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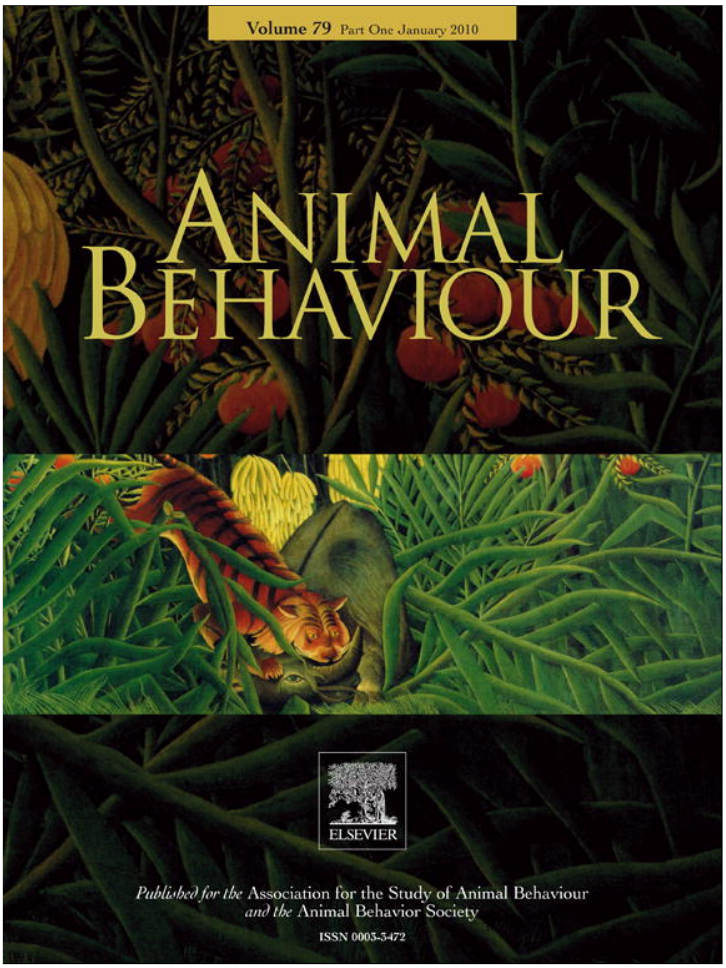

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Mate choice in response to dynamic presentation of male advertisement signals in túngara frogs

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Signalling is a dynamic process that often occurs over brief timescales, particularly in the acoustic modality. Numerous studies of mate choice and acoustic communication have identified signal parameters essential for species recognition and mate preferences, although these studies have rarely considered the dynamic nature of these processes. Here we investigate mate choice behaviour in female túngara frogs, *Physalaemus pustulosus*, in response to temporally dynamic presentations of male advertisement calls. Our results demonstrate that females are sensitive to the location of preferred call types on a moment-to-moment basis, and that responses are influenced by the continued presence, complexity, sound pressure level and inherent attractiveness of individual male signals. In general, our results support the notion that decision making during mate choice is an open-ended process that is sensitive to interruption and persuasion from competing signallers. We show that for a species in which females actively compare multiple signallers simultaneously, the criterion that predicts the degree of commitment to an initial mate approach is whether there is a state change in the complexity of signals.
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There are few decisions in life more important than the selection of a mate. Sexually reproducing animals ensure passage of their genes to the next generation by choosing compatible mates, which often rests on species-specific perception of communication signals. The most significant criterion that must be met when selecting a mate is whether the individual belongs to the same species, as the costs of hybridization are often significant (Dobzhansky 1951; Mayr 1963). Once a female has classified a pool of courting males as belonging to her species, she typically selects the most attractive member. Although much research has explored what constitutes 'attractive' by determining which particular male traits females use to estimate male quality (Lande 1981; Hamilton & Zuk 1982; Bateson 1983; Andersson 1994), the process of reproductive decision making remains relatively unexplored.

The conventional approach to studying mate choice decision making is to identify whether females use a sample- or threshold-based decision rule (sensu Valone et al. 1996). Sample-based decision rules (e.g. 'best of *n*': Janetos 1980; 'sequential comparison': Wittenberger 1983) posit that a female's decisions are based on her sampling of available males, whereas threshold-based decision rules (e.g. 'fixed threshold' and 'one-step': Janetos 1980) posit that females select mates that exceed some threshold of suitability. This framework has the following limitations: (1) these two categories of rules are overly simplified and typically construed as mutually exclusive; (2) for certain lek-breeding species, in which females compare multiple males simultaneously, a strictly threshold-based decision rule is untenable: females frequently compare multiple males simultaneously. Valone et al. (1996) suggested that to distinguish adequately between sampling- and threshold-based decision rules requires presenting females with only a single male at a time, yet such a protocol is clearly unnatural for certain lek species. Moreover, at the level of the mate choice process, decisions are considered to have two steps (sensu Valone et al. 1996). First, sensory information is gathered and used to discriminate between mates. Second, a decision rule is engaged to select the optimal option. Here we address this general issue with a different approach: rather than testing the fit of sample- and threshold-based decision rules, we describe the decision-making process itself (i.e. its flexibility and time frame) as an empirical window into the potential rules that may govern mate choice. We suggest that the dynamic signalling environments common to

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species such as acoustically advertising lek breeders compel females to iterate the two-step process mentioned above, and in doing so execute decisions though a process of 'temporal updating'. Here we use 'temporal updating' to mean the adjustment of a behavioural decision during the execution phase following a change, perceived or real, in the available choice options, which is distinct from the use of this phrase in studies of human memory (see Zwaan 1996; Blaisdell et al. 1999).

