Supplementary Materials for

Irrationality in mate choice revealed by túngara frogs

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Materials and Methods

We conducted these experiments from June-October, 2010-2012 at the Smithsonian Tropical Research Institute in Gamboa, Republic of Panama (9°07.0 N, 79°41.9 W). Gravid female túngara frogs were collected in amplexus from the field, and tested in phonotaxis trials the same night. We conducted the phonotaxis trials between 2130 and 0430 h in a darkened sound-attenuation chamber (2.7 x 1.8 x 1.78 m, L x W x H; Acoustic Systems, Austin, TX, U.S.A) maintained at approximately 27°C. The chamber was illuminated only by 850 nm infrared lighting and monitored remotely via an infrared camera. At the beginning of a trial, each female was separated from her male and placed in the center of the phonotaxis chamber under a mesh funnel. Stimuli were broadcast antiphonally (such that there was no temporal overlap of calls) at 82 dB SPL (re. 20 μPa) at the center of the chamber from (two or three, depending on the test) ADS L210 speakers for a 2 minute acclimation period, after which the female was remotely released from the funnel and monitored. A positive "choice" was scored if a female approached within 10 cm of a broadcasting speaker in less than the maximum trial duration of 10 minutes. Additional details for phonotaxis experiments are provided elsewhere *(14, 17)*.

The 3 stimuli (A, B, C) were composed of two traits (DIM-1, DIM-2). In order to assign a relative attractiveness value for each composite stimulus, we completed a series of preliminary experiments in which we measured the relative attractiveness of each of the 6 trait levels [DIM-1 call types: (A_1, B_1, C_1), DIM-2 rates (A_2, B_2, C_2)] (Table S1, Fig. S1).

For DIM-1 (static attractiveness), we selected three natural whine-chuck calls whose relative attractiveness was measured in a previous study *(14)* and replicated the tests in our preliminary study here. These calls had originally been recorded in the field from different males and were identified as 3 of 9 male calls whose static call characteristics represented the multidimensional acoustic variation present in the Gamboa population [Corresponding call IDs: A_1 = Sa (2003); B_1 = Sb (2003); C_1 = Sd (2003)]. Female phonotaxis preferences were also measured in that study, thus for our study we were able to predict the relative values of these calls based on previous results. We tested 18 females on all three dyads between the 3 call types (A_1, B_1, C_1) (Table S1).

In general, female frogs show a preference for faster call rates. For the DIM-2 (call rate), we chose call rates centered on the average natural call rate measured in previous studies of 1/2 *calls/sec (26)* [C_2= 1/4, A_2= 1/2, and B_2= 1/1, *calls/sec*]. To calculate the value of DIM-2, we tested 20 females on each of the 3 static call types (A_1, B_1, C_1) for each DIM-2 rate (A_2, B_2, C_2): n= 180 trials, n=20*3= 60♀ in each of 3 stimulus categories. This also allowed us to determine whether the relative preference for faster call rates were dependent on any of the three static call types used. The distribution of choices to the faster stimulus across DIM-1 call types was analyzed using a 3 (B_2 vs C_2, B_2 vs A_2, A_2 vs C_2) * 3 (A_1, B_1, C_1) contingency table.

We calculated binomial z-ratios separately for each DIM based on the proportional preference out of the total choices in the 3 stimulus set and derived total attractiveness values (Fig. 1C, shown geometrically in Fig. 1D) by summing the DIM z-scores (Table S2). Binomial z-scores enabled direct and scale-free comparisons between levels of the two traits.

For the decoy experiments, our large sample sizes permitted us to apply the normal approximation to the binomial distribution for binary pairwise contrasts (A vs B) [all $n > 30$, Rosner's Rule *(27)*]. In replicated studies with these and other independent traits, we see consistent group-level preferences and find no among-individual preference polymorphisms *(28)*; nonetheless, here we tested the same individuals on both binary and trinary tests in order to avoid sampling bias. Given that for each experiment, the same females were tested in both the binary and trinary tests, the difference in the proportion of choices between {A, B} and {A, B | C} was compared using McNemar's tests for paired data. To meet the requirements for the McNemar's tests, three females who did not successfully make a choice in both tests were excluded from the analysis (experiment 2: final n=77). Exact p-values are presented and our criterion for statistical significance was $\alpha = 0.05$. All statistical tests were conducted in SPSS v22.0.

