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The Development of Sexual Behavior in Túngara Frogs (*Physalaemus pustulosus*)

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We examined the emergence of a critical component of sex, response to sexual signals—phonotaxis—in male and female túngara frogs (*Physalaemus pustulosus*). We determined the ontogenetic trajectories of phonotactic responses as animals developed from metamorphic froglets to reproductive adults. The results demonstrated that species-typical phonotaxis emerges quite early during postmetamorphic development, well before sexual maturity, suggesting that a developmentally early bias in the auditory system for species-typical signals might be a more general phenomenon than previously thought, and that the neural circuits responsible for processing and responding to conspecific advertisement signals in a species-typical manner might develop long before the coordinated behavior is demanded of the organism.

Keywords: acoustic communication, locomotor, ontogeny, phonotaxis, sexual behavior

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An inclusive investigation of behavior must consider at least four major features: evolution, adaptation, causation, and development (Tinbergen, 1951, 1963; Bolhuis & Verhulst, 2009). One issue in animal communication that unifies Tinbergen's approaches is vocal recognition (Gentner & Margoliash, 2002; Ryan, 2005). For a signal to transmit information effectively, the receiver must be able to interpret predictably some of the variability in the signal. An intrinsic bias in sensory organs and the central nervous system for detecting and perceiving conspecific vocalizations can aid in this process, contributing to physiological parity between sender and receiver. In this way, animals often exhibit behaviors with strong predispositions to specific sensory stimuli that are likely to result from the activation of developmentally programmed circuits (Balaban, 1997; Gottlieb, 1965; Gottlieb, 1991; Long, Kennedy, & Balaban, 2001). While past arguments about the nature–nurture dichotomy have yielded to a more inclusive consensus that organisms represent a complex union of plastic and fixed traits, we lack examples in animal communication of behavioral development in receivers that occurs in the absence of socially mediated learning. Examining when a coordinated behavior first emerges, rather than when it is first exhibited in its full form, is especially relevant for behaviors that result from stimulus processing, and in particular stimuli of social relevance such as species specific mating signals, because it also informs our understanding of the emergence of species recognition (Burghardt, 1977).

Many animals exhibit certain behavioral predispositions, and these are often expressed during different developmental stages. Vocal recognition in songbirds is one example in which auditory predispositions are thought to prepare animals for communication later in life. Shortly after the seminal discovery by Thorpe (1958) and Marler (1963) that songbirds acquire their vocalizations through vocal learning, it became apparent that, in addition to an experience dependent aspect of vocal behavior, there were also important predispositions (Thorpe, 1961). Most of these early investigations concentrated on the sender by examining the song produced by adult males as a function of early experience (Marler, 1998). There were, however, some studies of early development of song recognition. In these studies, physiological (e.g., cardiac orienting response) and behavioral (e.g., begging call rate) measures demonstrated that naïve fledgling songbirds respond more strongly to conspecific song, as compared with alternatives such as heterospecific song (Braaten & Reynolds, 1999; Dooling & Searcy, 1980; Hauber, Russo, & Sherman, 2001; Nelson & Marler, 1993; Whaling, Solis, Doupe, Soha, & Marler, 1997). In white crowned sparrows, this bias for conspecific song appears to be

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based on selectivity for acoustic features (e.g., frequency) rather than more complex features such as syntax (Whaling et al., 1997). In songbirds it has been proposed that such biases function to guide conspecific vocal learning (Nelson & Marler, 1993). This functional suggestion, however, would likely not explain biases in animals which lack vocal and auditory learning. Similarly, psychological studies have shown that human neonates respond preferentially to human vocal sounds (Eimas, Miller, & Jusczyk, 1987), and even express unlearned preferences for consonant over dissonant musical sounds (Zentner & Kagan, 1996).

Although there is a good understanding of auditory ontogeny in the context of learned responses (e.g., birds, humans), there is little known about how the expression of responses to acoustic signals proceeds when there is a lack of learning. This is a major oversight as there is little evidence that learning is involved in shaping acoustic pattern recognition in many major taxa that utilize acoustic communication, such as insects (Greenfield, 2002; for an exception see: Bailey & Zuk, 2008), frogs (Gerhardt & Huber, 2002), most nonpasserine birds (Catchpole & Slater, 1995) and most primates (Newman & Symmes, 1982; for exceptions see: Seyfarth & Cheney, 1999). Thus in these taxa we do not even know if predispositions to conspecific sexual signals are expressed behaviorally in the absence of vocal or auditory learning, or if any sex differences seen in adults are manifest ontogenetically.

In the present study, phonotaxis in frogs was used as a behavioral measure of acoustic pattern recognition analogous to the approaches used in avian and human recognition studies. Phonotaxis in reproductive female anurans has been used extensively to assess mate choice preferences, species recognition and signal detection (Gerhardt & Huber, 2002; Ryan, 2001). Phonotaxis in male anurans is less frequently examined and it is assumed that in chorus breeding species this behavior functions to guide males to aggregations and in territorial species to repel rival male intruders (Hödl, Amézquita, & Narins, 2004). Sex differences in phonotactic responsiveness and underlying locomotor differences have not been examined previously. It is important to note that phonotaxis is an unlearned behavior; no animal training is required and the stereotypic nature of the calls and consistency of phonotactic preferences have led to the suggestion that experience plays virtually no role (Gerhardt & Huber, 2002). In the present study, we examined the development of conspecific phonotaxis in the túngara frog, *Physalaemus pustulosus*—the only anuran species in which the absence of vocal and auditory learning has been confirmed directly through acoustic isolation experiments, demonstrating that neither vocalizations (males) nor phonotactic responses to them (females) are affected by conspecific acoustic isolation, heterospecific, conspecific, or noise stimulation (Dawson, 2007; Dawson & Ryan, 2009). Thus, unlike in studies of songbirds, frogs do not have to be socially and acoustically isolated to examine experience-independent contributions to behavior. Therefore, responses to sexual signals by sexually immature individuals, or premature responses, might suggest that intrinsic biases are a more general feature of the vertebrate auditory system than has previously been appreciated.

Despite the prevalence of auditory biases, there are significant lacunae in our understanding of their developmental bases, including sex differences in the expression of such predispositions (Dooling & Searcy, 1980; Nelson & Marler, 1993). Given the extent of research on túngara frog adults (reviewed in Ryan, 1985; Ryan & Rand, 2003a; Ryan, in press), we are positioned to address questions regarding the development of auditory, and in this case, sexual behavior. As a system, túngara frogs provide an important advantage: a stimulus-elicited behavior for a task relevant to sexual reproduction is shared by both sexes, making possible the dual examination of behavioral ontogeny in both males and females. This is the first study, to our knowledge, to examine phonotaxis in subadult anurans. We propose two competing hypotheses for the developmental time course of phonotaxis and we refer to these as "graded" and "threshold" patterns. First, if the auditory and motor circuits for processing and responding to species-typical signals are present after metamorphosis and phonotactic responses to conspecific signals are simply a function of circulating gonadal steroid levels, then phonotactic responses might emerge early in development when the gonads begin secreting hormone, and increase in frequency gradually as animals grow to adulthood. Alternatively, phonotaxis might only be expressed at sexual maturity, either because the circuitry for this behavior is absent in sexually immature animals, or because only at sexually maturity does a threshold level of steroid content activate this circuitry and motivate responses.

The System

During the breeding season (May–December) adult male túngara frogs vocally advertise to females using a species-typical call, known as the 'whine,' or simple call (Ryan, 1985). Males can embellish facultatively the whine with one to seven suffixes known as "chucks" thereby producing what is known as the complex call. These whine-chuck calls are preferred compared to whines in two-choice phonotaxis tests by reproductively active females collected in the field (Ryan, 1985; Ryan & Rand, 2003a). While these reproductively mature females respond with robust phonotaxis toward conspecific mating calls, adult females in between cycles of ovulation ("nonreproductive") also exhibit recognition and discrimination of conspecific signals, although the overall frequency of phonotactic responses is diminished (Lynch, Rand, Ryan, & Wilczynski, 2005a). Further, reproductive adult males collected in the field have been shown to exhibit positive phonotaxis to conspecific mating calls with a similar preference for the complex call (Bernal, Rand, & Ryan, 2009).

