

Alouatta pigra males ignore *A. palliata* loud calls: A case of failed rival recognition?

Dawn M. Kitchen^{1,2,*}, Liliana Cortés-Ortiz³, Pedro A. D. Dias⁴,

Domingo Canales-Espinosa⁴, Thore J. Bergman^{3,5}

¹Department of Anthropology, The Ohio State University, Columbus, OH 43210, USA

²The Ohio State University-Mansfield, Mansfield, OH 44906, USA

³Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA

⁴ Primate Behavioral Ecology Lab, Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz CP 91000, Mexico

⁵Department of Psychology, University of Michigan, Ann Arbor, MI 48109, USA

Text pages with Bibliography: 29 (20 without); Figures: 3; Tables: 3.

Abbreviated title: Failed rival recognition in howler monkeys

KEYWORDS: hybrid zone, loud call, phenotype matching, playback experiment, species recognition

Correspondence to: Dawn M. Kitchen, Department of Anthropology, The Ohio State University, 174 West 18th Avenue, Columbus, OH, 43210; Phone: 419-755-4027; FAX: 419-755-4367; E-mail: kitchen.79@osu.edu.

Grant Sponsor: National Science Foundation Grants: BCS-0962807 and BCS-0962755.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of record](#). Please cite this article as [doi:10.1002/ajpa.23443](https://doi.org/10.1002/ajpa.23443).

Abstract

Objectives: When closely related species overlap geographically, selection may favor species-specific mate recognition traits to avoid hybridization costs. Conversely, the need to recognize potential same-sex rivals may select for lower specificity, creating the possibility that selection in one domain constrains evolution in the other. Despite a wealth of data on mate recognition, studies addressing rival recognition between hybridizing species are limited to a few bird species. Using naïve populations, we examine the extent to which failed rival recognition might have affected hybridization patterns when two species of howler monkeys (*Alouatta pigra* and *A. palliata*) first met after diverging in allopatry.

Methods: We simulated first contact between naïve subjects using playback experiments in allopatric populations of the two purebred species. Using linear mixed models, we compared their look, move, and vocal responses to conspecific and heterospecific loud calls.

Results: Although not different in overall response strength to playbacks, the two species differed in reaction to heterospecific callers. Male *A. pigra* ignored calls from male *A. palliata*, but the reverse was not true.

Discussion: Despite striking differences in vocalizations, *A. palliata* respond equally to calls from both species whereas *A. pigra* respond only to conspecifics. This apparent

failure of *A. pigra* males to recognize interspecific rivals might have biased hybridization (F1 hybrids = male *A. palliata* x female *A. pigra*), a pattern previously hypothesized based on genetic analysis of hybrids. Given that *A. pigra* males could be losing reproductive opportunities to heterospecific males, our findings add to growing evidence of potential costs for overly-specific species recognition.

Author Manuscript

Hybridization may be costly for individuals if hybrid offspring have low fitness (reviewed in Burke & Arnold, 2001). Consequently, where closely-related species overlap, selection generally favors visual, vocal and/or olfactory based species recognition that is highly specific to avoid the costs of hybridization (e.g., Grether et al., 2009; Wilkins et al., 2013). Most research examining the effects of selection on these cues has focused on mate recognition, where greater specificity means avoiding heterospecific mating (e.g., Seddon, 2005). In contrast, the need to recognize potential same-sex rivals may select for *less* specificity. Indeed, a few studies suggest that overly specific rival recognition could contribute to hybridization if individuals fail to recognize heterospecifics as rivals and, consequently, make little attempt to exclude them from mating opportunities (e.g., *Dendroica* warblers: Pearson & Rohwer, 2000; *Puffinus* shearwaters: Curé et al., 2010; *Ficedula* flycatchers: Qvarnström et al., 2010). This suggests that the demands of rival recognition may be different from those of mate recognition, creating a possible conflict during the evolution of recognition cues used by males vs. females. However, what little we know about rival recognition between hybridizing species comes only from birds (e.g., Baker, 1991; Collins & Goldsmith, 1998; Secondi et al., 1999; Ceugniet & Aubin, 2001; McDonald et al., 2001; Patten et al., 2004; den Hartog et al., 2007; Dingle et al., 2010), limiting the taxonomic generality of any conclusions that can be drawn.

Research in rival recognition is biased towards birds presumably because they are highly vocal and therefore amenable to experimental manipulation in the field. However,

many mammalian species also use vocalizations both to attract mates and repel rivals (reviewed in: Delgado, 2006), making it possible to explore species recognition experimentally. We conducted playback experiments of rival recognition in howler monkeys (*Alouatta* spp.), a taxon named for the loud vocalizations they produce during male-male competitive interactions (reviewed in da Cunha et al., 2015).

We studied two closely-related howler monkey species, *A. palliata* (mantled) and *A. pigra* (black), that are known to hybridize in a small area of overlap in southern Mexico, likely as a result of secondary contact after divergence in allopatry (Cortés-Ortiz et al., 2007). The vocalizations of these species are highly divergent including the most salient loud call, the “roar,” produced during howling bouts (Bergman et al., 2016). Rival recognition may play a role in hybrid zone dynamics if resident males impede takeover, immigration, or sneaky copulation attempts by some males but not by others. The inability to recognize some males as a competitive threat could contribute to a bias in interspecific mating hypothesized for this hybrid system (Cortés-Ortiz et al., 2007), in which interspecific crosses of purebred *A. palliata* males and purebred *A. pigra* females seemed to be more likely than the opposite to produce hybrid offspring.

Although genetic and physical incompatibilities likely play a major role in the unidirectional formation of many hybrid systems, behavior also has the potential to influence hybridization patterns. For example, in hybridizing doves (*Streptopelia vinacea* x *S. capicola*) both types of crosses produce viable F1 offspring in the lab, but due to behavioral differences of the two species, only unidirectional hybridization and introgression occur in the wild (den Hartog et al., 2010). Similarly, although

hybridization can occur in both directions between different species/subspecies of baboons (hamadryas, *Papio hamadryas* x olive, *P. anubis* x yellow, *P. cynocephalus* x kinda, *P. kinda* x chacma, *P. ursinus*) and thus phenotypic differences do not act as absolute reproductive barriers, behavioral and life history differences can affect predominant mating patterns seen in different types of groups (e.g., Bergman and Beehner, 2004; Bergman et al., 2008; Jolly et al., 2011; Charpentier et al., 2012).

