



Playbacks revisited: asymmetry in behavioural response across an acoustic boundary between two parapatric bird species

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Accepted 12 July 2015; published online 27 August 2015

Abstract

Behavioural barriers to gene flow can play a key role in speciation and hybridisation. Birdsong is well-known for its potential contribution to such behavioural barriers as it may affect gene flow through an effect on territorial and mating success across population boundaries. Conspecific recognition and heterospecific discrimination of acoustic variation can prevent or limit hybridization in areas where closely related species meet. Here we tested the impact of song differences on territorial response levels between two adjacent *Henicorhina* wood-wren species along an elevational gradient in Colombia. In an earlier study, playback results had revealed an asymmetric response pattern, with low-elevation *H. leucophrys bangsi* responding strongly to any conspecific or heterospecific song variant, whereas high-elevation *H. anachoreta* birds discriminated, responding more strongly to their own songs than to those of *bangsi*. However, in that study we could not exclude a role for relative familiarity to the song stimuli. In the current study we confirm the asymmetric response pattern with song stimuli recorded close to and on both sides of the distinct acoustic boundary. Furthermore, we also show a previously unnoticed divergence in singing style between these two wood-wren species, which may contribute to an acoustically guided barrier to hybridization in this secondary contact zone.

Keywords

birdsong, elevational gradient, gene flow, species boundary, secondary contact.

1. Introduction

There is a long history of interest in understanding the origin and maintenance of species over evolutionary time (Panhuis et al., 2001; Sobel et al., 2010; Abbot et al., 2013). Theoretical studies suggest that divergence of sexual traits among populations and the behavioural responses to these traits are likely to play a key role (e.g., Dieckmann & Doebeli, 1999; van Doorn et al., 2009; Servedio et al., 2011). However, empirical studies testing the responsiveness to sexual signals and examining the congruence between signal divergence and genetic variation are necessary to link behavioural mechanisms to evolutionary processes. A considerable number of studies have addressed acoustic variation and the evolution of reproductive divergence in birds (Price, 1998; Slabbekoorn & Smith, 2002a; Tobias et al., 2010; Derryberry et al., 2012), including experimental tests of response to playbacks of vocalizations in natural populations (e.g., Irwin et al., 2001; Patten et al., 2004; Dingle et al., 2010).

In many bird species song development relies partly on learning through auditory experience but, also in these species, songs are both genetically and culturally transmitted across generations (Marler & Tamura, 1964; Slater, 1986). This may give rise to distinct geographic variants with gradual changes over distance as well as to sharp acoustic boundaries where distinct geographic song variants persist even in adjacent territories (Ellers & Slabbekoorn, 2003; Handley & Nelson, 2005; Podos & Warren, 2007). Acoustic boundaries may reflect population divergence but also affect behaviour and can therefore further promote reproductive divergence between acoustically distinct populations (Slabbekoorn & Smith, 2002a; Hudson & Price, 2014). However, song differences are also known to converge between overlapping populations, despite clear differentiation in allopatry (e.g., Tobias & Seddon, 2009; Kenyon et al., 2011), and individuals in contact zones may respond to both conspecifics and heterospecifics (Jankowski et al., 2010).

When song differences in contact zones fade, discrimination between species cannot be guaranteed. In many situations acoustic boundaries act as 'leaky barriers' which restrict and direct gene flow to a variable extent. Consequently, divergence in learned song may have little relevance to decreasing gene flow in some cases, but in other cases it may promote reproductive isolation, especially when geographic variation in song is related to ecological variation (Slabbekoorn & Smith, 2002a). For example, little greenbills

(*Andropadus virens*) in tropical Africa exhibit song variation related to both habitat and distance between populations (Slabbekoorn & Smith, 2002b), but playbacks revealed that far-away songs from the same habitat triggered similar response levels, whereas relatively nearby song recordings from different habitats triggered lower response levels (Kirschel et al., 2011). Only birds from ecotone forest showed this pattern of song discrimination, as birds from the rainforest responded equally strongly to all variants. Such asymmetric response patterns appear to be common among bird species (e.g., Robinson & Terborgh, 1995; Martin & Martin, 2001; Colbeck et al., 2010; Dingle et al., 2010; Ruegg et al., 2012; Caro et al., 2013; McEntee, 2014).