Examining juvenile behavior prior to reproductive maturity will provide a window into the origin of auditory recognition. Here we pursue early recognition by tracking acoustically guided behavior during postmetamorphic growth in male and female frogs to determine the developmental time course of this sexual behavior, which is critical to species recognition, and furthermore to examine sex differences therein. We began by characterizing the fully developed behavior more closely in adults so that we have a standard with which the behavior of the developing animals can be compared (Bekoff, 1978).

Method

Animals and Experimental Design

Between February 2006 and April 2007 we tested 24 male and 18 female túngara frogs (*Physalaemus pustulosus*) throughout

their entire postmetamorphic (froglet to adult) development for phonotaxis using a within-subject design at six developmental time points, including three juvenile and three adult time points (see Figure 1). Males express a secondary sexual trait (a visible vocal sac) at the adult time points and begin calling while females begin to show external signs of egg maturation. In the field, the smallest adult male collected at a breeding site was 24 mm snout-to-vent length (SVL) and the smallest female was 27 mm (Ryan, 1985). Davidson and Hough (1969) found that in the laboratory male túngara frogs are potentially capable of reproduction when they exceed 20 mm SVL.

Development of Conspecific Recognition Behavior

Subjects ($N = 42$) were from a breeding colony at the University of Texas at Austin and collected as larvae from seven broods derived from seven different adult mated pairs and reared until metamorphosis in brood-housed aquaria. During postmetamorphic development, animals were housed in the same room as a colony of adults and were thus exposed to conspecific acoustic signals throughout development. Again, such experience has been shown previously to have no effect on vocalizations or phonotactic responses to them (Dawson, 2007; Dawson & Ryan, 2009). At metamorphic climax (tail fully resorbed at end of Gosner stage 46; Gosner, 1960), animals were transferred to terrariums where they were group-housed until the first testing time point, and housed individually thereafter. We tested animals at six time points. All subjects could not be tested on a single day, thus the testing for each time point encompassed several days. The six time points included the following in days following metamorphic climax (mean ± *SD*): (1) 13 ± 5; (2) 51 ± 4; (3) 89 ± 6; (4) 221 ± 21;

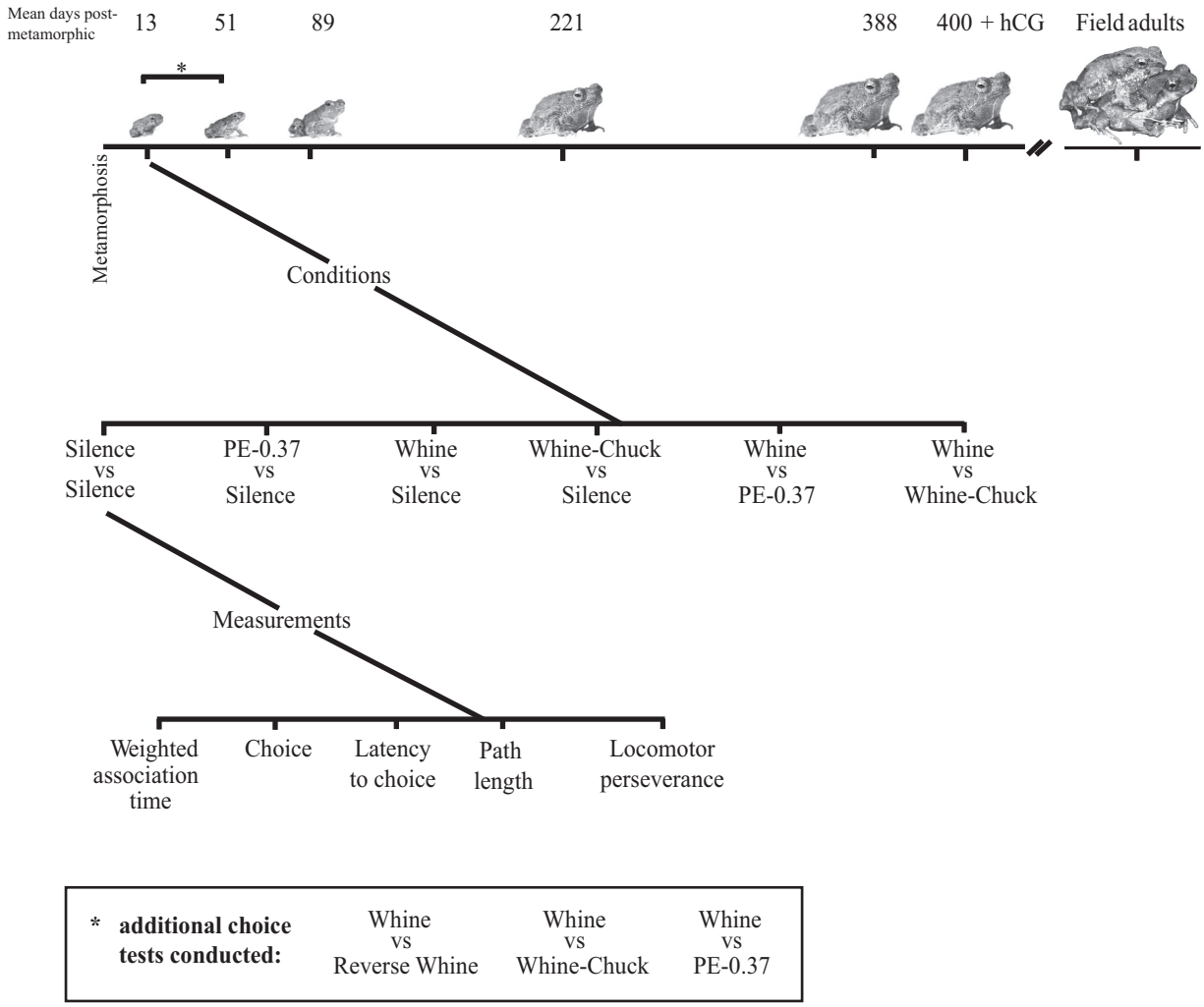

Figure 1. Developmental timeline of study that shows the six time points evaluated during postmetamorphic development in lab-reared frogs ($N = 42$) and an identical battery of tests conducted on field-caught, reproductive adults ($N = 24$). At each time point the six tests depicted were conducted and five behavioral measurements were recorded in each, resulting in a total of 1,656 trials. Additionally, three two-choice tests were conducted in 2008 on young (approx. time points 1 and 2) juveniles ($N = 20$; 10 males and 10 females; 60 choices). In total, this study comprised 422.1 hours of behavioral observation.

(5) 388 ± 22; and (6) 400 ± 21. At this final time point, the frogs were adults and injected with human chorionic gonadotropin (hCG), which acts as a ligand for luteinizing hormone receptors, stimulating the production of gonadal hormone. We used this hormone to ensure that animals in the final testing time point are sexually competent.

An identical testing procedure was conducted on field-collected reproductive male ($N = 12$) and female ($N = 12$) frogs at facilities of the Smithsonian Tropical Research Institute in Gamboa, Panamá in order to compare the behavior of the adult lab-reared animals to that of wild caught adults in which phonotaxis behavior is fully developed (see Figure 1); the phonotaxis chambers in Gamboa, Panamá and Austin, Texas are the same dimensions and model (Acoustic Systems).

At each time point, six phonotaxis tests were conducted, including (1) conspecific whine-chuck versus silence, (2) conspecific whine versus silence, (3) an intermediate whine (PE-0.37, see Stimuli below) versus silence, (4) silence versus silence, (5) whine versus intermediate whine (interspecific discrimination), and (6) whine-chuck versus whine (intraspecific discrimination). Recognition was ascribed when animals performed significantly more phonotaxis (choices and association time) in response to the conspecific stimuli (whine and whine-chuck) compared to silence. Subjects were measured for mass and SVL at each time point. Animals were fed fruit flies and pinhead crickets ad libitum three times per week throughout development. For the final adult time point (time point 6), all subjects (female and male) were injected intraperitoneally with a 300-International Unit (IU) dose of hCG (Sigma) to induce oviposition in females and ensure that the final time point represented a gravid, reproductive condition. A previous study demonstrated that hCG treatment in adult female túngara frogs increases phonotactic responsiveness, including robust increases in the frequency of phonotactic choices, decreases in the latency to choice, and increases in permissiveness (Lynch, Crews, Ryan, & Wilczynski, 2005b); similar effects are observed in females that are naturally cycling through reproductive conditions (Lynch et al., 2005a). Using age- and size-matched frogs in a previous study, we determined that a dosage of 300 IU was effective at stimulating approximately 90% of females to oviposit within 24 hours of injection (Baugh, unpublished data). On average, females were tested 19.9 hours (±2.1 hours *SD*) postinjection and males were tested at 21.0 hours (±2.0 hours *SD*) postinjection. We recorded the presence and timing of oviposition for all females. Males have not previously been examined for behavioral responses following hCG treatment—we did so here to examine if such effects exist and in order to maintain a symmetrical sex difference study.

