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No pre-existing biases for heterospecific call traits in the frog *Physalaemus enesefae*

Z. TÁRANO* & M. J. RYAN†
*Postgrado en Ciencias Biológicas, Universidad Simón Bolívar
†Section of Integrative Biology C0930, University of Texas at Austin

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Previous phonotaxis studies with two species of the *Physalaemus pustulosus* species group indicated that female preferences for several heterospecific call traits resulted from sensory biases inherited from a common ancestor. In phonotaxis experiments, we determined whether *Physalaemus enesefae,* a distant relative of the *P. pustulosus* group, showed similar preferences for call traits not present in conspecific males. We presented females with a choice between the typical conspecific advertisement call and the same call to which we digitally appended a chuck from *P. pustulosus,* a squawk from *P. freibergi*, and an amplitude-modulated prefix from *P. pustulatus.* In addition we presented the advertisement call in doublets, a trait peculiar to *P. coloradorum.* We also analysed male vocal behaviour evoked in response to the same suite of stimuli. *Physalaemus enesefae* females did not prefer the calls of their own males with appended heterospecific traits over unmodified calls, nor conspecific calls in doublets over single calls. The lack of preference among females was not the result of a behavioural polymorphism. Female responses to repeated presentations of the same stimulus pair were not consistent. Males also did not show an enhanced vocal response to altered calls relative to the typical conspecific call. Consequently, there are no pre-existing biases for these heterospecific call traits in *P. enesefae*; pre-existing preferences in the *P. pustulosus* group could have been inherited from an ancestor not shared with *P. enesefae*, but data from other closely related species are needed to confirm this conclusion.

Most hypotheses for the evolution of female mating preferences suggest that preferences evolve because of their direct or indirect effects on female fitness (Kirkpatrick & Ryan 1991; Andersson 1994; Ryan 1997). These hypotheses involve processes in which male trait and female preference coevolve (Kirkpatrick & Ryan 1991). The hypothesis of sensory exploitation, however, proposes that current female preferences for male traits became established for reasons unrelated to current influences on mate choice (Endler & McLellan 1988; Ryan 1990, 1998; Endler 1992; Shaw 1995; Endler & Basolo 1998). The preference or bias may be common or fixed when the male trait arises, and the subsequent evolution of the male trait may occur with or without appreciable change in the female preference. This hypothesis can be tested with a combination of appropriate phylogenetic analysis and behavioural experimentation (Ryan et al. 1990; Shaw 1995; Basolo 2000). If a current preference for a male trait results from a pre-existing bias, the preference must be present not only in the species showing the attractive trait but also in related species lacking the trait. The hypothesis of sensory exploitation has been supported by the results of studies in several taxa including frogs, platyfish, water mites, auklets and spiders (reviewed in Ryan 1998).

Traits involved in female choice often elicit male responses as well (Jones & Hunter 1993; Morris & Ryan 1996; Ryan & Rand 1998). Male responses often parallel female preferences and involve an escalation of display: males increase the intensity, the duration or the complexity of their mating displays, especially when confronted with attractive males (Narins & Capranica 1978; Clutton-Brock & Albon 1979; Wells & Schwartz 1984; Ryan 1985; Wagner 1989; Bosch & Márquez 1996). Sexes, however, may differ in their responses to extant or novel signal traits (Narins & Capranica 1976; Morris & Ryan 1996; Witte & Curio 1999), especially when sexes differ in their ecological requirements and are subject to different selective pressures. Differences between sexes in pre-existing sensory biases might also influence the evolution of mating traits.

Correspondence and present address: Z. Tárano, Instituto de Biología Experimental, Universidad Central de Venezuela, Apartado 47106, Caracas 1041–A, Venezuela (email: ztarano@reacciun.ve) M. J. Ryan is at the Section of Integrative Biology C0930, University of Texas at Austin, Austin, TX 78712, U.S.A.

