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Inter-signal interaction and uncertain information in anuran multimodal signals

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Abstract Disentangling the influence of multiple signal components on receivers and elucidating general processes influencing complex signal evolution are difficult tasks. In this study we test mate preferences of female squirrel treefrogs *Hyla squirella* and female túngara frogs *Physalaemus pustulosus* for similar combinations of acoustic and visual components of their multimodal courtship signals. In a two-choice playback experiment with squirrel treefrogs, the visual stimulus of a male model significantly increased the attractiveness of a relatively unattractive slow call rate. A previous study demonstrated that faster call rates are more attractive to female squirrel treefrogs, and all else being equal, models of male frogs with large body stripes are more attractive. In a similar experiment with female túngara frogs, the visual stimulus of a robotic frog failed to increase the attractiveness of a relatively unattractive call. Females also showed no preference for the distinct stripe on the robot that males commonly bear on their throat. Thus, features of conspicuous signal components such as body stripes are not universally important and signal function is likely to differ even among species with similar ecologies and communication systems. Finally, we discuss the putative information content of anuran signals and suggest that the categorization of redundant versus multiple messages may not be sufficient as a general explanation for the evolution of multimodal signaling. Instead of relying on untested assumptions concerning the information content of signals, we discuss the value of initially collecting comparative empirical data sets related to receiver responses [Current Zoology 57 (2): 153–161, 2011].

Keywords Inter-signal interaction, Multimodal signaling, Signal detection, Signal weighting, Squirrel treefrog, *Hyla squirella*, Túngara frog, *Physalaemus pustulosus*, Vocal sac

A first step to understanding the evolution of multimodal signals is disentangling the influence of individual signal components (as well as composite signals) on receiver responses. This challenging task is made even more difficult by our inability to identify salient signal components for experimental manipulation. Many animal signals may not be obvious to human observers due to sensory limitations, such as our lack of visual sensitivity to UV, poor low-light visual sensitivity or our inability to hear ultra/infrasonic acoustic communication. Furthermore, morphological traits that appear conspicuous to the human observer may not be important signal components (Tinbergen, 1951; Morris et al., 2001; de Luna et al., 2010). Once receiver responses to salient signal components are quantified, an additional problem exists in that signal components cannot be assumed to have similar functions across species, even within a particular clade. For example, receivers of closely related species often exhibit markedly different

responses to signal components of complex displays, such as the visual and vibratory displays of wolf spiders (Hebets and Uetz, 2000; Hebets, 2008).

A common approach to understanding signal function is to classify signals based on their putative information content (e.g. redundant information vs. multiple messages). Unfortunately, the information content of a signal is often either assumed or poorly defined (Dall et al., 2005). Rendall et al. (2009) made this lack of specificity clear by outlining 15 different characterizations of information in studies of animal communication. In most studies, it is not clear to what “information” refers: a precise quantity, as in Shannon’s Information Theory (Shannon, 1948); aspects of the signaler’s genotype and phenotype as required in honest signaling (Zahavi and Zahavi, 1999; Searcy and Nowicki, 2005); or a more metaphysical quantity in the sense that if a receiver responds to a signal then the signal must carry information. These various definitions raise some problems in

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testing hypotheses about the function and evolution of multimodal signals when such hypotheses are heavily invested in a particular notion of information.

In most anuran species, male vocalizations are the primary signal modality of sexual communication (Ryan, 1985; Gerhardt and Huber, 2002), and its use in a lek-like mating system is often similar. Males typically congregate at a pond and produce advertisement calls (the chorus), competing for the relatively small number of sexually receptive females. The number of males in a chorus may vary widely, but densities are often high. Dense choruses result in significant call overlap and produce discrimination challenges for female receivers (Gerhardt and Klump, 1988; Schwartz, 1993; Wollerman, 1999; Ryan, 2001; Gerhardt and Huber, 2002; Schwartz et al. 2001; Bee and Micheyl, 2008).

Many species also signal visually (Hödl and Amézquita, 2001; Narins et al., 2003) using stereotyped limb motions (Lindquist and Hetherington, 1996; Amézquita and Hödl, 2004) or by displaying conspicuous color patterns (Summers et al., 1999; Hirschman and Hödl, 2006; Vasquez and Pfennig, 2007). In males of most species, conspicuous vocal sac and abdominal inflation also accompany the courtship call, providing widespread opportunity for the vocal sac to be co-opted as a visual signal.

In the squirrel treefrog *Hyla squirella* (Anura: Hylidae), Taylor et al. (2007) showed that females preferentially responded to courtship displays that include a call and a visual cue of model frog with a vocal sac over the call alone. In addition, females preferred male models possessing conspicuous lateral body stripes. Using a robotic frog, Taylor et al. (2008) demonstrated that female túngara frogs *Physalaemus pustulosus* (Anura: Leptodactylidae) also prefer the multimodal signal of a call and vocal sac over the call alone. In both species, the visual component interacts with the dominant acoustic signal to modulate female responses (inter-signal interaction).

