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Female Preferences Are Not Altered by Early Acoustic Experience in the Neotropical Frog *Physalaemus pustulosus*

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Abstract.—**Female mate choice preferences may be the result of innate factors, experience, or an interaction between the two mechanisms. Understanding the relative contribution and possible interaction between these mechanisms is important for identifying sources of variation in behaviors under sexual selection. In this study, we evaluate the contribution of early experience on the development of mate choice preferences in the Neotropical Túngara Frog, *Physalaemus pustulosus*. We reared frogs from tadpole stage through sexual maturity in four acoustic treatments: the first group heard a conspecific chorus of Túngara Frogs, the second group heard a heterospecific chorus of the closely related sympatric congener *P. enesefae*, the third group heard no frog sounds, and the fourth group heard only broadband white noise. At sexual maturity, we tested each female's preferences for conspecific complex vs simple calls and discrimination against calls of the sympatric congener. Female choices in all of these tests were consistent with those in previous studies of wild-caught and laboratory-reared specimens of this species. The acoustic rearing environments in this study did not alter the preferences of females for complex conspecific calls or the discrimination of females against the sympatric congener. This study supports the hypothesis that early experience does not alter the mate choice preferences of female *P. pustulosus*.**

Animals acquire adult mating behaviors through innate processes (i.e., genetics), by environmental processes (i.e., experiential), or through an interaction of these processes. Understanding the relative contributions of genetics and environment in expression of mating behaviors is important for identifying sources of variation that contribute to the process of sexual selection (Andersson, 1994). Genetically determined mate preferences are more reliable across generations but often lack variation needed to respond to environmental change. Learned mate preferences provide an opportunity for species to adapt to changing acoustic environments but require a guided juvenile experience and can be susceptible to influences such as the introduction of new species.

The effect of early experience on female mate choice has been studied in a variety of taxa, with mixed results. Female Zebra Finches (*Taeniopygia guttata*) preferentially recognize songs to which they were exposed as juveniles (Miller, 1979) and prefer the specific song variant to which they were exposed during development (Clayton, 1988; Braaten and Reynolds, 1999). In female Cowbirds (*Molothrus ater artemisiae*), experience with the local dialect may explain their preference for calls of local males (O'Loghlen and Rothstein, 1995). Female White-crowned Sparrows (*Zonotrichia leucophrys*) reared hearing songs from their home dialect respond almost exclusively to males singing that dialect (Baker et al., 1981), although another study of the same species found no such effect (Baptista and Morton, 1982). In contrast, female Cardinals (*Cardinalis cardinalis*) do not show a preference for the songs of males to which they were exposed during development (Yamaguchi, 1999). These studies demonstrate the possible influence of early experience on female preference in songbirds; however, the timing of the experience and its interaction with innate mechanisms is not understood (Riebel, 2003). In invertebrates, female Wolf Spiders (*Schizocosa uetzi*) are more likely to mate with males that exhibit the same secondary sexual traits of males to which they were exposed before reaching sexual maturity (Hebets, 2003). More research across a wider range of taxa is needed to clarify the complex interaction of genes and environment in the development of mating preferences of females.

Anurans are a classic model system for studies of acoustic communication. Male advertisement calls typically serve as the basis for female mate choices. Much is known about the causation, function, and evolution of male and female mating behaviors (Ryan, 2001; Gerhardt and Huber, 2002; Wells, 2007). Less is known about the development of preferences for certain mating behaviors and the relative roles of innate and experiential factors. Male anurans may show minor variations in their advertisement call based on early experience (Dawson and Ryan, 2009). To date, no research has been published on the possible role of early experience in the formation and variation of mate choice preferences by female anurans.

Here, we study the contribution of early experience to the female preferences for male mating calls in the Neotropical Túngara Frog, *Physalaemus pustulosus*. Males of this species produce either simple calls with no suffix, or complex calls that consist of a simple call followed by one or more suffixes; females typically prefer the complex call (Ryan, 1985). Females do not respond to the male advertisement call of closely related heterospecifics (Ryan and Rand, 1993). In a test of discrimination, females were studied using conspecific calls, heterospecific calls, and calls representing the acoustic transect between conspecific and heterospecific calls (Ryan et al., 2003). Recognition of conspecific calls halted at a specific acoustic mid-point; this intermediate call represents the threshold of discrimination between conspecifics and heterospecifics and is the intermediate call used in this study.

