

Female túngara frogs vary in commitment to mate choice

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Mate choice studies most often examine female preferences based on population responses, thus potentially overlooking individual differences in behavior. Moreover, such studies typically use invariant stimulus conditions to infer preferences. By using population responses and static stimulus presentations, it is difficult to thoroughly understand the complexity of the mate choice process, including variation present between individuals. Here, we investigated phonotactic mate choice behavior in female túngara frogs (*Physalaemus pustulosus*) in response to temporally dynamic presentations of male advertisement calls. We tested females on repeated trials to examine individual differences and found considerable variation in the extent to which females update their mate choice decisions. Females in our study were bimodally distributed and thus broadly classified as either committed or uncommitted to an initial mate approach. We compared body condition measures of females differing behaviorally and determined that females with larger residual body masses were more committed to initial mate choices than less massive individuals, despite the fact that all females were in reproductive condition and field collected in amplexus. Our results suggest that anuran phonotaxis, once considered to be a highly stereotyped behavior, is more complex and variable than previously thought. *Key words*: body condition, choosiness, dynamic behavior, individual differences, mate choice, phonotaxis. [*Behav Ecol*]

Communication in nature is dynamic and acoustic signaling in particular usually occurs over brief timescales. Most studies of auditory behavior, however, use repetitive trains of identical stimuli to simplify the experimental conditions under which receiver behavior is observed. Such designs, although useful, limit the examination of the temporal dynamics involved in signal–receiver interactions. This is especially true in mate choice studies wherein acoustic signals act as a key trait used by receivers in nature to select mates and especially in lek-breeding species because multiple signalers often compete simultaneously for female attention and signal attractiveness can vary within a call bout (Gerhardt and Huber 2002; Ryan 2009).

Furthermore, most mate choice studies examine preferences using population assays, rather than individual responses. In some cases, this has perpetuated a typological thinking about behavior promoting the idea that there is “a female response” (sensu Jennions and Petrie 1997). This limits a thorough understanding of individual variation in sexual behavior and thus sexual selection. In studies that have explored individual variation in sexual behavior, the focus has typically been on examining differences in male traits and their relationship to mating success (reviewed in Andersson 1994). Such studies often focus on trade-offs between the costs of maintaining elaborate traits and the benefits such traits engender through increased attractiveness to females (Møller and de Lope 1995). A much smaller fraction of research has explored the role of individual differences in female choice, even though such patterns might be expected given individual variation in attributes such as age, experience, and body condition among females (Höglund and Alatalo 1995, reviewed in Cotton et al. 2006). Condition-dependent expression of

preferences, though not addressed in most studies, might optimize female investment in the mate choice process (Jennions and Petrie 1997).

Female preferences might vary in 2 fundamental ways: First, there may be variation in “preference functions,” the multidimensional landscapes that describe how females rank prospective mates (Kirkpatrick et al. 2006); second, females may vary in “choosiness,” the effort an individual is willing to invest in mate assessment (Jennions and Petrie 1997). In this way, the costs associated with choosiness can affect mating patterns without appreciable variation in preference functions. If these costs vary by individual, then patterns of mate choice may vary, whereas preference functions remain fixed. It is important to consider such sources of variation as they can affect, among other things, the rate and direction of evolution by sexual selection and provide a window into the mechanistic basis of mate choice. The proximate causes of variation in female preferences might arise through genetic differences (i.e., polymorphisms), environmental conditions (e.g., predation risk), or differences in reproductive condition, such as the urgency with which females must mate.

A few studies have examined genetic polymorphisms in female preferences and others have described phenotypic polymorphisms. Morris et al. (2003) demonstrated the former by showing that variation among females in their preferences for vertical bar patterns in male swordtail fish (*Xiphophorus cortezi*) was correlated with the presence of the same visual traits in females, suggesting that trait and preference are correlated through a genetic mechanism. Phenotypic polymorphisms have been demonstrated in satin bowerbirds (*Ptilonorhynchus violaceus*), wherein individual differences in preference are due to female age (Coleman et al. 2004); older females attended to male display intensity, whereas their younger counterparts selected males based on their bower ornamentation. Similarly, Moore and Moore (2001) showed that female cockroaches (*Nauphoeta cinerea*) become less choosy as they age. In addition to age effects, female preferences may differ due to their reproductive condition. In midwife toads, for example,

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female preferences were most consistent during the mated stage. Consistency of phonotactic preferences for the 1.8-kHz call over the 1.5-kHz call were lower at pre and postmating time points compared with the mated time point (Lea et al. 2000). In standard phonotaxis tests, female túngara frogs (*Physalaemus pustulosus*) prefer complex to simple calls and this preference is shared by all females (i.e., there are no repeatable individual differences; Kime et al. 1998; Ryan et al. 2003). Further, willingness to accept a less attractive male call is greatest in mated female túngara frogs compared with pre and postmated time points (Lynch et al. 2005).