Temporal updating, or dynamic reproductive decision making, provides a practical solution for animals that make decisions in social and physical environments that are in rapid flux. In lek-breeding animals, such as many insects, anurans and birds, females make mate decisions in the midst of temporally variable social signals. Males gather, often in high densities, to court females using short-duration calls that can vary between repetitions in dynamic features such as complexity (e.g. presence of call embellishments), amplitude and call rate, traits that are known in many systems to influence a signal's attractiveness (Darwin 1871; Andersson 1994; Gerhart & Huber 2002; Greenfield 2002; Searcy & Nowicki 2005). Given this environment, we asked: as a reproductive female compares advertising males, at what point does she make a decision and how does she execute such a choice when the available signals are changing? Hence, rather than attempt to distinguish between two classes of decision rules (that do not necessarily apply to certain lek species and would require unnaturalistic testing conditions), we take a more psychophysical approach by attempting to gain stimulus control over females such that we, as experimenters, can predict adequately the final mate choice that a female makes. This approach, while not incompatible with either class of decision rules, has the added advantage of providing detailed information about the signalling parameters essential for male persuasion during female choice, and therefore makes predictions about optimal behaviour in signallers.

Much of the experimental analyses of mate choice document the degree to which females select males based on temporally 'static' traits (e.g. male size or plumage coloration) compared to 'dynamic' traits (e.g. courtship display vigour). Static traits are relatively invariant within an individual, while dynamic traits often vary considerably (Gerhardt et al. 1996; Rosenthal et al. 1996; Hill et al. 1999). In addition to temporal variation in signals, receivers face spatial variation. For example, as receivers approach an acoustic source, the source generally becomes more intense. Interestingly, experimental tests of preferences for both static and dynamic traits are often conducted with static rather than dynamic presentations. Here, we used dynamic presentations in which signals varied temporally to test preferences for two dynamic properties of acoustic advertisement signals (amplitude and complexity) and to ascertain the details of when and how receivers make decisions in variable environments. By doing so we were able to address which specific decision rule explains and predicts commitment during mate choice.

We examined the mate choice behaviour of female túngara frogs, *Physalaemus pustulosus*, in response to dynamic playbacks of male advertisement calls by broadcasting two male calls of similar or dissimilar attractiveness and adjusting the two signals as females made an approach. By doing so we addressed the following questions. (1) Do females make their final mate decisions before they begin an approach? (2) At what spatial approach distance is an irreversible decision made? (3) Which features of acoustic signals influence female decision-making behaviour over brief time periods?

THE SYSTEM

Túngara frogs are small anurans (ca. 30 mm snout-to-vent length) distributed throughout much of Mesoamerica (Weigt et al. 2005). Males advertise vocally to females during the breeding season (May–December) using a species-typical call, known as the 'whine' or simple call (Ryan 1985). Males can ornament the whine with one to seven suffixes know as 'chucks', thereby producing what is known as the complex call or whine-chuck. In nature, females use calls to localize an individual male amongst a chorus and then select a mate by making physical contact, after which the male mounts and clasps the female in a posture known as amplexus. In laboratory two-choice phonotaxis tests, the whine-chuck calls are strongly preferred to whine calls (85% preference strength; Ryan 1985; Ryan & Rand 2003a). Female túngara frogs also show strong preferences for calls of higher amplitude over lower-amplitude alternatives, which presumably results in attraction towards nearer males and thus reduces travel time (Ryan & Rand 1990). Males, however, are also known to adjust their call amplitude over the course of a call bout (Pauly et al. 2006) and, therefore, call amplitude has both passive and active dynamic properties. While females prefer complex to simple calls, for a given complex call (natural or synthetic) they do not appear to prefer a more complex version (e.g. whine with four chucks) to a less complex version (e.g. whine with two chucks) (Bernal et al. 2009).

Individual male túngara frogs differ in the attractiveness of their calls. Ryan & Rand (2003b) studied female mate choice in response to recorded natural calls from males in the study population (Gamboa, Panamá) and showed that the complex calls of some males are consistently more attractive than those of others. Here we use this natural variation in attractiveness to examine how such intrinsic differences influence the extent to which females commit to a given male in the face of dynamic changes in call complexity.

GENERAL METHODS

Animals

We conducted all 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 amplectant mated pairs from breeding aggregations between 1900 and 2200 hours and performed behavioural testing between 2000 and 0500 hours. Animals were held in small plastic bags in dark, quiet conditions before testing. To prevent resampling, we marked individuals with a unique toe-clip combination, measured the mass and snout-to-vent length and returned them to their original site of collection within 12 h. In marking frogs, we followed the Guidelines for the Use of Live Amphibians and Reptiles in Field Research, compiled by the American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL), the Society for the Study of Amphibians and Reptiles (SSAR), and our methods were approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin and La Autoridad Nacional del Ambiente in Panamá. In total, 373 females were tested in 1264 trials, resulting in 982 successful choices (i.e. 77.7% of trials resulted in choices).

