Multimodal weighting differences by bats and their prey: probing natural selection pressures on sexually selected traits

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Multimodal communication has received increasing attention in recent years. While much is understood about how intended receivers (such as potential mates) respond to multimodal displays, less is known about how eavesdropping predators perceive and interpret these cues. The male túngara frog, *Physalaemus pustulosus*, is a neotropical anuran that attracts females with an acoustic call and a dynamically inflating/deflating vocal sac. However, the túngara frog's multimodal courtship display also attracts eavesdropping predators, such as fringe-lipped bats, *Trachops cirrhosus*. We utilized robotic frog models to expose fringe-lipped bats to multimodal túngara frog courtship displays. The models varied in call amplitude and/or the presence of vocal sac cues. In a two-choice test, we show that fringe-lipped bats more often attack higher-amplitude calls. Additionally, coupling the inflating vocal sac cues to the lower-amplitude frog call increased the probability that a bat would attack this less attractive call. Previous studies have demonstrated that vocal sac cues do not increase the attractiveness of low-amplitude calls to female *P. pustulosus*. Thus, although natural selection, through the bats, and sexual selection, through the female frogs, exert counter-selection forces on the male's sexual display, the strength of these forces are not symmetrical. We discuss possible explanations for why this might be the case. This study underlines the importance of understanding the contribution of both intended and unintended receivers on signal evolution, and it helps explain how selection pressures might vary across sensory modalities.

Courting animals often exhibit elaborate multimodal displays that increase signal detection and attractiveness to mates (Hebets & Papaj, 2005; Partan & Marler, 2005). Some female frogs, for example, attend to the male's body movements while listening to his calls (Preininger, Boeckle, Sztatecsny, & Hodl, 2013; Taylor, Buchanan, & Doherty, 2007). However, mate attraction can be costly, as multimodal displays can also increase detection by eavesdropping predators (Halfwerk, Dixon, et al., 2014; Rhebergen, Taylor, Ryan, Page, & Halfwerk, 2015).

While single-modality mating signals between a signaller and its intended receivers (mates) is well understood, few studies have attempted to understand how multimodal displays are perceived by eavesdropping predators (Halfwerk & Slabbekoorn, 2015; Hebets et al., 2016). Furthermore, it is unclear how predators use prey cues, especially since they not only attend to the same cues as mates, but can also perceive display components via different sensory modalities (Halfwerk, Dixon, et al., 2014; Halfwerk, Jones, Taylor, Ryan, & Page, 2014). Additionally, multiple cues often interact, which can alter a receiver's behavioural response to a single cue presented alone (Partan & Marler, 1999; Partan, 2013). For example, poison-dart frogs modulate their response to acoustic cues of intruding frogs in the presence of visual cues (Narins, Grabul, Soma, Gaucher, & Hodl, 2005; Narins, Hodl, & Grabul, 2003). However, cues do not necessarily interact in the same way. Thus, determining the relevance of multimodal display components can be difficult. Rather than assigning 'absolute' values to cues, receivers have evolved ways to compare cues with one another (Bateson & Healy, 2005; Lea & Ryan, 2015) and assign 'relative' values to them. This process, termed 'signal weighting', allows receivers to compare multiple cues among signalling individuals and make decisions based on cue importance.

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The fringe-lipped bat, *Trachops cirrhosus*, is a predator that eavesdrops on mating calls of frog and insect prey (Tuttle & Ryan, 1981). One of its preferred prey species, the male túngara frog, *Physalaemus pustulosus*, often calls in large choruses. When choruses are loud, males increase the amplitude of their calls (Halfwerk, Lea, Guerra, Page, & Ryan, 2016), which makes them more attractive to both female frogs and bat predators (Tuttle & Ryan, 1981). During call production, male frogs inflate and deflate their vocal sacs as a by-product of recycling air. These dynamically moving vocal sacs make males more visually attractive to females (Taylor & Ryan, 2013; Taylor, Klein, Stein, & Ryan, 2008, 2011) and more echo-acoustically attractive to frog-eating bats (Halfwerk, Dixon, et al., 2014). However, for female frogs, the visual cue increases attractiveness only when differences in male call amplitude are miniscule. In other words, amplitude is more important in female decision making than the perception of an inflating vocal sac (Stange, Page, Ryan, & Taylor, 2016). While bat predators are more attracted to calling frogs when inflating vocal sac cues are perceptually available (Halfwerk, Dixon, et al., 2014), it is unclear how this cue interacts with call amplitude, and what importance bats place on each of these cues during foraging.