Here we present additional experimental results and compare data between these two species showing that: 1) morphologies that appear as conspicuous signals to the human observer may not be salient communication features, 2) the information content contained within a signal component is not always clear, and 3) receiver responses often differ among species that share similar ecologies and communication systems. We argue that classifying signals based solely on information content may hinder our understanding of complex signaling. Instead, we suggest that studies of signal function should first be directed at understanding how the signals

influence receiver responses.

1 Materials and Methods

1.1 Multimodal signal weighting in the squirrel treefrog

Squirrel treefrogs *H. squirella* for this study were collected at two field sites in southern Louisiana. The frogs were housed in 38 L aquaria in a laboratory at the University of Louisiana Lafayette and fed crickets ad libitum until they were used in experiments. We transported the frogs to an outdoor enclosure near the University for testing. We placed 105 frogs (70 males : 35 females) into the enclosure prior to dawn and allowed them to acclimate to the enclosure during the day before the night of testing. During the afternoon, we turned on a garden hose and sprinkler attached to the enclosure and showered the frogs to simulate a rain event. At dusk, we broadcast a recording of squirrel treefrog vocalizations. This process stimulated males inside the enclosure to call. After two hours, we entered the enclosure and collected all the frogs. Pairs in amplexus (indicating a sexually receptive female) were separated and those females were placed into plastic containers for testing. It was necessary to use a headlamp to collect frogs, therefore we placed females in their individual containers into a light-safe cooler for a minimum of 1 h prior to testing. This ensured that their eyes were dark-adapted and had regained low-light sensitivity. For testing, we removed females from their containers without a headlamp (by feel) to ensure that their eyes maintained a dark-adapted state. For an extensive description of collecting locations, housing conditions, and experimental procedures, see Taylor et al. (2007).

We presented females with a two-choice test. The acoustic stimulus for this experiment was a digitally-synthesized call based on average parameters from natural calls recorded in the population. Females were presented with this stimulus from two speakers broadcasting the call antiphonally but at different rates, 80 calls min^{-1} versus 120 calls min^{-1} . Previous experiments showed that females express a five-fold preference for the faster call rate. They also prefer model frogs with larger than average body stripes, but still within their natural size range (Buchanan, 1994; Taylor et al., 2007). In this experiment, a model of a squirrel treefrog male was placed in front of the speaker broadcasting the call at the slow rate of 80 calls min^{-1} , creating a multimodal signal combining relatively attractive visual and relatively unattractive acoustic components. The speaker broadcasting the faster call rate did not have a visual

component associated with it.

The model frog used for the multimodal stimulus was constructed from plaster and sculpted and painted to match live male frogs. The frog model used in this experiment had a larger than average stripe size, but was within the range of natural variation (Buchanan, 1994). A latex condom was glued to the throat of the model and attached to a rubber bulb via aquarium air-line tube. The model's vocal sac was then inflated synchronously with the call at the speaker by squeezing the rubber bulb and forcing air into the artificial vocal sac (Taylor et al., 2007).

For each trial, a female was placed into a cage constructed of plastic mesh with a Petri dish floor and lid. This allowed the female to both hear and see the stimuli. Once a female was in the cage, the playbacks were started and females remained in the cage for at least 2 min. After this acclimation period, the lid of the cage was lifted remotely giving females the opportunity to approach a speaker. We scored a choice when a female approached to within 5 cm of a speaker or speaker/model frog. The positions of the multimodal and unimodal stimuli were switched between trials to avoid position bias.

The test arena was open to natural light from above. All tests were conducted on clear, moonless nights with light levels less than 1×10^{-3} lux (lowest reading on a Science & Mechanics light meter, model 102). Human observers were unable to see test females in these conditions so viewing was done with an infrared viewer (Night Owl Optics monocular viewer). For detailed information regarding call properties, light conditions, model frog construction, and experimental design see Taylor et al. (2007).

We tested the hypothesis that the visual stimulus increases the attractiveness of a relatively slow call. We analyzed the choice data using a one-tailed binomial test with an expected distribution of $P = 0.86$ and $q = 0.14$ (corresponding to the distribution exhibited by females to fast versus slow calls in a previous phonotaxis test). A one-tailed test was justified based on female preferences for visual cues in previous experiments (Taylor et al., 2007). The p value of the binomial test is the probability that the observed distribution differed significantly from the expected distribution.

1.2 Multimodal signal weighting in the túngara frog

All túngara frogs *P. pustulosus* were collected in the field around Gamboa, Panama. We placed each pair found in amplexus into an individual plastic bag and placed the bags into a light-safe cooler. We transported the frogs to the laboratory at the Smithsonian Tropical

Research Institute. As with the squirrel treefrogs, we allowed the frogs to remain in dark conditions for a minimum of 1 h prior to testing so that their eyes would be dark-adapted at the time of testing. For testing, we handled the frogs in near total darkness to preserve the visual sensitivity of their eyes. For each trial, we separated a female from her male and placed her into the arena for testing. After each night of testing, we toe-clipped the frogs (to prevent retesting on subsequent nights) and released them at the location where they were collected.