In amphibians, reproductive females might differ individually in a manner that is not detected using static mate choice conditions. As female anurans mature their clutch, they face a diminishing window of time with which to find a suitable mate, and in some cases, oviposition eventually occurs in the absence of a male (Pope 1931; Coe 1974), including in túngara frogs (Baugh AT, personal observations). Consistent with this is the prediction that females facing greater urgency to mate will be less choosy (i.e., less willing to invest time in protracted mate assessment or sampling) without necessarily any change in their preference function. In other words, more urgent females might still evince a preference for a more attractive mate over a less attractive alternative if the costs of selecting either mate are equal—if, however, the costs associated with the more attractive mate are high, only those females that do not urgently need to mate will persistently express such a preference.

In a previous study we found that females of a neotropical frog (*P. pustulosus*) are sensitive to changes in timing and location of preferred male call types. Females reversed their phonotactic approach in approximately one-half of trials in which the preferred signal was diminished naturally in attractiveness, whereas the nonpreferred call was simultaneously enhanced in attractiveness between 2 opposing speakers (Baugh and Ryan in review). In the remaining one-half of trials, females continued on the trajectory of their initial approach despite the stimulus manipulation, thus maintaining their commitment to the same calling male even though his call became less attractive. Here, we explore individual differences in this behavior by repeatedly testing females.

Only a few studies have pursued dynamic mate choice, all of which have used frogs. Dyson et al. (1994) found that female reed frogs (*Hyperolius marmoratus*) will reverse direction after initially approaching (by one body length) a preferred stimulus (the leading call) if the stimuli are switched. Similarly, Gerhardt et al. (1996) examined the preference for pulse number in *Hyla versicolor* and demonstrated that females, after initially approaching a high pulse number call, would reverse directions if this stimulus were suddenly switched with the less preferred call. These studies provide evidence that at least in a few species of frogs, decision making involves temporal updating. The extent to which this type of behavior varies individually has, to our knowledge, not been addressed except in the present study. We believe there is much to learn about female variation by closely examining not only the preferences evinced by females but also the process of mate choice itself. One central question is when are mating decisions made and to what extent is the process open ended? This question can be answered by manipulating the attractiveness of a female's options during the decision-making process.

The system

Túngara frogs are small anurans (ca. 30-mm snout–vent length, SVL) distributed throughout much of Central America (Weigt et al. 2005). During the breeding season (May through

December), males vocally advertise to females using a species-typical call, known as the “whine” or simple call (Ryan 1985). Males can produce a complex advertisement call by ornamenting the whine with 1–7 suffixes known as “chucks” producing what are known as “whine-chuck” calls. Females use advertisement calls to localize and select a male among a chorus; by making physical contact with a male, a female selects a mate, after which the male mounts and clasps the female in a posture known as amplexus. In laboratory 2-choice phonotaxis tests, the whine-chuck calls are strongly preferred to whine calls (85% preference strength in the study population; Ryan 1985; Ryan and Rand 2003a). In addition, female túngara frogs exhibit strong preferences for calls of higher amplitude over lower amplitude, presumably resulting in attraction toward nearer males and thus reducing the travel time and distance required to reach the chosen male (Rand et al. 1997).

MATERIALS AND METHODS

Animals

We conducted experiments during the breeding season between the months of June and September in 2007 and 2008 at facilities for the Smithsonian Tropical Research Institute in Gamboa, Panamá (9°07.0'N, 79°41.9'W). We collected frogs as amplexant mated pairs from breeding aggregations between 1900 and 2200 h and performed behavioral testing between 2000 and 0400 h; all females were tested and measured before oviposition. Animals were held in small dry plastic bags in dark, quiet conditions before testing. We marked individuals with a unique toe-clip combination to prevent resampling, following the Guidelines for the Use of Live Amphibians and Reptiles in Field Research, compiled by the American Society of Ichthyologists and Herpetologists, The Herpetologists' League, and the Society for the Study of Amphibians and Reptiles. After behavioral testing, we measured body mass using a digital balance (to the nearest 0.1 g) and SVL using digital calipers (to the nearest 0.01 mm) before returning females to their original site of collection within 12 h. In total, we tested 105 females in 338 trials; the final data set that resulted included 60 responsive females and 260 successful mate choices (i.e., 76.9% of trials resulted in choices).