Contact zones between different species and subspecies of wood-wrens (Troglodytidae, *Henicorhina*) in South America have proven to be suitable study systems to gain insight into the impact of song variation on reproductive isolation. One established contact zone concerns two subspecies of grey-breasted wood-wren in Ecuador, *Henicorhina leucophrys leucophrys* (hereafter *leucophrys*) and *H. l. hilaris* (hereafter *hilaris*). Dingle et al. (2008) showed that the two subspecies exist across an ecological gradient with *leucophrys* occurring at high elevation in the Andes and *hilaris* at lower elevations. They sing different songs and their acoustic divergence is maintained in a narrow contact zone at mid elevation (Dingle et al., 2010). Playback experiments have shown that *leucophrys* has little or no response to *hilaris* songs, whereas *hilaris* responds strongly to both their own and to *leucophrys* songs (Dingle et al., 2010).

A remarkably similar situation has been found with two different wood-wren populations in Colombia, which are now recognized as two distinct species (Cadena et al., data not shown): *Henicorhina anachoreta* (hereafter *anachoreta*) and *H. leucophrys bangsi* (hereafter *bangsi*). Caro et al. (2013) reported acoustic variation in the songs of *anachoreta* and *bangsi* along an elevational gradient in the Sierra Nevada de Santa Marta, a mountain system separated from the main Andean ridges. The spectral song divergence in Colombia between the high- and low-elevation populations (i.e., higher-pitched songs at higher elevations) is congruent with the habitat-dependent pattern in Ecuador and suggests the existence of ‘acoustic ecotypes’ reflecting adaptation to similar acoustic environments (Dingle et al., 2010; Caro et al., 2013). Playback response results were also highly similar across studies: *anachoreta* discriminated, with low response levels to *bangsi* songs, while *bangsi* responded strongly to both their own and *anachoreta* songs (Caro et

al., 2013). An important difference, however, was that *bangsi* individuals did not discriminate at mid-elevation but did at low elevation: the low-elevation individuals were further away from the contact zone and responded stronger to *bangsi* songs than to *anachoreta* songs.

At the time of the earlier playback experiments in the Sierra Nevada de Santa Marta (Caro et al., 2013), the distribution of *anachoreta* and *bangsi* genotypes over the elevational gradient was not clear. As a consequence, *anachoreta* at high elevation and *bangsi* at mid- and low elevation were all tested with song stimuli recorded either at the high-elevation *anachoreta* or the low-elevation *bangsi* population (Caro et al., 2013). Therefore, playback response patterns may have been affected by the relative familiarity to song stimuli that were recorded at varying distances from the playback territory. High-elevation *anachoreta* and low-elevation *bangsi* responded stronger to songs of their own species than to songs of the other, but species differences were confounded by distance: ‘own population’ was compared to ‘far-away other’. In contrast, populations in Ecuador were never tested with song stimuli from their own population and stimuli origins were selected to compare ‘far-away own’ to ‘nearby other’ to avoid a confounding effect of familiarity or distance on subspecies recognition (Dingle et al., 2010, cf., Ruegg et al., 2012). The playbacks in the mid-elevation *bangsi* population were done comparing ‘nearby-own’ to ‘nearby-other’ (Caro et al., 2013), which may be biologically realistic but less conservative than the test in Ecuador.

In this study, we revisited two of the three earlier playback sites in Colombia to confirm that the asymmetric response pattern was not due to the confounding influence of familiarity with local songs. We went back to *anachoreta* and *bangsi* territories at high and mid elevations, respectively, to examine song response patterns across the now established contact zone. All the songs we used for playback originated from the populations that we played back to and the resulting comparison between responses to ‘nearby own’ and ‘nearby-other’ songs provides an optimally balanced reciprocal test that is also biologically realistic (because birds with both kinds of song variation are now within likely dispersal distance). Figure 1 illustrates the difference between the playback design in our study compared to the previous experiments of Caro et al. (2013). Furthermore, we analysed vocal response patterns in more detail than before. Grey-breasted wood-wrens can sing in a ‘fast’ or ‘slow’ mode, but previous studies have only focused on the former category (Dingle et al., 2010; Caro et al., 2013). The current study