In our study, we used robotic frog models to investigate how a bat predator weights individual cues of a multimodal frog mating display. Because bats and frogs use different sensory modalities to perceive the male frogs' vocal sac inflation, we hypothesized that predators and mates would also differ in their weighting of male cues. Teasing apart the influences that multimodal display cues have on bat predators is important in our understanding of how natural selection pressures have shaped the evolution of the male frog's sexual display.

METHODS

Animal Care

Fringe-lipped bats ($N = 16$) were caught in Soberanía National Park, Panamá, between September 2014 and May 2015 with mist nets set in the forest or near known roosts. The bats were caught 0—4 h after sunset and were housed in an outdoor flight cage ($5 \times 5 \times 2.5$ m) in Gamboa, Panamá, where they had access to water ad libitum. The bats were given at least one night to acclimate to the flight cage and, on subsequent nights, training and testing took place in this same flight cage. Bats began to exhibit natural foraging behaviour very quickly (generally by the night after capture), suggesting they were comfortable with our experimental set-up and initial handling. Furthermore, animals were handled as minimally as possible; once they were placed in the flight cage, they were not caught or moved by researchers for the duration of the experiment, and thus, were only handled during initial catching and final release back into the wild. Prior to release, each bat was injected with a subcutaneous passive integrative transponder (Trovan, Ltd, http://www.trovan.com) to prevent the retesting of wild individuals. All necessary permits were obtained from the Government of Panamá (ANAM SE/A-86-14), and all research complied with the Institutional Animal Care and Use Committee (IACUC 2014-0101-2017, 2015-0209-2018) protocols from the Smithsonian Tropical Research Institute (STRI).

Experimental Set-up

Two frog models (a robotic model and a control model) were used in a two-choice test (Klein, Stein, & Taylor, 2012; Taylor et al., 2008). Each frog model was placed on the centre of a smooth-surfaced Plexiglas circular platform (height = 9 cm, diameter = 33 cm) that echo-acoustically mimics a water surface

(Siemers, Stilz, & Schnitzler, 2001). Speakers (Tymphany Peerless, 6 cm; powered by a Pyle PCA2 Stereo Power Amplifier 2×40 W) were placed directly below each platform to broadcast a synthetic túngara frog whine plus one chuck call (for details on the creation of the synthetic whine-chuck call see Ryan & Rand, 2003). Holes were drilled into the Plexiglas below the frog model to allow for sound propagation from the speaker directly below.

Frog models had an inflatable silicon balloon in front of the frogs (which mimicked a frog vocal sac), and an air tube that ran under the Plexiglas and through the wall to an adjacent room (refer to Gomes et al., 2016; Laird, Clements, Hunter, & Taylor, 2016 for more details on vocal sac construction). One of the models (hereafter referred to as the robotic model) had connection to a gas-relay station via the air tube, which allowed for vocal sac inflation. The air tube for the control model ended after ~1 m and was not connected to the gas-relay station.

The two frog models were always placed 0.8 m from each other (on centre), and the pair of models were set 2.4 m, 3.2 m or 4 m from the perched bat on any given trial. Both frog models were placed randomly in an array of nine paired positions, resulting in a total of six possible locations for the pair of platforms (i.e. positions 1 and 2, 2 and 3, etc.). Two additional Plexiglas platforms, which lacked frog models, were placed randomly in one of the remaining seven positions (for an illustration of the platform array, see supplementary methods in Gomes et al., 2016).

Speakers from both frog models simultaneously broadcast synthetic túngara frog calls on a 2 s cycle (Ryan & Rand, 2003). The silicon vocal sac on the robotic model inflated in synchrony with the túngara frog call via a 19 kHz actuation signal (sent from a laptop, Dell Latitude E4300, via Adobe Audition to the gas-relay station). All three audio channels (control model speaker, robotic model speaker and 19 kHz inflation signal) came from the same laptop and were split to independent outputs using a multichannel set-up (Edirol FA-101).

The control frog model always broadcast the túngara frog call at 76 dB SPL (re. 20 μPa; at 1 m; C weighting; set to Max and Fast), while the robotic model played back at 76 dB, 73 dB, 70 dB or 67 dB, corresponding to an amplitude difference of 0 dB, −3 dB, −6 dB or −9 dB, respectively. While the control model did not have an inflating vocal sac (only a deflated, stationary one), the robotic model had the option of having the inflating vocal sac turned off (similar to the control model; unimodal trials) or turned on (multimodal trials) by muting the 19 kHz channel on Adobe Audition. This allowed for eight robotic model treatments (vocal sac inflation: on/off; playback amplitude difference: 0 dB, −3 dB, −6 dB, −9 dB), which were repeated four times per bat, resulting in 32 total trials per bat. The robotic frog treatments were randomly presented throughout the duration of the experiment to control for effects of learning and habituation.