Failure to recognize rivals could have evolutionary consequences, as has been demonstrated in two sets of hybridizing birds. Using playback experiments, Curé and colleagues (2010) found that Balearic shearwater (*Puffinus mauretanicus*) did not respond as strongly to the sound of the smaller Yelkouan shearwater (*P. yelkouan*) males as to conspecific callers, whereas Yelkouans responded equally to both species. The failure of Balearic shearwater to identify a threat might explain the biased pattern of colony invasion by Yelkouan shearwater. Similarly, hermit (*Dendroica occidentalis*) and Townsend (*D. townsendi*) warblers differ from each other in their responses to heterospecifics (Pearson & Rohwer, 2000). Researchers argue that the strong and equal aggression toward both species by Townsend but not by hermit warblers affords the former a competitive advantage (Pearson, 2000; Pearson & Rohwer, 2000) and might explain how Townsend have been genetically swamping hermit warbler populations (Rohwer et al., 2001). The evolutionary impact of rival recognition on hybridizing systems has otherwise rarely been tested directly. Thus, our study represents an important test case among mammalian taxa. If *A. pigra* males fail to recognize smaller *A. palliata* males as potential threats while *A. palliata* males defend their females against both

species, this difference could have an effect on hybridization patterns in the howler monkey hybrid system.

To assess the potential role of rival recognition in the dynamics of early *A. pigra* x *A. palliata* hybridization, we used playback experiments on subjects of each parental species, far away from the hybrid zone. Tests in these allopatric populations allowed us to simulate first contact between naïve subjects of both purebred species. Based on the biased hybridization inferred for first generation hybrids (Cortés-Ortiz et al., 2015), we predicted that *A. pigra* would have a weak response to the heterospecific call and a strong response to the conspecific call, whereas *A. palliata* should recognize both species as rivals. To be clear, we did not conduct this study in the hybrid zone, but a similar study with hybrid zone animals is currently underway.

METHODS

Study sites and subjects

Subjects were the adult males in six groups of *A. palliata* at three locations within one population in southern Veracruz and in six groups of *A. pigra* at three locations within one population near Escárcega, Campeche (Fig 1). Playback experiments were conducted from January-March, 2012 and March-April, 2013 for *A. palliata* and *A. pigra*, respectively (Table 1). All sites consisted of relatively small, fragmented forests surrounded by cattle ranches, cocoa and banana plantations, villages, or roads. *A. pigra* groups contained an average of 7.3 individuals (range 5–9) including 1–3 adult males (mean = 1.7), whereas *A. palliata* groups had an average of 16.5 individuals (range 8–30)

including 2–12 adult males (mean = 4.7). All six *A. palliata* and four of six *A. pigra* groups had small infants present in the group; however, the presence or absence of small infants did not change between trials on the same group. Groups were part of ongoing census and behavioral studies (e.g., Ho et al., 2014) and males were thus identifiable based on distinctive markings (e.g., coloration of testicles; light colored sections of fur on tail or feet; scars) and photographs taken during prior capture (Cortés-Ortiz et al., 2015).

[Figure 1 approx here]

[Table 1 approx here]

Playback stimuli

From 2008–2011 we recorded natural loud calls opportunistically from groups in both purebred populations (Fig. 2). We used Sennheiser ME66 directional microphones (Wennebostel, Germany) and Marantz PMD660 compact flash recorders (Tokyo, Japan).

From recordings, we chose high-quality calls (i.e., relatively free of background noise with high signal-to-noise ratio) for playback stimuli. Using Cool Edit (Syntrillium Software, AZ, USA), we created sequences from each population where the number, type, and order of calls and pauses were nearly identical to each other and matched patterns seen in natural howling bouts. All sequences started with grunts, followed by an inhaled introductory syllable, an approximately 2 s roar, 4 barks (with short pauses of <0.5 s in between), a 7.5 s pause, an approximately 2 s roar, 5 more barks, a 9 s pause, 2.5 s of barking (5 single and 2 double barks), and so on (see examples in supplemental materials). Although we held inter-call intervals constant, the duration of roars varied

within and between individuals and species; thus, the overall sequence durations varied slightly (mean duration \pm SE, *A. pigra*: 139.6 ± 1.0 s; *A. palliata*: 157.0 ± 8.7 s). Each sequence was made up of multiple different calls from the same caller from one of the two different purebred populations (*A. pigra* or *A. palliata*).

[Figure 2 approx here]

Experimental protocol

We followed suggested procedures for making playbacks realistic and avoiding habituation (Fischer et al., 2013). We played calls from a 4th generation iPod Touch (Apple, CA, USA) through a Kenwood KAC-5204 350-watt car amplifier (CA, USA), powered by a 12-volt rechargeable battery (Power-Sonic PS-1270, CA, USA) and broadcast out a Bose 151 speaker (MA, USA). We calibrated call amplitude with a Realistic Sound Pressure Level Meter (RadioShack, TX, USA). The maximum intensity (mean = 84 dB at 5 m for all sequences) was within the normal range of this genus (e.g., *A. pigra* mean natural amplitude = 88 dB at 5 m: Kitchen, 2004). Additionally, all sequences sounded realistic to experienced human observers.

The speaker was mounted on a collapsible fiberglass surveyor's pole (CST/berger, IL, USA), raised approximately 8 m, and obscured behind natural vegetation. The speaker was an average of 61.1 m (SE: ± 1.4) from the subjects (range: 50.0–75.0 m), a distance that naturally prompts intergroup encounters (e.g., Whitehead, 1987). However, we could not ensure that speaker-subject distance was the same between

trials on the same group due to habitat constraints, so we included this as a potentially confounding effect in our statistical model.

Each of the 12 groups (six groups from each species) was presented with one conspecific and one heterospecific sequence. Trials were evenly distributed so that an equal number of groups of each species heard each of the two types of sequences (conspecific or heterospecific) first (Table 1). Subjects were unfamiliar with all callers they heard in playbacks (i.e., groups were never presented with recordings made of callers that lived in their group or in a neighboring group). To reduce habituation, trials were conducted at a low rate (once every 6.2 days on average; range = 3–16 days) and human observers frequently followed groups and setup broadcasting equipment on days when no playback occurred.

Trials were conducted between 06:30–17:00 hours and approximate time of day did not vary between trials on the same group. We observed the group for at least one hour before and one hour following each trial. No playbacks were conducted if audible howling occurred or if another group was encountered in the previous hour. We waited to conduct trials until subjects were alone (>2 m from any other animal), feeding or resting but not sleeping or traveling, and not engaged in social interaction. Behavior did not vary between trials on the same group. Observers were in contact via two-way radios so that trials could be aborted if necessary.

One observer was stationed with each male with the exception of the very largest *A. palliata* groups, in which case observers were spread out and assigned several males at once. Because we were interested in comparing maximum responses per group, we used

data from the male with the strongest response (based on a composite index, see Statistics below) rather than focus on a randomly chosen focal male. Likewise, although most or all of the males in small *A. palliata* and *A. pigra* groups frequently join in a response, only a few males typically respond in the larger *A. palliata* groups (pers. obs.) so we did not use average responses. However, we did include group size as a potential confound in our statistical model. Group size was used because adult females and subadults/large juvenile males sometimes join inter-group vocal interactions (e.g., Briseño-Jaramillo et al., 2017). However, results remained the same if number of adult males and/or females was substituted for group size in the model and if sex ratio was added to the model (see Statistics below).