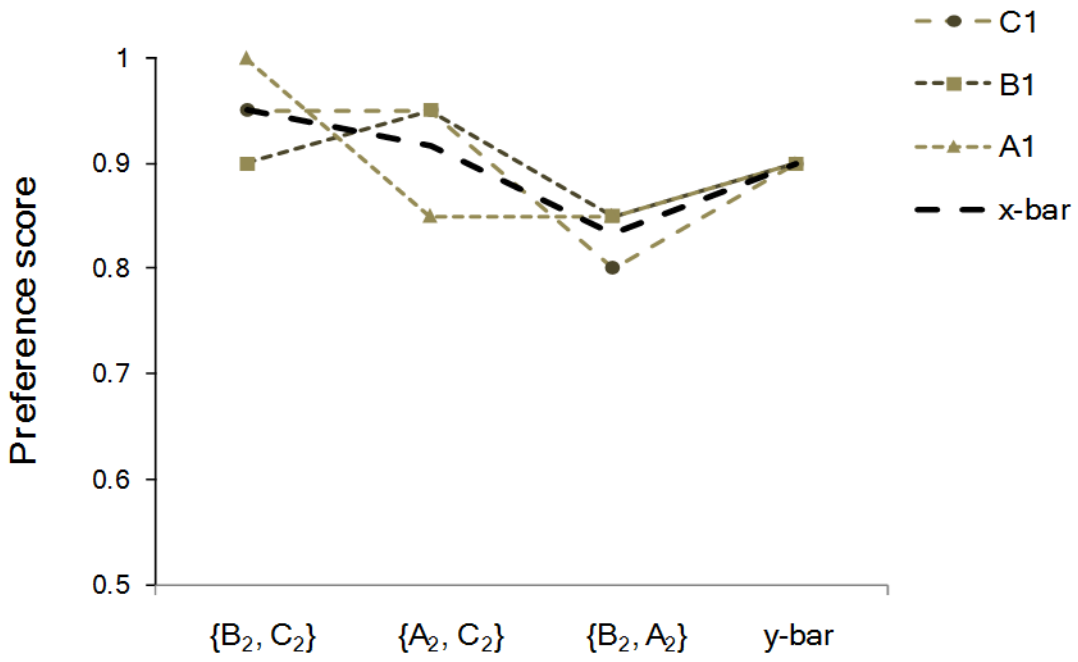

Fig. S1.

Results of preliminary tests measuring call rate preferences [DIM-2 trait values]. DIM-2 preferences were highly skewed toward the fastest call rate in each dyad and independent of the static call type [DIM-1; (A_1, B_1, C_1)]. Preference score indicates the proportion of choices to the faster stimulus in each pair. There was a significant preference for the faster call rate in each of the 9 tests [2-tailed binomial probabilities; all $p<0.05$]. We found no differences between DIM-1 call types on the magnitude of the preference for faster call rates [$X^2=4.81$, $p=0.09$] nor any differences between stimuli in the total number of choices across the three contrasts; the average proportion of choices for each of the three stimuli (y-bar) was exactly equal to 90% [$X^2=0$, $p=1.0$].

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Table S1.

Results of preliminary tests measuring static attractiveness [DIM-1] trait values. Relative preferences of 18 female túngara frogs for 3 call types (A_1, B_1, C_1). Preference score indicates the proportion of choices for the preferred stimulus out of the total (n=18). Both A_1 and C_1 were significantly more preferred than B_1 but there was no significant difference between the static attractiveness of the target (A_1) and that of the decoy (C_1). P-values indicate the two-way binomial probabilities that the number of choices to each stimulus was equal. Significant contrasts at $\alpha = 0.05$ are indicated with an asterisk (*).

Trait	**A_1 vs. B_1**	**A_1 vs. C_1**	**B_1 vs. C_1**	$\sum_{(choices)}$
Choices [A_1]	15	6	n/a	21
Choices [B_1]	3	n/a	1	4
Choices [C_1]	n/a	12	17	29
$\sum_{(choices)}$	18	18	18	54
Preference score	0.83 [A_1]	0.67 [C_1]	0.94 [C_1]	
p	< 0.01*	0.24	< 0.001*	

Table S2.

Independent valuation of trait dimensions in the preliminary study. Each trait is valuated based female preferences. We measured the subjective values of attractiveness for each trait level by comparing the relative proportion of females' choices that each stimulus garnered in total from the two pairwise tests in which it participated in the round-robin set $\{A_t$ vs $B_t\}$,$\{B_t$ vs $C_t\}$,$\{A_t$ vs $C_t\}$. Each female was tested in 3 round-robin style tests in which each stimulus participated in 2 of the 3. The sum of choices to each stimulus from both pairwise tests was divided by the total number of trials to calculate the proportional relative preference and binomial z-scores. Given that each stimulus was tested in 2 of 3 pairwise tests, the sum of proportions for the three stimuli was 1.50 while the maximum possible attractiveness for a given stimulus was 1.0. Final stimulus values were calculated by summing the two corresponding trait z-scores [$B = B_1 + B_2 = +3.99$; $A = A_1 + A_2 = +1.65$; $C = C_1 + C_2 = -5.90$] (Fig.1C, shown geometrically in Fig. 1D).

Trait	Level	Sum (choices)	Total Possible	Proportion (choices)	Binomial Z-score
DIM-1 (static) (n=54)	B_1	4	36	0.111	-4.500
	A_1	21	36	0.583	+0.830
	C_1	29	36	0.806	+3.500
DIM-2 (rate) (n=180)	B_2	107	120	0.892	+8.490
	A_2	65	120	0.542	+0.820
	C_2	8	120	0.067	-9.400
Total Value (DIM-1 + DIM-2)	B (competitor)				+3.990
	A (target)				+1.650
	C (decoy)				-5.900

Additional Data table S1 (separate file)

Raw data results of phonotaxis assays for the two decoy experiments. Rows represent individual female frogs. Columns indicate the experiment number that is consistent with the article and the date on which the female was tested. All females in experiment 1 (floor speaker design, decoy available) were tested in each of the 4 tests. In experiment 2 (ceiling speaker design, unavailable decoy), 50 females were tested in all 4 tests and 30 were tested only in the focal binary [A vs B] and trinary [A vs B w/C] tests. Corresponding choices for each test are indicated by the numbered suffix. A = target, B = competitor, C = decoy.