ethology international journal of behavioural biology

RESEARCH PAPER

The Responses of Duetting Antbirds to Stereo Duet Playback Provide Support for the Joint Territory Defence Hypothesis

Julianne Koloff & Daniel J. Mennill

Department of Biological Sciences, University of Windsor, Windsor, ON, Canada

Correspondence
Julianne Koloff and Daniel Mennill, Department of Biological Sciences, 401 Sunset Avenue, Windsor, ON, Canada N9B 3P4.
E-mails: j.koloff@gmail.com and dmennill@uwindsor.ca

Received: December 10, 2012
Initial acceptance: January 20, 2013
Final acceptance: February 16, 2013
(S. Foster)

doi: 10.1111/eth.12084

Abstract

In multiple animal taxa, including many birds and primates, members of mated pairs produce coordinated acoustic displays known as duets. By observing the behaviour of territorial animals as they respond to playback-simulated duets of rivals, we can gain insight into the behavioural significance of vocal duets. Playback experiments, however, have been conducted across a very narrow range of duetting animals. Furthermore, many studies have been conducted with single-speaker playback, whereas stereo-speaker playback offers more spatially realistic simulation of duets. Moreover, by evaluating the reactions of animals to separate loudspeakers broadcasting male and female duet contributions, we can study the interactions of both males and females with same-sex vs. opposite-sex rivals. We used a paired experimental design to broadcast duet stimuli through a single-speaker and a stereo-speaker apparatus to 30 pairs of duetting barred antshrikes *Thamnophilus doliatus* in Costa Rica. Our goals were (1) to evaluate whether territorial antbirds respond more aggressively to male vs. female duet components and (2) to assess aggressive responses of antbirds towards single-speaker vs. stereo-speaker playback. Neither males nor females differentiated between the loudspeaker simulating the male vs. female duet contribution during stereo-speaker playback trials. Barred antshrikes displayed significantly stronger responses to stereo-speaker playback compared with single-speaker playback. Males displayed stronger playback responses than females with closer, quicker and more vocal responses. These results provide evidence for a joint resource defence function of antbird duets given that pairs responded together with equivalent intensity to male and female simulated intruders. This is the first study to show that although duetting is an aggressive territorial signal, birds do not necessarily respond to sex-specific components of duets. Our results support the idea that spatially realistic stereo presentation of duet stimuli is critical for experimental duet research.

Introduction

Vocal duetting behaviour is a joint signalling strategy that has evolved multiple times in diverse taxa, most notably in tropical birds and primates (Farabaugh 1982; Haimoff 1986). Duetting occurs when two individuals, usually a male and female of a mated pair, combine their vocalizations in a temporally coordinated acoustic display (Hall 2004, 2009). Studies of duetting behaviour have enhanced our understanding of how individuals communicate with their breeding partner and how pairs communicate with nearby conspecific animals. Only a very small proportion of the taxonomic breadth of duetting animals have been studied with an experimental approach (reviewed in Hall 2009; Douglas & Mennill 2010).

Duetting behaviour has been studied most intensively in birds, where acoustic playback experiments have provided insight into duet function (Douglas & Mennill 2010). The vast majority of playback studies of duetting behaviour have been conducted on oscine songbirds, and this collective work has produced

evidence supporting the joint resource defence and mate-guarding hypotheses for duet function (with some additional evidence for the Signalling Quality, Signalling Identity, Maintaining Contact and Pair Bond Maintenance hypotheses; reviewed in Hall 2004, 2009). Evidence supporting the joint resource defence hypothesis (i.e. pairs perform duets to cooperatively defend their breeding territory against rivals; Seibt & Wickler 1977) and Mate Guarding hypothesis (i.e. birds perform duets to acoustically guard their mate; Sonnenschein & Reyer 1983) includes playback experiments where birds respond aggressively to playback-simulated intruders (reviewed in Logue 2005; Douglas & Mennill 2010; Dahlin & Wright 2011). Although more research is needed, several studies support the idea that duets likely serve multiple functions in different contexts (e.g. Grafe & Bitz 2004; Mennill & Vehrencamp 2008; Benedict 2010).