Acoustic Discrimination in Juveniles

In addition to evaluating recognition (approach toward conspecific signals), in 2008 we conducted experiments to evaluate acoustic discrimination in juveniles (preferential approach toward certain conspecific signal variants over others simultaneously presented; age postmetamorphic, mean ± *SD*: males, 31 ± 17 days; females, 26 ± 13 days; approximately time points 1 & 2; see Figure 1). Animals were reared from two unique broods and housed under identical conditions to those used in the 2006–2007 study. We performed three separate two-choice tests: (1) whine versus reversed whine; (2) whine versus intermediate whine; and (3) whine-chuck versus whine. We measured choices by testing 71 froglets across 378 trials, which resulted in 20 froglets that responded in each of the three testing conditions (i.e., $N = 60$ choices; 15.9% of trials resulted in a choice).

Stimuli

Four acoustic stimuli were used in this study (see Figure 2). The whine-chuck and whine are natural stimuli recorded in Panamá from a male with call properties centered near the mean for the study population (for spectrograms/oscillograms see stimulus 'M' in Ryan & Rand, 2003b). These two natural stimuli are identical except that the whine has the chuck component excised. Female and male túngara frogs preferentially approach the whine-chuck stimulus in a whine versus whine-chuck choice test (Ryan, 1985). The intermediate whine (PE-0.37) is a synthetic call intermediate between the dominant frequency of the average synthetic túngara whine and the dominant frequency of a related species, *P. enesefae* (Ryan, Rand, Hurd, Phelps, & Rand, 2003). For this stimulus, seven call parameters were adjusted by the same percentage to synthesize this call as an intermediate between each of the species' calls. PE-0.37 represents a signal that has an "acoustic distance" that is 37% similar to *P. enesefae* and 63% similar to *P. pustulosus.* For example, PE-0.37 has a fall time of 381 ms, which is 37% different from the *P. pustulosus* fall time (343 ms) and 63% different from *P. enesefae* fall time (446 ms). This linear method was used for the following seven parameters: rise time, fall time, maximum frequency, final frequency, time to one half the final frequency, time to one half the peak amplitude, and time from the call's peak amplitude to one half the peak amplitude during the fall. Female túngara frogs preferentially approach the conspecific whine in a PE-0.37 versus whine choice test (Ryan et al., 2003). The reverse whine is the identical whine stimulus with the temporal order reversed. Field-collected adult females discriminate against this stimulus when paired with the normal whine (Ryan, unpublished data).

All stimuli were adjusted to the same peak amplitude and broadcast using CoolEdit, 2000 (Syntrillum Software, Scottsdale, AZ) at a sampling rate of 44.1 kHz and 16 bit depth. During all treatments both speakers, including "silent" speakers, were amplified to control for any low frequency sound present in the system. SPLs of "silent" speakers were inaudible and below the threshold for detection using a GenRad SPL meter (General Radio Corporation, West Concord, MA; Model No. 1982).

Measurements

As túngara frogs are nocturnal, phonotaxis tests were conducted at night (1800–0200 hours) and filmed under infrared light. All tests were conducted in sound-attenuating chambers (Acoustic Systems, Austin, TX; dimensions: 1.8 m × 2.7 m; see Supplementary Material S1) with two speakers (Cambridge Audio, Texas; ADS, Panamá) at opposite poles and a ceiling-mounted wide-angle infrared camera (Fuhrmann Diversified, Inc.). Speakers were calibrated at the beginning of the night to 82 dB SPL (re. 20 μPa) at 0.5 m and recalibrated between each animal using a continuous 500 Hz tone and a GenRad SPL meter. In order to measure association time, the chamber was demarcated into equal thirds

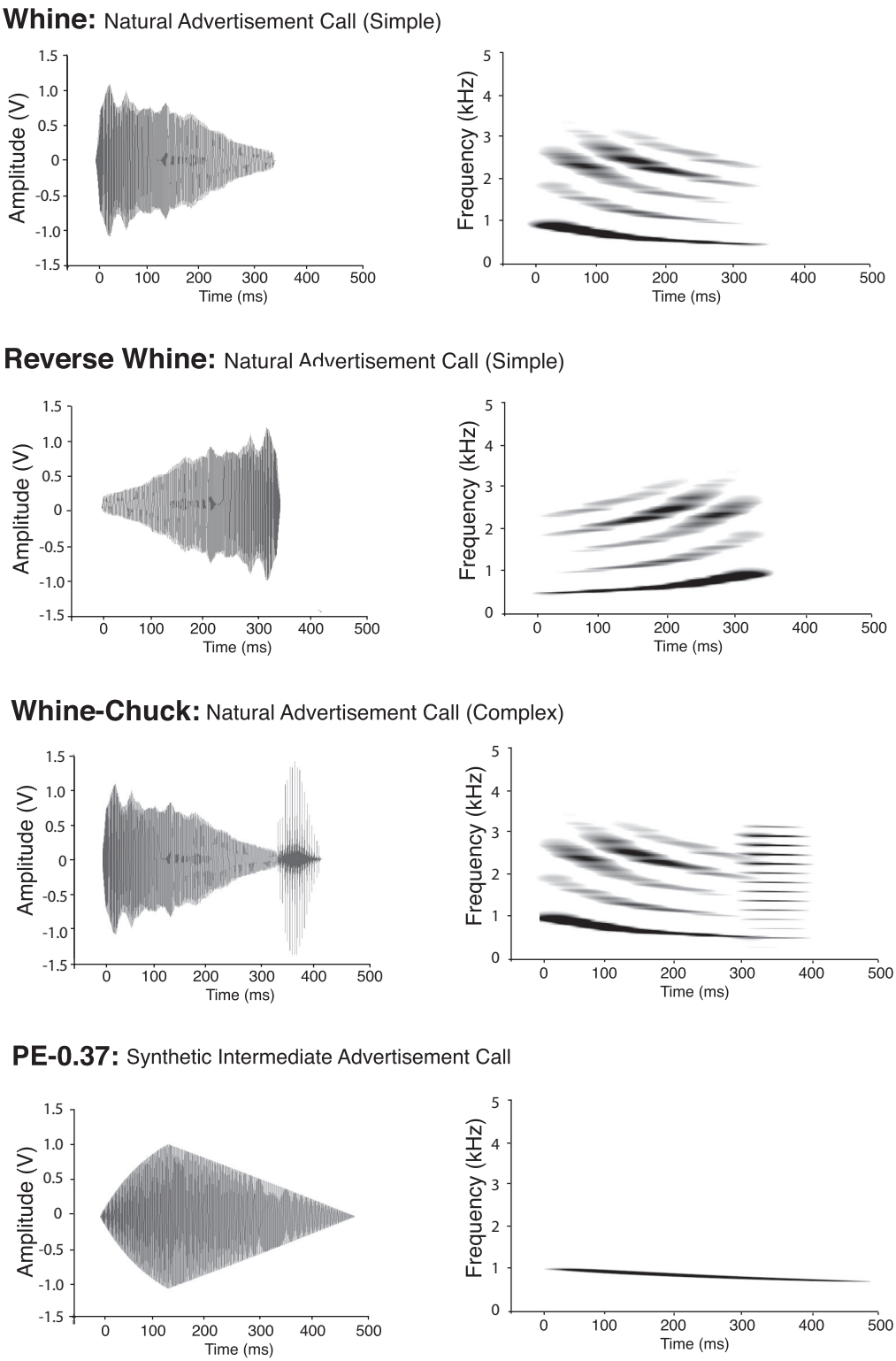

Figure 2. Acoustic stimuli used in study as oscillograms (left) and spectrograms (right).

such that each pole had a zone separated by a neutral center zone. The two terminal one-thirds were subdivided further into equally spaced concentric half circles radiating from the speakers representing the five weighted association zones (z_1–z_5). To achieve a fine-grained measure of affiliation, we recorded the time spent in these five zones and multiplied these values by each zone's weight (proximal to distal distances from the release point: 0.2, 0.4, 0.6, 0.8, and 1.0). Therefore, time spent directly adjacent to a speaker (e.g., z_5 weight = 1.0) was weighted more heavily than time spent at a more distant location (e.g., z_1 weight = 0.2). Time spent in the neutral third of the chamber arena did not contribute to association time. Weighted association time measures have not been used previously in anuran phonotaxis studies; we did so here because it is possible that in juveniles approaching a conspecific signal does not involve a near approach (<10 cm—the diameter of the choice zone) and could therefore potentially be a more sensitive metric of recognition in young animals. In a similar way, Narins and Capranica (1976) used an approach-distance measurement to obtain finer resolution of auditory behavior in *Eleutherodactylus coqui*.