In each trial, bats made a choice between the control model (amplitude always 76 dB; no vocal sac inflation) and the robotic model, which was either unimodal (amplitude varying across trials; no vocal sac inflation) or multimodal (amplitude varying across trials; with the dynamically inflating vocal sac). Thus, when the robotic model was played back at 76 dB (0 dB difference) and had the inflating vocal sac turned off (unimodal), it was equal, in all ways, to the control model. This treatment served as an additional control, in which we would expect 50% of the attacks to be to either model (random attacks; see Fig. 1).

Behavioural Observations

In all trials, bats were initially perched in a 60×60 cm roost made of black cloth that was located in one corner of the flight cage. Túngara frog call playbacks (and the inflation of the vocal sac) were
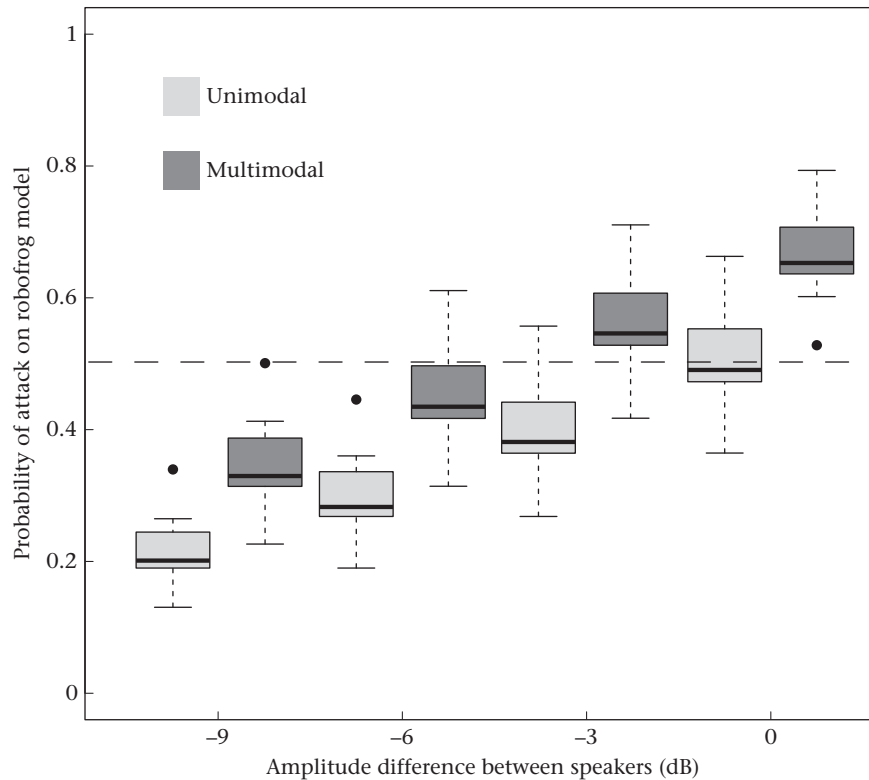
Figure 1. Box plots showing the proportion of attacks on the robotic model when the inflating sac was turned off (unimodal; light grey) or on (multimodal; dark grey) relative to the difference in amplitude between the two speakers (proportion of attacks to the control model = 1 minus the attacks to the robotic model). Box plots show 25% and 75% quartiles (boxes), medians (lines in the boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles). The robotic model was always quieter than the control model unless the amplitude difference was zero. Note that in unimodal trials with 0 dB difference, both models were equal and a 50% attack rate was observed, as expected.

continued until a bat made an attack or until 1 min passed without flight from the roost by the bat. The observer rewarded every third trial by placing baitfish on both (robotic and control) models, such that bats would not learn to associate food with one model or the other. We designated an 'attack' as the bat's successful retrieval of baitfish (in the rewarded trials), or an attempted bite to a frog model, landing on a frog model, or continued hovering over the model by the bat (in unrewarded trials). Trials that did not contain an obvious attack to one platform or the other (i.e. bats circled around the collection of platforms or flew in figure eights around two or more platforms) were discarded and repeated, which happened very infrequently (19 of 531 trials). Attack sequences were recorded with a home security camera system (Conrad) and three infrared-sensitive cameras: one CMOS 6 mm camera fixed to the flight cage wall to capture the side view of the platform array; one CMOS 6 mm camera fixed to the ceiling in front of the bat to record when the bat took flight; one Sony CCD pinhole 2.8 mm camera fixed to the ceiling to provide a top view over the platform array. Videos were analysed to confirm the bat's platform choices and to quantify the bat's flight latency: time from the onset of the frog playbacks to the bat's flight from its perch.