A decade ago, behavioural researchers proposed a two-speaker playback design for experimentally testing duet function (Langmore 2002). This technique, now known as 'stereo duet playback', involves broadcasting duet stimuli in a spatially realistic manner; the left and right channels of stereo sound files are played through separate loudspeakers, so that the male and female duet components originate at two different point sources (Logue & Gammon 2004; Rogers et al. 2004; Mennill 2006; Molles & Waas 2006). A recent review by Douglas & Mennill (2010) argued that stereo-speaker playback is important for studying species in which pairs regularly perform duets while far apart from their partner; in these species, only a two-speaker design offers a spatially realistic simulation of a duet. Douglas & Mennill (2010) also argued that stereo-speaker playback is critical for studying duetting animals where one individual is capable of performing both components of a duet; in these species, a duet stimulus broadcast through a single-speaker apparatus simulates a single individual, whereas the same stimulus broadcast through a stereo-speaker apparatus simulates two individuals.

One major advantage of stereo duet playback is that it allows researchers to independently assess the behavioural responses of the duetting male and female towards speakers broadcasting same-sex vs. opposite-sex duet contributions. Stereo-speaker playback thereby facilitates the testing of unique predictions of hypotheses for duet function. If duetting animals are cooperatively defending their territories against rivals (i.e. the joint resource defence hypothesis), both pair members should respond aggressively to loudspeakers broadcasting duets, and the pair should approach the simulated intruders together. Researchers have found support for this idea and have observed pair behaviours such as flights over loudspeakers together and approaches towards the loudspeakers in tandem (Hall 2009; Douglas & Mennill 2010). Conversely, if duetting animals are acoustically guarding their mate (i.e. the Mate Guarding hypothesis), they should respond more aggressively towards the loudspeaker broadcasting the same-sex duet contribution (Douglas & Mennill 2010). Researchers have found support for this idea and have observed playback subjects exhibiting greater aggression towards same-sex intruders (Hall 2009; Douglas & Mennill 2010). Therefore, not only does stereo-speaker playback permit simulation of vocal duets with spatial realism, it also allows researchers to distinguish between cooperative and competitive hypotheses for duet function.

In this study, we use both single-speaker and stereo-speaker playback to assess the responses of barred antshrikes *Thamnophilus doliatus* to simulated duets. Both male and female barred antshrikes sing, and they can produce songs as solos or combine their songs as vocal duets (for a detailed description, see Koloff & Mennill 2013). Both sexes can create duets by overlapping their partner's song and pairs routinely perform duets while <5 m apart (Koloff & Mennill 2011b, 2013). A previous single-speaker playback experiment demonstrated that male and female barred antshrikes respond aggressively towards duet playback regardless of whether the male or female sings the first component and that females display significantly more aggressive behaviour towards playback of female solo songs than duets or male solo songs (Koloff & Mennill 2011a). The current study offers the opportunities to further investigate female–female aggression using a spatially realistic experimental design.

Barred antshrikes provide interesting study subjects for a stereo-speaker playback experiment for several reasons. First, suboscine birds in general – and antbirds in particular – have received far less attention than oscine songbirds despite the fact that duets are known to occur in many suboscines, including antbirds (Farabaugh 1982). Second, barred antshrikes perform duets in relatively close proximity to their duet partner, usually <5 m (Koloff & Mennill 2011b), whereas the subjects of previous stereo-speaker studies (see Douglas & Mennill 2010) perform duets with highly variable distances of separation. Consequently, the present study provides the opportunity to evaluate whether species that perform duets with small distances of separation are able to distinguish between stereo and single-speaker playback. Third, a previous

single-speaker experiment showed that female barred antshrikes respond very aggressively to a loudspeaker simulating a female solo vs. duets (Koloff & Mennill 2011a); we were interested in exploring whether a female-biased response exists when duets were simulated with male and female duet contributions in separate locations.

This stereo-speaker playback experiment with barred antshrikes allowed us to address two main questions. (1) Do barred antshrikes respond more aggressively to same-sex or opposite-sex duet contributions when responding to a rival duetting pair? If duets serve a cooperative function in joint resource defence, we predicted that both sexes would display equivalent aggressive responses to both male and female loudspeakers. Conversely, if duets serve a competitive function in acoustic mate guarding, we predicted that barred antshrikes would respond more strongly to the loudspeaker broadcasting the same-sex duet contribution. (2) Do barred antshrikes respond differently to single-speaker vs. stereo-speaker playback? Based on previous studies of oscine songbirds (Rogers et al. 2004; Molles & Waas 2006), we predicted that duet stimuli broadcast through stereo speakers would elicit stronger responses than the same duet stimuli broadcast through a single loudspeaker. This is the first study to test single-speaker vs. stereo-speaker playback in a species that regularly perform duets in very close proximity to their partner. It is also the first stereo-speaker duet playback study of a suboscine songbird or an antbird; these are biodiverse tropical taxa where duetting is a relatively common, but poorly studied behaviour.