Stimuli and experimental design

We used 2 synthetic stimuli (whine, whine-chuck), which share the identical whine component (Figure 1). We synthesized these stimuli based on the average values for the parameters

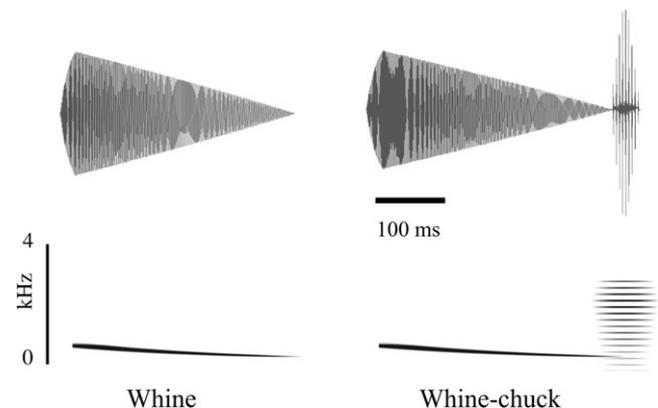


Figure 1
Stimuli used in this study. Synthetic Whine and Whine-chuck oscillograms (top) and spectrograms (bottom).

of the calls in the study population by shaping sine waves using custom software (J. Schwartz, Pace University at Pleasantville, NY; sample rate 20 kHz and 8 bits). We calculated mean values for the population based on the calls from 50 males recorded in July 1996 with a Marantz PMD 420 recorder (Mahwah, NJ) and a Sennheiser ME 80 microphone with K3U power module (Sennheiser Electronic Corporation, Old Lyme, CT) on magnetic cassette tape. Additional information on the call parameters used and the synthesis procedure can be found in Ryan and Rand (2003b). All playbacks were done using a 2-choice design (Figure 2). Stimuli were matched for the peak amplitude of the whine before playback and broadcast from small ADS L210 speakers (Vista, CA) located in the center of the 2 poles of the arena. The chuck of the complex call was twice the peak amplitude of the whine. The whines in these signals consist of only the fundamental frequency; it has been shown previously that the upper harmonics of the whine do not significantly contribute to mate choice in the laboratory (Ryan and Rand 1990; Rand et al. 1992).

Frogs were tested under infrared light in a sound-attenuating chamber (Acoustic Systems, Austin, TX) measuring $2.7 \times 1.8 \times 1.78$ m. Before each subject was tested, we calibrated both speakers to 82 dB sound pressure level (SPL; re. 20 μ Pa, peak, flat) at the center of the arena (1.35 m from each speaker) using the whine stimulus and a GenRad 1982 SPL meter. At the beginning of each trial, a subject was placed under a cone at the center of the acoustic chamber (i.e., release point; Figure 2) for 3 min while the 2 stimuli were broadcast antiphonally at a rate of 1 call per 2 s from each speaker. The cone was then raised remotely and the phonotactic behavior was monitored via a ceiling-mounted infrared camera (Fuhrman Diversified, Inc., Houston, TX) connected to a television monitor located outside the chamber.

The chamber was divided symmetrically by boundaries (hereafter “approach boundaries”) at a distance of 75 cm from the poles of the chamber (ca. 20 body lengths from release point, e.g., configuration see Figure 2). In a previous study using static presentations of whine versus whine-chuck stimuli, we showed that on crossing the boundary nearest the whine-chuck speaker, responsive female túngara frogs continued their approach trajectory selecting the whine-chuck speaker in 100% of trials ($N = 40$ choices, Baugh and Ryan in review). These dashed lines were visible only to the human observer (outlined by transparencies on the monitor). In the present

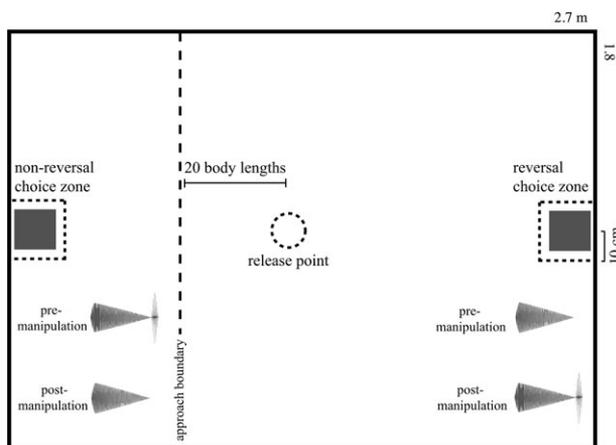


Figure 2 Phonotaxis chamber showing arena dimensions and position of release point (midpoint of chamber), speakers, choice zones, and approach boundary (75 cm from speaker, ca. 20 body lengths from release point). This figure depicts 1 of 2 symmetrical configurations.