This mate recognition system makes the Túngara Frog a good model for investigating the role, if any, of early experience in the acquisition of female mate choice in anurans. In addition, juvenile Túngara Frogs have been observed at ponds when adults are mating (Ryan, pers. obs.) and so have the opportunity to experience the mating behaviors of adult conspecifics.

In this study, we evaluate the role of early acoustic environment in the mate preferences of female Túngara Frogs by rearing females in varying acoustic environments and testing their preferences as adults. If female preferences are altered by acoustic experience, then the mating success of males living in the wild might change with changing environmental conditions, such as with the introduction of a previously allopatric species.

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If the females reared in these environments demonstrate similar mating preferences, then future studies looking for sources of variation in female preferences should focus on innate sources such as variation in genes, anatomy, and physiology.

Materials and Methods

All frogs in this study came from a colony of captive-bred *P. pustulosus* originally from Panama and maintained at the University of Texas at Austin. We randomly assigned 300 frogs from 20 broods into acoustic treatment groups by dividing each egg mass into four smaller masses. We maintained the animals in these treatment groups from tadpole stage to sexual maturity (approximately 10 months). Experiments began when the individuals reached sexual maturity and lasted 3 months.

The acoustic treatments were as follows: 1) a recording of a natural chorus of male Túngara Frogs; 2) a recording of a natural chorus of a related species, *P. enesefae*, 3) no frog calls (isolation), and 4) broadband white noise. For groups 1 and 2, the stimulus was broadcast for the 12 h corresponding to night. For 24 h each day, group 3 heard no stimulus and group 4 heard broadband white noise. All frogs were kept in enclosures that were 32 cm in width, 60 cm in length, and 18 cm in height. Animals were maintained at the same temperature and humidity with a 12-h day/night light cycle and were fed ad libitum. There was minimal mortality (<10/group). Sound levels in groups 1, 2, and 4 were 81 dB sound pressure level ([SPL]; approximately 20 μPa). All enclosures were sound attenuated by at least 35 dB from one enclosure to the next, bringing the sound of adjacent acoustic treatments to a level well below the threshold for hearing of this species (Wilczynski et al., 2001). During the 12-h day cycle, enclosures were opened to provide air circulation. This species needs water to call; no standing water was provided in the enclosures, so males were not able to call.

As each female reached sexual maturity (25 mm snout–vent length), we tested her in two-choice phonotaxis tests. These tests were performed in an acoustic chamber (2.7 × 1.8 m) illuminated by ceiling-mounted infrared lights and equipped with an infrared camera to allow undisturbed observations of the female's behavior from a video monitor outside the chamber. We placed each female in the center of this arena and kept her immobile under a funnel while two stimuli were played sequentially through speakers at each side of the chamber at a sound level of 81 dB SPL (approximately 20 μPa). After 3 min, the funnel was raised, allowing the female to move freely. A female made a choice if she met all of the following criteria: 1) she moved from the center within 5 min, 2) she continued to move at least every 2 min, 3) she moved within 10 cm of a speaker within 15 min of being released. We injected females with 250 IU of human chorionic gonadotropin per 2.45 g body mass 12–18 h before being tested to simulate the hormonal states of female *P. pustulosus* in the wild (Lynch et al., 2006). Observers for all female phonotaxis tests were unaware of the treatment each female received.

All females were tested with four pairs, using five stimuli (Fig. 1). The call stimuli were synthesized using the mean characteristics of a simple call produced by *P. pustulosus* (simple call), a simple call followed by a broadband chuck as produced by *P. pustulosus* (complex call), a call produced by *P. enesefae* (heterospecific call), or the call characteristics intermediate between the two (intermediate call; see Ryan et al., 2003). The fifth stimulus consisted of noise with the amplitude envelope of the conspecific simple call (amplitude-modulated noise). All females were first tested for their preference for conspecific simple vs. complex calls. Females that did not make a choice in the initial test were retested later that night; females that failed to make a choice in the second test were not retested. For all tests, we scored whether each female chose the amplitude-modulated noise stimulus or whether they chose the simple call, the heterospecific call, or the intermediate call. The final test was always another test of the simple vs. complex calls, and each female was required to make a choice in that final test for her choices in the prior tests to be included (data not shown but similar to the first stimulus pair in Table 1). Each female was tested only once and each female was tested completely in one night. At the completion of testing, each female was monitored for health during the subsequent day cycle then returned to the enclosure from which she came.