Methods

General Field Methods

We studied a population of barred antshrikes in Sector Santa Rosa of the Guanacaste Conservation Area, a neotropical dry forest in northwestern Costa Rica (10°40′N, 85°30′W). Barred antshrikes are common at this site and are readily located by their loud vocal duets (Koloff & Mennill 2013). This study took place during the end of the dry season and the start of the rainy season from 15 May to 9 Jun. 2010 (the first long, sustained rain of the year was 23 May 2010). At this time of year, the subjects were actively defending territories and had begun nesting activities (Koloff & Mennill 2011b).

We successfully captured some of the playback subjects in mist nets and gave them unique colour band combinations to facilitate identification. Of the 30 pairs of barred antshrikes in our experiment, there were two pairs where we banded both pair members, and nine pairs where we captured and banded only the territorial male. Previous studies demonstrate that barred antshrikes are territorial and site-faithful (Koloff & Mennill 2011a, 2013), and our field observations of the colour-banded birds in this study showed that birds were routinely found in the same territories throughout the 2010 breeding season. Therefore, we are confident that the unbanded birds in this study, distinguished on the basis of territory location, represent distinct pairs and that no animals were sampled repeatedly. Barred antshrikes are strikingly sexually dimorphic based on plumage features (Koloff & Mennill 2011b), and therefore, the sex of both banded and unbanded birds was easily identified during playback. The songs of male and female duet contributions are superficially similar, but are readily distinguishable on the basis of fine structural features during visualization of sound spectrograms; male songs are longer, have more syllables, and are lower pitched than female songs (e.g. Fig. 1; Koloff & Mennill 2013).

Playback Stimuli

To create playback stimuli, we used recordings of pairs of antshrikes in our population collected between 1 May and 20 May 2010. We selected recordings with a high signal-to-noise ratio and minimal heterospecific interference (assessed visually from sound spectrograms). Recordings were highpass filtered at 500 Hz and lowpass filtered at 15 000 Hz using AUDITION software (Adobe, San Jose, CA, USA). We created duet stimuli by overlapping our recordings of male and female solos, following the protocol suggested in Douglas & Mennill (2010; previous analyses confirm that barred antshrikes use the same songs to produce both solos and duets and that duets consistently involve a single song from each of the participating birds; Koloff & Mennill 2013). When combining male and female songs to create the stimuli, we pasted the second bird's song, so that there was a delay of 1.5 s between the start of the first bird's song and the start of their partner's, resulting in duets where the second bird overlapped approximately the terminal 25% of the first bird's song (this is a typical degree of overlap for this species; Koloff & Mennill 2013). We varied the delay in onset of the second bird's song by ±0.2 s around an average 1.5 s interval to create realistic vocal bouts where the duets did not have identical amounts of overlap. Each stimulus was 4.0 min long, and each stimulus contained alternating male-created

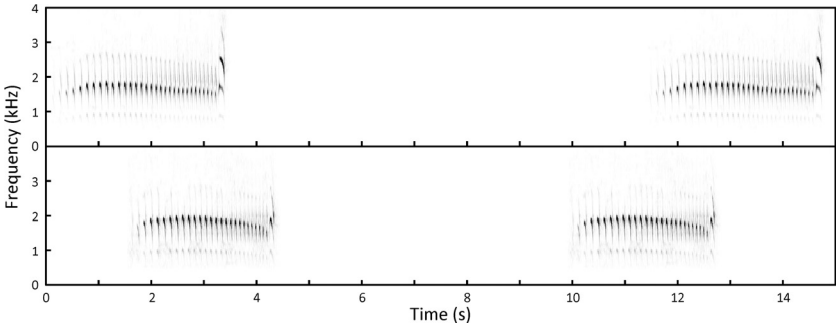

Fig. 1: Sound spectrogram showing the first 15 s of one of eight stereo sound files used to simulate the duets of Barred Antshrikes. The male's song is in the upper channel and the female's song is in the lower channel of this stereo sound file; when broadcast through stereo loudspeakers, the male and female duet contributions originate from different point sources. Duet stimuli featured alternating duet roles, with each sex responding to their partner's song in alternation.

duets (duets where the male overlapped the female song) and female-created duets (duets where the female overlapped the male song) occurring every 10 s. These stimuli were realistic; prior analyses confirm that both sexes of barred antshrike create duets by responding to their partner's song (a previous playback experiment revealed that the sexes do not respond differently to male or female-created duets; Koloff & Mennill 2013), and our field observations revealed that birds often sang in bouts where duets were repeated several times in succession, including both male-and female-created duets (pers. obs.).