Each animal at a given time point was tested under all six tests conditions and subjects were tested in a random order without the naïve observer's knowledge of the animal's sex during the subadult time points. Test order was assigned randomly for each night of testing and the order was reversed between each frog. Finally, for a given test condition, stimulus position was alternated between the two speakers. Each test began with the subject under a cone in the center of the chamber (release point) while the test stimuli were broadcast for 2 min. The cone was then raised remotely and the animal was free to behave for 13 min. The summed duration of time spent in the weighted association zones (weighted association time = $\Sigma\ 0.2(T_{z1}) + 0.4(T_{z2}) + 0.6(T_{z3}) + 0.8(T_{z4}) + 1.0(T_{z5})$, see Supplementary Material S1) was recorded as well as choice (ascribed when a subject enters within 10 cm of an active speaker without simply traveling along the perimeter of the chamber). Additionally, we measured latency to enter choice zones, total path length and path lengths within the choice zone (hereafter 'locomotor perseverance'; Hyde & Jerussi, 1983). All of these measurements were recorded for behavior at either pole of the chamber, irrespective of whether stimuli were broadcast from both speakers. Total path lengths and locomotor perseverance path lengths were measured by tracing each animal's path in a subsequent viewing of the video-recorded trial (monitor dimensions: 34 × 28 cm) and quantified by placing a grid (1 cm^2) on the tracing and counting the number of intersections. This value was then converted to actual distances using a conversion factor derived empirically. Prior to data quantification, this grid was tested using a range of path lengths varying from simple to complex and these grid dimensions accurately captured real world path lengths in a manner unbiased by the complexity of the path.

Analysis

Development. We used repeated measures analysis of variance (ANOVA) on choice and weighted association time data to evaluate the onset of species recognition [two within-subject factors: Time point (1–6) and Stimulus (Conspecific and Silence)]; and, one between-subjects factor: Sex). Due to the relatively infrequent occurrence of phonotactic responses at the subadult time points we collapsed the results for choice and association time from the six tests into two categories: conspecific (whine and whine-chuck) and silence control (for full results see Supplementary Material S2–S3). Across the six testing conditions there were four opportunities each for subjects to select a conspecific or a silent speaker/zone (see Figure 1). Therefore the ANOVA on the choice results evaluates the number of conspecific (out of four) versus silent choices (out of four) within a subject. Likewise, the ANOVA on the association time results evaluates the weighted association time summed across the four conspecific speakers versus four silent speakers.

Path lengths, latency to choice and locomotor perseverance were also examined with repeated measures ANOVAs (for full results see Supplementary Material S2–S3). Because choice and weighted association time data were positively skewed due to an abundance of zeros at early time points, we performed parameter-free bootstrapped resampling (10,000 simulations/analysis) of repeated measures analyses of variance (R statistical package) to complement the canonical parametric ANOVAs (SPSS 16.0). Statistical *p* values derived from the bootstrapping approach were in broad agreement with the canonical outcomes. In the instances in which there were minor discrepancies (that did not affect statistical significance), the canonical *p* values were more conservative than the bootstrapped values and for this reason we report the canonical results here.

Acoustic discrimination in juveniles. We analyzed juvenile choice behavior in the three two-choice discrimination tests with two-tailed binomial and Fisher's exact tests.

Adults. The effects of hCG were evaluated using an ANOVA for the pre- and postinjection time points within subjects. Interspecific (whine vs. PE-0.37) and intraspecific (whine vs. whine-chuck) preferences were explored in males and females before and after hCG treatment. Path lengths, latency to choice and the proportion of choices made by field-caught adult males and females were examined for sex differences using a student's *t* test. Additionally, the nonparametric K-S test was used to compare the distribution of latencies of field-caught males and females. A repeated measures ANOVA was used to assess sex differences in perseverance for field-collected adults.

An alpha criterion of 0.05 was applied to all statistics used in this study and all predictions are two-tailed. All *p* values for tests involving multiple comparisons were adjusted and are reported after Holm-Bonferroni correction.

Results

Baseline Adult Responses

Before examining the developmental time course of lab-reared frogs using the protocol outlined above, we applied this same protocol to field-collected adults, which are known to exhibit robust species-typical phonotaxis and therefore could provide some ground-proofing of this method, particularly weighted association time measures. Field-collected adults responded in weighted association times as anticipated: females and males spent considerably more time associating with conspecific signals compared to the alternatives (silence, intermediate whine) demonstrating that the protocol described here has the potential to yield

reliable information during development (Supplemental Material S4). Analyses of these field-collected animals are discussed below (see sections on adults).

Development Overview

The development study of lab-reared frogs was conducted over the course of more than 1 year. The body mass and SVL followed an asymptotic growth curve (see Supplementary Material S5), as predicted for a species with both indeterminate growth and growth rates that decrease with size (Ryan, 1985). Froglets from the 2008 discrimination study were within juvenile size ranges (mean ± *SD* SVL: males, 13.75 ± 1.13 mm; females, 13.37 ± 0.90 mm; mass: males, 0.23 ± 0.06 g; females, 0.23 ± 0.05 g).

The principal behavioral result of this study is that species recognition was present in juvenile frogs at early time points and persisted throughout development, as seen in both of the choice and association time measures (Figure 3, Figure 4). We observed sex differences in developmental trajectories of locomotor activity, and in particular the locomotor activity directly adjacent to call broadcasting speakers.

Choices

Field-collected adults made significantly more conspecific compared to silent choices (Wilcoxon's Signed Ranks: males, $Z = 2.83$, $p = .005$; females, $Z = 2.95$, $p = .003$).

In general for the developmental time series, juveniles (time points 1–3) encountered conspecific choice zones in 10.2% of trials and silent choice zones in 2.4%; adults (time points 4–6), encountered conspecific choice zones in 27.8% of trials and silent choice zones in 4.4%. The repeated measures ANOVA returned significant main effects for the two within-subject factors: Time point ($F_{5,36} = 13.63$, $p = 1 \times 10^{-6}$) and Stimulus ($F_{1,40} = 62.28$, $p < 1 \times 10^{-6}$). The main effect of Sex was not significant ($F_{1,40} = 0.57$, $p = .46$). There was a significant Stimulus-by-Time point interaction ($F_{5,36} = 8.51$, $p = .00002$); at all six time points frogs selected conspecific speakers (C) more often than silent speakers (S) yielding statistically significant responses at time points 2–6, and the strength of this response increased gradually during development (C:S, time point 1: 10:2, $p = .06$; time point 2: 11:3, $p = .049$; time point 3: 30:7, $p = .003$; time point 4: 29:8, $p = .04$; time point 5: 52:11, $p = .00004$; time point 6: 59:4, $p < 1 \times 10^{-6}$). The Stimulus-by-Sex interaction was not significant ($F_{1,40} = 2.16$, $p = .15$). Finally, males and females did not differ in the number of conspecific to silence choices at any time point (time point 1: $p = .46$; time point 2: $p = .41$; time point 3: $p = .91$; time point 4: $p = .102$; time point 5: $p = .416$; time point 6: $p = .095$). In general, these results show that juvenile túngara frogs exhibit conspecific choices and increasingly perform this species-typical behavior throughout postmetamorphic development.