Twenty-two females in each treatment group completed all of the phonotaxis tests. A logistic regression score was used to compare the choices made in each two-choice phonotaxis test (outcome) across the early acoustic treatment each female received (predictor).

Results

In all tests, there were no differences in the choices made based on the females' early experience (Table 1). More females in all the groups chose the conspecific complex call over the simple call, and there were no significant differences across rearing groups ($P = 0.95$). When presented with amplitude-modulated noise vs. the simple conspecific call, females more often chose their own species call, but there were no differences among rearing groups ($P = 0.42$).

Females in all rearing groups chose intermediate calls or calls of the sympatric congener *P. enesfae* only rarely. When tested for their choices between the amplitude-modulated noise stimulus and the intermediate call, females rarely chose the intermediate call; there was no effect of rearing group ($P = 0.43$). When tested for their choices between the amplitude-modulated noise stimulus and the calls of the sympatric congener *P. enesefae*, only two or three females in each rearing group chose the *P. enesefae* call; there was no effect of rearing group ($P = 0.93$).

Discussion

The results of this study support the hypothesis that early experience does not alter female mate choice in *P. pustulosus*. Females reared in four acoustic environments demonstrated similar choices in a series of two-choice behavioral tests. Females showed strong preference for the conspecific advertisement call and preferred the complex call to the simple call, consistent with results of previous studies of this species in both laboratory-reared and wild-caught animals (Ryan, 1980; Ryan and Rand, 2003; Gridi-Papp et al., 2006). The percentage of females in all rearing groups that chose the complex call over the simple call is similar to that seen in much larger data sets of female phonotaxis in this species (Gridi-Papp et al., 2006). In particular, the fact that females reared in acoustic isolation showed no difference in mate choices supports the conclusion that early exposure to the conspecific call is not necessary for female *P. pustulosus* to acquire species-typical preferences.

This experiment also shows that early experience does not alter the discrimination of female *P. pustulosus* against the calls of the sympatric heterospecific *P. enesefae*, even in female *P. pustulosus* reared hearing calls of *P. enesefae*. The strong