Female túngara frogs express a five-fold preference for a complex call ("whine" plus one or more "chuck" components appended to the "whine") over the simple call ("whine" only) (Ryan, 1985; Gridi-Papp et al., 2006). In the present study, we also presented female túngara frogs with a two-choice test between a simple and complex call, but we placed a robotic frog (robofrog, described below) in front of the speaker broadcasting the simple call. This created a multimodal signal composed of visual stimuli and the less attractive call. Thus, females were allowed to choose between the multimodal robofrog/less attractive call and the unimodal, more attractive call. The positions of the stimuli were switched between trials to avoid position bias.

The test arena consisted of walls made from acoustic foam and acoustic ceiling tiles supported on a PVC frame. The acoustic foam and tiles limited acoustic reverberations inside the lab. Lighting was provided by a GE brand night light (model no. 55507; Fairfield, CT, U.S.A.) suspended over the arena such that the full width of the arena received equal lighting coverage. We placed duct tape over the majority of the surface of the light to reduce intensity. We used an International Light, model IL 1700 research radiometer with an SHD033/W high gain detector to measure irradiance. We adjusted the irradiance of the arena to approximately 8.57×10^{-10} W/cm², roughly equivalent to a moonless night on a forest edge.

The robofrog was sculpted and painted to mimic a live male. An inflatable vocal sac consisted of a painted urological catheter (Gold Foley latex 30 cc, Teleflex Medical, Rusch Division, Research Triangle Park, NC, U.S.A.) attached to a pneumatic pump. The pump was activated by an audio signal from a laptop computer controlling the acoustic playback signals. This synchronized the inflation/deflation of the robofrog's vocal sac with the call broadcast from the speaker, mimicking a live, calling male. For each trial a female was restrained for two minutes under a funnel consisting of plastic ribs

wrapped with polyethylene food wrap which is effectively transparent to visual and acoustic stimuli. After this acclimation period, the funnel was raised and a choice was scored when females approached to within 5 cm of a speaker/robofrog. For details on the robofrog design and construction, see Taylor et al. (2008).

We tested the hypothesis that the visual stimulus increases the attractiveness of the simple call when the alternative is the complex call. We analyzed the choice data using a one-tailed binomial test with an expected distribution of $P = 0.856$ and $q = 0.144$ (corresponding to the distribution exhibited by females to complex versus simple calls in previous phonotaxis tests). A one-tailed test was justified based on female preferences for visual cues in previous experiments (Taylor et al. 2008). The p value of the binomial test is the probability that the observed distribution differed significantly from the expected distribution.

1.3 Stripe preference in the túngara frog

In this experiment, we presented females with a two-choice test where the identical complex call was broadcast from each speaker. A robofrog was placed in front of each speaker and the vocal sac was inflated/deflated synchronously with the playback call, mimicking a live male. A robofrog at one speaker had a vocal sac painted with a conspicuous white stripe and the other robofrog lacked a white stripe. Túngara frogs in the population at Gamboa, Panama where these tests were conducted express natural variation in stripe size ranging from conspicuous to absent (Fig. 1). This experiment held call properties constant, but allowed females to choose based on presence or absence of a stripe. As in previous experiments, females were restrained for two minutes under a funnel, the funnel was lifted remotely and females were allowed to approach the robofrog or speakers to make a choice.

We tested the hypothesis that females express a preference for the presence of a white vocal sac stripe. We analyzed the choice data using a two-tailed binomial test with an equiprobable distribution (corresponding to the probability that females will choose among identical calls equally). The P value of the binomial test is the probability that the observed distribution differed significantly from the expected distribution.

2 Results

2.1 Multimodal signal weighting in the squirrel treefrog

In this study where the visual cue was coupled with the less attractive slow call rate, 9 females chose the

multimodal stimulus with the less attractive call and 14 females chose the more attractive unimodal call. The visual stimulus of the frog model significantly increased the attractiveness of the slow call rate ($n = 23$, $P = 0.0026$). In this experiment, the addition of the visual signal component rendered the slow call rate nearly 3 times more attractive (14% visual absent : 39% visual present), significantly modulating female responses (Fig. 2).



Fig. 1 Variation in male stripe patterns

A. Male túngara frog with conspicuous white stripe on the vocal sac (uninflated in this photo). **B.** Male túngara frog lacking conspicuous white stripe on the vocal sac. **C.** Male squirrel treefrog showing prominent yellow labial and lateral stripes.