study, one of the 2 speakers was selected randomly to initially broadcast the whine-chuck stimulus; thereafter, we alternated the initial position of stimuli between both tests and females to minimize any potential side bias (see Results) while the opposite speaker broadcast the whine. When the subject crossed the approach boundary nearest the preferred stimulus (whine-chuck), the human observer pressed a key on the playback computer's keyboard that initiated a custom program in the acoustic software program Signal (Engineering Design, Belmont, MA); these programs introduced a 500-ms delay to prevent the premature occlusion of a stimulus, and then each program performed a custom operation suited to the question addressed in the given experiment.

We scored a phonotactic choice when a frog approached and entered within a 10-cm radius of one of the speakers without simply following the perimeter of the wall. A frog failed to exhibit a phonotactic choice if it was motionless for the initial 5 min after the cone was raised or during any 2-min interval thereafter or if the animal failed to make a choice within 15 min after the cone was raised. In approximately 85% of trials, females initially approached the whine-chuck stimulus, a necessary prerequisite for these experiments. In the remaining instances, in which females approached and selected the whine, these responses were not analyzed in the present study.

Experiment 1: individual differences in reversal frequency

In Experiment 1, we addressed individual differences in the likelihood of commitment to an initial approach. Here, the approached stimulus (whine-chuck) became a whine, whereas the unapproached whine simultaneously became a whine-chuck and the amplitude of this distant stimulus was increased by an amount that equilibrated the mean peak amplitude along the approach boundary (2 dB; this was determined empirically; Baugh and Ryan in review). We demonstrated previously that manipulating call type but not amplitude also results in reversal behavior but that reversals do not occur if only amplitude is manipulated (ibid).

Three behaviors were scored and a fourth one was calculated: 1) choice (whether the subject made a choice and if so, whether it involved a reversal or a choice for the initially approached speaker; a nonreversal), 2) the latency to the approach boundary (time lapsed between lifting the cone and crossing the approach boundary nearest the target speaker), 3) the overall latency to choice (total time lapsed between lifting the cone and the choice), and 4) the latency to choice following stimulus manipulation (overall latency minus latency to approach boundary).

We tested each female in multiple trials back-to-back until 6 trials resulting in choices were completed (requiring a mean of 6.44 trials/female) and measured the number of reversal choices that each female performed across these 6 trials (minimum = 0, maximum = 6; $N = 40$ females). To explore the shape of this reversal distribution, we determined its kurtosis and compared that with a sampling distribution of kurtosis values drawn from a Monte Carlo simulation (R statistical package) of 10 000 replicates. Kurtosis provides a measure of the strength of a single peak in a sample distribution (small kurtosis values indicate a weak single peak). From a linear regression of body mass versus SVL for subjects in Experiment 1, we calculated residual body mass for each female (SVL and body mass were not measured for 6 females in this experiment). Also, a body condition index was calculated by obtaining the residual values from a linear regression of the cubed root body mass on SVL and dividing those values by the SVL, following the approach used by Baker (1992), Howard et al. (1997), Howard and Young (1998), and Leary et al. (2008). We used linear regression to evaluate further the degree of reversibility and its correlation with body condition.

We determined if female condition predicted commitment. We classified females dichotomously as either highly reversible (more than 3 reversal choices, i.e., “uncommitted”) or weakly reversible (less than 3 reversal choices, i.e., “committed”) and compared the SVL, body mass, residual body mass, and body condition index for these groups using a multivariate analysis of variance (MANOVA; SPSS 16.0). The 3 females that reversed on exactly 3 trials were omitted from this analysis. To ensure that repeatedly testing females did not influence behavior in any systematic way across trials, we tested for a trend of increasing or decreasing latency in later trials using a repeated measures MANOVA (SPSS 16.0) for the 3 latency measures and a nonparametric Cochran’s Q test for the dichotomous reversal/nonreversal choice data. Finally, we examined the time costs of reversal behavior with paired t -tests (2 tailed) on each of the 3 latency measurements between trials involving a reversal to trials involving a nonreversal within females.