Stimuli and Experimental Design

We performed 19 phonotaxis experiments. We used nine stimuli throughout this study and all playbacks were done using a two-choice design (Fig. 1). All nine stimuli were matched for the peak amplitude of the whine before playback and broadcast from small speakers located at the two poles of an arena (Fig. 2). We used three synthetic stimuli: a simple whine (W), a whine with one chuck (Wc) and a whine with three chucks (W3c). The W3c stimulus simply has the chuck from Wc in triplicate. The chuck on these

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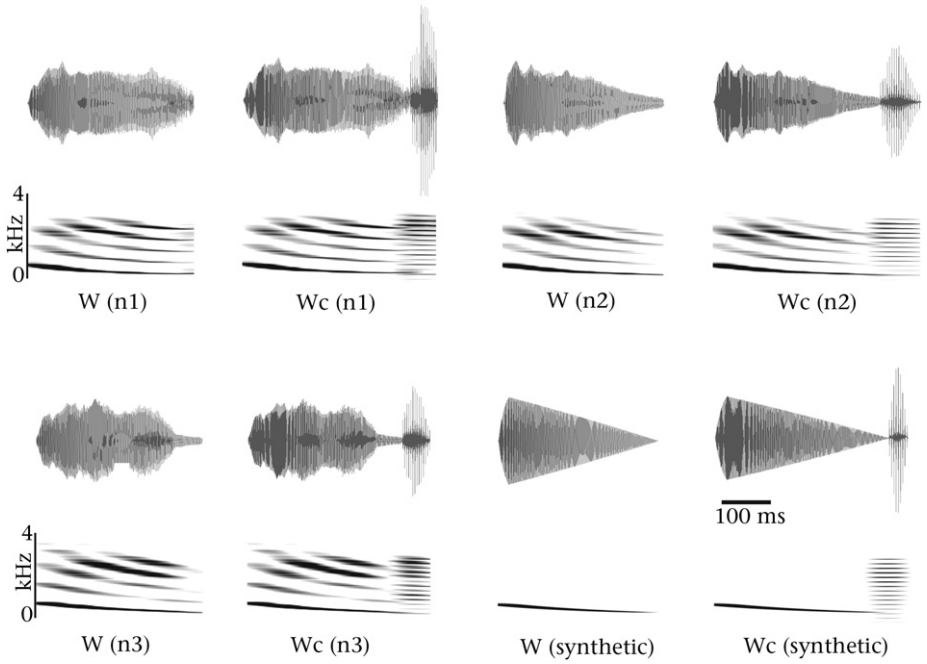

Figure 1. Stimuli used in two-choice phonotaxis tests to examine mate choice decisions of female túngara frogs in response to variation in natural and synthetic male advertisement signals. Stimuli included three natural whine-chucks, Wc (n1), Wc (n2) and Wc (n3), three natural whine-chucks with the chuck digitally excised to leave just the whine, W (n1), W (n2) and W (n3), and three synthetic stimuli, a simple whine (W), a whine with one chuck (Wc) and a whine with three chucks (W3c; not shown), which is the same as a whine-chuck with two identical chucks and interchuck intervals (4 ms) appended.

two complex calls (Wc and W3c) is 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 influence mate choice in the laboratory (Ryan & Rand 1990; Rand et al. 1992), and that these synthetic stimuli are as attractive as natural signals (M. J. Ryan, unpublished data). We synthesized these stimuli based on the mean values for the parameters of the calls in the population by shaping sine waves using custom software (J. Schwartz, Pace University at Pleasantville, NY, U.S.A.; sample rate 20 kHz and 8 bit). We calculated mean values for the population based on the calls from 50 males recorded in July 1996 with a Marantz PMD 420 recorder and a Sennheiser ME 80 microphone with K3U power module on magnetic cassette tape. We assume that the calls sampled in 1996 capture a relevant portion of the present-day call variation. Additional information on the call parameters used and the synthesis procedure can be found in Ryan et al. (2003). The use of this mean synthetic signal has allowed comparisons among a variety of studies conducted in this

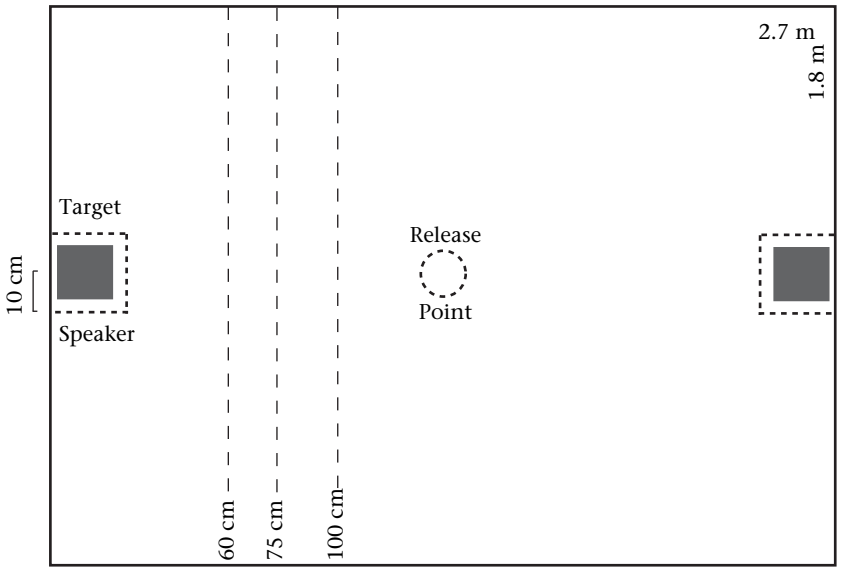

Figure 2. Phonotaxis arena showing dimensions and the placement of the three switch boundaries. A symmetrical placement of these boundaries was present but not shown here for simplicity. The switch boundaries located at 60 cm and 100 cm from the release point were used only for experiments 4 and 5, respectively, while the 75 cm boundary was used for all remaining experiments.