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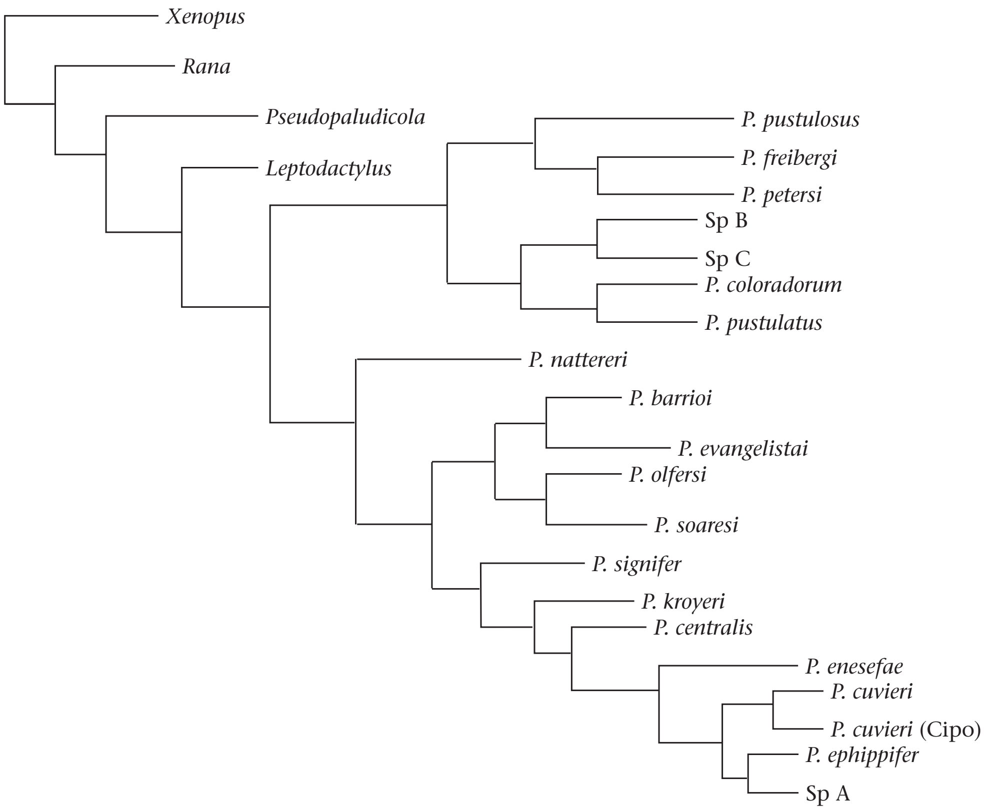

Figure 1. Phylogenetic relationships in the genus *Physalaemus.* Species A, B and C are undescribed species. The phylogeny was based on morphological characters, allozymes and mitochondrial DNA sequences (D. Cannatella, M. Holder, D. Hills, A. S. Rand & M. J. Ryan, unpublished data).

The Study System

The mate recognition systems of the túngara frog, *Physalaemus pustulosus,* and its close relatives have been the subject of intense investigation (e.g. Rand & Ryan 1981; Ryan 1985; Ryan & Rand 1990, 1993a, b; Kime et al. 1998). The *P. pustulosus* group originally consisted of four species: *P. pustulosus*, *P. petersi*, *P. pustulatus* and *P. coloradorum* (Cannatella & Duellman 1984). Three new species may also exist in the species group, (*P. freibergi*, sp. B temporarily referred to as *P.* 'caicai' in Cannatella et al. 1998, and sp. C an undescribed species from northwestern Ecuador, in Fig. 1). Preliminary phylogenetic analysis of the genus suggests that *Physalaemus* consists of two monophyletic groups, the *P. pustulosus* species group and all other congeners (D. Cannatella, M. Holder, D. Hills, A. S. Rand & M. J. Ryan, unpublished data; Fig. 1). *Physalaemus enesefae* is in the *P. cuvieri* group that includes *P. kroyeri* and all its descendants. Species A is an undescribed species from the state of Roraima in northern Brazil (see Ryan & Rand 1995). The advertisement calls of the members of the *P. pustulosus* species group have been described (Ryan & Drewes 1990; Ryan & Rand 1993a, b).

Male *P. pustulosus* have a complex call, which consists of a frequency-modulated whine followed by one to six chucks (Ryan 1985). The whine is necessary and sufficient to elicit female phonotaxis, but chucks increase call attractiveness (Rand & Ryan 1981; Rand et al. 1992). Ryan (1985, 1990) suggested that chucks evolved because they exploited a bias in the sensory system of the females. Ryan & Rand (1993a, b) analysed the response of *P. coloradorum* females, a species whose males do not produce chucks, to conspecific calls modified by the addition of a terminal chuck. *Physalaemus coloradorum* females preferred the modified calls to unmodified conspecific calls. Ryan & Rand (1993a, b) concluded that the preference for adorned calls observed in both species was inherited from a common ancestor. In addition, Ryan et al. (1990) compared the tuning of the inner ear of *P. pustulatus* and *P. coloradorum* females and found no significant difference, with sensitivity peaks of 2.13 and 2.23 kHzs respectively. A more detailed study, with more statistical power, compared the tuning of eight species, five in the species group and three outgroups, and also concluded that there was no evidence that preference for chucks in *P. pustulosus* involved any evolutionary change in the peripheral auditory system (Wilczynski et al. 2001).

Other species of the group produce complex calls: in several populations of *P. petersi* complex (*P. petersi* plus *P. freibergi*; Fig. 1) males produce a whine-like component followed by a suffix (squawk), *P. pustulatus* calls are preceded by an amplitude modulated prefix, and *P. coloradorum* males can produce calls in doublets and triplets (Ryan & Rand 1993a). Phonotaxis experiments with *P. pustulosus* females (with all of these heterospecific call traits) and with *P. coloradorum* females (with chucks of *P. pustulosus)*, showed that there are preferences for several call features lacking in conspecific males in both species (Ryan & Rand 1993a). The researchers concluded that there existed ample opportunity for sensory exploitation in this group of frogs as all of these traits seem to have evolved after the corresponding preferences (Ryan & Rand 1993b).