Experiment 2: variation among reversible females

In this experiment, we addressed a different source of variation in female commitment—variation in the extent of reversibility among reversible (uncommitted) females. A similar stimulus manipulation operation carried out in Experiment 1 was conducted in Experiment 2 with exceptions: 1) Here, we repeatedly alternated which speaker broadcast the whine-chuck each time a female crossed an approach boundary nearest the whine-chuck during the course of a single trial until a choice was eventually made and 2) at no point were stimuli adjusted for amplitude. Provided a female reversed after the initial stimulus manipulation, we repeatedly manipulated stimuli after every approach boundary crossing until the female eventually chose the whine. We scored 2 behaviors for each subject ($N = 20$ females): 1) total number of reversals performed before a choice was made and 2) overall latency to choice. We used a linear regression to determine if the number of looped reversals was related to female body condition. We also used a linear regression to examine the continuous relationship of latency to choice and the number of reversals. Unlike in Experiment 1, in which females were dichotomized as highly reversible or weakly reversible depending on if they reversed more or less than the median, respectively, females in Experiment 2 were considered reversible if they reversed on their first approach. This protocol allowed us to minimize screening females in Experiment 2, which might potentially lead to subject fatigue.

Experiment 3: oviposition in responsive and unresponsive females

We also determined if reproductive state influenced whether females make a mate choice. We estimated reproductive condition simply as whether or not the females mated and constructed a nest with a male at the cessation of testing. In 2008, we tested 144 females using static conspecific phonotaxis trial conditions (2 choice tests using natural and synthetic whines and whine-chucks). Of this sample, 132 responded by making a choice in at least 1 trial and the remaining 12 females never responded. After testing, all females were reunited with the male that they were collected in amplexus with, and each pair was placed in a small plastic container with 1 cm of water and held in a dark, room temperature (26 °C) cooler for 12 h. After this holding period, we recorded which pairs oviposited (túngara frogs produce a “foam nest” on the water surface; Ryan 1985).

RESULTS

From a separate study conducted simultaneously (Baugh and Ryan in review) using a static mate choice design and identical stimuli at both speakers (whine vs. whine, whine-chuck vs.

whine-chuck), we found no evidence of a side bias (left:right choices: 122:113; 2-tailed exact binomial $P = 0.60$).

Experiment 1: individual differences in reversal frequency

Choices

The frequency of trials resulting in a reversal in Experiment 1 was 47.9% (i.e., 52.1% nonreversal). This was similar to the reversal frequency found in a separate study in which females were tested in the same acoustic condition but only in 2 back-to-back trials rather than 6 (44.3% reversal frequency, $N = 140$ choices; Baugh and Ryan in review). Dichotomizing females resulted in 45% of females classified as “highly reversible” (>3 reversals), 47.5% of females as “weakly reversible” (<3 reversals), and 7.5% of females (3 subjects) that were not assigned to a category because they reversed exactly 3 times; because these 3 subjects were not categorized, they were not included in the MANOVA evaluating the relationship between the body condition measures and the reversibility category.

From the distribution of these responses (Figure 3), it appears that this approximately 50% reversal frequency was due to an underlying bimodal distribution, in which one-half of the females tend to be highly reversible, whereas the other one-half tend to be weakly reversible. The observed kurtosis of this distribution was quite small (1.55), placing it in the tail end of the Monte Carlo sampling distribution of kurtosis values ($P = 0.06$). Of the 6 opportunities for each female to reverse, the 2 most common outcomes were females that reversed either 1 or 5 times (Figure 3).

To ensure that there was no effect of repeated testing on the probability of reversal, we performed the nonparametric repeated measures Cochran’s Q test and found that the probability of reversal was not influenced by trial number ($Q_5 = 5.043$, $P = 0.411$; see Supplemental Figure S1[d]).

The linear regression for cubic body mass by SVL was significant (Pearson’s $R = 0.617$, $N = 34$, $P = 1.0 \times 10^{-4}$ [2 tailed]). For the dichotomized females, we compared body condition, body mass, residual body mass, and SVL using a MANOVA; 6 females oviposited prior to mass measurements and were thus excluded as egg mass contributes significantly to female mass, yielding a final sample of 31 females (>3 reversals, $N = 16$; <3 reversals, $N = 15$). Results of this analysis indicated that highly and weakly reversible females differed significantly overall (omnibus $F_{3,27} = 3.333$, $P = 0.034$), and in the univariate tests of body condition index ($F_{1,29} = 7.703$, $P = 0.010$, Figure 4a), body mass ($F_{1,29} = 6.267$, $P = 0.018$) and residual