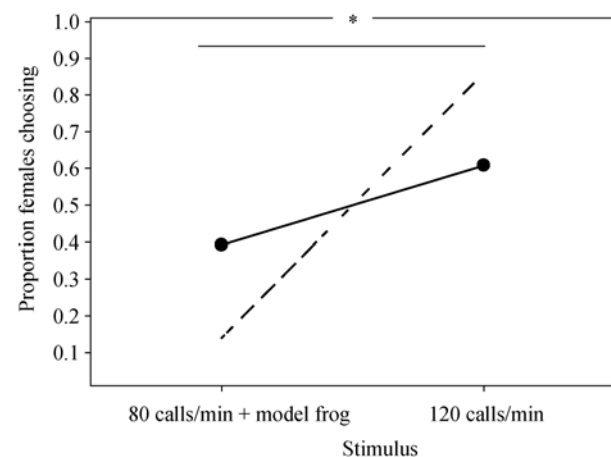


Fig. 2 Preference function for female squirrel treefrogs

Female preference for multimodal stimulus (slower call rate plus model frog) versus unimodal stimulus (faster call rate only). The line connecting two solid points represents female responses to the alternative stimuli ($n = 23$). Significant difference between observed (solid line) and expected distribution ($P = 0.86$, $q = 0.14$; dotted line) indicated by an asterisk.

2.2 Multimodal signal weighting in the túngara frog

In this experiment, female responses did not deviate from the expected 85.6% preference rate for complex calls; 3 females chose the multimodal stimulus with the less attractive simple call and 17 females chose the more attractive complex call ($n = 20$, $P = 0.595$). The addition of the visual signal component did not modulate female responses, and the less attractive simple call remained less attractive when the alternative was a complex call (Fig. 3).

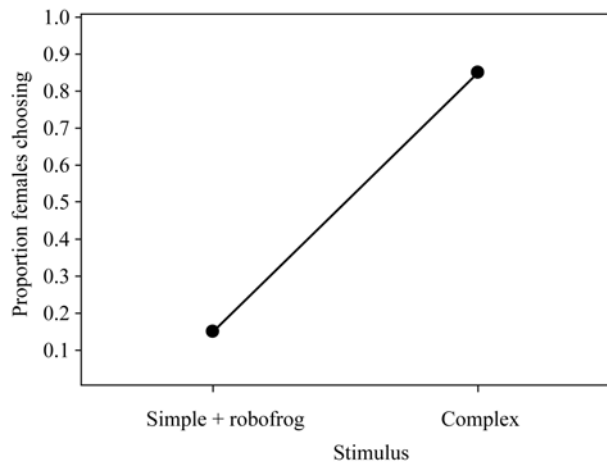


Fig. 3 Preference function for female túngara frogs

Female preference for multimodal stimulus (simple call plus robofrog) versus unimodal complex call. The line connecting two solid points represents female responses to alternative stimuli ($n = 20$). No significant difference between observed (solid line) and expected distribution ($P = 0.856$, $q = 0.144$; hidden by observed value).

2.3 Stripe preference in the túngara frog

Females failed to express any preference for a robofrog possessing a conspicuous white stripe when the alternative was a robofrog lacking a white stripe (two-tailed binomial test, $n = 30$, $P = 0.572$). Fifteen females chose the robofrog with a large stripe and 15 females chose the robofrog with no stripe (Fig. 4).

3 Discussion

Túngara frogs and squirrel treefrogs are only distantly related, but both species share similar courtship behaviors and communication challenges. Both species breed in temporary pools on warm nights with males vocalizing at ground/water-level from the edges of the pool. The density of breeding choruses is often high, with males competing for acoustic space and both species often experiencing high predation pressure. The dense chorus that is typical of both species results in substantial call overlap, producing a cocktail party problem that challenges female discrimination (Cherry,

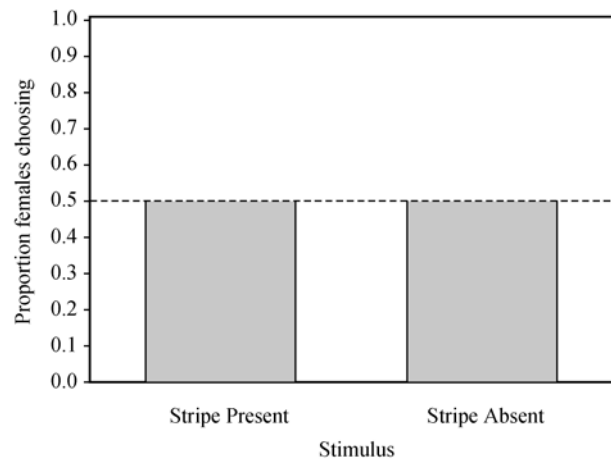


Fig. 4 Female túngara frog preference for vocal sac stripe

Female preference for a robofrog with a white vocal sac stripe versus a robofrog lacking a vocal sac stripe in a two-choice test ($n = 30$). Dotted line represents equal probability. Each robofrog had a vocal sac inflating synchronously with a call at the speaker, and each speaker broadcast the same call.

1953; Bee and Micheyl, 2008). Similar ecological problems often result in evolutionary convergence of traits, so we might expect similar patterns of behavior and signal function between these two species (Trillmich and Trillmich, 1984; Robert et al., 1992; Foster and Ratnieks, 2001; Bernal et al., 2006). The data from this study coupled with previous work (Rosenthal et al., 2004; Taylor et al., 2007; Taylor et al., 2008) show that females of both squirrel treefrogs and túngara frogs attend to multimodal signals. The visual signal component salient for female attraction and the process by which signal components interact to influence female responses are markedly different, however.