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system. Other studies have addressed signal variation within the population (Ryan 1985; Ryan & Rand 2003b). In addition to using the mean synthetic call, we also conducted choice tests with six natural advertisement calls (three whine-chucks, Wc (n1), Wc (n2) and Wc (n3), and the identical three whine-chucks with the chuck digitally excised to leave just the whine, W (n1), W (n2) and W (n3)) recorded from three males in Gamboa, Panamá (Fig. 1; also see Results, Table 1). These stimuli have been used previously to explore female preferences in the Gamboa túngara population and the relative attractiveness of these calls for females has been determined (Ryan & Rand 2003b). Natural whine-chucks Wc (n1) and Wc (n3) are equivalently attractive, while Wc (n2) is significantly more attractive than Wc (n1). In a few experiments (12–15) we used these natural differences in attractiveness as leverage for examining the role of inherent male call qualities in influencing female commitment.

Frogs were tested under infrared light in a sound-attenuating chamber (2.7 × 1.8 × 1.78 m, L × W × H; Acoustic Systems, Austin, TX, U.S.A.; Fig. 2). Before each subject was tested, we calibrated both speakers to 82 dB SPL (re. 20 μPa) at the centre of the arena (1.35 m from each speaker) using the W stimulus (GenRad 1982 SPL meter, peak amplitude, flat weighting). Each trial began with the subject placed under a cone at the centre of the acoustic chamber (i.e. release point; Fig. 2) for 3 min while the two stimuli were broadcast antiphonally at a rate of one call per 2 s from each of two ADS L210 speakers opposite one another. The cone was then lifted remotely and the phonotactic behaviour was monitored via a ceiling-mounted infrared camera and television monitor outside the chamber. The chamber was divided symmetrically by boundaries (hereafter 'switch boundaries') at given distances from the speakers (dashed lines, Fig. 2). These dashed lines were visible only to the human observer (outlined by transparencies on the monitor). For most of the experiments, one of the two speakers initially broadcast a preferred stimulus (this 'target' speaker was selected randomly and then alternated between tests and females to minimize potential side bias; see Results) while the opposite speaker broadcast the less preferred call of the pair. When the subject crossed the switch boundary nearest the preferred stimulus (as measured from the face of the speaker), 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, U.S.A.); 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 specific question addressed in the given experiment. For many of the experiments the two stimuli were switched and the amplitude of the distant stimulus (the one that was not initially approached) was amplified by a factor that equilibrated the mean peak amplitude along the switch boundary (this was determined empirically by averaging the peak amplitude differential at six equally spaced points along the switch boundary transect). For each experiment, we scored the same three behaviours, and we calculated a fourth behaviour as follows: (1) we noted whether the subject made a choice and if so, whether it involved a reversal or nonreversal of choice for the initially approached speaker; (2) we noted the latency to the switch boundary (time lapsed between lifting the cone and crossing the switch boundary nearest the target speaker); (3) we noted the overall latency to make a choice (total time elapsed between lifting of the cone and the subject's choice); and (4) we calculated the latency to make a choice following stimulus switching (overall latency minus boundary latency).

We scored a phonotactic choice when a frog approached one of the speakers within a 10 cm radius without simply following the wall. Frogs that were motionless for the initial 5 min after the cone was raised or during any 2 min interval thereafter, or that failed to make a choice within 15 min after the cone was raised were scored as failing to show a phonotactic choice. A prerequisite for these tests was that females must initially approach the preferred stimulus (complex call). Frogs that were unresponsive in any experiment or that failed to initially approach the preferred stimulus in experiments involving initially a simple versus complex call (all experiments except 8–11), and instead initially approached and selected the less preferred stimulus (simple call), were scored as showing 'no response'. In the uncommon instances in which females were responsive but approached the less preferred stimulus initially, we re-tested these subjects. Almost invariably these subjects approached the preferred stimulus on a subsequent trial (ca. 85% of the time).

We tested each female twice in the same condition (two replicates) and used the nonparametric Mann–Whitney *U* test (two

Table 1
Results of two-choice tests examining phonotactic responses (reversal, nonreversal, no response) of female túngara frogs to natural and synthetic male advertisement signals

Experiment	Initial stimuli	Final stimuli	Amplitude boost (dB)	Switch boundary to speaker (cm)	Reversal choices	Nonreversal choices	No response	Total choices	*N*	% Reversals
1	W–Wc	W–Wc	2	75	0	40	18	40	20	0
2	W–Wc	Wc–W	0	75	24	56	41	80	40	30.0
3	W–Wc	Wc–W	2	75	62	78	26	140	70	44.3
4	W–Wc	Wc–W	6	60	17	23	7	40	20	42.5
5	W–Wc	Wc–W	1.33	100	26	14	3	40	20	65.0
6	W–Wc	Wc–W	4	75	18	22	15	40	20	45.0
7	W–Wc	W–W	2	75	5	35	10	40	20	12.5
8	Wc–Wc	Wc–W	2	75	15	25	0	40	20	37.5
9	Wc–Wc	Wc–Wc	2	75	4	36	4	40	20	10.0
10	W–W	Wc–W	2	75	21	19	3	40	20	52.5
11	W–W	W–W	2	75	3	37	10	40	20	7.5
12	W (n1)–Wc (n3)	Wc (n1)–W (n3)	2	75	18	42	14	60	30	30.0
13	W (n3)–Wc (n1)	Wc (n3)–W (n1)	2	75	23	37	30	60	30	38.3
14	W (n1)–Wc (n2)	Wc (n1)–W (n2)	2	75	17	43	10	60	30	28.3
15	W (n2)–Wc (n1)	Wc (n2)–W (n1)	2	75	38	24	33	62	31	63.3
16	W–W3c	W3c–W	2	75	18	42	19	60	30	30.0
17	W–W3c	W3c–W	2	75	11	29	14	40	40	27.5
18	W–Wc	W–silence	2	75	40	0	10	40	20	100
19	W–Wc	Silence–silence	N/A	75	0	5	15	20	20	0