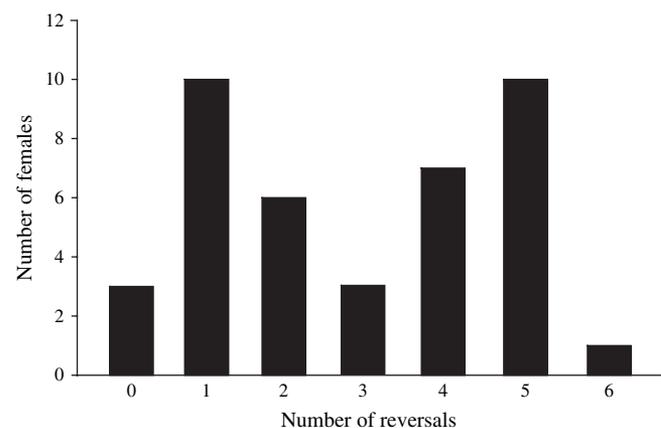


Figure 3

Histogram of number of reversals (of 6) for each female ($N = 40$) suggests a bimodal distribution of females in the study population. The population reversal frequency was 47.9%.

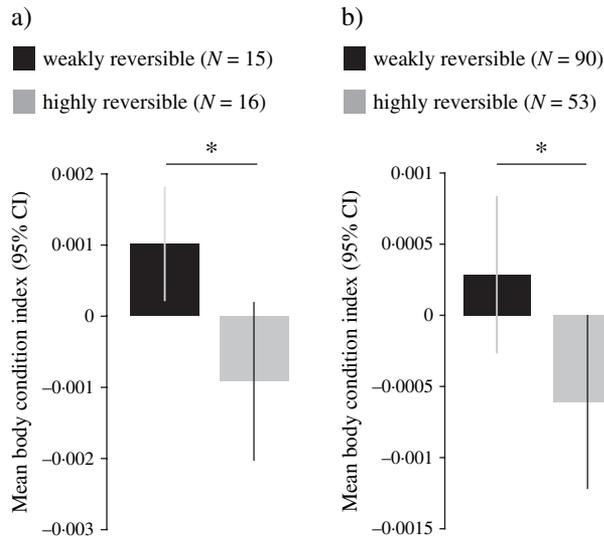


Figure 4
Mean body condition (\pm 95% confidence interval) and female reversibility. Females with lower body condition were more likely to be reversible both in (a) Experiment 1 wherein females were characterized as committed or uncommitted after 6 trials ($F_{1,29} = 7.703$, $P = 0.010$) and (b) in an independent study wherein females were characterized after 2 trials ($F_{1,141} = 4.177$, $P = 0.043$).

body mass ($F_{1,29} = 7.976$, $P = 0.008$), but not in SVL ($F_{1,29} = 0.380$, $P = 0.543$), suggesting that behavioral differences were not simply due to differences in age (túngara frogs, like many ectothermic vertebrates, have indeterminate growth). To provide an additional test of this relationship between reversibility and body condition, we examined data from an independent study of dynamic mate choice in this system (Baugh and Ryan in review). Although this previous study was designed to address a different set of questions, it used a similar protocol in which females were tested on 2 back-to-back dynamic choice trials of whine versus whine-chuck stimuli for which reversal frequencies observed were similar to that seen in the present study. Here, the linear regression for cubic body mass by SVL was also significant (Pearson's $R = 0.456$, $N = 143$, $P < 1 \times 10^{-6}$ [2 tailed]). We dichotomized these females as nonreversible (zero out of 2 reversals; $N = 90$) and reversible (2 of 2 reversals; $N = 53$; thus excluding the intermediate category of 1 of 2 reversals). This larger data set yielded similar results as Experiment 1, in which highly and weakly reversible females differed significantly and in the same direction in body condition index ($F_{1,141} = 4.177$, $P = 0.043$; Figure 4b), body mass ($F_{1,141} = 3.938$, $P = 0.049$), and residual body mass ($F_{1,141} = 3.943$, $P = 0.049$). Likewise, these 2 groups did not differ in SVL ($F_{1,141} = 0.213$, $P = 0.645$).

Finally, we performed linear regressions for the data set of 6 repeated trials to examine further if females making a greater number of reversals had smaller mass measurements. We found negative correlations in all 3 measures with a significant effect for the number of reversals performed and body mass (Spearman's $\rho = -0.345$, $N = 34$, $P = 0.046$ [2 tailed]), and nonsignificant correlations for residual body mass (Spearman's $\rho = -0.327$, $N = 34$, $P = 0.059$ [2 tailed]), and body condition (Spearman's $\rho = -0.313$, $N = 34$, $P = 0.072$ [2 tailed]); see Supplemental Table S1).