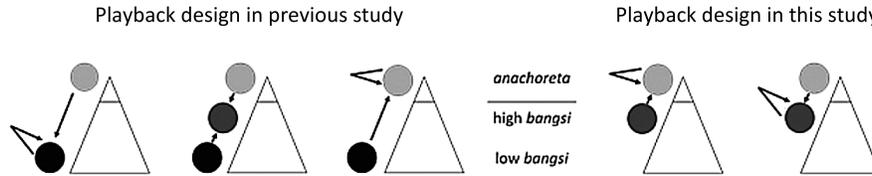


Figure 1. Playback design in the previous study from Caro et al. (2013) compared to our own playback design. The black circle represents the lowest *bangsi* population. The dark grey circle represents the high *bangsi* population, which during the playbacks of Caro et al. (2013) was of undetermined species type. The light grey circle represents the *anachoreta* population. Arrows originate in the source of the stimuli and indicate where the playback was conducted.

sheds light on species differences and the potential for a signalling function for these two singing modes that may also affect behavioural interactions between the species.

2. Methods

2.1. Study sites and subjects

We collected data on the San Lorenzo slope of the Sierra Nevada de Santa Marta mountain range, northern Colombia (11°07'46"N, 74°04'39"W; Caro et al., 2013). Two populations of the grey-breasted wood-wren replace each other on the elevational gradient. The populations were recently recognized as separate species, based on divergence in genetic, acoustic, and behavioural response data (Cadena et al., data not shown). *Anachoreta* territories are found at high elevation (range 2270–2810 m) in the thick undergrowth of relatively low, upper-montane forest. *Bangsi* territories are found at lower elevation (range 1100–2270 m) in the variable undergrowth of multi-layered, lower-montane forest (Caro et al., 2013). We sampled individual birds of both species on both sides of a narrow contact zone located around an elevation of 2270 m (Caro et al., 2013).

Both species of wood-wren from Santa Marta sing repetitions of stereotypic note sequences, which are referred to as their song. Each individual has a small to moderate repertoire of different song types that consist of different note sequences. They sing several bouts of one song type before switching to another (eventual variety singers). Males are most active in territorial behaviour, but both males and females sing solo or in duets. Song sequences can be sung in 'fast' mode or 'slow' mode. Fast mode songs are distinct clusters of rapid trains of the same song type, in which song duration is longer

than the short and typically consistent interval between subsequent songs. Such a cluster is referred to as a song bout and a song interval of 5.0 s or more is considered to separate one bout from the next. Slow mode songs are sung more or less separately with longer and more variable intervals.

2.2. Playback stimuli and procedure

We used recordings made in May and June 2009 during a previous study by Caro et al. (2013) to make the stimuli for our own experiments. We selected high-quality solo songs recorded in 10 *anachoreta* and 10 *bangsi* territories. We assumed that we used male songs because females rarely sing solos. We focused on the ‘fast’ singing mode because songs in this mode are often also the loudest and thought to be most important for territorial advertisement (Dingle et al., 2010; C. Dingle, pers. commun.). We generated 20 unique playback stimulus pairs for trials in 20 *anachoreta* territories and 20 *bangsi* territories. We randomly paired each of the ten *anachoreta* stimuli with one of the ten *bangsi* stimuli. Each pair of stimuli was used in two different orders: once starting with *anachoreta* song and once with *bangsi* song (Dingle et al., 2010; Caro et al., 2013).

We edited the song recordings of both species in the same way with Audacity software, version 2.0.0 (Unicode) for Windows 7. We composed series of four song bouts that each consisted of four to ten songs (number randomly assigned) taken from the same recording, which reflects the natural bout structure in grey-breasted wood-wrens and has been used before (Dingle et al., 2010; Caro et al., 2013). The intervals between songs within the bouts were kept the same as in the original recordings. Each playback stimulus lasted 2 min from the start of the first to the end of the last song. All recordings were band-pass filtered at the high and low end of the song frequency range to remove any sounds that were not wren song. Each stimulus was normalized to the same dynamic range, which enabled broadcasting of all recordings at an equal amplitude during our playbacks.