Using recording equipment (which allowed us to collect time stamped data without having to look away to take notes), we measured the latency to and duration of all look (i.e., orientation toward or scanning the area around the speaker), move (>1m), and vocal behaviors produced following a playback trial. Latency to respond was measured from onset of trial with a value of 3600 s used if no response occurred within 1 hour (following Kitchen, 2004). For vocal responses, we noted both the latency to onset of the first vocalization, typically a low amplitude grunt (83.3% of 12 vocal responses in 24 trials), as well as the latency to the first loud call (a roar, a bark, or an “oodle”: da Cunha et al., 2015). We also noted the duration of grunting that preceded loud calls, the duration of total loud calling (including short pauses of less than 1 min, following Van Belle et al., 2013), and the number of roars produced. For move responses, we measured the total distance moved during a trial and we calculated “approach” as the percent of

speaker-subject distance covered during a trial in order to differentiate movement away from and movement toward the speaker. Animals that approached never interacted or even looked directly at the speakers. The maximum value assigned to any approach was 100%. In one trial, the focal group approached and then continued to move past the speaker; however, results remained the same if we used a value of 127%, instead of 100%, in this case. Finally, we measured the latency to return to baseline behavior as the time from first orientation toward the speaker until the subject stopped scanning the area and the group returned to prior behavior of resting or feeding.

Statistics

Because response variables were correlated (Spearman correlation: between all responses except grunt duration: $P < 0.022$; including grunt duration: $P < 0.068$), we combined all nine response variables (i.e., excluding look latency) using a Principal Components Analysis (PCA; McGregor, 1992) in SPSS 24.0 (IBM SPSS Statistics, NY, USA). We focused analyses on the component that explained the most variance (hereafter, PC1) following McGregor (1992). We used linear mixed models (LMM) with group identity and caller identity as random factors, focal species identity (*A. pigra* or *A. palliata*) and focal species-caller type (heterospecific or conspecific) interaction as fixed factors, and the potentially confounding effects of speaker-subject distance and group size as covariates. We ran a likelihood ratio test (LRT) to ensure that the addition of fixed effects was an improvement over a model based on just intercept and random effects only (Dunteman & Ho, 2006). All analyses were two-tailed and alpha was set at 0.05.

RESULTS

Because subjects looked immediately at the speaker at the onset of the trial in all but one case, we did not consider latency to look in analyses. In all cases where a trial elicited a move and/or vocal response, subjects began responding before completion of the playback stimuli (Table 2).

A PCA of the nine remaining response variables (i.e., look duration, move latency, move distance, percent of speaker-subject distance covered during approach, grunt latency, grunt duration, loud call latency, loud call duration, total number of roars) resulted in three principal component scores (hereafter, PC1, PC2 and PC3) with eigenvalues greater than 1.0 (Table 3). All variables had strong loadings on PC1 except grunt duration; thus, large PC1 values indicated a strong response (i.e., short latencies, long durations, etc.). Large PC2 values indicated strong grunt responses and PC3 was not strongly correlated with any responses. Because PC1 explained >50% of the variance (Table 3), PC2 and PC3 were not considered further (see Table 2 for mean values based on focal species and caller type).

[Table 2 approx here]

[Table 3 approx here]

An LMM with fixed effects of focal species, **focal species by caller type, subject by speaker distance**, and group size (full model: $N = 24$ trials, $F_{1,16.6} = 6.8$, $p = 0.019$) was better at explaining results than a model based on only intercept and random effects (LRT: 15.6, $p < 0.010$). Within this model, there was no effect of focal species on PC1

($F_{1,9.7} = 1.5$, $p = 0.252$); in other words, *A. pigra* males were not more likely to produce a stronger response than *A. palliata* or vice versa. However, there was an interaction effect of focal species by caller type ($F_{2,11.1} = 5.0$, $p = 0.028$). Although both *A. pigra* and *A. palliata* callers elicited a strong response in *A. palliata* subjects, only *A. pigra* callers elicited a strong response in *A. pigra* subjects (Fig. 3; Table 2). In fact, following onset of a heterospecific *A. palliata* call, *A. pigra* subjects never grunted or loud called, and moved on only one occasion, for a short distance (1.5 m toward the speaker and then 1.5 m lower in the tree).

There was no relationship between PC1 and group size ($F_{1,8.9} = 2.0$, $p = 0.193$), but there was with speaker-subject distance ($F_{1,16.8} = 9.3$, $p = 0.007$). Subjects had a stronger response (i.e., traveling farther, starting to respond sooner) when the speaker was farther away but still within the range of threat from intergroup encounters.

[Figure 3 approx here]

DISCUSSION

We simulated vocal contact between naïve *A. palliata* and *A. pigra* using playback experiments on allopatric populations. We found no strong evidence that overall intensity of responses was different between the subject species. However, we found evidence that the phenotypic match between caller and subject predicted responses in *A. pigra*.

Heterospecific callers not only elicited weaker responses by purebred *A. pigra* subjects than conspecific callers did, *A. pigra* virtually ignored *A. palliata*. In stark contrast, calls from heterospecific and conspecific callers elicited equally strong responses among

purebred *A. palliata* subjects (see also observational studies on another pair of syntopic howler monkeys, *A. caraya* and *A. guariba* in Argentina: Holzmann et al., 2012).

Assuming that vocal and approach responses thwart takeovers or extra pair copulations (Van Belle et al., 2014), the strong response of *A. palliata* males to the calls of *A. pigra* males might help restrict access by *A. pigra* males to *A. palliata* females in a contact zone. On the other hand, the weak response of *A. pigra* males to *A. palliata* calls suggests that they are not identifying these males as potential rivals to be confronted/defended against. Ignoring the calls of *A. palliata* males might therefore contribute to pairings of *A. palliata* males with *A. pigra* females, which is the hypothesized directional bias of F1 hybrid offspring in sympatry (Cortés-Ortiz et al., 2007; 2015). The lack of response by *A. pigra* males to *A. palliata* males could have influenced hybridization patterns when these two species first encountered each other in southern Mexico, and might continue affecting the dynamics of hybridization in the current hybrid zone.

Naïve *A. pigra* males clearly heard the stimuli in all our trials (they immediately looked to the speaker in all but one trial), yet they had very weak responses to only the *A. palliata* calls. It remains unclear which acoustic features *A. pigra* subjects use to differentiate calls and why *A. palliata* subjects do not respond to these differences. There are a number of striking differences in the loud roar vocalizations of these closely-related species (Bergman et al., 2016): *A. palliata* have multiple, short syllables produced with no amplitude modulation, whereas *A. pigra* have two syllable calls that reach a crescendo in amplitude during the longer syllable. *A. pigra* males also have a larger hyoid apparatus (the hyoid bone and associated bulla create a resonant chamber used in producing loud

vocalizations: Schön, 1971) than *A. palliata* (Dunn et al., 2015; Youlatos et al., 2015). Because the hyoid apparatus contributes to features of vocalizations that highlight the larger body size of *A. pigra* (Dunn et al., 2015; Bergman et al., 2016), the hyoid size difference between the species could make the calls of *A. pigra* sound more intimidating. For example, *A. pigra* have noisier/less tonal roars with lower fundamental frequencies, lower formant dispersion, and wider emphasized frequency ranges than *A. palliata* (Bergman et al., 2016). Given these differences, we hypothesize that *A. pigra* roars sound like exaggerated *A. palliata* roars. If so, the weak *A. pigra* response to *A. palliata* could be proximately explained if *A. palliata* calls mimic a less motivated, smaller, or inferior *A. pigra* rival such as a subadult male. For example, in a review of vocal development in nonhuman primates, Ey and colleagues (2007; see also guenons: Bouchet et al., 2012) highlight age and size related changes in vocalizations (e.g., differences in syllable duration, frequency components, formant dispersion) that also differ between these two howler species (Bergman et al., 2016). The best way to test whether these adult male *A. palliata* calls could be confused for subadult male *A. pigra* roars would be to compare features of their calls. However, such an analysis would be logistically difficult because subadult males rarely produce isolated loud calls and instead call with other group members as part of a chorus (e.g., Briseño-Jaramillo et al., 2017; pers. obs.).