Weighted Association Times

For field-collected adults, weighted association times for conspecific were greater than silence (see Figure 4). The results of the repeated measures ANOVA from choices and weighted association times during development were largely in agreement with each other. As with choices, the main effects of the within-subject factors were significant, Time point: $F_{5,36} = 20.81$, $p < 1 \times 10^{-6}$; Stimulus: $F_{1,40} = 81.04$, $p < 1 \times 10^{-6}$. In contrast to the choice analysis, the main effect of Sex was also significant, $F_{1,40} = 5.97$, $p = .02$. As with choices, the Stimulus-by-Time point was also significant: $F_{5,36} =$

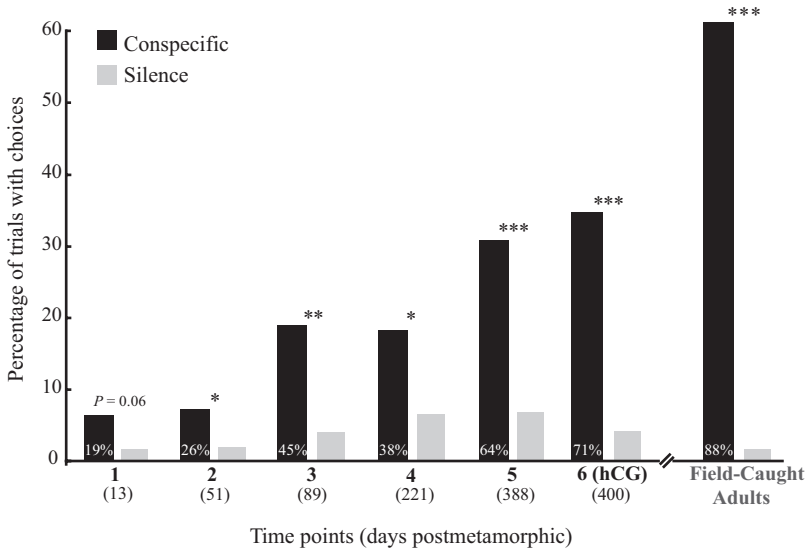

Figure 3. The percentage of trials in which phonotactic choices were made by frogs during development (sexes combined). The frequency of conspecific choices increased gradually during postmetamorphic development reaching a maximum in the reproductive adult state. Percentage values inside bars indicate the fraction of animals that made at least one conspecific choice (out of four opportunities) at each time point. These values were calculated because sample sizes were not equal between the lab-reared animals ($N = 42$) and field-caught animals ($N = 24$; bar at far right). NS = Not Significant. * $p < .05$. ** $p < .01$. *** $p < .001$.

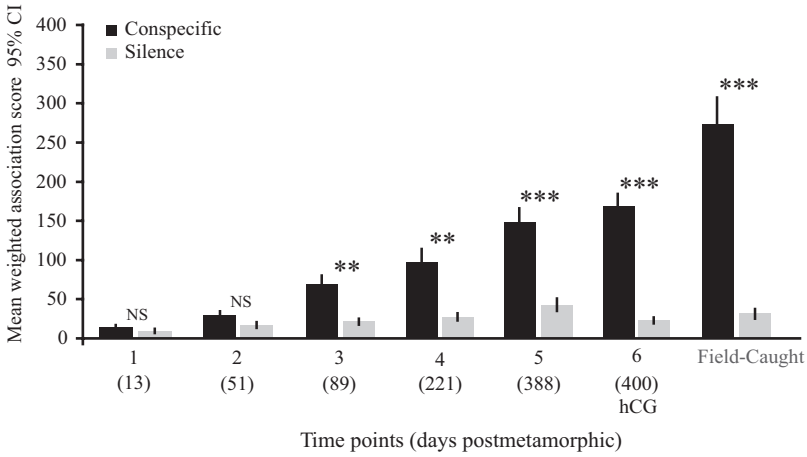

Figure 4. Mean weighted association time scores for developing frogs (sexes combined). Significance tests for time points 1–6 were carried out with pairwise comparisons within a repeated-measures ANOVA. For field-caught animals, a paired *t* test was conducted. NS = Not Significant. * $p < .05$. ** $p < .01$. *** $p < .001$.

18.6, $p < 1 \times 10^{-6}$. At all six time points frogs associated more strongly with conspecific speakers than silent speakers; this yielded statistically significant responses at time points 3–6, and as with choices the strength of this response increased gradually during development (time point 1: $p = .23$, Cohen's $d = 0.253$, effect size $r = .126$; time point 2: $p = .20$, Cohen's $d = 0.339$, effect size $r = .167$; time point 3: $p = .004$, Cohen's $d = 0.727$, effect size $r = .342$; time point 4: $p = .006$, Cohen's $d = 0.850$, effect size $r = .391$; time point 5: $p = .00001$, Cohen's $d = 1.07$, effect size $r = .472$; time point 6: $p < 1 \times 10^{-6}$, Cohen's $d = 1.84$, effect size $r = .677$). The Stimulus-by-Sex interaction was significant ($F_{1,40} = 10.32$, $p = .003$). Finally, males and females did not differentially respond to conspecific compared to silence, except at time points 4 and 5 (time point 1: $p = .63$; time point 2: $p = .40$; time point 3: $p = .28$; time point 4: $p = .02$; time point 5: $p = .02$; time point 6: $p = .82$). Thus, similar to the choice data these results suggest that túngara frogs exhibit positive phonotaxis toward the conspecific signals as juveniles and increasingly perform this species-typical behavior throughout postmetamorphic development.

Path Lengths

Path length analysis was conducted on total path length [total distance traveled (cm) during each 13 min trial] and path length restricted to the choice zone (see Locomotor perseverance below). The analysis of total path length addressed whether there were general locomotor differences during development, both between the sexes and in response to the six different acoustic conditions, irrespective of whether such locomotor behavior was guided by the presence of stimuli. The ANOVA for total path length returned a significant main effect of Time point ($F_{5,36} = 13.24$, $p < 1 \times 10^{-6}$) and Acoustic Condition ($F_{5,35} = 2.64$, $p = .017$) as animals traveled greater distances as they developed (see Figure 5). The main effect of Sex was not significant ($F_{1,40} = 0.003$, $p = .98$). The interaction between

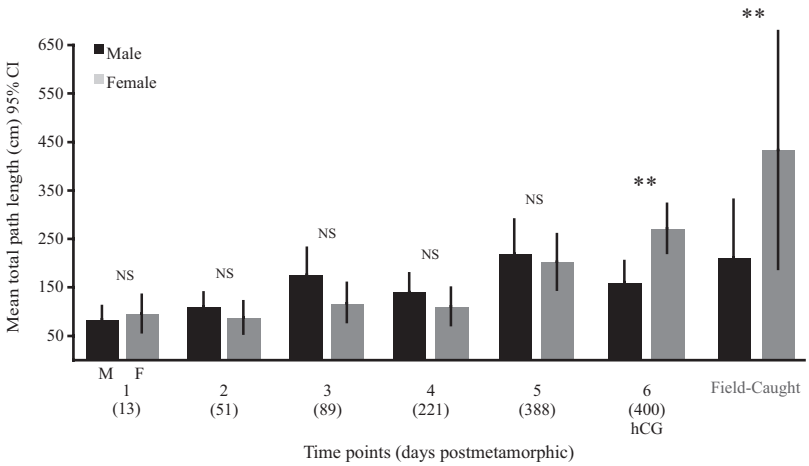

Figure 5. Mean total path length for males and females during development for all six acoustic conditions.

Time Point-by-Sex was significant ($F_{5,36} = 4.06$, $p = .002$; Figure 5), as was the interaction between Acoustic Condition-by-Sex ($F_{5,35} = 4.11$, $p = .001$) and Time point-by-Acoustic Condition ($F_{30,11} = 1.85$, $p = .004$). The three-way interaction, Time point-by-Acoustic Condition-by-Sex, was also significant ($F_{30,11} = 1.51$, $p = .04$). With respect to sex, there were no simple sex differences in baseline locomotor behavior, as demonstrated by a lack of difference under the silence versus silence control condition (overall $F_{1,40} = 0.342$, $p = .562$; pairwise comparisons: time point 1, $p = .98$, Cohen's $d = 0.183$, effect size $r = .09$; time point 2, $p = .71$, Cohen's $d = 0.012$, effect size $r = .006$; time point 3, $p = .73$, Cohen's $d = 0.274$, effect size $r = .135$; time point 4, $p = .25$, Cohen's $d = 0.455$, effect size $r = .222$; time point 5, $p = .61$, Cohen's $d = 0.164$, effect size $r = .081$; time point 6, $p = .94$, Cohen's $d = 0.022$, effect size $r = .011$). Likewise, there were no sex differences in total path length for field-caught adults in response to the silence versus silence control ($t_{22} = 0.756$, $p = .457$, Cohen's $d = 0.309$, effect size $r = .152$). More generally, there were no sex differences in total path length from the combined acoustic conditions except at time point six ($p = .002$, Cohen's $d = 1.01$, effect size $r = .453$; Figure 5) and in field-collected adults ($F_{1,22} = 9.41$, $p = .006$, Cohen's $d = 0.650$, effect size $r = .310$; Figure 5); in both instances this is when females exhibit a large increase due to locomotor perseverance at the choice zone (see Figure 6 and Locomotor Perseverance).