As control stimuli, we used recordings of ambient forest noise. The control stimuli contained vocalizations from animals common at our study site, including blue-crowned motmots *Momotus momota*, long-tailed manakins *Chiroxiphia linearis* and thicket tinamous *Crypturellus cinnamomeus*, but no barred antshrikes. The control stimuli were broadcast from one loudspeaker during the trials when antshrike duets were broadcast from the other loudspeaker, thereby ensuring that it was not the mere presence of any sound out of the two loudspeakers that drove differences in response between the two treatments.

For stereo-speaker playback trials, we created stimuli where male and female duet components were contained in separate channels of the stereo sound files, so that the male and female songs were broadcast separately through the left and right loudspeakers (Fig. 1). For single-speaker playback trials, we created single-channel versions of the stereo stimuli (i.e. single-channel stimuli were identical to the stereo stimuli), so that the male and female contributions of a duet originated from the same loudspeaker while we simultaneously broadcast ambient forest noise out of the second loudspeaker. To mitigate the effects of pseudoreplication, we created eight different duet stimuli from recordings of eight pairs (16 individuals) in our population and eight different ambient forest noise stimuli from eight different locations at our study site. For each stimulus recording, we created multiple versions of the same file, allowing us to broadcast the male and female contributions, or the duets and ambient noise, out of either the left or the right loudspeaker. For each subject pair, the same source recordings were used for both the stereo and single-speaker treatments. Throughout the thirty trials, the eight stimuli were used in either three or four trials, and we tested whether the stimulus recording had an effect by including stimulus identity as an effect in our analysis (see below). We alternated whether the male or female contribution (or the duet stimulus or ambient noise recording) came from the left or right loudspeaker across subsequent trials with the same stimulus. We saved all stimuli as 16-bit WAV files with a sampling rate of 44.1 kHz and normalized all stimuli to −1 dB.

Playback Technique

Our playback simulated a pair of duetting barred antshrikes performing duets inside the territories of n = 30 subject pairs. Each subject pair received two treatments: a stereo-speaker treatment (male and female duet contributions broadcast through separate loudspeakers) and a single-speaker treatment (male and female duet contributions broadcast through one loudspeaker, with a second loudspeaker playing ambient forest noise).

We conducted playback experiments in locations where we had previously observed pairs of antshrikes vocalizing during the dawn chorus. Taking into account the very small territory sizes of barred antshrikes (0.36 ± 0.08 ha; Koloff & Mennill 2011b), we placed loudspeakers close to the centre of a pair's territory to minimize interference from neighbours (territories were mapped by following the focal pairs during dawn chorus recordings on the days prior to playback while carrying a hand-held global positioning system; model: Garmin GPS

60CSx, Garmin, Olathe, KS, USA). We mounted two loudspeakers (Sony SRS-A37, Sony, Tokyo, Japan) on 1 m poles (1 m is a common perch height for this species) and oriented the speakers upwards to minimize any effects of speaker directionality. All stimuli, including antshrike duets and ambient noise, were broadcast at an amplitude of 85 dB (assessed with a Radioshack Sound Level Meter held at 1.0 m horizontal distance from the upwards-oriented loudspeaker; settings: C-weighting, fast response). This sound level was comparable to that of naturally singing birds, judged by comparing our loudspeaker output to birds in the field. Volume was held constant across all trials. The two loudspeakers were positioned 3 m apart, a distance typical of the positions of male and female antshrikes while duetting, although partners regularly perform duets much closer than this, and occasionally farther apart. We connected the loudspeakers to a portable digital audio player (Apple iPod classic, Apple, Cupertino, CA, USA). We hung flags at 1 and 2 m from each loudspeaker on both sides of the stereo set-up, and at the midpoint between the two speakers, to serve as references when observing the birds' responses. One observer sat 15 m away at a point equidistant from the speakers to avoid any asymmetrical influences on the subjects' behaviour. The playback trial was recorded using a directional microphone (Audiotechnica AT815b, Audiotechnica, Tokyo, Japan) mounted to a tripod and attached to a digital recorder (Marantz PMD-660, Marantz, Kanagawa, Japan). The observer, seated next to the recording apparatus, quietly dictated the activities of the birds, noting the distance of each bird to each loudspeaker, the number of flights by each bird over each loudspeaker and the sex of the bird producing each vocalization.