The previous studies concluded that there was a pre-existing preference ancestral to the *P. pustulosus* species group for some of these acoustic traits. It was not possible to determine when the pre-existing preference appeared. For example, it might have occurred in the immediate ancestor of the species group, which is the latest it could have occurred if the previous interpretation is correct, or this preference might be ancestral to the genus. To address these questions we determined whether another member of the genus, *P. enesefae*, shows similar preferences for novel traits as do *P. pustulosus* and *P. coloradorum*. The tuning of the basilar papilla of *P. enesefae* is similar to that of *P. pustulosus* (Wilczynski et al. 2001). This is the inner ear organ whose sensitivity matches the chuck‘s dominant frequency in *P. pustulosus*. Given the relationships within the genus (Fig. 1), parsimony would suggest that if *P. enesefae* shares the same preferences for heterospecific adornments to their conspecific call as *P. pustulosus* and *P. coloradorum*, the pre-existing preference occurred in the ancestor of the entire genus. Alternatively, if *P. enesefae* does not share these same preferences, the pre-existing preference occurred in the most immediate ancestor of the *P. pustulosus* species group (Fig. 1).

We investigated the responses of female *P. enesefae* to several heterospecific acoustic traits appended to the conspecific call. *Physalaemus enesefae* mating calls have been described elsewhere (Tárano 2001). We also determined the consistency of female responses to heterospecific traits. If pre-existing preferences could not be detected, consistency estimates would allow us to determine whether the lack of preference is an individual or a population effect; that is, whether lack of a preference by the population derives from lack of preference or a polymorphism in preference among individuals. Kime et al. (1998) showed that the patterns of female permissiveness for complex call traits found in *P. pustulosus* resulted from females finding natural and adorned calls equally attractive and not from a polymorphism in female call preferences. Finally, we investigated sexual differences in pre-existing biases by determining the extent to which males and females responded to the same heterospecific traits.

METHODS

We conducted the study at Hato Masaguaral, a cattle ranch located in the central llanos (savannas) of Venezuela, 50 km south of the town of Calabozo in Guárico State, from June to August during 1996–1998. The study site is a seasonal savanna that floods during the rainy season (Monasterios & Sarmiento 1968). The breeding season of *P. enesefae* coincides with the rainy season, and lasts from June to August. *Physalaemus enesefae* is sympatric with *P. pustulosus* in the llanos, the only area in which *P. pustulosus* is sympatric with another congener (La Marca 1992).

We collected amplectant pairs of *P. enesefae* from choruses between 1900 and 2200 hours. We performed phonotaxis experiments indoors, between 2300 and 0500 hours, in the facilities of the ranch. We did not observe any perceptible effect of duration of captivity on female response. There was no correlation between duration of captivity and latency to phonotaxis (unpublished data). Most females were tested within 3 h after capture. We allowed males and females an acclimatization period of 30 min prior to testing, during which time we broadcast a natural conspecific chorus. Individuals were tested the same evening of capture and subsequently released at the site of capture.

Ethical note

Because *P. enesefae* lack natural markings that reliably distinguish individuals, we toe-clipped subjects prior to release to allow individual identification (to ensure that subjects were not retested). Toe clipping was minimal, only one toe-tip was removed, and was preformed according to the guidelines for the use of live amphibians in research. Other methods of marking either interfered with male calling (waistbands) or were not functional (fluorescent paint marks) because frogs were quite small (mean weight=1.2 g). We did not observe any adverse effects of toe clipping on locomotion or survival: recaptured males did not show weight loss nor movement impairment. In addition, male behaviour was not noticeably affected because recaptured males were found calling or in amplexus.

Female Choice Experiments

We performed phonotaxis experiments using a portable arena 2 m in diameter. The walls of the arena were formed by sheets of foam 1.5 cm thick and 80 cm high, supported by a semirigid octagonal frame. We performed the experiments under dim light provided by a 25-W light bulb above the centre of the arena.

We placed two speakers (JBL M5) at opposite sides of the arena, facing the centre. We broadcast the stimuli from an analogue tape recorder (JVC TD-W209) or a Macintosh PowerBook 1400 cs, connected to an amplifier (Pioneer 104–A). We set sound intensity at the centre of the arena at a sound pressure level (SPL) of 82 dB (re 20 μPa) with a sound pressure level meter (Radio Shack Digital Meter, flat weighing, fast response). An intensity of 82 dB SPL corresponds to the average intensity of a conspecific call measured at 50 cm from a male.

We placed a female at the centre of the arena under a plastic cone that could be lifted by pulling a string. After 3 min of stimulation we lifted the cone and the female was free to move within the arena. One experimenter observed the arena from the outside. To avoid experimenter biases the observer was acoustically isolated while conducting the tests by hearing music through headphones. The observer scored a response when the female touched a speaker, provided it had not reached it by following the walls of the arena. A no-response was scored when the female: remained immobile at the centre of the arena for 5 min after raising the funnel, stopped at any site of the arena for more than 5 min, or hopped around the arena without reaching a speaker within 15 min. Thus trials were a maximum of 15 min in duration.