Total path lengths do not allow us to parse out more specific locomotor differences during development and between the sexes. Thus we also analyzed path lengths inside the 10-cm choice zone around the speaker to examine fine scale patterns of locomotion that could potentially inform our understanding of why adults of both sexes, especially males, perform phonotaxis in the first place.

Locomotor Perseverance

We refer to movement inside the choice zone as locomotor perseverance because we observed that after an animal enters the choice zone in response to a conspecific signal it restricts most of its movement in the near vicinity of the broadcasting speaker (Hyde & Jerussi, 1983). This locomotor activity was comprised principally of circling the selected speaker repeatedly until stimulus offset. Path length inside the conspecific or silence choice zones was quantified and analyzed with a repeated measures ANOVA and t tests. For the developmental series, perseverance behavior was only exhibited by adult females following hCG injection (see Figure 6), resulting in significant main effects and interaction terms from the ANOVA (see Figure 6): Time point ($F_{5,36} = 24.7$, $p < 1 \times 10^{-6}$); Stimulus ($F_{1,40} = 41.44$, $p < 1 \times 10^{-6}$); Sex ($F_{1,40} = 18.8$, $p = 9.6 \times 10^{-5}$); Time point-by-Sex ($F_{5,36} = 21.6$, $p = 1.2 \times 10^{-5}$); Stimulus-by-Sex ($F_{1,40} = 17.6$, $p = 1.5 \times 10^{-4}$); Time point-by-Stimulus ($F_{5,36} = 24.8$, $p < 1 \times 10^{-6}$) and Time point-by-Stimulus-by-Sex ($F_{5,36} = 22.4$, $p < 1 \times 10^{-6}$).

Locomotor perseverance was not exhibited in the silence choice zones and therefore we conducted the ANOVA on perseverance inside the conspecific choice zones only: Time point ($F_{5,36} = 24.9$, $p = 1 \times 10^{-6}$); Sex ($F_{1,40} = 18.4$, $p = 1.1 \times 10^{-4}$); and Time point-by-Sex ($F_{5,36} = 22.1$, $p < 1 \times 10^{-6}$). From Figure 6 it is evident that significance in these analyses is driven by the fact that only reproductive females (time point 6) perform perseverance and only in response to conspecific vocalizations ($t_{28} = 4.02$, $p = .0004$, Cohen's $d = 1.60$, effect size $r = .626$; Figure 6). Likewise, field-collected adult females exhibit perseverance but males do not ($t_{19} = 4.38$, $p = .0003$, Cohen's $d = 1.96$, effect size $r = .701$; Figure 6).

Acoustic Discrimination in Juveniles

Because juveniles exhibited conspecific recognition early in development, we also performed an independent experiment in 2008 to evaluate the degree of discrimination present. Here the choice results demonstrated that recognition of the conspecific whine depended on the direction of the frequency sweep as juveniles strongly preferred

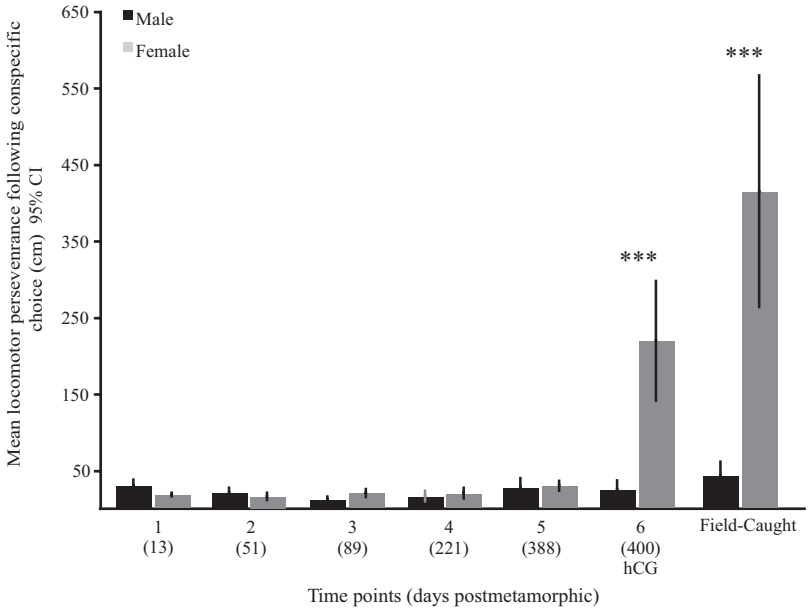

Figure 6. Mean path length for males and females inside conspecific choice zones (perseverance) after a choice was made. NS = Not Significant. * $p < .05$. ** $p < .01$. *** $p < .001$.

the conspecific whine when paired with the temporally reversed stimulus (choices whine:reverse whine = 19:1, binomial: p = .00004). Juveniles also exhibit the species-typical preference for the whine-chuck stimulus to the whine (whine-chuck:whine = 16:4, binomial: $p = .01$) and the whine compared to the intermediate whine (whine:intermediate whine = 15:5, binomial: $p = .04$). There were no sex differences in responsiveness (of the 20 responding froglets 10 were male and 10 were female), nor preferences (Fisher's exact test: whine:reverse whine: males: 10:0, females: 9:1, $p = .99$; whine-chuck:whine: males: 9:1, females: 7:3, $p = .58$; whine:intermediate whine: males: 8:2, females: 7:3, $p = .99$).

Sex Differences in Adults

Females and males collected in amplexus in Panamá exhibit similar phonotactic readiness at sexual maturity, as demonstrated by similar choice frequency (males: 27 choices [45% of trials]; females: 33 choices [55% of trials]; $t_{22} = 0.86$, $p = .39$). Despite this fact, the sexes differ markedly in locomotor perseverance in response to conspecific stimuli, with females but not males exhibiting perseverance ($F_{1,22} = 14.7$, $p < .001$). This perseverance behavior is not only sex specific but also developmentally specific, occurring only during a period of reproductive competence in adult females. Again, no appreciable perseverance behavior, nor developmental pattern in perseverance behavior, was observed in males (see Figure 6). Perseverance thus shows both quantitative and qualitative differences between the sexes and ages.

Latency to choice in field-collected adults also differs between the sexes, with females having significantly shorter mean latencies ($t_{57} = 3.27$, $p < .01$). This difference in latency was also observed in the distributions, with males exhibiting a positive skew compared to females (Kolmogorov–Smirnov test: $D = 0.366$, $p < .05$). Finally, differences in locomotor perseverance are not simply manifestations of general locomotor differences between the sexes, since path lengths en route to the choice zones did not differ between the sexes ($t_{22} = 0.58$, $p = .57$). In a separate study we show that perseverance behavior in females scales positively with the attractiveness of the male call (Baugh, unpublished data).

Finally, sex differences appear to be present in the effects of hCG treatment on weighted association time preferences. An ANOVA for the intraspecific discrimination condition (whine vs. whine-chuck) demonstrated a significant effect of hCG on females (Supplementary Material S6): the ANOVA produced a significant interaction of hormone state (pre/post) and stimulus (whine vs. whine-chuck; $F_{1,17} = 5.45$, $p = .032$) wherein the preference for the whine-chuck is evident only in posthCG females. In males, this same interaction was not significant ($F_{1,23} = 0.49$, $p = .49$), although there was a tendency toward a similar pattern of preference for the whine-chuck after hCG treatment (Supplementary Material S6).