In squirrel treefrogs, females exhibit a significant preference for a model male possessing a relatively large lateral body stripe when call properties are held constant (Taylor et al., 2007). In túngara frogs, the throat of both males and females may possess a conspicuous white stripe, but we found the presence of this stripe did not affect female choice. Individuals vary greatly, ranging from no stripe to having a relatively large, conspicuous stripe (Fig 1). Given the stripe preference exhibited by squirrel treefrogs, and the variability of stripe size in túngara frogs, we expected that female túngara frogs would prefer the conspicuous white vocal sac stripe on courting males. Unexpectedly, they expressed no such preference. Although nocturnally-active frogs retain high visual sensitivity in low light (Cummings et al., 2008), the sparse photon “rain” available on dark nights likely presents some level of visual discrimination challenge by reducing image

resolution (Land and Nilsson, 2001). Conspicuous and variable color patterns are often favored by sexual selection, especially when they improve signal detection. Contrasting stripe patterns could potentially improve visual detection of calling male frogs in low-light and thus we might expect them to be favored by female receivers. Data from the present study and Taylor et al. (2007) suggest that this is true in squirrel treefrogs, but not in túngara frogs (Table 1).

Table 1 Female responses to variation in signal components demonstrating inter-signal interaction

	Squirrel treefrog	Túngara frog
Preference for contrasting stripe	Yes*	No
Visual cue enhances less attractive call	Yes	No
Temporal synchrony required for attraction	?	Yes†

Temporal synchrony refers to the inflation of the vocal sac occurring simultaneously with the vocalization during experimental playbacks. * indicates data from (Taylor et al. 2007), † indicates data from (Taylor et al., in press).

How can we explain this difference in response to pattern variation between the two frogs? In squirrel treefrogs, when a visual cue of a calling male (model frog) is coupled with a relatively slow call rate (less attractive), the probability of female attraction to the slow call rate is increased. The vocalization alone is sufficient for mate attraction (Taylor et al., 2007), but the modulation of female choice by the added visual cue suggests that females place some level of differential weighting on the two signal components or that the multimodal stimulus generates a novel perceptual stimulus. Richardson et al. (2010) demonstrated a similar phenomenon, using video playback, where the vocal sac enhanced less attractive calls in the European treefrog *Hyla arborea*. The same interaction does not hold for túngara frogs, however. Compared with the complex call, a simple call remains less attractive even when coupled with the visual stimulus of a calling male. Slow/fast call rates and simple/complex calls represent different acoustic discrimination tasks, but two lines of evidence suggest that comparing the influence of visual cues with these calls is appropriate. First, in both cases the preference strength for the more attractive acoustic signal is the same, approximately 85% (Ryan, 1985; Taylor et al., 2007). Second, call rates above 120 min⁻¹ (Taylor et al., 2007) or increased numbers of chucks appended to a call (Bernal et al., 2009) do not make the calls even more attractive. These acoustic stimulus pairs

represent upper and lower bounds of the female preference function for their respective species and are within the range of what females experience in the field. Our data suggest that the different modulation effect between the species may result from differences in signal function. Alternatively, differences in visual and acoustic perceptual abilities (e.g., receiver psychology, see Rowe, 1999; Rundus et al., 2007) could affect the evolution of complex signaling in anurans. For example, female túngara frogs may not have the same visual sensitivity, resolution, or chroma discrimination as squirrel treefrogs, and this could constrain the evolution of male stripe pattern through female choice (sensu Cummings, 2007).

The redundant/non-redundant information framework (Johnstone, 1996; Partan and Marler, 1999; Partan and Marler, 2005) posits that if receivers exhibit equivalent responses to isolated unimodal signal components, then each component transmits redundant information. If receivers exhibit different responses, then each conveys different information (but see Partan and Marler, 2005, for discussion of non-informative signals in this framework). Evolution may act to shape a multimodal signal in more complex ways, however, and the categorization of signals into redundant versus non-redundant messages may not be sufficient to explain the evolution of multimodal signaling in all cases.

As mentioned in the introduction, the concept of information is often an imprecise one that may not always add clarity to the challenge of understanding multimodal signals. For example, in most frog species, the vocalization is correlated with species identity, male location in the chorus, male size, and potentially male quality (Welch et al., 1998; Gerhardt and Huber, 2002). Are these correlations enough to demonstrate that calls transmit information about each of these states? We can calculate such correlations, and thus derive measures of the signal's predictability, reliability, honesty, or information content (all of these terms are often used synonymously), but does that necessarily tell us anything about the receiver and by extension about the selection pressures that have enforced these signal-phenotype relationships?