Each session consisted of control (C) and experimental (E) trials arranged as follows: C-E-E-C-E-E-C. Control and experimental trials consisted of one stimulus pair each. In control trials we exposed each female to a typical natural conspecific call and to white noise of the same duration as the call. In experimental trials we exposed each female to a conspecific call (unmodified call) and to the same call to which an heterospecific trait was appended (modified call). We prepared the stimuli with SoundEdit 16 v. 2 and Sound Designer software. Each sound of a stimulus pair, either control or experimental, was placed on a separate track on a sound file, antiphonally, thus they were broadcast alternately without interference. Thus, in control trials one speaker broadcast one natural call, from one male, and the other broadcast white noise, and in experimental trials one speaker broadcast one modified call and the other one unmodified call. Each sound of the pair was repeated at 3.8-s intervals, the average intercall interval of the species (Tárano 2001). We alternated the speaker from which a particular sound was broadcast between tests to control for side bias. The time interval between tests was that required to place the female under the funnel and begin to broadcast a different pair of stimuli, and was usually about 2 min.

We used control trials to test female responsiveness before and after each experimental trial. Females that did not respond to the control preceding or following an experimental trial were not used for the experiments. Thus, we included only female responses to experimental trials that were followed and preceded by positive control trials. Positive controls also ensured that females were not behaving randomly during the preference trials.

We tested female *P. enesefae* responses to four heterospecific call traits appended to the species-specific whine: chucks from *P. pustulosus*, squawks from *P. freibergi*, prefixes from *P. pustulatus* and double calls (a trait typical of *P. coloradorum;* Figs. 2, 3). Call traits of *P. pustulatus*, *P. freibergi* and *P. pustulosus* used to prepare the stimulus were from Ryan & Rand (1993a, b). We used 10 natural *P. enesefae* calls recorded in the field in the population under study to prepare the experimental stimuli and five natural calls to prepare the control stimuli. In addition we used 15 *P. pustulosus* chucks from 15 different males, 12 *P. pustulatus* prefixes from the same number of males, and one *P. freibergi* squawk. Thus, each female heard only one natural call (modified versus unmodified) from one male on each trial, but different females heard calls from different males.

We randomized the sequence of trials on the experimental session for each female to control for any order effect. We used each female in several consecutive tests, but we never tested a female more than once with the same pair of stimuli (except for consistency experiments, see below). Results for each test are interpreted separately, thus no assumption regarding statistical independence was violated by using the same females in multiple tests. We used at least 20 different females on each test.

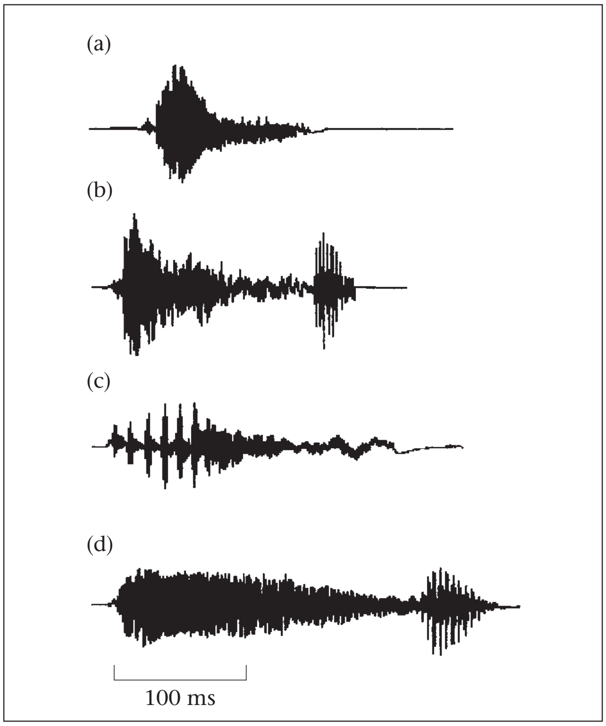

Figure 2. Oscilograms of the advertisement calls of (a) *Physalaemus coloradorum*, (b) *P. freibergi*, (c) *P. pustulatus* and (d) *P. pustulosus.* Time scale is the same in all oscilograms.

Consistency Tests

We conducted consistency tests to determine whether variation in stimuli preferred by females indicated a behavioural polymorphism or, as in túngara frogs, resulted from random variation between females (Kime et al. 1998). In consistency experiments a female was presented twice with the same stimulus pair. We analysed the consistency of female responses to whine versus whine-chuck, whine versus whine-squawk and whine versus prefix-whine stimulus pairs. During the second presentation of the stimulus pairs, we alternated the stimulus between the speakers (used the opposite speaker to broadcast each stimulus). The experiments described in this study were included in experimental sessions that contained other tests. Thus, between the first and the second presentation of the stimulus pair, several different tests were performed with the same female. The number of unrelated tests between presentations of the same stimulus pair varied from two to five ($\overline{X} \pm \mathrm{SD}=3.2 \pm 1.5$, $N=55$), and time between them varied from 10 to 50 min ($\overline{X} \pm \mathrm{SD}=20.2 \pm 12.7$, $N=55$).