In the interspecific discrimination condition (whine vs. PE-0.37), however, female preferences were unaffected by hCG treatment: there was not a significant interaction between hormone state (pre/post) and stimulus (whine vs. PE-0.37; $F_{1,23} = 0.001$, $p = .489$). In this instance it appears that hCG might increase the strength of the preference but that the preference is there before hCG (main effect of Stimulus: $F_{1,17} = 9.91$, $p = .006$; Supplementary Material S7). Preferences for males were also unaffected by the hCG treatment in the interspecific discrimination condition: the ANOVA for males showed no effect for the interaction between hormone state and stimulus ($F_{1,17} = 0.50$, $p = .98$)—that is, the preference for the whine stimulus was there before and after hCG (main effect of Stimulus: $F_{1,23} = 23.4$, $p = 7 \times 10^{-5}$; Supplementary Material S7).

More generally, the choice results suggested that hCG treatment in females elevated conspecific responsiveness (Supplementary Material S8; compare 388 and 400 days), whereas hCG appears to diminish responsiveness in males (Supplementary Material S8; compare 388 and 400 days). The bidirectionality of the effect (elevated choices in females and diminished in males) of hCG on conspecific choices for the sexes was significant for the interaction of hormone state and sex ($F_{1,40} = 6.89$, $p = .012$). This sex difference was also seen in latencies to choice, wherein females were faster to respond after hCG (mean ± *SD*: posthCG = 344.0 ± 151.8 s) than before (per subject mean ± *SD*: prehCG = 421.9 ± 105.2 s) and males showed the opposite effect (prehCG = 297.6 ± 155.3 s; posthCG = 415.6 ± 166.0 s).

Comparison of Lab-Reared and Field-Collected Adults

Lab-reared, hCG injected (time point 6) adult females expressed a similar responsiveness to conspecific stimuli in choice behavior compared to females collected in amplexus in the field (89% vs. 92% responders, respectively; Supplementary Material S8). In this study, 15 of the 18 females injected with hCG successfully oviposited and did so with an average latency after injection of 24 hours (±7.1 hours *SD*). Males, however, were less responsive as lab-reared adults than as field-collected adults (58% vs. 83% responders; Supplementary Material S8).

In response to conspecific stimuli (whine vs. silence, whine-chuck vs. silence, whine vs. whine-chuck, and whine vs. PE-0.37), the average total path length of lab-reared frogs at time point 6 (mean ± *SD*: males, 116.2 ± 137.9 cm; females, 319.2 ± 247.5 cm) was approximately half of that observed in field-collected animals (males: 204.7 ± 197.5 cm; females: 614.4 ± 440.5 cm). Males exhibited a decrement in phonotactic choice, association time and total path length after injection of hCG, so we also compared the total path lengths of time point 5 males (males: 202.5 ± 279.6 cm), which were similar to field-collected males. An ANOVA of total path lengths pooled across all six acoustic conditions from field-collected versus time point 6 males and females returned significant main effects (Lab/Field: $F_{1,62} = 16.9$, $p = 1.1 \times 10^{-4}$; Sex: $F_{1,62} = 43.2$, $p < 1 \times 10^{-6}$) and the interaction between these factors was significant ($F_{1,62} = 4.9$, $p = .03$). This was driven by the fact that field-caught females differed from field-caught males more than did their lab-reared counterparts. Because males at time point 6 (posthCG injected) experienced a decrement in phonotaxis, we also substituted time point 5 males in place of time point 6 in the ANOVA—this returned similar main effects (Lab/Field: $F_{1,62} = 7.74$, $p = .007$; Sex: $F_{1,62} = 24.26$, $p < 7 \times 10^{-6}$) and interaction ($F_{1,62} = 7.5$, $p = .008$). These total path length differences between the sexes and localities were due to differences in locomotor perseverance. Males did not exhibit significant perseverance regardless of time point (Lab: time point 5, 28.2 ± 29.5 cm; time point 6, 25.5 ± 28.9 cm) or locality (Field: 43.7 ± 31.3 cm; Figure 6), whereas females collected from the field performed

more perseverance (451.6 ± 292.1 cm) than lab-reared females at time point 6 (238.0 ± 185.1 cm; Figure 6).

Discussion

Many studies of ontogeny, especially in songbirds and primates, have explored behavioral changes in species-typical signaling (Hauser, 1989; Hollén & Manser, 2007; Rose et al., 2004; Seyfarth & Cheney, 1986; Tchernichovski, Mitra, & Nottebohm, 2001), including the influence of unlearned forces on the trajectories of song development (Soha & Marler, 2001). The present study is unique in that it is one of the most detailed studies of the ontogeny of a sexual behavior in a receiver, and in this case a "wild" animal. Collectively, we have demonstrated that a well-studied behavior—conspecific phonotaxis in frogs—is present well before sexual maturity, but that the full adult form of the behavior for females emerges only at reproductive competence. A gradual increase in the expression of conspecific-directed phonotaxis is mirrored by gradual increases in total locomotion in response to sound. This result supports the "graded" hypothesis of behavioral development. And although both sexes complete maturity with an overlapping but distinct collection of related behaviors as adults (e.g., females have shorter latencies to phonotactic choice), males and females also exhibit a nonoverlapping feature: perseverance locomotor behavior. This particular behavior in females is consistent with the observation that females actively update signaler information during an "evaluation" phase, and males may use female proximity information to adjust their calling strategy to maximize female commitment during this crucial, brief window of time (Baugh & Ryan, in press; Akre, pers. comm.). Further, while this particular detail of anuran phonotaxis (or of mate choice in general) has not been reported previously, it is in accord with a longstanding interpretation that females are performing phonotaxis to seek a mate; males, on the other hand, are likely doing so to join a chorus or simply find a calling location rather than localizing the individual calling (Ryan, 1985). In that sense, male phonotaxis might be a form of conspecific cueing—using the presence of conspecifics as an indicator of habitat quality (Keister, 1979; Stamps, 1988, 1991).

Young frogs not only approach conspecific signals, they also do so with the same selectivity observed in reproductive adults. The whine versus reverse whine result confirms that juveniles do not simply approach sound generally or sounds in the frequency range of the conspecific call as the reverse whine has all of the acoustic attributes of the conspecific call except for the direction of the frequency sweep. Likewise, young froglets selectively approach the complex whine-chuck stimulus when paired with the simple whine, demonstrating that the high frequencies contained in the chuck elicit a greater response, presumably due to stimulation of the basilar papilla in the frog's inner ear. Finally, also like adults, froglets prefer the conspecific whine to the intermediate whine, suggesting again a surprising level of selectivity for an immature animal. Methodologically, weighted association time and choice measures produced similar outcomes, suggesting that the more efficient measure (choice) is sufficient to examine recognition and discrimination in this species.

One of the most studied areas of behavioral development is play behavior (Burghardt, 2005). Although the premature expression of conspecific phonotaxis described here does not meet the criteria to qualify as play behavior, it has some parallels. Sex differences in the development of play behavior have been demonstrated in diverse groups, including rodents, primates, and reptiles. Adaptive explanations for play behavior have been suggested, such as the idea of preparatory development for predation (e.g., object play in felines). Both sexes of túngara frogs must perform phonotaxis as adults, yet the early expression of this behavior during development might signal some form of preparatory development or constraint. As has been suggested in certain instances of play behavior (Williams, 1991), if the costs of performing premature phonotaxis in frogs are low enough, the maturation of intermediate behavior will occur if the benefits of having the behavior immediately available for a rapid onset are great. This is a conceivable scenario for seasonal anurans that rely heavily on rainfall patterns and for whom reproductive success is tied so closely with attendance and effort at the breeding site (Ryan, 1985).

Function of Juvenile Phonotaxis

Our study of behavioral development has identified a previously unknown behavior—juvenile phonotaxis. While the present study did not examine the potential function of this behavior, it is possible that such behavior has an ecological and adaptive basis. For example, approaching and residing in the vicinity of an active chorus could serve to enable vocal (males) or auditory learning (males and females). We can reject a narrow form of this possibility because sexual behaviors in both sexes (calls of males and phonotaxis in females) are not influenced by acoustic isolation or stimulation during development (Dawson, 2007; Dawson & Ryan, 2009). Of course, this does not preclude a possible role for auditory learning not evinced through phonotaxis or other forms of auditory learning (e.g., predatory cues).