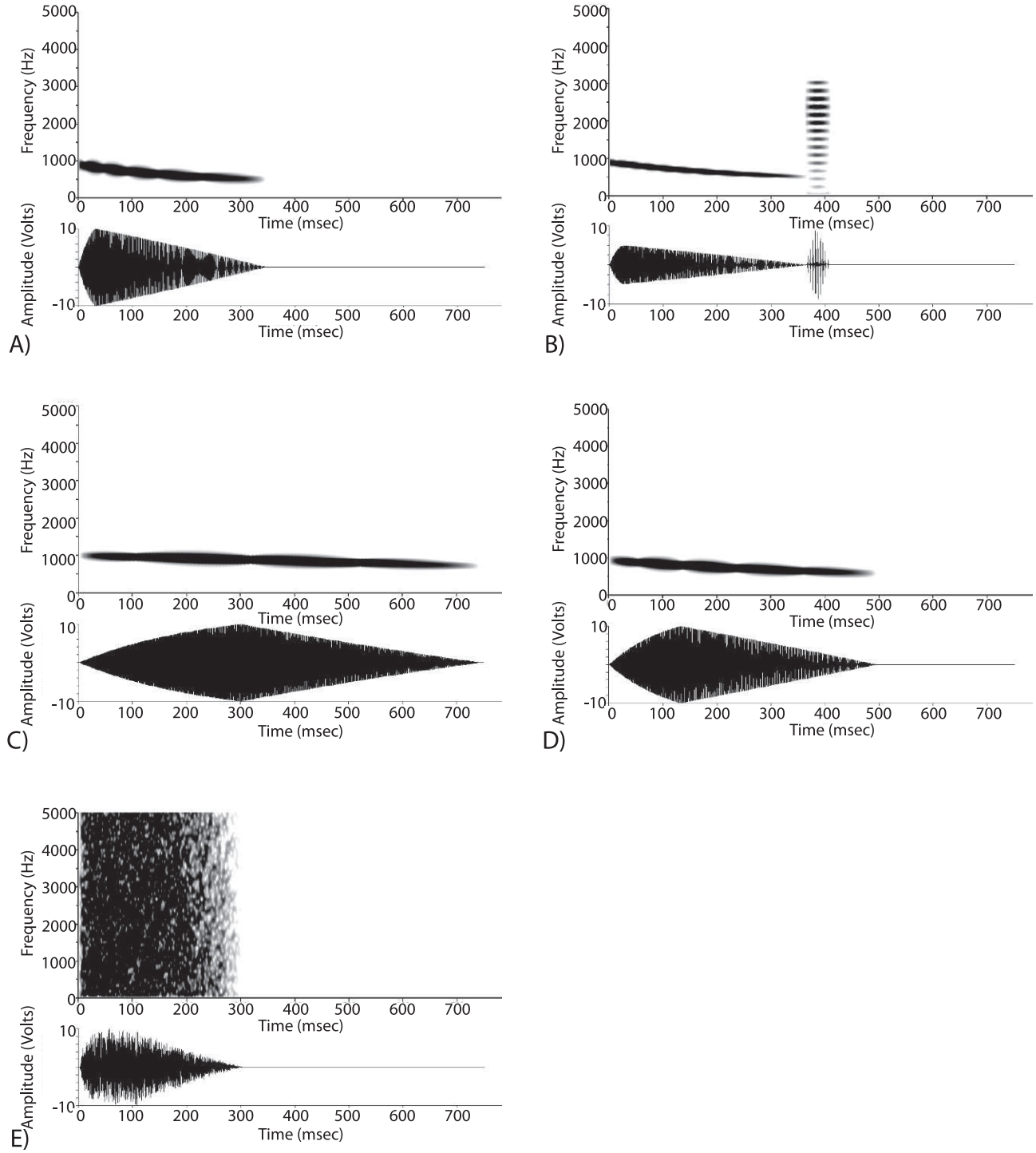

FIG. 1. Five stimuli were used to test female choices: conspecific simple call (A), conspecific complex call (B), heterospecific call (C), intermediate call (D), and amplitude-modulated noise (E). Spectrograms (top) and waveforms (bottom).

discrimination against sympatric congeners suggests that this discrimination may be the result of intrinsic factors, in which case this discrimination should reinforce mating isolation in *P. pustulosus* when congeners are present.

These results are consistent with models of sexual selection and with the sensory bias hypothesis. Models of sexual selection predict that female mating behaviors will show less variation than male behaviors (Andersson, 1994). In this species, males show alterations in the structure of their mating call when reared in the same environments as in this study ((Dawson and Ryan, 2009). If female mate choices had shown an effect of experience, then environmental variation such as the introduction of non-native species or the alteration of the physical habitat could alter early experience and potentially change female preferences. Instead, our data indicate that female mate choices are invariant in the face of early experience. These results are also consistent with the sensory bias hypothesis (Ryan and Rand, 1990). This hypothesis predicts the evolution of male traits to exploit the receiver's preexisting sensory biases. In this model, female sensory biases should be relatively

TABLE 1. Responses of female *P. pustulosus* reared in four acoustic treatments, to stimulus pairs presented in a two-choice experiment (sample size in each treatment group = 22, df = 3).

Stimulus 1	Stimulus 2	Rearing treatment	Chose stimulus 2	Logistic regression score	*P*
Conspecific simple call	Conspecific complex call	*P. pustulosus*	18	0.34	0.95
		P. enesefae	19		
		Isolation	18		
		Noise	19		
Noise	Conspecific simple call	*P. pustulosus*	16	2.81	0.42
		P. enesefae	20		
		Isolation	19		
		Noise	18		
Noise	Intermediate call	*P. pustulosus*	7	2.62	0.45
		P. enesefae	5		
		Isolation	7		
		Noise	10		
Noise	*P. enesefae* call	*P. pustulosus*	3	0.45	0.93
		P. enesefae	2		
		Isolation	2		
		Noise	3		

unchanged by environmental factors such as developmental experience.

Future work is needed to further explore the possibility that early experience may influence mate choice in female *P. pustulosus*. Experiments that use calls from all species in the *Physalaemus* genus group as early acoustic treatments might elucidate the boundaries and strength of conspecific discrimination. Also, this study does not address possible influences of age or sexual experience on variation in female preference. For example, females might alter their mate choices if exposed to varying acoustic treatments for a longer duration, or across multiple seasons. To understand whether permissiveness changes in mated females, females reared in varying treatments could be mated (ideally without exposure to conspecific acoustic signals) and then tested.

This study provides empirical evidence to support the hypothesis that acoustic experience before mating does not alter mate choice in females of this species of anuran. With early experience eliminated as a major influence in female mate choice, future studies of variation in female mate preference should focus on intrinsic factors, such as physiology, anatomy, and genetics.

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