Signal detection by receivers is critical for communication and individual signal detection within acoustically complex environments is a difficult task for many animals (Bee and Micheyl, 2008) and especially problematic for túngara frogs (Farris et al., 2002; Farris et al., 2005). In the frog's multimodal display, the vocalization is dominant, but the vocal sac seems to enhance the

ability of a female to correctly assign the acoustic signal to individual callers (Taylor et al., 2011). Although we do not have data on female responses of squirrel treefrogs to a non-calling model frog, Taylor et al. (2011) showed that female túngara frogs who respond readily to an acoustic playback failed to respond to a robofrog with inflating vocal sac but no call. This differential response would be indicative of multiple messages in the redundant/non-redundant framework. Alternatively, these non-equivocal responses to acoustic versus visual components might occur simply because the vocal sac improves acoustic signal detection against the background noise rather than each component transmitting distinctly different messages. A reliance on the untested assumption that responses are based on specific information content may lead the investigator to overlook alternatives such as selection for signal detection. A more valuable paradigm would be to test receiver responses to various signal combinations, elucidating signal function or perception. We suggest that the interesting, but inherently challenging analysis of signal meaning be relegated to subsequent analyses.

One of the most intriguing aspects of our data is that both species exhibit strong inter-signal interaction (Hebets and Papaj, 2005). In inter-signal interactions of complex displays, signal components do not act independently but interact to affect receiver responses. Our data show that for frogs, the visual signal component alters female responses to the acoustic component. In túngara frogs (and probably squirrel treefrogs) the vocalization is dominant and is both necessary and sufficient for mate attraction (Taylor et al., 2011). The visual components interact with the dominant acoustic signal and modulate female responses in both species, but in different ways. In female squirrel treefrogs, the visual stimulus of male stripes is attractive and visual cues enhance a less attractive call; this is not true for túngara frogs. For túngara frogs, the presence of a stripe on the vocal sac (inflating vocal sac being the salient visual cue, see Taylor et al., 2008) does not enhance call attractiveness, but temporal synchrony of the visual/acoustic components is critical. A perceived temporal asynchrony between the components renders the multimodal signal significantly less attractive than the call alone, effectively reversing female preference (Taylor et al., in press). In sum, similar morphological patterns or behaviors (e.g. stripes and acoustic communication) are not always indicative of equivalent signal function or perception by receivers.

Hebets and Papaj (2005) outlined several hypotheses

to explain the evolution of multimodal signals and two of these seem to be important in anuran communication. The “Context” hypothesis states that the presence of one signal component provides a context in which the other signal component can be recognized. Data from Taylor et al. (2011) show that female túngara frogs do not respond to the isolated vocal sac as a sexual signal, supporting the “Context” hypothesis of inter-signal interaction. The “Attention-altering” hypothesis states that one component influences the information filtering mechanism of the receiver, focusing attention on another signal component. For example, visual cues can increase comprehension and alter perception of human speech (Sumbly and Pollack, 1954; McGurk and MacDonald, 1976). In acoustically complex communication systems, such as anuran communication in which auditory stream analysis presents a particular problem (Farris et al., 2002; Bee and Micheyl, 2008), auditory filtering is important for discrimination of individual signalers and thus should result in strong selection favoring attention-altering signal components (Gerhardt et al., 2001). The most parsimonious explanation for the incorporation of the visual component into frog courtship signals is that it evolved as an attention-altering component (sensu Hebets, 2005; Hebets and Papaj, 2005) within the context of the vocalization. That is, male signaling efficacy is improved by increased discrimination/filtering of individual callers in an acoustically complex environment. The data presented in this study (Figs. 2B, 3) indicate, however, that the degree to which individual signal components are weighted, differs dramatically among species. This suggests that signal function or perception within the Order Anura differ (Table 1), rendering a generalized classification based on information content problematic.

The anuran vocal sac probably evolved in response to selection for increasing calling efficiency (Bucher et al., 1982; Pauly et al., 2006) and was first incorporated into the multimodal signal as a cue. As a visual cue it probably carried little additional information content early in signal evolution. Additional information may have become incorporated in the visual component of anuran multimodal signals in some species (e.g., color patterns or shape variation correlated with body condition or developmental history), thereby evolving as a signal under content-based selection pressures. For example, the carotenoid-based stripes in squirrel treefrogs (Fig. 1) could indicate some aspect of male condition. Data are currently lacking to document if additional information content (beyond that contained in the vocalization) is

communicated through visual signal channels, however, and at least one study failed to find a correlation between male condition and luminance of male throats (Sztatecsny et al., 2010).

Animals that communicate acoustically often exhibit similar responses to variations in signal properties (Gerhardt and Huber, 2002). Receiver responses often become more variable with increasing signal complexity, however, making it difficult to generalize the evolution of multimodal signals. We argue for an approach that tests hypotheses based on receiver responses to elements of complex signals (unimodal + multimodal) without underlying assumptions of signal meaning (e.g. efficacy-based approaches and inter-signal interaction in Hebets and Papaj, 2005). Quantifying responses to multimodal signal components across species is likely to reveal commonalities as well as important differences (Framenau and Hebets, 2007; Hebets 2008), and these data can then provide the foundation for analyzing signal information content.