Each playback trial had three phases. Phase 1 was a silent control period, where we recorded all activities and vocalizations of the birds for 5 min prior to broadcasting playback. Phase 2 involved the broadcast of the playback stimuli for 4 min. Phase 3 was a post-playback period, where we recorded all activities and vocalizations of the birds for 5 min. Each playback trial lasted 14 min in total. All playback sessions were conducted between 0630 and 1100 h.

Each pair of subjects received the single-speaker and stereo-speaker playback treatments on two different days, with the order of treatments varying between subject pairs. We conducted the second treatment for each pair on a non-consecutive day, placing the speakers in the exact same location. We used the following guidelines for conducting playback treatments: (1) pairs were given 48 or 72 h recovery time between subsequent treatments (average delay between the first and the second trial was 53.5 ± 1.9 h); (2) trials were never conducted on nearby territories within 48 h (minimum distance of 300 m between playback sites within a 48-h period); and (3) pairs were presented with stimuli that originated >500 m from their territory to minimize any effects of familiarity with the stimuli. Given their small territories and a high population density at our study site, we felt that this >500 m separation would ensure that the birds were not familiar with the simulated pair.

Response Measures

To quantify responses to playback, we documented both physical and vocal behaviours of each male and female. We assessed the following behaviours: (1) number of passes over each loudspeaker; (2) closest approach to each loudspeaker (in m); (3) latency from the start of playback to first approach to within 2 m of each loudspeaker (in seconds); (4) amount of time spent within 2 m of each loudspeaker (in seconds); (5) latency to first song (in seconds); (6) number of independent songs (i.e. for males: male solos plus duets where male sang first and female joined him to create a duet; for females: female solos plus duets where the female sang first and male joined her to create a duet); and (7) number of duets created (i.e. the number of times the bird overlapped its partner's song to create a duet). If the birds did not respond during a trial, we recorded the latency to approach to 2 m and latency to first song as 240 s, the length of a playback trial.

We visualized recordings of playback trials using SYRINX-PC sound analysis software (J. Burt, Seattle, WA, USA). We annotated all vocalizations from the focal pair and transcribed observations of the birds' physical behaviour, as dictated by the observer, to produce time-synchronized annotations of the pair's activities. We extracted the above response measures from these annotations. We compare the average values across the two silent pre-playback periods to the birds' responses to the two playback treatments.

Statistical Techniques

To compare antshrike responses to the different loudspeakers simulating male and female vocalizations during stereo playback trials, we used paired *t*-tests of the four physical response measurements (response measurements 1–4, above; we could not include the vocal response measurements 5–7, above, because it was not obvious which of the two speakers the birds' songs were directed towards).

To evaluate antshrike behaviour during the pre-playback silent period, single-speaker playback, and stereo-speaker playback, we summarized the birds' behaviour using principal component analysis (PCA) conducted on the correlation matrix of the four physical response measurements and the three vocal response measurements (average ± SE correlation coefficients among the seven response measurements was 0.59 ± 0.04). We report only principal component scores with eigenvalues >1.0 (Bryant & Yarnold 1995). This analysis yielded a single principal component (PC1) that explained 71.2% of the variation in the original seven variables. PC1 had a strong positive correlation with total number of passes over the loudspeakers (0.85), amount of time within 2 m of the loudspeaker (0.91), number of independent songs (0.63) and number of duets created (0.63); and a strong negative correlation with closest approach to the loudspeaker (−0.90), latency to approach within 2 m of the loudspeaker (−0.89) and latency to first song (−0.84). Therefore, individuals with high PC1 scores responded quickly, closely and vocally.

We evaluated birds' responses to single- vs. stereo-speaker playback using a linear mixed model where the three fixed effects were treatment (i.e. silent pre-playback, single-speaker playback or stereo-speaker playback), sex of the responding animal, the interaction between treatment and sex, and playback stimulus (i.e. the eight stimuli used in playback), and pair identity was a subject variable with random effects (to account for the fact that each pair was assessed in the three treatments). Fixed effects were estimated using the restricted maximum likelihood method; the subject effects were estimated using a variance components covariance structure. We conducted *post hoc* Tukey's tests of honestly significant differences to evaluate differences between all statistically significant fixed effects.

All values are presented as means ± standard error. All tests are two-tailed. We conducted all statistical analyses using JMP 10.0 software (SAS Institute, Cary, NC, USA).