Latencies

Two of the 3 latency measurements differed between reversal and nonreversal trials. Latency to choice after stimulus manip-

ulation was significantly greater for trials involving a reversal choice (\pm standard error of mean, SEM, s; 72.9 ± 6.3) compared with a nonreversal (34.0 ± 5.0 ; $t_{29} = 6.28$, $P = 1 \times 10^{-6}$). Likewise, latency to overall choice was greater for reversal choices (137.2 ± 13.2) compared with nonreversals (94.2 ± 10.7 ; $t_{29} = 5.50$, $P = 6 \times 10^{-6}$). Latency to the approach boundary, however, did not differ between reversals (64.7 ± 8.9) and nonreversals (60.3 ± 7.8 ; $t_{29} = 0.61$, $P = 0.54$).

To ensure that repeated testing did not influence latency responses, we examined our 3 measures of latency across the 6 repeated trials using repeated measures MANOVA. Mauchly's test of sphericity was violated and therefore we used the Greenhouse–Geisser correction (adjusted degrees of freedom) to generate P values. We found that the main effect of trial number on latency was not significant ($F_{11,22} = 1.116$, $P = 0.395$) and that none of the 3 individual outcomes were significant (boundary latency: $F_{5,160} = 1.974$, $P = 0.121$; latency to choice after stimulus manipulation: $F_{5,160} = 1.133$, $P = 0.344$; and overall choice latency: $F_{5,160} = 1.979$, $P = 0.105$). This suggests that repeatedly testing females does not affect their latency behavior (see Supplemental Figure S1[a–c]).

Experiment 2: individual differences among reversible females

In this experiment, females were required to reverse after the first initial stimulus manipulation; therefore, the minimum number of reversals was one. Of the 20 females in this study, 10 females reversed only a single time before making a choice, 3 females reversed 2 times, 1 female reversed 3 times, 1 female reversed 4 times, 3 females reversed 5 times, 1 female reversed 9 times, and 1 female reversed 16 times (mean = 3.15, median = 1.5, mode = 1.0, range = 1–16). Unlike the results from the studies in Experiment 1, we did not find a significant correlation between female condition and the number of looped reversals performed (residual body mass: Spearman's $\rho = 0.226$, $N = 18$, $P = 0.368$ [2 tailed]; body condition: Spearman's $\rho = 0.241$, $N = 18$, $P = 0.335$ [2 tailed]).

We also measured overall latency to choice in this experiment and found a large range (mean \pm SEM (s): 250.6 ± 44.87 ; range: 70–773). Not surprisingly, females generally had longer latencies given a greater number of reversals (Spearman's $\rho = 0.602$, $N = 20$, $P = 0.005$ [2 tailed]). In other words, greater choosiness is more costly.

Experiment 3: oviposition in responsive and unresponsive females

Across all females, 90.2% produced a foam nest when reunited with the mate they had chosen in the field. The majority of females were responsive, performing conspecific phonotaxis in at least one trial (132 compared with 12 nonresponsive frogs). Responsive females were significantly more likely to produce a foam nest (94.6% produced foam nests) compared with nonresponsive females (58.3% produced foam nests; Fisher's exact test, 2-tailed: $P = 0.002$) despite the fact that all females were collected at breeding sites in amplexus with a male.

DISCUSSION

Because the vast majority of acoustic mate choice studies use static stimulus presentation designs and population assays, important elements of behavior potentially go unnoticed. Here, we demonstrate that female frogs update their decision making in real time as advertisement signals change and report, for the first time, that the process of executing a mate choice can vary strikingly between individuals; in our study, females were bimodally distributed in their commitment to an initial mate

approach, and these 2 behavioral classes of females differed in body condition, wherein less committed females had lower body masses compared with more committed females. When females are tested in the looped stimulus switching test condition (Experiment 2), this negative relationship between body condition and number of reversals was not observed. This might suggest that within the time frame of a typical mate approach, females are more constrained by factors such as attention.