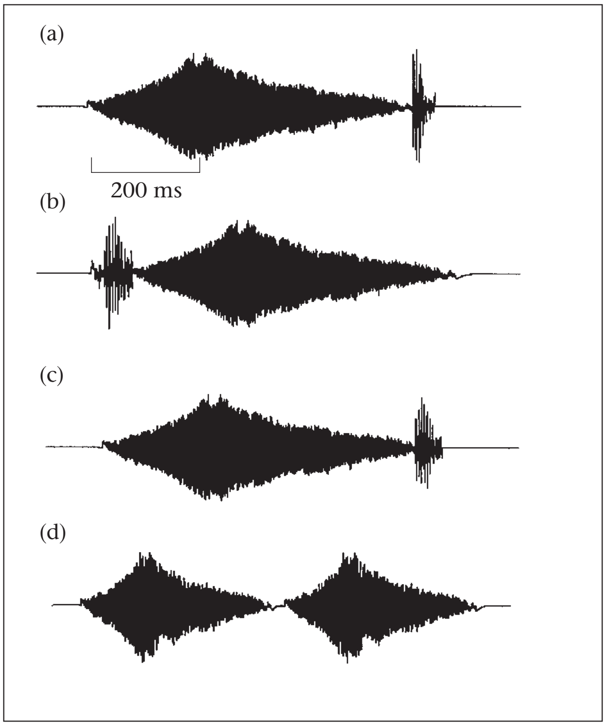

Figure 3. Oscilograms of an advertisement call of *P. enesefae* to which has been appended (a) a chuck typical of *P. pustulosus*, (b) a prefix typical of *P. pustulatus,* (c) a squawk typical of *P. freibergi*, or (d) that has been arranged in a doublet (a peculiar trait of *P. coloradorum*). Time scale in (d) is half that in other oscilograms.

Male Playback Experiments

We placed individual males, usually four, each in a plastic bag with water, on a table, 50 cm apart from each other. Ryan & Rand (1998) showed that there was little acoustic distortion of sound under these conditions. We placed a speaker (JBL M5 or SONY) in front of the males, at 60–80 cm, and broadcast a natural chorus with a PowerBook 1400 cs connected to an amplifier (Pioneer 104–A) and the software SoundEdit 16 v. 2. When a male called in response to the chorus, we carefully removed the other males from the table, thus only one male was on the table during the tests. We positioned the speaker, if necessary, in front of the calling male and placed a microphone (Sennheiser ME62/K6) on the table at mid-distance between the speaker and the male, oriented to the male. We adjusted sound intensity at the male to 82 dB SPL. We connected the microphone to an analogue tape recorder (Marantz PMD 201) and used metal tapes (Maxell MX90, Sony UX) to record male responses during the experiments. We maintained the male in the plastic bag during the experiments.

We tested males singly and usually only one male each night. The experimental session consisted of an initial control stimulus followed by several experimental stimuli and a final control. The control stimulus consisted of one natural conspecific call 860 ms in duration and 960 Hz dominant frequency broadcast at 3.8–s intervals. Experimental stimuli were the same modified calls used in the female preference trials: calls with appended chucks, squawks or prefixes. We presented the control stimulus to the test male for 2 min and recorded his responses during the following 2 min. Then we presented the male one experimental stimulus for 2 min and recorded his response during the following 2 min, and repeated the same procedure until we presented the male all the experimental stimuli. Time interval between subsequent stimuli was that necessary to retrieve the file containing it, and usually lasted less than 1 min. Calls, either control or experimental, were broadcast while recording, thus, both the stimulus and the male response were recorded. We tested each male with all the experimental stimuli, so that the experimental session of one male that responded to all the sounds presented (control and experimental stimuli) lasted around 20 min. Experimental stimuli were presented at a random sequence during each experimental session to control for any order effects. Males that stopped responding during or after a test were stimulated to resume calling by playing the chorus stimulus. We finished the session when the male did not resume calling after 10 min of stimulation.

We digitized male recordings with a PowerBook using SoundEdit 16 (44 kHz, 16 bit). We measured call duration, dominant frequency and intercall interval in male responses to all stimuli. Call duration and dominant frequency were measured in 5–10 calls randomly chosen from the recording of the male response to each stimulus. Spectral information was obtained by performing a Fast Fourier Transform (FFT, frequency resolution 176 Hz, width 512 points) with Canary 1.2.1 software for Macintosh (Charif et al. 1995). Intercall interval was measured from the oscilogram of the 2-min recording for each stimulus with SoundEdit 16.