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References

- Amézquita A, Hödl W, 2004. How, when, and where to perform visual displays: The case of the Amazonian frog *Hyla parviceps*. *Herpetologica* 60: 420–429.
- Bee MA, Micheyl C, 2008. The cocktail party problem: What is it? How can it be solved? And why should animal behaviorists study it? *J. Comp. Psychol.* 122: 235–251.
- Bernal X, Rand AS, Ryan MJ, 2006. Acoustic preferences and localization performance of blood-sucking flies *Corethrella coquillett* to túngara frog calls. *Behav. Ecol.* 17: 709–715.
- Bernal XE, Akre KL, Baugh AT, Rand AS, Ryan MJ, 2009. Female and male behavioral response to advertisement calls of graded complexity in túngara frogs *Physalaemus pustulosus*. *Behav. Ecol. Sociobiol.* 63: 1269–1279.
- Buchanan BW, 1994. Sexual dimorphism in *Hyla squirella*: Chromatic and pattern variation between the sexes. *Copeia* 1994: 797–802.
- Bucher TL, Ryan MJ, Bartholomew GW, 1982. Oxygen consumption during resting, calling and nest building in the frog *Physalaemus pustulosus*. *Physiol. Zool.* 55: 10–22.
- Cherry EC, 1953. Some experiments on the recognition of speech, with one and two ears. *J. Acoust. Soc. Am.* 25: 975–979.
- Cummings ME, 2007. Sensory trade-offs predict signal divergence in surferperch. *Evol.* 61: 530–545.
- Cummings ME, Bernal XE, Reynaga R, Rand AS, Ryan MJ, 2008. Visual sensitivity to a conspicuous male cue varies by reproductive state in *Physalaemus pustulosus* females. *J. Exp. Biol.* 21: 1203–1210.
- Dall SRX, Giraldeau LA, Olsson O, McNamara JM, Stephens DW, 2005. Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.* 20: 187–193
- de Luna AG, Hödl W, Amézquita A, 2010. Colour, size and movement as visual subcomponents in multimodal communication by the frog *Allobates femoralis*. *Anim. Behav.* 79: 739–745.
- Farris HE, Rand AS, Ryan MJ, 2002. The effects of spatially separated call components on phonotaxis in túngara frogs: Evidence for auditory grouping. *Brain Behav. Evol.* 60: 181–188.
- Farris HE, Rand AS, Ryan MJ, 2005. The effect of time, space and spectrum on auditory grouping in túngara frogs. *J. Comp. Physiol.* 191: 1173–1183.
- Foster KR, Ratnieks FLW, 2001. Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proc. R. Soc. Lon. B* 268: 169–174.
- Framenau VW, Hebets EA, 2007. A review of leg ornamentation in male wolf spiders, with the description of a new species from Australia, *Artoria schizocoides* (Araneae, Lycosidae). *J. Arachnol.* 35: 89–101.
- Gerhardt HC, Huber F, 2002. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. University of Chicago Press: Chicago.
- Gerhardt HC, Huber F, Schwartz JJ, 2001. Auditory tuning and frequency preferences in anurans. In: Ryan MJ ed. *Anuran Communication*. Washington DC: Smithsonian Institution Press.
- Gerhardt HC, Klump GM, 1988. Masking of acoustic signals by the chorus background noise in the green treefrog: A limitation on mate choice. *Anim. Behav.* 36: 1247–1249.
- Gridi-Papp M, Rand AS, Ryan MJ, 2006. Complex call production in the túngara frog. *Nature* 441: 38.
- Hebets EA, 2008. Seismic signal dominance in the multimodal courtship display of the wolf spider *Schizocosa stridulans* Stratton 1991. *Behav. Ecol.* 19: 1250–1257.
- Hebets EA, 2005. Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behav. Ecol.* 16: 75–82.