W = synthetic whine; Wc = synthetic whine-chuck; W (n1) = d1 natural whine 'Oc' (Ryan & Rand 2003b); Wc (n1) = d1 natural whine-chuck; W (n2) = d3 natural whine 'M' (Ryan & Rand 2003b); Wc (n2) = d3 natural whine-chuck; W (n3) = d8 natural whine 'Sd' (Ryan & Rand 2003b); Wc (n3) = d8 natural whine-chuck; W3c = synthetic whine with three chucks.

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tailed) to compare the number of reversals across treatments (females could show reversal of choice 0–2 times within a test condition). To ensure independence, we used a unique pool of females in all the experiments in which we made pairwise comparisons. An alpha criterion of 0.05 was applied to all statistical tests. We additionally used paired *t* tests to examine differences in female latencies (latency to switch boundary, latency to make a choice following stimulus switching, and overall latency to make a choice) between trials in which a given female reversed choice compared to the identical test condition in which she did not reverse. To achieve a sufficient sample size to examine these latency differences between reversals and nonreversals within females (i.e. females that reversed on one of the two trials only), we used data from experiment 3 and from experiments 12–15 (i.e. all experiments that involved a simple versus complex call followed by stimulus switching at 75 cm and a boost of 2 dB).

To ensure that testing females on sequential trials did not influence their behaviour, we performed several analyses on reversal behaviour and overall latency to make a choice in the largest single experiment (experiment 3), including (1) a paired *t* test of overall latency to choose between the first and second trials for females that showed no reversals during the two trials, and (2) a paired *t* test of overall latency to make a choice between the first and second trials for females that showed reversals on both trials. It would not be informative to perform an analysis of latency by trial number for females reversing on one of the two trials since latencies were consistently higher when females reversed their choices (see Results). We extended this latency analysis across all experiments by performing a repeated measures ANOVAs on the overall latency from the two replicates for females that made no reversals and for females that made two reversals (one within-subject factor (trial number) and one between-subject factor (experiment type)). Finally, to ensure that testing females twice in successive trials did not influence the probability of reversal, we performed a binomial exact test on reversal/nonreversal results for trials one and two.

RESULTS

To identify any potential for side bias in the acoustic chamber, we performed trials ($N = 235$) in which both speakers broadcast the identical standard call (W versus W or Wc versus Wc). We found no evidence of a side bias in either 2007 or 2008 (left:right choices: 122:113; $P = 0.60$). Furthermore, a post hoc analysis showed that reversal behaviour was not influenced by whether subjects required additional testing because of one or more unsuccessful ('no response') trials (reverals/nonreversals: zero no-choice trials = 57/68; >1 no-choice trials = 5/10; Fisher's exact test: $N = 70$, $P = 0.41$, two tailed).

Reversals

Experiments 1–7: effects of complexity and amplitude across space using synthetic signals

In the control condition (experiment 1) females were initially faced with the synthetic whine versus whine-chuck. Once the female crossed the switch boundary (75 cm, ca. 20 body lengths) towards the whine-chuck, there was a 500 ms pause in playback and then the signals were rebroadcast from their original location (i.e. the female was still facing the whine-chuck), but now the distant stimulus (whine) was amplified +2 dB, thus equilibrating the peak amplitude of the two options at the switch boundary. Then we recorded whether the female continued on her original trajectory selecting the whine-chuck, or reversed course and selected the distant stimulus (whine) behind her. In this condition, females reversed in 0% of the trials (Table 1), suggesting that a female's decision is immutable at a minimum of 75 cm from the approached speaker provided her options do not change. This condition also confirms that the brief interruption of the playback program did not itself interfere with the decision-making process.

Experiment 2 followed the identical protocol of experiment 1 except we switched the whine and whine-chuck stimuli after the female crossed the switch boundary towards the whine-chuck, and then we rebroadcast the stimuli without any amplitude change (i.e. the near stimulus, which had become the whine, was approximately 2 dB louder because of its proximity). In this condition, females reversed directions and selected the distant whine-chuck in 30% of trials, and therefore maintained their original trajectory selecting the near whine for the remaining 70% of trials. The reversal frequency in this condition differed significantly from that of experiment 1 (Mann–Whitney *U* test: $U = 210$, $N_1 = 40$, $N_2 = 20$, $P < 1 \times 10^{-6}$).

Experiment 3 followed the same protocol as experiment 2 except that the distant whine-chuck stimulus was amplified +2 dB after switching, thus equilibrating the peak amplitude of the two options at the switch boundary as in experiment 1. Females in experiment 3 reversed significantly more frequently (44.3%) than females in experiment 2 ($U = 1080$, $N_1 = 70$, $N_2 = 40$, $P = 0.031$; this *P* value was not significant when corrected for multiple comparisons) and in experiment 1 ($U = 220$, $N_1 = 70$, $N_2 = 20$, $P = 4.5 \times 10^{-4}$). Experiment 3 was used as a standard to compare several of the other conditions in this study; we performed nine planned comparisons against experiment 3 ($N = 70$ females) using 10 independent groups of frogs (i.e. number of comparisons $\leq k - 1$; Tabachnick & Fidell 2001), and we conducted these tests over two summer field seasons to provide statistical power and to minimize the likelihood of an unrepresentative sample. To be conservative, however, we report *P* values corrected for multiple comparisons (Holm–Bonferroni) for contrasts in which statistical significance was affected.