Statistical Analysis

For female responses, we analysed overall female preferences for unmodified versus modified conspecific calls using a two-tailed binomial probability test on the pooled data for each stimulus pair. We analysed female responses in consistency tests with a chi-square test. Females could have shown four different choice sequences to the two types of calls (modified and unmodified) in the first and the second test. Thus, females could have chosen the unmodified call in the first presentation and the unmodified call in the second (unmodified–unmodified sequence), the modified call in the first presentation and again the modified call in the second presentation (modified–modified sequence), the unmodified call in the first presentation and the modified call in the second (unmodified–modified sequence), and the modified call in the first presentation and the unmodified call in the second presentation (modified–unmodified sequence). Chance probabilities for each sequence were calculated from the binomial expansion of the proportion of females choosing each of the two stimuli during the previous female choice tests. We compared observed versus expected values for each sequence to test the hypothesis that females would choose randomly in successive tests; that is, that female responses during the first

Table 1. Female responses to modified and unmodified conspecific calls

Test	*N*	Unmodified call	Modified call	*P* (binomial)
Whine+chuck versus whine	27	15	12	0.84
Whine+squawk versus whine	26	15	11	0.54
Prefix+whine versus whine	25	15	10	0.42
Double versus single call	20	11	9	0.82

Values represent the number of females that chose each stimulus of the pair. *N* represent the number of females used on each test.

presentation would not be related to their responses during the second presentation.

For male responses, we compared the average call duration, dominant frequency and intercall interval of male responses to the control stimulus with their responses to each experimental stimulus using a two-tailed paired *t* test. Despite the fact that we used each male in several successive tests, this does not violate statistical assumptions of data independence, because we interpret the results of each experiment separately. Thus, comparisons were performed between sets of data obtained from several males and a male‘s response to one stimulus was independent of another male‘s response to the same stimulus. All statistical analyses were performed with Statgrafics 6.0.

RESULTS

Female Choice Experiments

We completed 98 tests with 46 females, and tested each female once in each experiment. Females did not discriminate between unmodified and modified conspecific calls for any of the novel traits used (Table 1). We note that these females were capable of making phonotactic discriminations in these experiments because all females tested showed a preference for the natural call versus white noise during previous and subsequent control tests. In addition, results are based on the responses of a high proportion of nonrandom females since we discarded only a small fraction of all attempted trials due to failure of the criterion of positive response in previous and subsequent control trials. In whine versus whine-chuck and in whine versus whine-squawk trials only 7% of attempted trials were discarded (2 out of 29, and 2 out of 28, respectively) and in whine versus prefix-whine trials only 10% of attempted trials were discarded (3 out of 28). Thus, on average, more than 90% of attempted tests were successful.

We used the results of previous tests (Table 1) to estimate the probability that a female chose one or the other stimulus for each stimulus pair. Thus, the probability that a female chose the whine-squawk stimulus was 0.42 (11/26) and the probability that she chose the unmodified call was 0.58 (15/26). These probabilities were used to estimate the number of females that, by chance, would repeat the choice or would change it during the second presentation of the same stimulus pair in consistency tests. We completed 108 tests with 36 females and tested each female twice. In tests of whine versus whine-squawk, 12 females repeated and 21 changed their choice. Observed frequencies for all possible sequences of response did not differ significantly from the ones expected by chance (chi-square test: χ^2_3=2.83, *N*=33 females, *P*=0.42; Fig. 4a), thus females were not consistent in their choice. Female responses in whine-chuck versus whine tests, and prefix-whine versus whine tests produced identical results (whine-chuck test: χ^2_3=1.94, *N*=10 females, *P*=0.58; Fig. 4b; prefix-whine test: χ^2_3=3.45, *N*=11 females, *P*=0.32; Fig. 4c). Differences in range of data presented in Fig. 4 reflect differences in the number of females tested and not differences in female responsiveness.

In this study we used a restricted subset of population females for testing preferences for several heterospecific traits. Drawing conclusions on pre-existing biases for different traits using this procedure could be unreliable if a female's probability of showing a given preference is not independent of the same female's probability of showing another preference in a subsequent test. We analysed whether a female's probability of showing a given preference (e.g. a preference for squawks) in a trial was dependent on her preference in a previous trial (e.g. a preference for chucks), and found that females that showed no preference for chucks in a previous trial were neither more nor less likely to show a preference for squawks (or prefixes) in a subsequent trial than were females that showed a preference in the previous trial (chi-square test: χ^2_3=1.5, NS).

Male Playback Experiments

We tested a total of 25 males; sample sizes differed between tests because several males did not respond in all tests. We did not find significant differences in call duration, dominant frequency or the intercall interval in male responses between the control stimuli and any of the experimental stimuli (Fig. 5).