Another explanation of juvenile phonotaxis is that the behavior functions to maintain natal pond philopatry for developing animals and that choruses provide froglets with an acoustic beacon for their natal pond. In accord, studies have shown that there is no sex bias in dispersal (Lampert, Rand, Mueller, & Ryan, 2003; Marsh, Fegraus, & Harrison, 1999; Marsh, Rand, & Ryan, 2000). If froglets disperse from the natal site, phonotaxis to other choruses might cause them to disperse to areas where breeding is likely. In both of these scenarios, juvenile phonotaxis could be a form of conspecific cueing (Donahue, 2006; Keister, 1979; Stamps, 1988, 1991). Although conspecific cueing might be a function of juvenile phonotaxis, we emphasize this is mere speculation. Conspecific affiliation in juveniles might reflect a developmental precursor to an adult endpoint that subserves this critical behavior in adults. We occasionally find juveniles that are days to weeks postmetamorphic at chorus aggregations, but we are uncertain of their natal pond origin, whether they are dispersing from or to the chorus.

A final possible interpretation is that this behavior in premature animals is present but not adaptive—yet at the same time is not significantly maladaptive—it is conceivable that this behavior is nonfunctional when expressed prior to sexual maturity. There are a few reasons to think there might indeed be a function. First, performing phonotaxis requires time and energy. Second, unnecessary movement in the vicinity of a frog chorus could increase predation risk in this predator dense environment. Third, the fact that animals express this behavior infrequently (either because it is

actively inhibited or minimally activated due to low levels of motivation) suggests that phonotaxis would not occur if there was no benefit.

One important suggestion provided by this study is that the neural circuits responsible for processing and responding in a species-typical way to conspecific advertisement signals might develop long before the coordinated behavior is appreciated. Therefore when we trace a behavior back to its ontogenetic origins we must not fail to consider antecedent behaviors. Behaviors may appear to develop de novo when in fact the underlying neural circuitry has developed gradually. As an example of such preparation, Bentley and Hoy (1970) demonstrated that the neural network for song generation in crickets is present during postembryonic development and "in place" before the actual sound-producing structures (the forewings) have developed. This network is suppressed until the final moult to adulthood (see also Hoy & Cassaday, 1978). Similarly, complex motor patterns such as walking and the righting response in silk moths (*Antheraea pernyi* and *Hyalophora cecropia*) that are typical of posteclosion adults are present in developing pupae but only seen if the pupal cuticle is removed. Shortly before eclosion these behaviors are inhibited and then released from inhibition by the eclosion hormone at the final pupation (Truman, 1975). A similar finding was demonstrated in *Antheraea polyphemus*, in which pupae exhibit flight and warm up motor patterns characteristic of adults in the week preceding pupation (Kammer & Rheuben, 1974). In a study of auditory and vocal ontogeny in the anabantoid fish *Trichopsis vittata*, Wysocki and Ladich (2001) found that auditory sensitivity preceded vocal capabilities and that vocalizations preceded acoustic communication, as juveniles are not sensitive to the dominant frequencies contained in conspecific sounds. These examples and the present study on frogs point toward ontogenetic precursors to adult behaviors that emerge potentially well before such behavior is demanded of the organisms.

Sexual Dimorphism in Adult Response to Gonadotropin

Besides the dimorphism in the expression adult perseverance locomotor behavior, there is another sexual dimorphism that bears some consideration. At the completion of maturation, both males and females were administered hCG prior to their final phonotaxis tests. This gonadotropin should lead to an increased production of sex steroids in females (primarily estrogen) and males (primarily testosterone and dihydrotestosterone) and thus should promote the expression of sexual behaviors. But hCG had a dimorphic effect on the sexes, with females experiencing an elevation in responsiveness and males exhibiting a diminution.

Lynch et al. (2005b) also showed that hCG administration increased phonotactic responsiveness in female túngara frogs using 500 and 1000 IU dosages and that these dosages resulted in significant increases in plasma estrogen that were similar to the concentrations seen in field-caught amplectant females (Lynch & Wilczynski, 2005). In contrast, plasma androgen concentrations in the test females (testosterone and/or dihydrotestosterone) were unaffected by hCG treatment and were similar to that seen in field-caught amplectant females, although there was a tendency toward a decrease in androgen with increasing hCG dosages (Lynch & Wilczynski, 2005; Lynch et al., 2005b). Here we show that a 300 IU dosage is sufficient to stimulate female responsiveness and oviposition. Lynch et al. (2005b) showed that a choice preference for conspecific whine was present after 500 or 1000 IU dosages of hCG in two-choice tests when the signal was paired with PE-0.50. Here we show that for females a preference for a signal of even greater likeness to conspecific (PE-0.37) is present both before and after hCG treatment in association time measurements. Further, we show that the preference for the complex call is present in females only following hCG treatment, despite significant recognition of both conspecific signals before hormonal treatment (Figure 3; time point 5); this result is somewhat similar to a finding in midwife toads (*Alytes muletensis*) by Lea, Halliday, and Dyson. (2000) in which females exhibit conspecific phonotaxis after mating yet do not express the species-typical preference for vocalizations of mean (1.8 kHz) over low (1.5 kHz) frequencies. As with the study by Lynch et al. (2005b), we show that latency to choice was similarly reduced after hCG injections in females. Because hCG acts as an agonist for luteinizing hormone receptors, it is possible that hCG directly induced receptive behavior in females. This possibility, however, is unlikely given the results of a study by Kelley (1982), which showed that luteinizing hormone releasing hormone was effective in evoking receptive behavior in female *Xenopus laevis* that were ovariectomized and steroid injected while hCG had no effect. The elevated receptive behavior in females following hCG could be caused by the action of hCG on other hormones such as prostaglandins (Schmidt, 1984, 1985), a combination of estrogen and progesterone (Kelley, 1982) or arginine vasotocin (Schmidt, 1985). It remains to be seen if phonotaxis can be artificially induced in subadult female frogs, but this could potentially help discriminate between direct and indirect effects of hCG if administered to females with immature gonads.

We also present the first evidence for the effects of hCG on male phonotaxis and describe how such treatment decreases choices and overall movement and increases latencies to choice. In a study by Marler and Ryan (1996), male túngara frogs with higher endogenous testosterone levels had a higher probability of engaging in vocal behavior and application of corticosterone decreased levels of testosterone and the likelihood of calling. In that study the behavior was calling, and not call-seeking, and it is conceivable that these two different responses might be promoted and inhibited, respectively, by androgens. This could be explored by allowing a male frog to choose between engaging in vocalizing or phonotaxis, and manipulating levels of androgens to explore whether such manipulations affect this decision.

Conclusion

Studies of sex differences in anurans have focused on gonadal development (Gramapurohit, Shanbhag, & Saidapur, 2000), the neural expression and hormonal facilitation of sexual behavior in adults (Boyd, 1992; Boyd & Moore, 1992; Boyd, Tyler, & De Vries, 1992; Boyd, 1994), and auditory processing and morphology in adults (Mason, Lin, & Narins, 2003; McClelland, Wilczynski, & Rand, 1997; Miranda, 2007; Narins & Capranica, 1976). Excepting the present study, the topic of ontogenetic changes in behavioral responses to social signals and sex differences therein are entirely unexplored (Shofner & Feng, 1981). Recent studies of the determinants of sexual behavior in adults have demonstrated that sex differences in some species are due largely to differences in the

expression of a single gene, such as the Trpc2 gene in the vomeronasal organ being responsible for male like sexual behavior of mice (Kimchi, Jennings, & Dulac, 2007), and the *fruitless* gene's role in male like sexual behavior and sexual orientation in *Drosophila* (Ryner et al., 1996). There are no such studies with amphibians, but given the sex differences that emerge at reproductive adulthood such an examination would be informative.

Previous authors have suggested that auditory predispositions to conspecific signals in songbirds might function to minimize the learned acquisition of heterospecific vocalizations (Nelson & Marler, 1993) or function more generally across vertebrates to guide learned perceptual preferences (Balaban, 1997). In túngara frogs, it appears that a developmental predisposition to conspecific signals is present, and because vocal and auditory learning are absent in this species (Dawson, 2007; Dawson & Ryan, 2009) we must consider explanations other than the avoidance of heterospecific vocal learning or more general effects on auditory learning. We suggest that predispositions for conspecific vocalizations are a more general feature of developing vertebrate auditory systems. While a functional explanation for this behavior is not presently available, we must consider the possibility that phonotactic behavior in premature animals is premature itself; it might not serve a function when first expressed, but its initial expression might be a prerequisite for a normal developmental trajectory.

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