- Hebets EA, Papaj DR, 2005. Complex signal function: Developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57: 197–214.
- Hebets EA, Uetz GW, 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). *Behav. Ecol. Sociobiol.* 47: 280–286.
- Hirschman W, Hödl W, 2006. Visual signaling in *Phrynobatrachus krefftii* Boulenger, 1909 (Anura: Ranidae). *Herpetologica* 62: 18–27.
- Hödl W, Amézquita A, 2001. Visual signaling in anuran amphibians. In: Ryan MJ ed. *Anuran Communication*. Washington, DC: Smithsonian Institution Press, 121–141
- Johnstone RA, 1996. Multiple displays in animal communication: “back-up signals” and “multiple messages.” *Phil. Trans. R. Soc. Lon. B* 351: 329–338.
- Land MF, Nilsson DE, 2001. *Animal Eyes*. Oxford: Oxford University Press.
- Lindquist ED, Hetherington TE, 1996. Field studies on visual and acoustic signaling in the ‘earless’ Panamanian golden frog *Atelopus zeteki*. *J. Herpetol.* 30: 347–354.
- McGurk H, MacDonald J, 1976. Hearing lips and seeing voices. *Nature* 264: 746–748.
- Morris MR, Elias JA, Moretz JA, 2001. Defining vertical bars in relation to female preference in the swordtail fish *Xiphoropus cotezi* (Cyprinodontiformes, Poeciliidae). *Ethology* 107: 827–837.
- Narins PM, Hödl W, Grabul DS, 2003. Bimodal signal requisite for agonistic behavior in a dart-poison frog. *Proc. Nat. Acad. Sci. USA* 100: 577–580.
- Partan SR, Marler P, 1999. Communication goes multimodal. *Science* 283: 1272–1273.
- Partan SR, Marler P, 2005. Issues in the classification of multimodal communication signals. *Am. Nat.* 166: 231–245.
- Pauly G, Bernal XE, Rand AS, Ryan MJ, 2006. The vocal sac increases call rate in the túngara frog *Physalemus pustulosus*. *Physiol. Biochem. Zool.* 79: 708–719.
- Rendall D, Owren MJ, Ryan MJ, 2009. What do animal signals mean? *Anim. Behav.* 78: 233–240.
- Richardson C, Gomez D, Durieux R, Théry M, Joly P et al., 2010. Hearing is not necessarily believing in nocturnal anurans. *Biol. Lett. On-line*.
- Robert D, Amoroso J, Hoy RR, 1992. The evolutionary convergence of hearing in a parasitoid fly and its cricket host. *Science* 258: 1135–1137.
- Rosenthal GG, Rand AS, Ryan MJ, 2004. The vocal sac as a visual cue in anuran communication: An experimental analysis using video playback. *Anim. Behav.* 68: 55–58.
- Rowe C, 1999. Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* 58: 921–931.
- Rundus AS, Owings DH, Joshi SS, Chinn E, Giannini N, 2007. Ground squirrels use an infrared signal to deter rattlesnake predation. *Proc. Nat. Acad. Sci.* 104: 14372–14376.
- Ryan MJ, 2001. *Anuran Communication*. Washington, DC: Smithsonian Institution Press.
- Ryan MJ, 1985. *The Túngara Frog: A Study in Sexual Selection and Communication*. Chicago: University of Chicago Press.
- Schwartz JJ, Buchanan BW, Gerhardt HC, 2001. Female mate choice in the gray treefrog *Hyla versicolor* in three experimental environments. *Behav. Ecol. Sociobiol.* 49: 443–455.
- Schwartz JJ, 1993. Male calling behavior, female discrimination and acoustic interference in the neotropical treefrog *Hyla microcephala* under realistic acoustic conditions. *Behav. Ecol. Sociobiol.* 32: 401–414.
- Searcy WA, Nowicki S, 2005. *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton: Princeton University Press.
- Shannon CE, 1948. A mathematical theory of communication. *Bell Syst. Tech. J.* 27: 623–656.
- Sumby WH, Pollack I, 1954. Visual contribution to speech intelligibility in noise. *J. Acoustic. Soc. Am.* 26: 1298–1319.
- Summers K, Symula R, Clough M, Cronin T, 1999. Visual mate choice in poison frogs. *Proc. R. Soc. Lon. B* 266: 1–5.
- Sztatecsny M, Ströndl C, Baierl A, Ries C, Hödl W, 2010. Chin up: Are the bright throats of male common frogs a condition-independent visual cue? *Anim. Behav.* 79: 779–786.
- Taylor RC, Buchanan BW, Doherty JL, 2007. Sexual selection in the squirrel treefrog *Hyla squirella*: The role of multimodal cue assessment in female choice. *Anim. Behav.* 74: 1753–1763.
- Taylor RC, Klein BA, Stein J, Ryan MJ, 2008. Faux frogs: Multimodal signalling and the value of robotics in animal behaviour. *Anim. Behav.* 76: 1089–1097.
- Taylor RC, Klein BA, Stein J, Ryan MJ, 2011. Multimodal signal variation in space and time: How important is matching a signal with its signaler? *J. Exp. Biol.* 214: 815–820.
- Tinbergen N, 1951. *The Study of Instinct*. Oxford: Oxford University Press.
- Trillmich F, Trillmich K, 1984. The mating systems of pinnipeds and marine iguanas: Convergent evolution of polygyny. *Biol. J. Lin. Soc.* 21: 209–216.
- Vasquez T, Pfennig KS, 2007. Looking on the bright side: Females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad *Scaphiopus couchii*. *Behav. Ecol. Sociobiol.* 62: 127–135.
- Welch AM, Semlitsch RD, Gerhardt HC, 1998. Call duration as an indicator of genetic quality in male gray tree frogs. *Science* 280: 1920–1930.
- Wollerman L, 1999. Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. *Anim. Behav.* 57: 529–536.
- Zahavi A, Zahavi A, 1999. *The Handicap Principle: A Missing Piece of Darwin’s Puzzle*. Oxford: Oxford University Press.