DISCUSSION

Physalaemus enesefae females did not have pre-existing preferences for any of the heterospecific traits we tested: *P. pustulosus* chucks, *P. freibergi* squawks, *P. pustulatus* prefixes, or calls arranged in doublets (*P. coloradorum*). The lack of preference for each heterospecific trait observed at the population level results from random

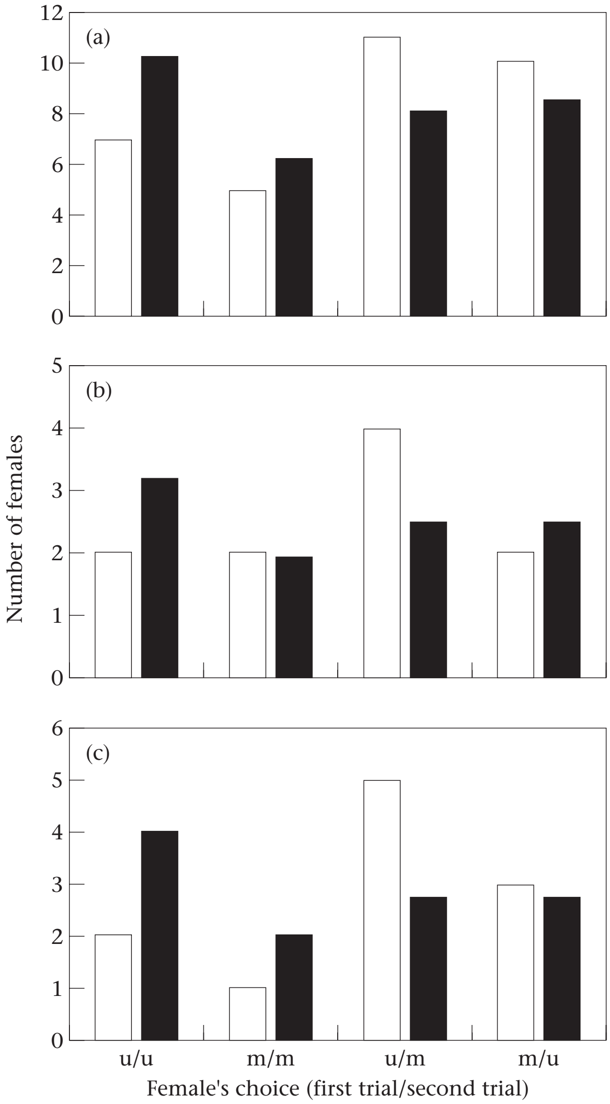

Figure 4. Female responses during consistency tests with (a) whine versus whine-squawk, (b) whine versus whine-chuck and (c) whine versus prefix-whine stimulus pairs. □: The observed choices for each of the four possible sequences of response. ■: The expected choices. u: unmodified call; m: modified call. The null hypothesis was analysed with a chi-square test. Differences were nonsignificant for all comparisons.

choices within females and not from a behavioural polymorphism among them. Male responses to the same suite of stimuli used in phonotaxis tests parallel those of females: males did not alter their vocal response to modified calls in relation to unmodified average calls.

We also showed that females did not discriminate against their own call to which we appended the *P. pustulosus* chuck. This might be expected since the chuck, when produced, is always a reliable indicator of a heterospecific and could be an additional cue for *P. enesefae* females to further discriminate against *P. pustulosus* males. Although *P. enesefae* females seem to be quite tolerant of modifications to conspecific calls, they do discriminate conspecific from heterospecific calls, specifically those from of the sympatric *P. pustulosus*. In another set of experiments (unpublished data) we tested *P. enesefae* responses to *P. pustulosus* calls and found that when given a choice between a typical *P. pustulosus* call and a *P. enesefae* call, *P. enesefae* females significantly chose the conspecific call. In addition, when given a choice between a *P. pustulosus* call and white noise (a no-choice situation), females did not respond to either sound.