Results

Male and female barred antshrikes responded to playback of conspecific duets with strong vocal output and rapid, close approaches to the loudspeakers. Responses were often accompanied by back-and-forth flights above the loudspeakers, and responding pairs consistently produced their stereotypical visual display as they performed duets in response to playback (display described in Koloff & Mennill 2011b).

Responses to Male vs. Female Loudspeaker during Stereo-Speaker Playback Trials

During stereo-speaker playback, both male and female barred antshrikes showed equivalent responses to the loudspeaker broadcasting the male and female duet contribution. Males showed equivalent responses to the loudspeakers in terms of number of flights over each loudspeaker, closest approach to each loudspeaker, latency to approach within 2 m of each loudspeaker and amount of time spent within 2 m of each loudspeaker (Table 1). Females also showed equivalent responses to the loudspeakers in terms of number of flights over each loudspeaker, closest approach to each loudspeaker, latency to approach within 2 m of each loudspeaker and amount of time spent within 2 m of each loudspeaker (Table 1).

Responses to Single-Speaker vs. Stereo-Speaker Playback

A linear mixed model revealed that the behaviour of barred antshrikes varied significantly with playback treatment, with the sex of the responding bird and with the interaction term between treatment and sex (Fig. 2). Barred antshrike behaviour varied significantly across the pre-playback silent period, and during single-speaker and stereo-speaker (treatment effect: $F_{2,145} = 173.2$, $p < 0.0001$); a *post hoc* test revealed significant differences in PC1 playback response scores between all three treatments, with the highest level of activity during stereo-speaker playback, a lower level of activity during single-speaker playback and the lowest level of activity during the pre-playback silent period (Fig. 2). Barred antshrike behaviour also varied with the sex of the responding animal (sex effect: $F_{1,145} = 41.1$, $p < 0.0001$) with significantly higher PC1 playback response scores for males (Fig. 2). The interaction term between treatment and sex was significant (interaction term: $F_{2,145} = 8.7$, $p = 0.0003$) where the responses of males and females to the control treatment were similarly low, but male responses to the experimental treatments were higher than female responses. Which of the eight stimulus sets were used in playback had no significant effect on PC1 playback response scores (stimulus effect: $F_{7,22} = 1.0$, $p = 0.44$).

Discussion

Barred antshrikes responded strongly to conspecific territorial intrusions simulated through both single-speaker

Table 1: Responses of barred antshrikes towards the male and female loudspeaker during stereo-speaker playback

Variable	Mean ± SE						
	Male loudspeaker	Female loudspeaker	*t*	p	Mean difference	Lower confidence interval	Upper confidence interval
Male # of flights over speaker	5.8 ± 0.5	5.2 ± 0.5	1.4	0.18	−0.6	−1.58	0.31
Female # of flights over speaker	1.7 ± 0.2	1.5 ± 0.2	1.1	0.30	−0.2	−0.58	0.18
Male closest approach (m)	2.1 ± 0.2	1.8 ± 0.2	1.5	0.14	−0.3	−0.62	0.09
Female closest approach (m)	5.7 ± 0.4	5.6 ± 0.4	0.5	0.64	−0.2	−0.89	0.56
Male latency to within 2 m (s)	120 ± 23	79 ± 23	1.8	0.08	−42	−88.0	4.8
Female latency to within 2 m (s)	293 ± 34	314 ± 34	0.6	0.54	21	−49	91
Male amount of time within 2 m (s)	111 ± 25	91 ± 25	0.8	0.44	−20	−72	32
Female amount of time within 2 m (s)	98 ± 34	113 ± 34	0.4	0.67	14	−54	83