Data Analyses

We analysed the bat's platform choice and flight latency using linear mixed models from the package 'lme4' in the program R v.2.15.1 (R Core Team, 2012). The number of attacks on the multimodal set-up was compared with the number of attacks on the control set-up using the binding function of the generalized linear mixed model, with a binomial error structure and a probit-link function. Latency was modelled using an inverse Gaussian error structure and 1/muˆ2 link function. We tested for significant effects of multimodal cue (vocal sac on or off), playback amplitude as well as their interaction. Models with and without the fixed effect of interest were compared using likelihood ratio tests (using maximum likelihood). Models always included bat ID as a random slope factor and trial number as a random intercept factor. Distance from perch to platform had no explanatory power for both response variables and was therefore left out of the models.

RESULTS

Bats more often attacked the robotic model when multimodal cues were present (inflating sac on) rather than absent (unimodal; inflating sac off), regardless of call amplitude ($\chi^2_1 = 11.919$, $N = 16$ bats, $P = 0.0006$; Fig. 1). This suggests that the vocal sac of a calling male, if perceptually available to a bat predator, makes that male more attractive or easier to locate. Likewise, call amplitude had a significant effect on bat preference ($\chi^2_3 = 27.561$, $P = 0.0001$; Fig. 1). We found no interaction effect between multimodal cues and call amplitude on bat attack preferences ($\chi^2_3 = 3.19$, $P = 0.36$). Additionally, we found no effect of either multimodal cue ($\chi^2_1 = 2.01$, $P = 0.16$) or call amplitude ($\chi^2_1 = 2.41$, $P = 0.12$) on latency to flight.

DISCUSSION

Bats were given the opportunity to attack a robotic frog that provided unimodal (sound only) or multimodal (sound plus vocal sac) cues. Frog calls from the robotic frog were broadcast at equal or

lower amplitudes than a control model (always unimodal and fixed amplitude). We found that bats preferred higher-amplitude play-backs, and that their preference scaled with amplitude: each 3 dB increase in call amplitude led to similar preference gains. Female frogs, in contrast, do not respond to call amplitude in a scaled fashion. Recent experiments have shown that females prefer higher-amplitude calls at a threshold around +3 dB, and that preference strength remains similar at +6 dB (Stange et al., 2016).

When vocal sac cues were coupled with lower-amplitude calls, bat preferences for the higher-amplitude control model decreased. Both call amplitude and vocal sac cues were important for bats in all situations. This is not the case for females, as vocal sac cues only make the display more attractive when amplitudes are similar. In other words, female frogs prefer higher-amplitude calls, irre-spective of vocal sac cues (Stange et al., 2016). Thus, call amplitude is a dominant aspect of the multimodal display for female frogs, while both cues are important for bats. This comparison is limited however, as female frogs make mate choice decisions (likely involving male quality), while bats may simply attack prey that are easier to locate.

Bats' latency to flight did not change across treatments, which suggests that bats did not use echo-acoustic cues or call amplitude to decide when to take flight. In previous studies, we found that use of multimodal cues can reduce flight latency, but only under complex acoustic background conditions when sound localization may be difficult (Gomes et al., 2016; Rhebergen et al., 2015).

Our results may help us understand frog calling behaviour. As male frog vocal sac cues are weighted more strongly by bat pred-ators than they are by female frogs, it may benefit males to limit perceptual access to this cue. Since echolocation is strongly affected by background clutter (Halfwerk, Jones, et al., 2014), frogs may escape predation by calling from dense vegetation. Female choice is likely unaffected, since visual cues are not important when call amplitudes differ (Stange et al., 2016), and call amplitudes can vary by 10 dB in the wild (Halfwerk et al., 2016). Thus, frogs may find that the release from predation by calling from dense vegetation is worth the cost of limiting visual access of their vocal sacs to mates.

It would seem that frog vocal sacs are costly in terms of predator attraction. However, vocal sacs likely evolved to recycle air during call production (Pauly, Bernal, Rand, & Ryan, 2006), and were later co-opted as relevant cues to females. Thus, frogs are likely con-strained by their evolutionary history and cannot easily lose such a vocal sac or the incidental movement cues that go with it. Further work is needed to understand how differences in female and predator responses influence the natural and sexual selection pressures acting on this multimodal display.

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