A previous study using a static mate choice design suggested that female túngara frogs do not exhibit individual differences in preferences for complex over simple calls (Kime et al. 1998). Our study shows, however, that choosiness does vary between individuals; under dynamic conditions, choosiness is reflected in the likelihood that females reverse their approach in order to select a signaler that has become more attractive, despite the fact that such behavior incurs a time cost and presumably energetic and conspicuousness costs as well (Rand et al. 1997). We also show that this choosiness is linked to variation in body condition. There are a few possible causes of body mass variation, including differences due to clutch size (i.e., number of ova), clutch mass, or both. In many anurans, clutch size correlates positively with SVL (reviewed in Duellman and Trueb 1986), including tropical anurans that make foam nests, where larger species (SVL) produce more eggs (Crump 1974). Because the 2 classes of females in our study did not exhibit differences in SVL, it is unlikely that body condition differences were due to variation in clutch size, although this possibility cannot be ruled out. Additionally, although clutch size in túngara frogs varies considerably, there is no evidence that it does so in a bimodal manner (Ryan 1985; Baugh AT, unpublished data). A more likely cause of body mass variation is differences in clutch mass, which has been shown in other anurans to be tied to clutch hydration, as evaporative water loss and intake can amount to as much as 20% of body mass (Sinsch 1983); thus, the body condition of a female may be strongly influenced by exactly how far she has progressed toward ovulation. In some species of tropical frogs, females lay multiple egg masses in a night of breeding and hydrate eggs during intermating intervals by partially immersing themselves in water (Duellman and Trueb 1986). Female túngara frogs, however, do not mate multiply within a breeding cycle but are often found prior to mating in the chorus ponds and also spend time in these pools while in amplexus before oviposition. It is unknown just how long females spend hydrating a clutch, or if clutch mass varies bimodally, but given that nonresponsive females were significantly less likely to oviposit (Experiment 3), it is conceivable that there is appreciable variation among amplexant females in the urgency with which females must mate. Importantly, mate choice appears to be strongly under female control in this species—there is no evidence of scramble competition mating in túngara frogs (Ryan 1985). Therefore, it is unlikely that amplexant females had been clasped by males without having selected them. Further, although there has been some debate about the assumptions of body condition measures that are based on residuals from length–mass correlations (Green 2001), these measures have been used widely, and in a recent meta-analysis appear to generate useful information (Schulte-Hostedde et al. 2005). Perhaps most importantly, these condition measures are related to behavior in the present study.

Our study showed that despite the lack of a robust phenotypic polymorphism in preferences for complex calls under static choice conditions (Kime et al. 1998), individual differences do arise due to variation in choosiness. This contrasts with levels of repeatable individual differences found in studies of mate choice generally (Bell et al. 2009) and specifically

with findings in other species of anurans. For example, studies of female preferences in midwife toads (*Alytes muletensis*) by Lea (2000) and cricket frogs (*Acris crepitans*) by Ryan et al. (1992) both found size-dependent preferences and attributed these differences to tuning properties of the inner ear that are known to change with body size. Similarly, using the African painted reed frog (*H. marmoratus*), Jennions et al. (1994) found size-dependent variation between females in their responses to calls differing in frequency, wherein large females appeared to be more sensitive to small differences in frequency and were more consistent in their preference for low-frequency calls than small females. In our study, body size did not differ between highly reversible (uncommitted) and weakly reversible (committed) females, and again, all females in our study approached the whine-chuck initially. It is unlikely that differences in frog behavior were due to age differences, as SVL in anurans often correlates positively with age, and no differences in SVL were observed (Halliday and Verrell 1988; Castellano et al. 1999). Instead, our data show that the temporal process of mate choice is correlated with differences in body condition among highly responsive and reproductively competent females (Experiment 3 showed that 94.6% of field-collected amplexant females that responded positively in at least one phonotaxis trial oviposited within 12 h). We suggest that females closer to the time at which they must oviposit are less choosy (i.e., more committed); this is congruent with a previous study that suggested that as female túngara frogs approach the terminal part of their reproductive cycle, they are willing to accept less attractive mates (Lynch et al. 2005).

Because vocal signaling in nature by male túngara frogs is a dynamic process in which males commonly transition between call types (Bernal et al. 2009) and amplitude (Pauly et al. 2006), the results of our study have implications for sexual selection. To maximize survival and reproductive success, male calling behavior should evolve an optimal compromise between the costs of complex call production (acoustic predators prefer complex calls; Ryan et al. 1982) and its benefits (female preference). In addition, we now show that a considerable subpopulation of females is attending to the timing and consistency of complex call production, thus adding another behavior that should be subject to selection. This challenges a popular conception of mate choice by “simple” vertebrates such as frogs, because multiple variables clearly influence the final choice outcome.

This is the first study to our knowledge that explores how body condition is related to updating and decision making during mate choice. Our results suggest that phonotaxis is not merely a 2-step procedure of evaluating signals and executing a decision but rather a flexible and open-ended process that varies between individuals.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>.

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