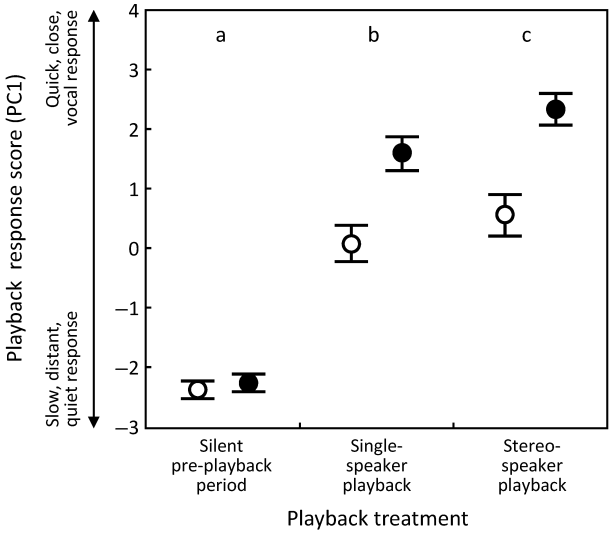

Fig. 2: Variation in playback response scores of barred antshrikes for n = 30 females (open circles) and n = 30 males (filled circles) in response to single-speaker and stereo-speaker playback. The playback response score is a principal component score summarizing variation in seven measurements of subjects' speed of approach, distance to loudspeaker and number of vocalizations (see text for details). Circles show means, and whiskers show standard error. Letters indicate the results of a Tukey *post hoc* test of the significant effect of playback treatment, where levels not connected by the same letter are significantly different.

and stereo-speaker playback of duets. The responses of territorial pairs of antshrikes included heightened vocal output and physical approach towards the loudspeakers. In response to stereo-speaker playback, we found no evidence that male or female barred antshrikes differentiated between loudspeakers broadcasting same-sex vs. opposite-sex duet contributions. In response to the same duet stimuli broadcast through a single loudspeaker vs. stereo loudspeakers, antshrikes exhibited significantly stronger responses to the stereo apparatus with 3.0 m between the male and female duet contributions.

Responses Towards Male vs. Female Duet Contributions

Male and female barred antshrikes responded with equal intensity to the two loudspeakers broadcasting same-sex and opposite-sex duet contributions during stereo duet playback. Territorial pairs approached the speakers together and although our linear model showed that males exhibited stronger overall playback responses than females, both sexes approached both loudspeakers closely and passed over both loudspeakers. These findings match predictions of the joint resource defence hypothesis; both birds in a pair defended their territory together. These findings fail to support predictions of the Mate Guarding hypothesis; the birds did not respond more strongly to the same-sex loudspeaker. We previously used playback to study duet function by broadcasting solos and duets to territorial pairs through a single loudspeaker (Koloff & Mennill 2011a). In line with the current study, our previous single-speaker study provided support for a joint resource defence hypothesis; we found no difference in male and female aggressive responses towards male- or female-created duets, and both pair members responded with similar levels of aggression towards all duet stimuli (Koloff & Mennill 2011a). Duets are clearly associated with aggressive interactions between rivals in barred antshrikes, yet birds do not appear to respond differently based on which sex creates the duet (Koloff & Mennill 2011a) or the specific position of the male and female contributor to the duet (current study).

Five previous investigations have used stereo duet playback to explore which sex of simulated intruder incites the strongest response from territorial duetting birds. Stereo duet playback to rufous-and-white wrens *Thryophilus rufalbus* (Mennill 2006; Mennill & Vehrencamp 2008), happy wrens *Pheugopedius felix* (Templeton et al. 2011), eastern whipbirds *Psophodes*

olivaceous (Rogers et al. 2006, 2007) and Steere's liocichlas *Liocichla steerii* (Weng et al. 2012) revealed that birds respond more strongly to the loudspeaker simulating the same-sex vocalization. A fifth study, involving stereo playback to black-bellied wrens *Pheugopedius fasciatoventris*, showed that females, but not males, responded more strongly to the loudspeaker simulating the same-sex vocalization (Logue & Gammon 2004). The asymmetry of response evident in these studies has been argued to provide support for the Mate Guarding hypothesis for duet function (Douglas & Mennill 2010; although it has also been argued to be a sex-specific division of labour during territory defence: Templeton et al. 2011). The current study of barred antshrikes is the first stereo-speaker investigation to show a lack of differentiation between the same-sex and opposite-sex rivals. That the current study has a larger sample size (30 pairs) than all of the previous studies (16.3 ± 1.9 pairs; range: 11–24; n = 6 investigations of five species) suggests that this is not an artefact of sample size. This difference may arise because antshrikes (a suboscine bird) presumably represent an independent evolution of duetting from the other five species studied (all oscine birds). For suboscine species, duets might serve only a territory defence function, but no mate-guarding function, whereas duets might serve both territory defence and mate-guarding functions in the five oscine species. Addressing this question requires further playback studies to determine whether our results hold true in other suboscines. Additionally, more thorough research on the social and genetic mating systems of both oscine and suboscine duetting birds will help us understand the importance of mate guarding. Interestingly, the only published investigation of paternity in a duetting suboscine bird showed no evidence of extra-pair paternity (the dusky antbird *Cercomacra tyrannina*, Fleischer et al. 1997), whereas the only three published studies of paternity in duetting oscine birds showed some extra-pair paternity (reviewed in Douglas et al. 2012). If duetting suboscines truly lack extra-pair paternity, duetting would not be expected to evolve a mate-guarding function.