Unidirectional pairings between these two howler monkey species might be further facilitated by other behavioral and morphological differences. For example, *A. palliata* males might be ignored visually as well as vocally; an *A. palliata* male (similar in size to female *A. pigra* and on average 31% lighter and 18% shorter than *A. pigra*

males: Kelaita et al., 2011) quietly approaching through thick vegetation might not appear to be a fully adult male and, consequently, might not be considered threatening by *A. pigra* males compared to the reverse. If ignored both vocally and visually, it would be possible that an *A. palliata* male might approach an *A. pigra* group because shadowing and joining an established group is a common dispersal mode observed in *A. palliata* (Glander, 1992). Once near a group, mating might be possible because *A. palliata* males frequently solicit sexual interactions with females (reviewed in Van Belle & Bicca-Marques, 2015), are known to sneak copulations (Jones & Cortés-Ortiz, 1998), and *A. pigra* females seem to leave their groups to engage in extra-group copulations (Horwich, 1983; Van Belle et al., 2008). Whether hybridization between these species is influenced by these additional factors should be the focus of future studies.

Such sneaker/satellite strategies (Gross, 1996) have been implicated in hybridizing systems of frogs (e.g., Gergus et al., 1999), fish (e.g., Garcia-Vazquez et al., 2002), and insects (Nomakuchi & Higashi, 1996). Candidate mammalian systems include an apparent hybridization bias favoring female red deer (*Cervus elaphus*) crosses with male sika deer (*C. nippon*). Male sika deer are small enough to be comparable to subadult male red deer and researchers suggest they may be sneaking into harems of red deer with no interspecific aggression by the larger harem-holder (reviewed in Wyman et al., 2011). Body size and behavior have also been implicated in the bias seen in female chacma baboon crosses with male kinda baboon (*P. ursinus* x *P. kinda*). Male kinda baboons are the size of juvenile chacma baboons and groom cycling females much more frequently than do adult male chacma baboons; the combination of body size and behavior might

explain the success of adult male kinda baboons fathering offspring in chacma groups (Jolly et al., 2011). However, although male-male competition is known to be mediated by loud call vocalizations in both deer (Clutton-Brock & Albon, 1979) and baboons (Kitchen et al., 2013), the role of vocal recognition has yet to be examined in these hybridizing systems.

Responses by female howler monkeys to loud calls are much weaker than responses by males, making it difficult to detect differences in male and female responses to playbacks of conspecific and heterospecific males. Thus, it remains unknown if *A. palliata* females share the lower specificity for species recognition observed in male *A. palliata*. However, the strong coupling between cue and response in male *A. pigra* is apparently not shared by female *A. pigra* given these females mate with *A. palliata* males. It is possible that howler females do not avoid heterospecific males and therefore do not rely solely or at all on vocal signals for mate selection, making a signal match irrelevant to them. For example, hybridizing female baboons do not seem to discriminate among hetero- and conspecific males (Phillips-Conroy et al., 1991; Alberts and Altmann, 2001; Charpeineter et al., 2012; see also intergeneric hybridization: Jolly, 1997) and may even prefer the novelty of heterospecific males (e.g., Colmenares and Gomendio, 1988).

Other hybrid zone research suggests that males and females attend to different visual and behavioral signals when assessing rivals and mates. For example, despite a documented fitness disadvantage and some genetic incompatibilities, unidirectional hybridization occurs between female collared flycatchers (*Ficedula albicollis*) and male pied flycatchers (*F. hypoleura*). Because one pied color morph resembles juvenile

collared, the former are ignored by the otherwise competitively dominant adult male collared flycatchers and allowed to set up territories (reviewed in Qvarnström et al., 2010). Although collared females do not prefer these pied males, they will mate with them under certain circumstances such as if the males sing a mixed species song (Qvarnström et al., 2010). Similar complex situations – with multiple signals under both intra- and intersexual selection regimes – may be at work in the *A. pigra* x *A. palliata* hybrid zone.

More research using playback trials on naïve populations of mammals such as we describe here would advance our understanding of the forces that shape traits used in species recognition. However, although our study uses allopatric populations to simulate the original conditions of first contact between two closely related species, it does not examine how selection for or against hybridization might affect call specificity in sympatry. Although our lack of long-term behavioral data will not allow direct testing for current mating bias between interspecific individuals in the hybrid zone, playback tests are currently underway on purebred and hybrid subjects in the hybrid zone to uncover how vocal signals affect responses.

In summary, our study is the first to use experimental simulation to show that different responses to heterospecific vocalizations might contribute to unidirectional hybridization between two primate species. This suggests that vocalizations might have an underappreciated role in shaping cross-species encounters in primates. Furthermore, given *A. pigra* males may be losing reproductive opportunities to *A. palliata* males, our findings add to growing evidence of potential costs for overly-specific species

recognition. These costs suggest that rival-recognition and mate recognition might favor differing recognition criteria in hybridizing systems.

ACKNOWLEDGMENTS

Our work was greatly assisted by Dara Adams, Rosario Candelero Rueda, Alejandro Coyohua, Pamela Cruz Miros, Jason Ferrell, Lucy Ho, Alfredo Gómez Martínez, Ana Peralta, Ariadna Rangel-Negrín and Cynthia Rodríguez Maldonado. We thank the editor and two anonymous reviewers for their valuable comments on this manuscript. We are grateful to the government of Mexico, La Flor de Catemaco, Rancho Jalapilla, and the many other landowners who allowed us to conduct this research. This study was conducted in compliance with laws in Mexico and the US, and was approved by The Ohio State University's Institutional Animal Care and Use Committee (IACUC protocol #2010A0012-R1) and the University of Michigan's University Committee on the Use and Care of Animals (UCUCA protocol 09319). This work was funded by a collaborative grant from the National Science Foundation (grant numbers BCS-0962807, BCS-0962755). This work was also supported by in-kind and financial assistance from The Ohio State University Columbus and Mansfield campuses to DMK, the University of Michigan to TJB and LCO, and Universidad Veracruzana to PADD and DCE.