All our playbacks were done between April and July 2012. Breeding activity is not easily spotted per individual territory and does not need to be synchronized, but tested birds may have been in any phase with respect to breeding throughout this period. We used a SME-APS portable field speaker to playback songs from a Marantz PMD661 portable Solid State Recorder. All songs were played back at 85 dB(A) measured at 1 m from the speaker with a Voltcraft SL-100 digital sound pressure meter. After locating a wood-wren territory, placing equipment, and taking positions for observations and

recordings by two observers (T.B. and T.P.), we had 10 min of silence which allowed the birds to habituate to our presence. During the subsequent 5 min of silence we began recording vocal responses and made behavioural observations of baseline behaviour. We continued the recordings and observations throughout the first playback, which consisted of 2 min of playback and 3 min of silence. We then repeated the entire sequence for the second stimulus, including another 10 min of habituation time and 5 min of pre-playback behaviour observations. Each trial therefore lasted 40 min. We randomised the order in which we conducted the 20 playback trials in *anachoreta* territories and the 20 playback trials in *bangsi* territories to avoid any bias in response due to seasonal effects or level of experience of the observers. Trials were abandoned or postponed if the bird targeted for playback was involved in obvious counter-singing with a neighbour. The stimulus selection for each territory was also random, but we always checked to ensure that birds were not tested with a song of their own or a neighbouring bird.

We measured the strength of the behavioural response to playback by evaluating the closest approach to the speaker and the vocal output. Closest approach distance was measured as the minimum distance between subject and speaker during the trial. Distance categories were 0, >16 m; 1, 8–16 m; 2, 4–8 m; 3, 2–4 m; 4, <2 m (cf., Nelson & Soha, 2004; Dingle et al., 2010; Caro et al., 2013). These distances were estimated after the trial by measuring the distance between the speaker and the nearest point where the subject was observed. We always assumed two birds being present, but we typically saw one and sometimes two birds at the time. We scored proximity for the closest individual. Vocal output was scored from the sound files recorded during the playback trial and we included the number of fast songs, the number of slow songs, and the number of songs sung in duet (for the 5-min pre-playback periods and during each of the 5 min associated with the first and second playback stimulus).

We also selected all individuals for which we had clear recordings of both pre- and post-playback solo vocal activity to further examine species differences and contextual variation in the relative tendency to use the fast and slow song mode. The length of all pre-playback and post-playback songs for these individuals was measured from the sonograms, as was the interval between these songs. The mean song/interval length ratio was calculated by dividing the mean song length by the mean interval length for each individual both before and after playback. In this way the rate at which each

subspecies sang in the pre-playback period and the after playback periods was compared.

2.3. Statistical analyses

All data were tested for normality using a Shapiro–Wilk test. The data were not normally distributed and transformations could not correct for this. We therefore used non-parametric Wilcoxon signed-rank tests to check for significant difference in the response levels to conspecific and heterospecific song stimuli. All tests were two-tailed and we used $\alpha = 0.05$ as the threshold level for significance. We also checked for order effects to take any carry-over effect into account (i.e., consistently different response levels in the second trial due to their experience in the first). We used Wilcoxon signed-rank tests again to determine if response behaviour was significantly different from the baseline behaviour in the pre-playback period. We only used the first playback of each trial for this test, which reduces the sample size but avoids an effect of the possible carry-over effect. We checked for any effect of exposure to the playback on behavioural changes for each of the three vocal response variables. We did not do this for the closest approach distance to the speaker because the subject was often not visible before playback triggered the response behaviour.

3. Results

3.1. Species recognition

The results from our playbacks show that *anachoreta* wood-wrens responded more strongly to conspecific songs than to heterospecific songs exhibiting a shorter approach distance ($W = 105$, $p < 0.001$, Table 1) and a higher number of fast mode songs ($W = 116$, $p = 0.001$) (Figure 2b) and duet songs ($W = 103$, $p = 0.015$) (Figure 2c), but not with slow mode songs ($W = 33$, $p = 0.232$) (Figure 2a) (Wilcoxon signed rank tests). *Bangsi* wood-wrens responded equally strong to songs of both species and did not respond more strongly to either con- or heterospecific songs for any of the response variables (Wilcoxon signed-rank tests: closest distance approach $W = 71.5$, $p = 0.241$, Table 1; slow mode songs $W = 97.5$, $p = 0.793$; fast mode songs $W = 76.5$, $p = 0.678$; duet songs $W = 22.5$, $p = 0.374$) (Figure 2a–c). Together, these results provide confirmatory evidence for clearly asymmetric response patterns across the acoustic boundary between the two species.