A previous playback study showed that female barred antshrikes responded most intensely towards playback of female solos, indicating heightened female–female aggression during barred antshrike territorial encounters (Koloff & Mennill 2011a). Yet we did not observe strong female–female aggression in the current investigation, even though there was the potential for females to respond more strongly to the loudspeaker broadcasting the female duet contribution. Lone female intruders may pose a greater level of threat than paired female intruders, because lone female intruders represent a challenge to the partnership, whereas a pair of intruders may represent a challenge to the territory. This difference may explain the increased physical aggression displayed towards solo female simulations in our previous study, but not towards the female speaker in the current study. The results of this stereo duet playback study show that females do not show heightened aggression towards a rival female if that rival is in the presence of a duetting partner.

Responses to Single-Speaker vs. Stereo-Speaker Playback

Pairs of duetting animals are known to perform their joint acoustic displays with variable distances of separation (Logue 2007; Mennill & Vehrencamp 2008). Quantifying the distance between animals as they perform duets is a challenging task, in part because many duetting animals live in thick tropical vegetation where visual tracking is difficult (Farabaugh 1982). Understanding whether animals respond differently to duets produced by pairs of animals that are far apart, vs. pairs of animals in close proximity, is an important area of investigation. To date, only two studies have directly compared aggressive responses of birds to duets broadcast using single-speaker and stereo-speaker playback. A playback study of kokako *Callaeas cinereus wilsoni*, involving duet stimuli broadcast through both a single- and stereo-speaker apparatus, revealed that birds displayed greater levels of aggression towards stereo-speaker playback (Molles & Waas 2006). A similar playback study of Australian magpie-larks *Grallina cyanoleuca* also revealed that birds display greater aggression towards stereo playback than single-speaker playback (Rogers et al. 2004). In our study, barred antshrikes displayed stronger responses to stereo duet playback than single-speaker duet playback, which was stronger still than behaviours exhibited during the pre-playback control period (as indicated through a composite principal component score). Therefore, our findings match the findings of these previous two investigations. The similar conclusions of these studies of three distantly related duetting birds offer compelling support for the idea that songs broadcast from two different point sources are more threatening territorial signals than songs broadcast from the same point source.

Barred antshrikes routinely perform duets in close proximity to their partner (Koloff & Mennill 2011b, 2013). To design a realistic playback experiment, we placed the speakers 3 m apart because this simulated a natural distance of separation for duetting barred antshrikes. In the other studies, the stimuli were broadcast through speakers placed $\geq$10 m apart (e.g. 12–15 m for magpie-larks, in addition to a 0.5–2 m treatment, Rogers et al. 2004; 10 m for kokako, Molles & Waas 2006; 16 m for rufous-and-white wrens, Mennill 2006). As advocated in a recent review of playback techniques for studying duets (Douglas & Mennill 2010), our results underscore the importance of stereo duet playback techniques for studying duets, regardless of the distance between duet partners. Rufous-and-white wrens, for example, perform duets with highly variable distances of separation (average: 19.2 m; range: 0.4–144.3 m) and duets sung in close proximity may communicate different information than duets sung far apart (Mennill & Vehrencamp 2008). This has been tested explicitly in magpie-larks, where pairs normally perform duets with separation distances of 1–15 m (Rogers et al. 2004). Rogers et al. (2004) compared responses to single-speaker playback to stereo-speaker playback with distances of 0.5–2 and 12–15 m and found that the distance between the speakers had no effect on the vocal behaviour of the subjects in response to playback, but that stereo-speaker playback elicited more flights towards the speakers compared with single-speaker playback (Rogers et al. 2004). Our data show that the same duet stimuli elicit different responses when broadcast from separate loudspeakers even in a species where birds regularly perform duets in very close proximity. Therefore, we recommend that all future investigations should endeavour to use a stereo playback approach, even when studying duetting animals that perform duets in close very near to their partner.

Acknowledgements

We thank the staff at Sector Santa Rosa of the Guanacaste Conservation Area for logistical support, particularly R. Blanco. We thank S. Foster, D. Gammon, and two anonymous referees for comments that improved the manuscript. Our research was funded by grants from the American Museum of Natural History's Frank M. Chapman Memorial Fund Grant to JK, and grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation (CFI), the Government of Ontario and the University of Windsor to DJM.

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