REFERENCES

- Alberts, S. C., & Altmann, J. (2001). Immigration and hybridization patterns of yellow and anubis baboons in and around Amboseli, Kenya. *American Journal of Primatology*, 53, 139–154.
- Baker, M. C. (1991). Response of male indigo and lazuli buntings and their hybrids to song playback in allopatric and sympatric populations. *Behavior*, 119, 225–242.
- Bergman, T. J., & Beehner, J. C. (2004). Social system of a hybrid baboon group (*Papio anubis* × *P. hamadryas*). *International Journal of Primatology*, 25, 1313–1330.
- Bergman, T. J., Cortés-Ortiz, L., Dias, P. A. D., Ho, L., Adams, D., Canales-Espinosa, & D., Kitchen, D. M. (2016). Striking differences in the loud calls of howler monkey sister species (*A. palliata* and *A. pigra*). *American Journal of Primatology*, 78, 755–766.
- Bergman, T. J., Phillips-Conroy, J. E., & Jolly, C. J. (2008). Behavioral variation and reproductive success of male baboons (*Papio anubis* × *P. hamadryas*) in a hybrid social group. *American Journal of Primatology*, 70, 136–147.
- Bouchet, H., Blois-Heulin, C., & Lemasson, A. (2012). Age- and sex-specific patterns of vocal behavior in De Brazza's monkeys (*Cercopithecus neglectus*). *American Journal of Primatology*, 74, 12–28.
- Briseño-Jaramillo, M., Biquand, V., Estrada, A., & Lemasson, A. (2017). Vocal repertoire of free-ranging black howler monkeys' (*Alouatta pigra*): Call types, contexts, and sex-related contributions. *American Journal of Primatology*, 79, e22630.

- Burke, J. M., & Arnold, M. L. (2001). Genetics and the fitness of hybrids. *Annual Review of Genetics*, 35, 31–52.
- Ceugniet, M., & Aubin, T. (2001). The rally call recognition in males of two hybridizing partridge species, red-legged (*Alectoris rufa*) and rock (*A. graeca*) partridges. *Behavioural Processes*, 55, 1–12.
- Charpentier, M. J., Fontaine, M. C., Cherel, E., Renoult, J. P., Jenkins, T., Benoit, L., Barthes, N., Alberts, S.C., & Tung, J. (2012). Genetic structure in a dynamic baboon hybrid zone corroborates behavioural observations in a hybrid population. *Molecular Ecology*, 21, 715–731.
- Clutton-Brock, T. H., & Albon, S. D. (1979). The roaring of red deer and the evolution of honest advertisement. *Behaviour*, 69, 145–170.
- Collins, S.A., & Goldsmith, A. R. (1998). Individual and species differences in quail calls (*Coturnix c. japonica*, *c. c. coturnix* and a hybrid). *Ethology*, 104, 977–990.
- Colmenares, F., & Gomendio, M. (1988). Changes in female reproductive condition following male take-overs in a colony of hamadryas and hybrid baboons. *Folia Primatologica*, 50, 157–174.
- Cortés-Ortiz, L., Agostini, I., Aguiar, L. M., Kelaita, M., Silva, F. E., & Bicca-Marques, J. C. (2015). Hybridization in howler monkeys: Current understanding and future directions. In: M. M. Kowalewski, P. A. Garber, L. Cortés-Ortiz, B. Urbani, & D. Youlatos (Eds.), *Howler monkeys: Adaptive radiation, systematics, and morphology* (pp. 107–132). New York, NY: Springer Press.

Cortés-Ortiz, L., Duda, T. J. Jr., Canales- Espinosa, D., García-Orduna, F., Rodríguez-Luna, E., & Bermingham, E. (2007). Hybridization in large-bodied New World primates. *Genetics*, 176, 2421–2425.

Curé, C., Mathevon, N., & Aubin, T. (2010). Intra-sex vocal interactions in two hybridizing seabird species: the Yelkouan and the Balearic shearwaters (*Puffinus yelkouan* and *P. mauretanicus*). *Behavioral Ecology Sociobiology*, 64, 1823–1837.

da Cunha, R. G.T., de Oliveira, D. A. G., Holzmann, I., & Kitchen, D. M. (2015). Production of loud and quiet calls in howler monkeys In: M. M. Kowalewski, P. A. Garber, L. Cortés-Ortiz, B. Urbani, & D. Youlatos (Eds.), *Howler monkeys: Adaptive radiation, systematics, and morphology* (pp. 337–368). New York, NY: Springer Press.

Delgado, R. A. (2006). Sexual selection in the loud calls of male primates: signal content and function. *International Journal of Primatology*, 27, 5–25.

den Hartog, P. M., de Kort, S. R., & ten Cate, C. (2007). Hybrid vocalizations are effective within, but not outside, an avian hybrid zone. *Behavioral Ecology*, 18, 608–614.

den Hartog, P. M., den Boer-Visser, A. M., & ten Cate, C. (2010). Unidirectional hybridization and introgression in an avian contact zone: evidence from genetic markers, morphology, and comparisons with laboratory-raised F1 hybrids. *Auk*, 127, 605–616.

Dingle, C., Poelstra, J.W., Halfwerk, W., Brinkhuizen, D. M, & Slabbekoorn, H. (2010).

- Assymmetric response patterns to subspecies-specific song differences in allopatry and parapatry in the gray-breasted wood-wren. *Evolution*, 64, 3537–3548.
- Dunn, J. C., Halenar, L. B., Davies, T. G., Cristobal-Azkarate, J., Reby, D., Sykes, D., & Knapp, L. A. (2015). Evolutionary trade-off between vocal tract and testes dimensions in howler monkeys. *Current Biology*, 25, 2839–2844.
- Dunteman, G. H., & Ho, M. H. R. (2006). *An introduction to generalized linear models*. Thousand Oaks, CA: Sage Publications.
- Ey, E., Pfefferle, D., & Fischer, J. (2007). Do age- and sex-related variations reliably reflect body size in non-human primate vocalizations? A review. *Primates*, 48, 253–267.
- Fischer, J., Noser, R., & Hammerschmidt, K. (2013). Bioacoustic field research: a primer to acoustic analyses and playback experiments with primates. *American Journal of Primatology*, 75, 643–663.
- Garcia-Vazquez, E., Moran, P., Perez, J., Martinez, J. L., Izquierdo, J. I., De Gaudemar, B., & Beall, E. (2002). Interspecific barriers between salmonids when hybridisation is due to sneak mating. *Heredity*, 89, 288–292.
- Gergus, E. W. A., Malmos, K. B., & Sullivan, B. K. (1999). Natural hybridisation among distantly related toads (*Bufo alvarus*, *Bufo cognatus*, *Bufo woodhousii*) in Central Arizona. *Copeia*, 199, 281–286.
- Glander, K. E. (1992). Dispersal patterns in Costa Rican mantled howling monkeys. *International Journal of Primatology*, 13, 415–436.
- Grether, G. F., Losin, N., Anderson, C. N., & Okamoto, K. (2009). The role of