Table 1.

A summary of the closest distance approach response for *anachoreta* and *bangsi* in response to con- and heterospecific playbacks.

Subspecies	Conspecific playback						Heterospecific playback							
	Distance categories					Median	SE	Distance categories					Median	SE
	0	1	2	3	4			0	1	2	3	4		
<i>anachoreta</i>	0	0	1	1	18	4	0.11	8	3	3	0	6	1	0.39
<i>bangsi</i>	4	1	1	3	11	4	0.37	5	2	4	3	6	2	0.36

The numbers of individuals recorded in each distance category are given, together with the median distance category and standard error. Distance categories: 0, >16 m; 1, 8–16 m; 2, 4–8 m; 3, 2–4 m; 4, <2 m.

We found no evidence for a significant carry-over effect in any of the tests, as there were no significant differences between response levels to the first and second stimulus in a trial (Wilcoxon signed-rank tests: *Anachoreta*: closest distance approach $W = 41$, $p = 0.483$; fast mode songs $W = 49$, $p = 0.55$, duet songs $W = 42$, $p = 0.318$; slow mode songs $W = 25.5$, $p = 0.765$. *Bangsi*: closest distance approach $W = 53.5$, $p = 0.975$; fast mode songs $W = 76.5$, $p = 0.678$; duet songs $W = 22.5$, $p = 0.374$; slow mode songs $W = 97.5$, $p = 0.794$). When comparing vocal response to pre-playback activity we only found a significant rise in the case of *bangsi* responding to conspecific song playback for songs sung in the fast mode ($W = 0$, $p = 0.036$). All other test results were non-significant, which may be due to the fact that singing activity was quite variable, both before and after onset of playback (*anachoreta* response to conspecific stimuli: fast mode songs $W = 5$, $p = 0.149$, duet songs $W = 4.5$, $p = 0.128$; slow mode songs $W = 0$, $p = 0.181$. *Anachoreta* response to heterospecific stimuli: fast mode songs $W = 0$, $p = 0.058$, duet songs $W = 6$, $p = 0.181$; slow mode songs $W = 0$, $p = 0.371$. *Bangsi* response to conspecific stimuli: duet songs $W = 2.5$, $p = 0.462$; slow mode songs $W = 2$, $p = 0.052$. *Bangsi* response to heterospecific stimuli: fast mode songs $W = 7$, $p = 0.271$, duet songs $W = 0$, $p = 0.174$; slow mode songs $W = 0$, $p = 0.059$).

3.2. Singing style

The variation in song and interval length showed clear differences in distribution patterns between the two species. *Anachoreta* sang very few songs in