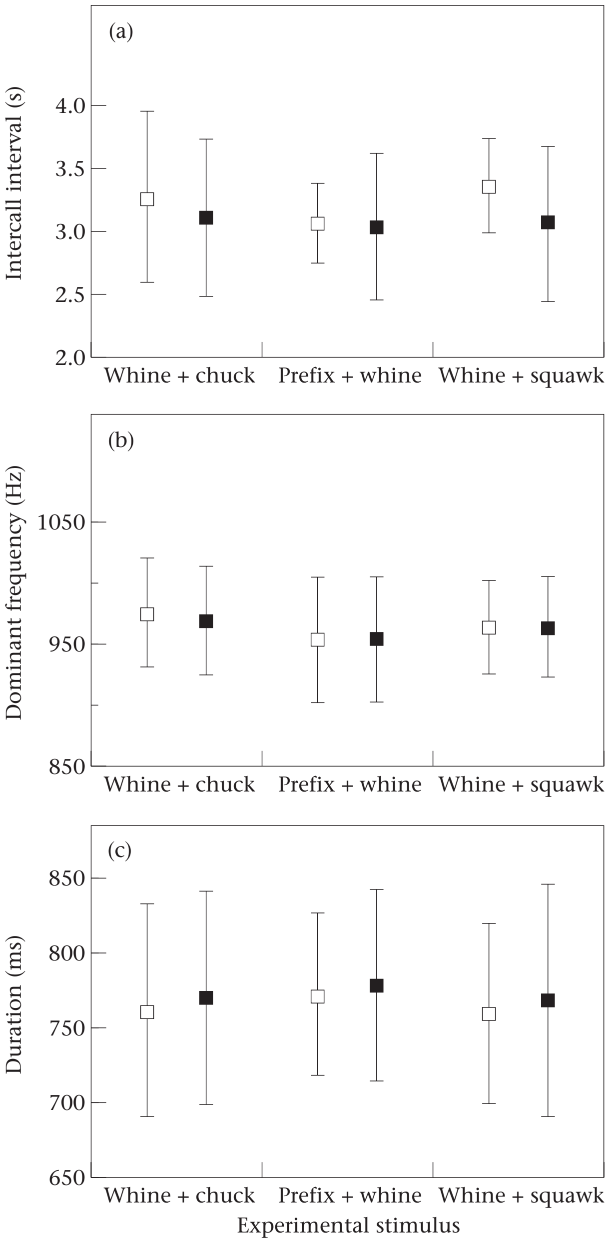

Figure 5. Male responses to control and experimental stimulus. (a) Intercall interval, (b) dominant frequency and (c) call duration. For each stimulus the box represents the mean and the line represents the standard error. ■: Response to the experimental stimulus. □: Response to the control stimulus. Differences between control and experimental stimuli were not significant for the variables studied (two-tailed paired *t* test, NS for all comparisons).

Female Responses and the Evolution of Complex Call Traits

Previous studies with *P. pustulosus* and *P. coloradorum* females support the hypothesis that chucks produced by *P. pustulosus* males evolved because they exploit a sensory bias among females (Ryan & Rand 1993a, b). Preferences of *P. coloradorum* females for their conspecific calls plus *P. pustulosus* chucks, and of *P. pustulosus* females for several heterospecific traits appended to conspecific calls found among other males of the genus suggest that preferences observed in both species are ancestral traits (Ryan & Rand 1993a, b). This explanation is more parsimonious than the hypothesis that preferences have evolved independently in *P. pustulosus* and *P. coloradorum*, although only by one step. Our results suggest that these females must have inherited the preference for chucks, squawks and prefixes from a more recent ancestor than the one they share with *P. enesefae* (i.e. this preference was not inherited from the ancestor of the entire genus or one older; Fig. 1).

An alternative, that the preference was present in the common ancestor of the genus and was then lost in *P. enesefae* is less parsimonious but only requires an additional evolutionary event. A recent description of the calls of *P. nanus*, a species of the *P. signifer* species group, suggests that the sensory biases found among females of the *P. pustulosus* group might be widespread in the genus, and might have evolved several times. Calls of *P. nanus* have a short prefix, with strong amplitude modulations (Haddad & Pombal 1998). This prefix is similar to the one produced by *P. pustulatus* males. If there is a preference for this prefix in *P. nanus*, this would suggest a shared preference with *P. pustulosus* through the common ancestor of the genus, and thus the secondary loss of the preference in *P. enesefae*. Additional data on female preferences in other species are required to draw more general conclusions about the prevalence of sensory biases in the genus. Although phylogenetic reconstruction of behaviour has made critical contributions to our understanding of behaviour evolution, this study as well as others should make it clear that interpretations should be considered with caution (Ryan 1996).

Our conclusion that *P. enesefae* females lack pre-existing preferences for the novel call traits analysed seem robust since we also demonstrated that female responses were random within females. The lack of consistency in female responses might be the result of neural processes that occur at different levels of their perceptual system: the frequencies contained in the novel traits may not excite the auditory system of females or, on the contrary, they do, but lack biological meaning. Nelson & Marler (1990) contrasted 'noticeable differences' perceived by an individual with 'meaningful differences' that affect an individual's behaviour. Undoubtedly, differences between doublets and single calls are noticeable but not meaningful for *P. enesefae* females. Spectral analyses of chucks, squawks and prefixes (Z. Tárano, unpublished data) indicate, however, that all of them have energy in several frequencies contained in the whines of *P. enesefae* (Tárano 2001), thus they might also be perceived by females. Also, Wilczynski et al. (2001) have shown that the tuning of the basilar papillae of *P. enesefae* is similar to that of *P. pustulosus* and thus should be sensitive to the higher (>1500 Hz) frequencies in chucks and squawks.

Sexual Differences in Response to Novel Traits

Male and female *P. enesefae* respond equally to modified calls, suggesting they share similar properties in their auditory systems or in their perception–decision systems. Other studies have found similar results (e.g. Burley 1986). Similarity between the sexes in perceptual mechanisms would offer a proximal explanation for the evolution of monomorphism, even in the presence of sexual selection (Moore 1987; Jones & Hunter 1993). There is also evidence of sexual differences in responses, both to extant and novel traits (Narins & Capranica 1976; Burley et al. 1982; Metz & Weatherhead 1991; Morris & Ryan 1996; Witte & Curio 1999).

The lack of sensory biases for a variety of call traits observed in both sexes of *P. enesefae* might retard the evolution of complex call traits in this species. Sensory biases may lead to rapid evolution of conspicuous signal traits because preferences so determined may be widespread or fixed when the male trait arises (Andersson 1994).

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