Auth Manuscript

- interspecific interference competition in character displacement and the evolution of competitor recognition. *Biological Reviews*, 84, 617–635.
- Gross, M. R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution*, 11, 92–98.
- Ho, L., Cortés-Ortiz, L., Dias, P. A. D., Canales-Espinosa, D., Kitchen, D.M., & Bergman, T.J. (2014). Effect of ancestry on behavioral variation in two species of howler monkeys (*Alouatta pigra* and *A. palliata*) and their hybrids. *American Journal of Primatology*, 76, 855–867.
- Holzmann, I., Agostini, I., & di Bitetti, M. (2012). Roaring behavior of two syntopic howler species (*Alouatta caraya* and *A. guariba clamitans*): Evidence supports the mate defense hypothesis. *International Journal of Primatology*, 33, 338–355.
- Horwich, R. H. (1983). Breeding behaviors in the black howler monkey (*Alouatta pigra*) of Belize. *Primates*, 24, 222–230.
- IUCN (2017). The IUCN Red List of Threatened Species. Version 2017.1. Retrieved from www.iucnredlist.org on 26 July 2017.
- Jolly, C. J., Woolley-Barker, T., Beyene, S., Disotell, T. R., & Phillips-Conroy, J. E. (1997). Intergeneric hybrid baboons. *International Journal of Primatology*, 18, 597–627.
- Jolly, C. J., Burrell, A. S., Phillips-Conroy, J. E., Bergey, C., & Rogers, J. (2011). Kinda baboons (*Papio kindae*) and grayfoot chacma baboons (*P. ursinus griseipes*) hybridize in the Kafue river valley, Zambia. *American Journal of Primatology*, 73, 291–303.

- Jones, C. B., & Cortés-Ortiz, L. (1998). Facultative polyandry in the howling monkey (*Alouatta palliata*): Carpenter was correct. *Boletín Primatológico Latinoamericano*, 7, 1–7.
- Kelaita, M. A., Dias, P. A. D., Aguilar-Cucurachi, M. S., Canales-Espinosa, D., & Cortés-Ortiz, L. (2011). Impact of intrasexual selection on sexual dimorphism and testes size in the Mexican howler monkeys *Alouatta palliata* and *A. pigra*. *American Journal of Physical Anthropology*, 146, 179–187.
- Kitchen, D. M. (2004). Alpha male black howler monkey responses to loud calls: effect of numeric odds, male companion behaviour and reproductive investment. *Animal Behaviour*, 67, 125–139.
- Kitchen, D.M., Engh, A.L. Cheney, D.L., Seyfarth, R.M, Moscovice, L.R., & Fischer, J. (2013). Male baboon responses to experimental manipulations of loud “wahoo calls”: Testing an honest signal of fighting ability. *Behavioral Ecology and Sociobiology*, 67, 1825–1835.
- McDonald, D. B., Clay, R. P., Brumfield, R. T., & Braun, M. J. (2001). Sexual selection on plumage and behavior in an avian hybrid zone: experimental tests of male-male interactions. *Evolution*, 55, 1443–1451.
- McGregor, P. K. (1992). Quantifying responses to playback: one, many, or composite multivariate measures? In: P. K. McGregor (Ed.), *Playback and studies of animal communication* (pp. 79–96). New York, NY: Plenum Press.

- Nomakuchi, S., & Higashi, K. (1996). Competitive habitat utilization in the damselfly *Mnais nawai* (Zygoptera: Calopterygidae) coexisting with a related species, *Mnais pruinosa*. *Research on Population Ecology*, 38, 41–50.
- Patten, M. A., Rotenberry, J. T., & Zuk, M. (2004). Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. *Evolution*, 58, 144–2155.
- Pearson, S. F. (2000). Behavioral asymmetries in a moving hybrid zone. *Behavioral Ecology*, 11, 93–101.
- Pearson, S. F., & Rohwer, S. (2000). Asymmetries in male aggression across an avian hybrid zone. *Behavioral Ecology*, 11, 93–101.
- Phillips-Conroy, J. E., Jolly, C. J., & Brett, F. L. (1991). Characteristics of hamadryas-like male baboons living in anubis baboon troops in the Awash hybrid zone, Ethiopia. *American Journal of Physical Anthropology*, 86, 353–368.
- Rohwer, S., Bermingham, E., & Wood, C. (2001). Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution*, 55, 405–422.
- Qvarnström, A., Rice, A. M., & Ellegren, H. (2010). Speciation in *Ficedula* flycatchers. *Philosophical Transaction of the Royal Society B*, 365, 1841–1852.
- Schön, M. A. (1971). The anatomy of the resonating mechanism in howling monkeys. *Folia Primatologica*, 15, 117–132.
- Secondi, J., Faivre, B., & Kreutzer, M. (1999). Maintenance of male reaction to the congeneric song in the *Hippolais* warbler hybrid zone. *Behavioral Processes*, 46, 151–158.

- Seddon, N. (2005). Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution*, *59*, 200–215.
- Van Belle, S., & Bicca-Marques, J. C. (2015). Insights into reproductive strategies and sexual selection in howler monkeys. In: M. M. Kowalewski, P. A. Garber, L. Cortés-Ortiz, B. Urbani, & D. Youlatos (Eds.), *Howler monkeys: Behavior, ecology, and conservation* (pp. 57–84). New York, NY: Springer Press.
- Van Belle, S., Estrada, A., & Garber, P. A. (2013). Spatial and diurnal distribution of loud calling in black howlers (*Alouatta pigra*). *International Journal of Primatology*, *34*, 1209–1224.
- Van Belle, S., Estrada, A., & Garber, P. A. (2014). The function of loud calls in black howler monkeys (*Alouatta pigra*): food, mate, or infant defense? *American Journal of Primatology*, *76*, 1196–1206.
- Van Belle, S., Estrada, A., & Strier, K. B. (2008). Social relationships among male *Alouatta pigra*. *International Journal of Primatology*, *29*, 1481–1498.
- Whitehead, J.M. (1987). Vocally mediated reciprocity between neighbouring groups of mantled howling monkeys, *Alouatta palliata palliata*. *Animal Behaviour*, *35*, 1615–1627.
- Wilkins, M. R., Seddon, N., & Safran, R. J. (2013). Evolutionary divergence in acoustic signals: causes and consequences. *Trends in Ecology and Evolution*, *28*, 156–166.
- Wyman, M. T., Charlton, B. D., Locatelli, Y., & Reby, D. (2011). Variability of female responses to conspecific vs. heterospecific male mating calls in polygynous deer: an open door to hybridization. *PloS One*, *6*, e23296.

Author Manuscript

Youlatos, D., Couette, S., & Halenar, L. B. (2015). Morphology of howler monkeys: a review and quantitative analyses. In: M. M. Kowalewski, P. A. Garber, L. Cortés-Ortiz, B. Urbani, & D. Youlatos (Eds.), *Howler monkeys: Adaptive radiation, systematics, and morphology* (pp. 133–176). New York, NY: Springer Press.