In experiment 4 we moved the switch boundary further from the release point (60 cm from the face of the target speaker, ca. 25 body lengths) and compensated for the distance by boosting the distant stimulus by +6 dB when the female crossed the switch boundary. Using the synthetic W versus Wc stimuli again, we found that the reversal frequency (42.5%) did not differ from that in experiment 3 ($U = 678.5$, $N_1 = 20$, $N_2 = 70$, $P = 0.82$). Experiment 5 was identical except the switch boundary was 100 cm (ca. 10 body lengths) from the face of the target speaker, and we boosted the distant stimulus by +1.33 dB to compensate. In experiment 5, we found a reversal frequency of 65.0%, which was significantly greater than the reversal frequency seen in experiment 3 ($U = 481$, $N_1 = 20$, $N_2 = 70$, $P = 0.020$; this *P* value was not significant when corrected for multiple comparisons) and marginally different from that in experiment 4 ($U = 134$, $N_1 = N_2 = 20$, $P = 0.054$). In experiment 6, we maintained the switch boundary at 75 cm but instead of boosting the distant Wc by +2 dB as in experiment 3, we boosted it by +4 dB to examine whether reversal frequencies would increase beyond the 44.3% seen in experiment 3. This was not the case; reversal frequencies remained approximately the same in both experiments (45.0%; $U = 697$, $N_1 = 20$, $N_2 = 70$, $P = 0.975$).

In experiment 7, we initially broadcast W versus Wc, but when the female crossed the switch boundary nearest the Wc, we changed it to a W while leaving the distant signal a W and amplifying it +2 dB to compensate for the distance. Here we found a significant decrease in reversal frequency (12.5%) compared to experiment 3 ($U = 352$, $N_1 = 20$, $N_2 = 70$, $P = 2 \times 10^{-4}$) but also a significant increase in reversal frequency over that of experiment 1 ($U = 150$, $N_1 = N_2 = 20$, $P = 0.018$).

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Experiments 8–11: adding and subtracting complexity to initially equivalent calls

In experiment 8, we initially broadcast the synthetic Wc from both speakers and then subtracted the chuck from the approached speaker after the female crossed the switch boundary (75 cm). Therefore, this experiment had different initial conditions but identical conditions at the switch boundary compared to experiment 3. Here we found a reversal frequency of 37.5%, which did not differ significantly from that of experiment 3 ($U = 621.5$, $N_1 = 20$, $N_2 = 70$, $P = 0.408$). However, in the control condition, experiment 9, which was identical to experiment 8 except the chuck was not removed, there was a decline in reversal frequency (10.0%) that was significantly different from that in experiment 8 ($U = 122$, $N_1 = N_2 = 20$, $P = 0.014$). Similarly, in experiment 10, in which we initially broadcast the synthetic W from each speaker and then added the chuck to the initially unapproached speaker once the female crossed the switch boundary (75 cm), we found a reversal frequency of 52.5%, which did not differ from that in experiment 3 ($U = 615.5$, $N_1 = 20$, $N_2 = 70$, $P = 0.371$), but did differ from that of the control version (7.5% reversals in experiment 11) of this experiment ($U = 71$, $N_1 = N_2 = 20$, $P = 9 \times 10^{-5}$).

Experiments 12–15: manipulating complexity using natural signals that vary in attractiveness

Here we used natural signals that varied in attractiveness to examine whether such inherent qualities influence reversal frequency in a predictable manner. We initially broadcast a simple natural whine versus a different male's complex whine-chuck and then subtracted that chuck once the female crossed the switch boundary nearest it and simultaneously added the distant male's chuck to his whine. In experiments 12 and 13, we used natural calls n1 and n3, which are known not to differ in attractiveness. Experiment 12 began with the n3 male using a complex call, whereas in experiment 13, we symmetrically began with the n1 male as complex. Reversal frequencies for experiments 12 (30.0%) and 13 (38.3) did not differ ($U = 410$, $N_1 = N_2 = 30$, $P = 0.518$). In experiments 14 and 15, the two natural calls (n1 and n2) differed in attractiveness, with n2 significantly preferred in standard phonotaxis tests. In a comparison of these conditions it did matter which signal began as complex. When the more preferred call was also the initially complex call (experiment 14), reversal frequencies were low (28.3%) compared to the reciprocal condition (experiment 15, 63.3%) and these reversal frequencies differed significantly ($U = 247$, $N_1 = 30$, $N_2 = 31$, $P = 0.001$). Lastly, results from these natural signal experiments corroborate our findings from the experiments using synthetic stimuli, and extend those findings because reversal frequencies were linked to signal attractiveness.

Experiments 16–17: do multiple chucks elicit greater commitment from females?

Experiment 16 was identical to experiment 3 except we used a synthetic whine followed by three chucks for the complex call. We found a marginal decrease in reversal frequency (30.0% versus 44.3% in experiment 3) in this condition ($U = 820$, $N_1 = 30$, $N_2 = 70$, $P = 0.057$). We repeated this experiment in experiment 17, but we tested 40 females once instead of in duplicate trials, and we found a similar result 27.5%, providing more evidence that multiple chucks elicit greater commitment, and given the similarity of the reversal frequencies between experiments 16 and 17, it appears that testing females more than once did not influence the outcome of these experiments (see Latencies below).

