Supplementary Materials for

Interactions of Multisensory Components Perceptually Rescue Túngara Frog Mating Signals

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Materials and Methods

Supporting Online Material

We conducted all experiments at facilities of the Smithsonian Tropical Research Institute in Gamboa, Republic of Panama. Mating pairs of frogs were collected at night from temporary pools using headlamps. We maintained frogs in total darkness for at least 1 h prior to testing to ensure visual sensitivity. Light levels in the test arena were adjusted to be similar to moonless nights (*10*). For each trial we placed a female under a cone equidistant (80 cm) from two speakers separated by 60° relative to female release point (*10*). One speaker broadcast a call (unimodal), the other speaker broadcast a call and had a robofrog placed in front (multimodal). Playbacks were adjusted to 82 dB SPL (re. μ20 Pa) at the initial position of the female and broadcast antiphonally. The call or vocal sac inflation at one speaker never overlapped with the call from the other speaker. Calls in experiments without a gap between whine-chuck were broadcast at 30 calls/min. In experiments where an acoustic gap was present, we broadcast calls at 18/min to ensure that the length of the gap between the whine-chuck in one call did not exceed the gap between separate calls. We started the playbacks and allowed females to acclimate to the arena for two min. Vocal sac inflation/deflation was produced by a pump, electronically triggered by the playback computer (*9*, *10,*). We released the female and allowed her to approach a speaker or speaker/robofrog. A choice was scored when the female approached to within 5 cm of the speaker or robofrog (*9*, *10*). In nature this behavior results in mating. After the trials we marked females with toe-clips to avoid retesting. Females were not re-tested within or across experiments, thus each trial represents a datum from a unique female. At the end of each night we released the male/female pairs where they were collected. The testing configuration here (60° speaker separation relative to the female) differs from previous phonotaxis tests with two speakers separated by 180° (e.g., *11*). We replicated some previous experiments using this new

configuration and obtained similar results (e.g., Fig. 1A-D). We compared the null hypothesis of no preference with a binomial test, and report the mid-level *P* value appropriate for tests with small sample sizes (*15, 16*). We conducted a one-tailed binomial test in the experiments in figures 1H-I because our perceptual rescue hypothesis predicts that the addition of the vocal sac inflation and the chuck in these non-salient positions will increase rather than decrease the signal's attractiveness. All research complied with IACUC protocols from Salisbury University, University of Texas, and the Smithsonian Tropical Research Institute. We obtained all required permits from the Government of Panama.