FIGURE LEGENDS

FIGURE 1. Distribution range of *A. palliata* and *A. pigra* in Central America (based on IUCN 2017) and locations of study groups involved in playback experiments (see Table 1): 1) Tormento South; 2) Tormento North; 3) Aguacate; 4) Oxcabal; 5) Alamo 1; 6) Alamo 4; 7) Flor 7; 8) Flor 9; 9) Jalapilla study; 10) Jalapilla House; 11) Agaltepec Main; 12) Agaltepec Punta.

FIGURE 2. Example spectrograms featuring roars, barks, and pauses from: A) a purebred *A. palliata* caller from Veracruz; B) a purebred *A. pigra* caller from Campeche. Spectrograms show frequency vs. time, with dark barks representing peak intensities.

FIGURE 3. Mean \pm SE responses by *A. pigra* (filled triangles) and *A. palliata* (open circles) subjects based on caller identity. PC1 is a composite value, with large values indicating strong overall responses (e.g., short latencies and long durations; see text).

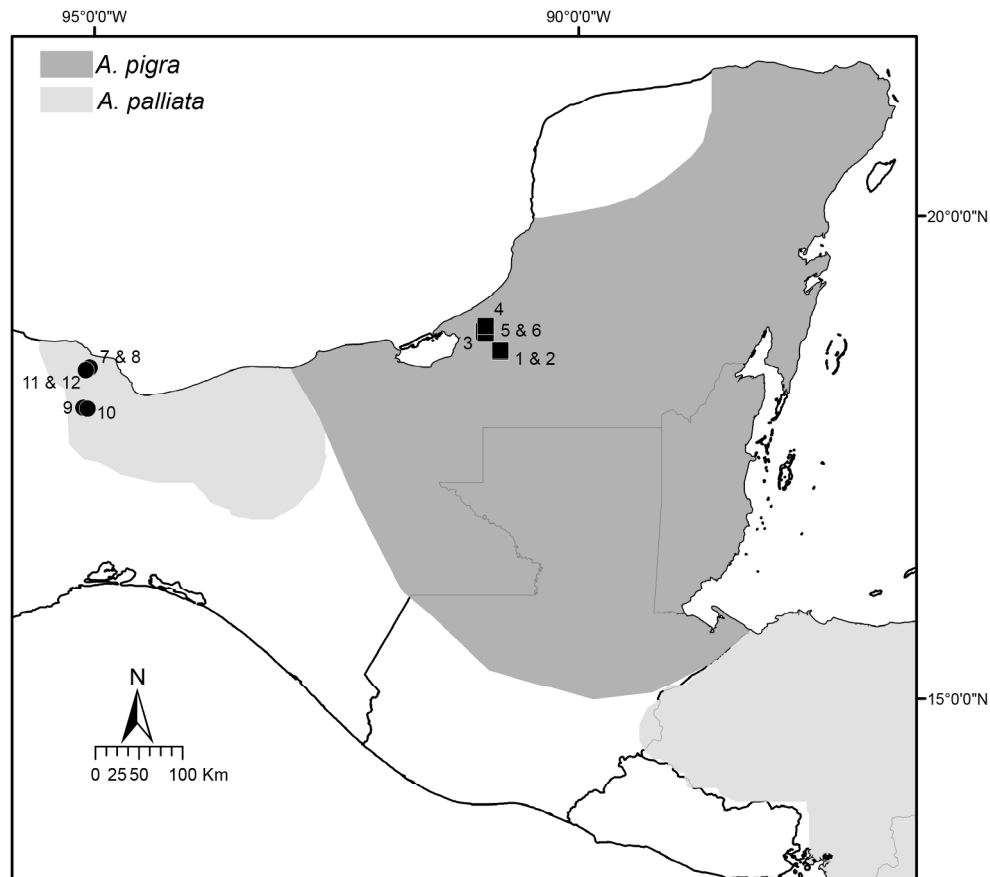


FIGURE 1. Distribution range of *A. palliata* and *A. pigra* in Central America (based on IUCN 2017) and locations of study groups involved in playback experiments (see Table 1): 1) Tormento South; 2) Tormento North; 3) Aguacate; 4) Oxcabal; 5) Alamo 1; 6) Alamo 4; 7) Flor 7; 8) Flor 9; 9) Jalapilla study; 10) Jalapilla House; 11) Agaltepec Main; 12) Agaltepec Punta.

629x552mm (96 x 96 DPI)

Auth

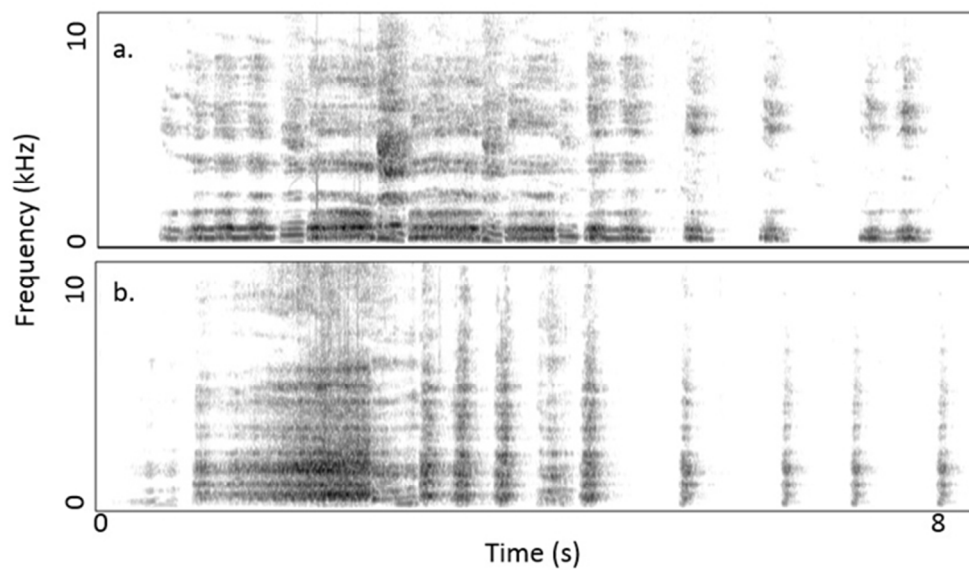


FIGURE 2. Example spectrograms featuring roars, barks, and pauses from: A) a purebred *A. palliata* caller from Veracruz; B) a purebred *A. pigra* caller from Campeche. Spectrograms show frequency vs. time, with dark bars representing peak intensities.