Experiments 18–19: manipulating call presence/absence

In experiment 18, we initially broadcast the synthetic W and Wc, but then we simply ceased broadcasting the Wc once the female crossed the switch boundary nearest it. In this condition, females reversed 100% of the time and chose the distant W. In experiment 19, we ceased both speakers and found that 0% of females reversed but that 25% of females continued on their original trajectory and selected the near, but then silent, speaker. The remaining 75% of females did not make a choice.

Latencies

Table 2 outlines the mean latency to the switch boundary, latency to make a choice after stimulus switching, and overall latency to make a choice with data pooled from the basic experiments involving a simple versus complex call and stimulus switching with a +2 dB boost (experiments 3, 12–15). From these experiments we selected females that reversed on one of the two trials and we performed paired *t* tests to determine whether these three latency measures differed for trials involving a reversal compared to those that involved a nonreversal. We found that the latency to the switch boundary did not differ between reversal and nonreversal trials ($P = 0.649$). The latency to make a choice following stimulus switching, however, differed significantly for reversals and nonreversals due to the longer distance that females travelled during a reversal ($P < 1 \times 10^{-6}$). Finally, the latency to overall choice differed significantly between reversal and nonreversal trials ($P = 2 \times 10^{-5}$).

We tested each female twice in these experiments (trial 1, trial 2). To ensure that female behaviour was unaffected by trial number, we analysed overall latency to make a choice for the first and second trials, comparing these latencies within subjects for females that either reversed or did not reverse on both trials in experiment 3, our single largest data set (again, we did not compare latencies across the two trials for females performing one reversal and one nonreversal because latencies differed significantly depending on whether a reversal occurred). In both instances, latency to make a choice was unaffected by trial number (paired *t* test: zero-reversal females: $t_{20} = 1.43$, $P = 0.168$; two-reversal females: $t_{12} = 1.09$, $P = 0.295$). To extend this analysis to all the experiments, we used repeated measures ANOVAs to examine latency to make a choice for females that either reversed or did not reverse on both trials (one within-subject factor: trial number; one between-subject factor: experiment type). In both analyses the main effect of trial number was not significant (zero reversals: $F_{1,212} = 1.56$, $P = 0.213$; two reversals: $F_{1,84} = 1.28$, $P = 0.260$), nor was the interaction term for either analysis significant (zero reversals: $F_{19,212} = 1.00$, $P = 0.453$; two reversals: $F_{15,84} = 1.14$, $P = 0.329$). Lastly, to ensure that the likelihood of reversal did not differ by trial number, we used an exact binomial test (two tailed) on the number of reversals performed on the first versus second trial for females making one of two reversals and found no effect in experiment 3 ($P = 0.07$) or across all experiments ($P = 0.14$). In summary, we conclude that testing females twice does not influence latency or reversal behaviour and is an acceptable method of data acquisition.

Table 2
Mean ± SE latency to the switch boundary (proxy for motivation), latency to make a choice after stimulus switching and overall latency to make a choice for female túngara frogs that showed reversal and nonreversal of phonotactic choice to natural male advertisement calls in experiment 3 and in experiments 12–15, and paired *t* test results for each measure

Latency measure	Nonreversal mean latency (s)±SE	Reversal mean latency (s)±SE	Paired *t* test
Boundary (75 cm)	82.7±11.5	78.0±9.6	t_{63}=0.46, P=0.649
Choice after switch	34.9±3.3	95.4±9.5	t_{64}=6.75, $P<10^{-6}$
Overall choice	132.6±14.4	177.5±14.7	t_{78}=4.51, P=0.00002

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DISCUSSION

Mate selection in túngara frogs provides a compelling model for examining the details of auditory behaviour, including temporal aspects of the mate choice process. The simplicity of conditions used in many studies of mate choice precludes a thorough appreciation of the variation in preferences that exist, which limits our understanding of the strength and direction of sexual selection. Our results demonstrate that: (1) frogs are sensitive to the spatial position of a preferred call and in many instances the reactions to altered calls are instantaneous (see Supplementary Material); (2) decisions are not always finalized prior to an approach: choices are flexible and depend on a consistent repetition of uninterrupted complex calls; (3) the complexity, amplitude and intrinsic attractiveness of signals influence the probability of reversing phonotaxis towards an alternative male; (4) a female's commitment to an initial choice increases as distance to the target decreases, or a female's commitment may increase as the distance she has travelled increases (i.e. her locomotor investment), or both; distinguishing between these possibilities will require additional experiments in which the position of speakers is adjusted; (5) mate choice in this species is an active process; females actively reject certain calls in favour of others despite a more intense stimulus from the rejected caller (experiment 2), contrasting with the predictions of passive mate choice (sensu Parker 1983).

We can now answer the question proposed at the beginning of this study regarding a specific decision rule that explains commitment during mate choice. Collectively interpreting results from experiments 1, 7, 9, 11 and 19, we deduce that females are strongly committed to an initial choice provided there is no change in signal complexity that leads to a simple versus complex contrast between the two sources, but that when there is such a change (experiments 2–6, 8 and 10) females update such information and change their mate choice decision 30–65% of the time. The frequency of reversals rests predictably on the acoustic parameters of the signals that are adjusted and the timing of the stimulus manipulation during approach.

