nei 1997; Salisbury & Kim 2001). My taxon sampling for the male trait included all known species of *Engystomops* and should be relatively robust. However, my sample of female preference was incomplete (~ 50% of the species in the ingroup) and it is conceivable that the reconstructions were an artefact of incomplete taxon sampling. However, at the species level, my sample of female preference was twice that of Ryan & Rand (2003a), and therefore, my estimates should be more robust. In addition, my analysis to address the effect of incomplete taxon sampling did not favour either hypothesis, suggesting that increasing taxon sampling is unlikely to result in support for the preference of the chuck at the root of *Engystomops*.

Despite sampling the same population, my results are inconsistent with Ryan & Rand's (1993a) finding of a preference for chuck-appended calls in *E. coloradorum*. It is possible that the discrepancy could be attributed to Ryan & Rand's choice of appending three chucks instead of one in their tests. I appended a single chuck in my experiments because a single chuck was appended in the experiments with *E. pustulosus* (e.g. Ryan & Rand 1990) and *P. enesefae* (Tárano & Ryan 2002). The choice of three chucks with *E. coloradorum* is problematic because it departs from the methodology used in the other species and alters excessively the energy content of the simple call. Adding

Table 4. Sensory-matching between male advertisement call and female ear frequency tuning in túngara frogs

	Ear BEF–BP (kHz)	Male call frequency at peak amplitude (kHz)	Difference BEF–call frequency (kHz)	Kruskal–Wallis <i>P</i>
<i>E. coloradorum</i>	2.229±0.353, <i>N</i> =7	2.204±0.078, <i>N</i> =5	–0.024	0.414
<i>E. petersi</i>	2.166±0.046, <i>N</i> =4	1.459±0.167, <i>N</i> =7	–0.707	0.010
<i>E. pustulosus</i> (E)	2.133±0.163, <i>N</i> =6	2.545±0.273, <i>N</i> =7	0.412	0.037
<i>E. randi</i>	2.549±0.056, <i>N</i> =4	2.628±0.206, <i>N</i> =7	0.079	0.385
<i>E. sp. B</i>	2.259*±0.140, <i>N</i> =9	1.890±0.427, <i>N</i> =7	–0.328	0.006
<i>P. enesefae</i>	2.157±0.060, <i>N</i> =9	0.878±0.105, <i>N</i> =7	–1.279	0.001

Best excitatory frequency (BEF–BP) represents the frequency to which the ears' basilar papilla (BP) is most sensitive (from Wilczynski et al. 2001). The values of call frequency (mean ± SD) are for the high frequency peak of the male call (calls have two peaks; Fig. 2). Kruskal–Wallis *P* values compare BEF–BP versus call frequency at peak amplitude. Except for *E. pustulosus*, all species produce simple calls.

*The value reported by Wilczynski et al. (2001) is in error (W. Wilczynski, personal communication).

a single chuck to the call of *E. pustulosus* and *E. coloradorum* increases energy by 7% and 29%, respectively; adding three chucks to the call of *E. coloradorum* increases energy content by almost 100%. The preference reported by Ryan & Rand (1993a) may result from a well-known tendency of frogs to prefer more energetic calls (Ryan & Keddy-Hector 1992). This tendency is directional and should be stronger when the difference between competing stimuli is larger. The preference for more intense calls can cancel or even reverse a preference that is based on other call variables (e.g. Gerhardt 1982; Ryan & Rand 1990). Thus, adding three chucks could have cancelled preferences expressed in the experiments that added only one chuck.

Importantly, the absence of support for the preference of chucks at the root of *Engystomops* persisted even when the reconstruction was carried out according to Ryan & Rand's (1993a) results with *E. coloradorum* (analyses not shown). Lack of support for sensory exploitation was not contingent on *E. coloradorum* experimental results.

Sensory Exploitation and Sensory Matching?

One significant contribution in the study of acoustic communication was Capranica's (1965) proposal of the matched-filter hypothesis, which posits that there is a general correspondence between AP and BP tuning, and the frequency bands emphasized in the conspecific advertisement call of frogs. Overall, it has been assumed that stimulation of the AP and BP are positive inputs that enhance the attractiveness of the conspecific advertisement call (Ryan & Rand 1990; Wilczynski et al. 2001). In other words, peripheral tuning should be an accurate predictor of female mate choice decisions.

The rejection of chuck-appended calls by female *E. randi* was unexpected because it shows the opposite effect: increased peripheral stimulation generating rejection of a courtship signal. This result adds to growing evidence (Wilczynski et al. 2001; Tárano & Ryan 2002; Bosch & Boyero 2003; Gerhardt et al. 2007a, b) showing that females do not necessarily prefer male call variants that stimulate more strongly the peripheral auditory system. Mate choice decisions in anurans cannot be predicted only on the basis of the strength of peripheral stimulation; an active role of central processing in mate choice decisions needs to be invoked to explain the observed results.

The sensory-matching hypothesis predicts that complex calls (whine-chuck) are more attractive than simple calls (whine-only) simply because they match the inner ear's sensory profile better (Wilczynski et al. 2001). My analyses highlight inconsistencies between the sensory-matching hypothesis and the sensory exploitation hypothesis. Contrary to expectations, there is no correspondence between the presence of the chuck and the amount of energy available for BP stimulation. For example, *E. petersi* from Yasuní has a complex call (Boul et al. 2007) and yet, only 13% of the call energy is above 1.5 kHz and available for BP stimulation. In contrast, *E. coloradorum* has a simple call but 38% of its call energy is above 1.5 kHz, with peak frequencies that match the best excitatory frequencies of the BP (Table 4, Fig. 2). The simple call of *E. coloradorum* has

more energy available for BP stimulation and a better tuning with the BP than does the *E. pustulosus* complex call.

The categorization of calls into 'simple' or 'complex' is problematic because the two criteria used to categorize the calls are incongruent with each other. According to the criterion based on the presence or absence of the chuck, only the calls of *E. pustulosus* and some populations of the *E. petersi* are complex; under the criterion of BP stimulation, calls of most species might be considered complex. Future studies could benefit from abandoning this categorization and instead analysing continuous variables derived from acoustic measurements of the calls.

My study addresses several issues in the evolution of complex male traits and female mate choice. First, I have documented coevolution between female preference and male trait. This pattern is compatible with processes of sexual selection, like Fisherian or good genes models, that previously have been dismissed as plausible explanations for the evolution of complex calls in *Engystomops*. Second, I show that both the male trait and the female preference have originated multiple times among closely related lineages and are strikingly homoplastic. Based on these results, I hypothesize that the clade Edentulus is a hot spot for the evolution of chucks and its corresponding female preference. Third, results from phonotaxis experiments highlight the role of central processing in mate choice decisions and show that increased peripheral stimulation by the courtship signal does not necessarily translate into additional attractiveness. Finally, I show unequivocally that the scenario of sensory exploitation reported by Ryan & Rand (1993a, 2003a), one of the most widely cited examples of sensory exploitation, lacks support. Taken together, these findings open new venues for the study of sexual selection and communication in this model system.

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