200x120mm (96 x 96 DPI)

Author N

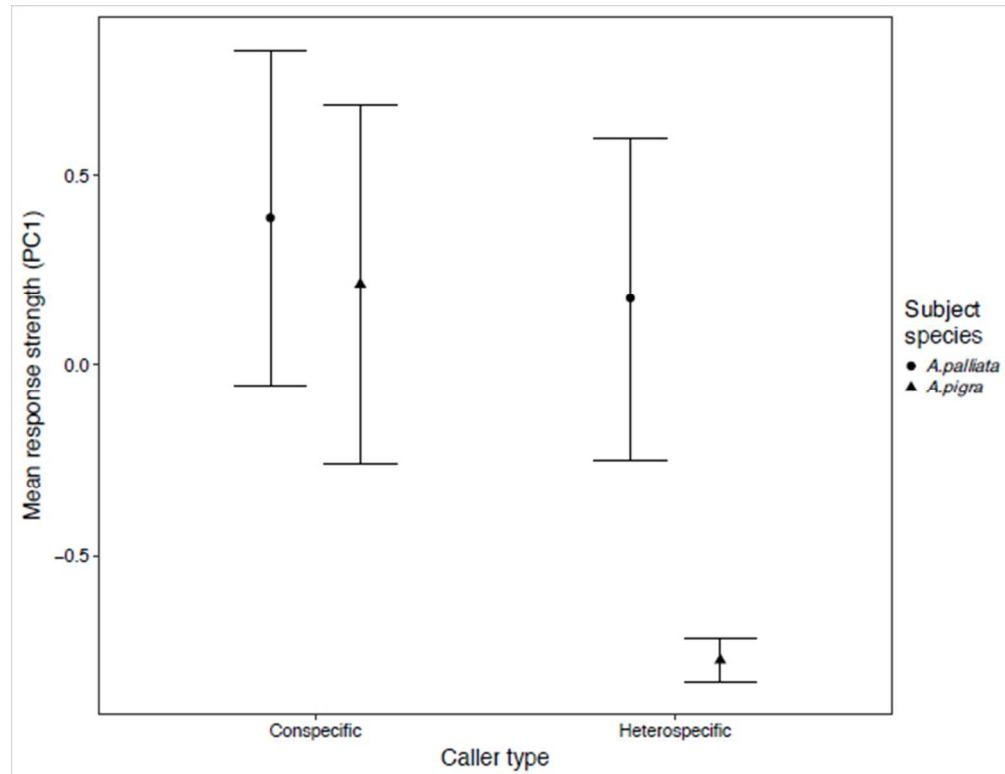


FIGURE 3. Mean + SE responses by *A. pigra* (filled triangles) and *A. palliata* (open circles) subjects based on caller identity. PC1 is a composite value, with large values indicating strong overall responses (e.g., short latencies and long durations; see text).

160x123mm (96 x 96 DPI)

Author

TABLE 1. Stimuli used and composition of study groups

Species	Site*	Group**	#Males	#Fems	Group Size	Trial Order	Caller Identity***	
							<i>A. palliata</i>	<i>A. pigra</i>
<i>A. pigra</i>	Tormento	South	1	1	9	Pal-Pig	B	B
<i>A. pigra</i>	Tormento	North	3	3	8	Pal-Pig	A	C
<i>A. pigra</i>	Sabancuy	Aguacate	1	2	5	Pig-Pal	B	C
<i>A. pigra</i>	Sabancuy	Oxcabal	1	2	7	Pig-Pal	A	A
<i>A. pigra</i>	El Alamo	Alamo 1	2	3	9	Pig-Pal	A	B
<i>A. pigra</i>	El Alamo	Alamo 4	2	2	6	Pal-Pig	B	A
<i>A. palliata</i>	La Flor	Flor 7	3	2	8	Pal-Pig	B	B
<i>A. palliata</i>	La Flor	Flor 9	2	3	8	Pig-Pal	A	B
<i>A. palliata</i>	Jalapilla	Study	5	9	26	Pig-Pal	A	A
<i>A. palliata</i>	Jalapilla	House	2	2	10	Pal-Pig	A	C
<i>A. palliata</i>	Catemaco	Agaltepec Main	12	12	30	Pal-Pig	B	A
<i>A. palliata</i>	Catemaco	Agaltepec Punta	4	9	17	Pig-Pal	B	C

*Groups within each population clustered in three general locations (see Fig.1).

**Groups named for farm, landmark, or conservation site where studied.

***Within each species, letters refer to different individual callers used to make stimuli.

TABLE 2. Mean \pm SE response of subjects to playback trials based on caller species.

	<u><i>A. pigra</i> subjects</u>		<u><i>A. palliata</i> subjects</u>	
	Conspecific (N=6)	Heterospecific (N=6)	Conspecific (N=6)	Heterospecific (N=6)
Return to baseline (s)	750.8 \pm 272.3	165.3 \pm 32.0	417.7 \pm 231.0	385.7 \pm 189.6
Move latency (s)	1820.0 \pm 796.1	3010.5 \pm 589.5	1,230.0 \pm 749.6	1,235.2 \pm 748.2
Distance moved (m)	5.1 \pm 2.6	0.5 \pm 0.5	27.2 \pm 16.5	16.5 \pm 10.0
Approach (%)	8.6 \pm 4.3	0.5 \pm 0.5	23.9 \pm 15.9	21.0 \pm 15.6
Grunt/vocalize latency (s)	1218.8 \pm 753.0	3600.0 \pm 0.0	1,227.5 \pm 750.3	1,205.7 \pm 757.2
Grunt duration (s)	136.3 \pm 87.7	0.0 \pm 0.0	11.8 \pm 5.6	40.5 \pm 28.1
Loud call latency (s)	2415.5 \pm 749.2	3600.0 \pm 0.0	1,239.2 \pm 746.6	1,818.2 \pm 796.9
Loud call duration (s)	407.8 \pm 266.4	0.0 \pm 0.0	481.5 \pm 198.7	263.3 \pm 190.9
Roars (#)	40.7 \pm 31.3	0.0 \pm 0.0	13.3 \pm 7.1	13.3 \pm 11.0
PC1*	0.26 \pm 0.50	-1.00 \pm 0.06	0.19 \pm 0.45	-0.03 \pm 0.44
PC2**	-0.78 \pm 0.64	0.48 \pm 0.07	0.46 \pm 0.37	0.08 \pm 0.40
PC3***	0.22 \pm 0.70	0.22 \pm 0.07	-0.32 \pm 0.48	-0.39 \pm 0.28

*Large PC1 values indicate overall strong responses; **Large PC2 values indicate strong grunt responses; ***PC3 values were not strongly correlated with any responses (see Table 3).

TABLE 3. The component loadings and variance explained by principal components

	PC1	PC2	PC3
Eigenvalue	5.2	1.4	1.2
Variance Explained	57.5%	15.2%	13.0%
	<u>Component Loadings*</u>		
Return to baseline (s)	0.794	0.225	0.371
Move latency (s)	-0.820	-0.189	0.322
Distance moved (m)	0.733	-0.510	-0.315
Approach (%)	0.757	-0.470	-0.241
Grunt/vocalize latency (s)	-0.819	-0.263	0.345
Grunt duration (s)	0.177	0.805	-0.428
Loud call latency (s)	-0.883	0.161	0.002
Loud call duration (s)	0.887	0.021	0.567
Roars (#)	0.705	0.230	0.387

*bold font indicates a strong loading (>0.700 or <-0.700)