A few other studies have pursued dynamic mate choice in anurans. In reed frogs, *Hyperolius marmoratus*, Dyson et al. (1994) found that females will reverse course after initially approaching (one body length) a preferred stimulus (leading call) if the stimuli are switched. Likewise, Gerhardt et al. (1996) tested the preference strength for pulse number in *Hyla versicolor* and found that females, after initially approaching a high pulse number call, reversed directions when this preferred stimulus was suddenly switched with a less preferred low pulse number alternative. By varying the pulse number of competing calls across experiments within the natural range of variation, and adjusting the distance and amplitude of the sources, Gerhardt et al. (1996) obtained reversal frequencies that averaged about 50%, and peaked at approximately 75% under conditions of greatest signal contrast. These previous studies provide strong evidence that decision making involves temporal updating in at least a few species of frogs. The extent to which this type of behaviour is influenced by other signal parameters and social conditions is explored further here, as well as one estimate of the cost of temporal updating (latency to make a choice and distance travelled increased during reversals).

Previous research in túngara frogs has shown that females prefer the complex call. Our study shows that this preference hinges critically on the timing of complex call production: females prefer males that produce a complex call. All males are capable of producing complex calls but choose to do so depending on the social environment, with complex calls more commonly produced (1) during bouts of vocal competition between neighbouring males (Bernal et al. 2009), (2) in the presence of females (K. L. Akre, personal communication), (3) when predation risk is perceived to be lower (Tuttle et al. 1982; Jennions & Backwell 1992; Phelps et al. 2007) and (4) when injected with arginine vasotocin (Kime et al. 2007), a neuropeptide that modulates social behaviour in many species (Moore & Miller 1983; Boyd 1994; Chu et al. 1998; Marler et al. 1999; Sanantgelo & Bass 2006). We show that the continued production of complex calls is critical when females are assessing and approaching potential mates, and therefore, males should be under selection to maintain complexity during peak female attendance at the chorus or when females are detected directly. Furthermore, in túngara frogs, the chuck attenuates over shorter distances than the whine (Ryan 1986), and thus there might be considerable pressure on males to selectively use complex calls when females are likely to be assessing them.

A study by Márquez et al. (2008) examined the related issue of mate choice as a function of call amplitude, using a treadmill to derive another metric of preference. In addition to investigating the role of amplitude, we suggest that the approach taken in the present study also provides a window into the dynamics of the decision-making process. We show that the reversibility of a particular decision can be titrated spatially, wherein females are more likely to maintain their present trajectory the closer they approach their target. The present study used distances of 10, 20 and 25 body lengths to demonstrate how the likelihood of a reversal decreases as the spatial investment increases or as the distance to the source decreases, or both. Collectively the dynamic studies of mate choice by Dyson et al. (1994), Gerhardt et al. (1996) and Márquez et al. (2008) and the present study decompose this decision-making process into spatial and temporal domains and in doing so demonstrate convincingly that anuran behaviour is more flexible than previously appreciated and that a dynamic mate choice paradigm could be applied to other systems wherein signallers simultaneously compete for receivers (e.g. acoustic signalling in crickets, multimodal signalling in manakins).

A continuous acoustic presence is essential for male success during female assessment. The call cessation experiments represent a reasonably natural scenario with which to examine this: multiple females at a chorus might be assessing the same male, and once the first female selects a given male, he is out of the pool of potential mates and his calling ceases abruptly. For other females nearby, continuing an approach after cessation would be of no value. Males also tend to cease calling when they detect predators (Tuttle et al. 1982; Jennions & Backwell 1992; Phelps et al. 2007), and therefore, it would be beneficial for females to restrict movement following call cessation. For many anuran species, including túngara frogs (Ryan 1983), a strong predictor of male mating success is chorus tenure (reviewed in Wells 2007); males that attend choruses and call more achieve more matings, thereby generating both important benefits and costs (e.g. predation, energetic demands) of persistent calling. From our call cessation experiment (experiment 19), it appears that some females, or all females some of the time (25%), commit to an initial decision to respond to a male's call, and once committed, they cannot be dissuaded from their choice or arrested in their movement towards that call even when the call ceases. Future studies will explore the extent to which individual variation in females provides an explanation for these results. Differences between reversal and nonreversal trials, however, do not appear simply to be explained by motivational differences because the latency to the switch boundary (a proxy for motivation) did not differ between reversal and nonreversal trials. For fickle females, the few moments before executing mate choice by physically contacting a male might be the crucial time window for male persuasion. This study demonstrates that such a time window exists and is perhaps narrower than previously thought (Schwartz et al. 2004).

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We suggest that mate choice behaviour in túngara frogs is not simply a two-step process of evaluating signals and applying a decision rule, as it is commonly framed in behavioural ecology (Valone et al. 1996); such conceptualizations ignore the role of executing the decision, including the iterative process of temporal updating during choice. By taking a psychophysical approach and dynamically manipulating signals during female choice, we show that the decision-making process is iterative and open-ended. The approach taken here places importance on achieving a level of stimulus control over a female during choice to parcel out experimentally the signalling conditions essential for commitment to an initial choice. We show that during mate approach, females continue to gather information about differences between males and there is a time window during which females use this information to modify their mate choice. In general, these results speak to the sensitivity of receivers and the dynamic nature of communication in a way that we believe expands our understanding of decision making generally and mate choice specifically. Future studies will benefit from extending this experimental design beyond dichotomous choice tests, thus providing an opportunity to evaluate female decision making in more complex social environments, and to determine saturation thresholds for female attention.

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Supplementary Material

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