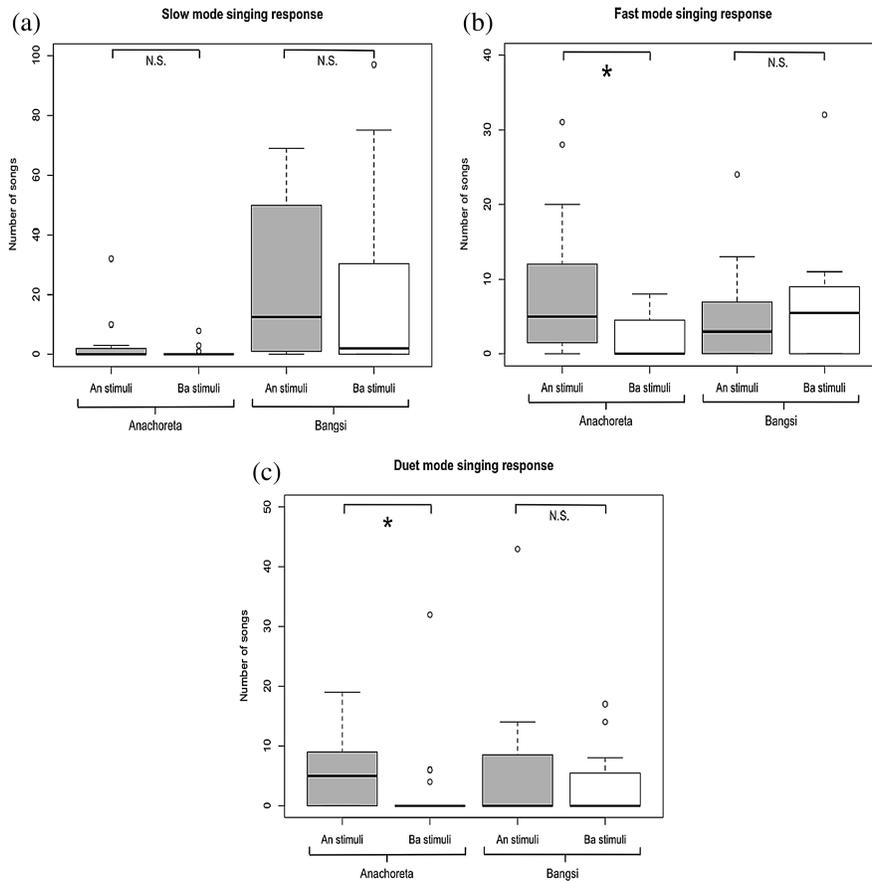


Figure 2. Results of the playback experiments showing total number of songs recorded from all individuals for each song type. Bars on the left represent *anachoreta* response. Bars on the right represent *bangsi* response. Bars in grey represent response to *anachoreta* stimuli and bars in white represent response to *bangsi* stimuli. Asterisks over brackets between pairs of bars indicate that response to con- and heterospecific song in that species differed significantly. $N = 4 \times 10$, the same 40 individuals for each graph: (a) slow singing response; (b) fast singing response; (c) duet singing response. Note that the y-axes differ between the graphs due to the variation in occurrence among singing modes.

the slow mode overall, whereas *bangsi* sang a similar number of songs in each mode. *Anachoreta* songs were significantly longer than *bangsi* songs (mean duration of *anachoreta* songs 1.71 ± 0.41 s, *bangsi* 0.84 ± 0.66 s; Mann–Whitney U -test: $W = 1171$, $p \leq 2.2e-16$). *Anachoreta* songs were 1.71 s on average and ranged between 0.6 and 3.2 s in a unimodal distri-

bution; the songs of this species were typically separated by short intervals that only very rarely lasted beyond 0.6 s. Figure 3a shows an example of a typical *anachoreta* fast mode song and Figure 3b shows a much rarer *anachoreta* slow mode song. *Bangsi* songs showed a clear bimodal distribution with peaks at 0.3 and 0.9 s within the range from 0.1 to 2.0 s. Figures 3c and 3d show typical fast and slow mode songs for *bangsi*, respectively. *Bangsi* song intervals were highly variable, with most in the range up to 0.8 s, but also often beyond with longer intervals up to 5.0 s becoming only gradually less common. The majority of *anachoreta* song intervals were between 0.1 and 0.6 s.

The relative frequency of fast and slow song mode can be derived from Figure 4. Values below 1.0 reflect slow mode songs and values above 1.0 reflect fast mode songs (based on 83 songs from 5 *anachoreta* individuals and 252 songs from 5 *bangsi* individuals). Many individuals were not suitable for this analysis for various reasons such as that recordings did not contain both pre- and playback solo singing activity or that the singing activity was masked by the playback and we were unable to take accurate measurements. It is clear that *anachoreta* individuals sing on average only fast mode, before and after on-set of playback, whereas *bangsi* individuals vary much more and can switch in bias from more slow mode to more fast mode songs and vice versa. There was no consistent change to singing faster or slower in response to playback for *anachoreta* or *bangsi* (Wilcoxon signed-rank test: $V = 5$, $p = 0.625$ and $V = 7$, $p = 1$, respectively).

4. Discussion

Our playback results confirm an asymmetric response pattern to heterospecific song between species of wood-wrens across a distinct acoustic boundary in the Sierra Nevada de Santa Marta in Colombia. High-elevation *anachoreta* wood-wrens discriminate and respond significantly less to mid-elevation *bangsi* songs than to their own. In contrast, *bangsi* wood-wrens do not discriminate and respond equally to *anachoreta* song and to their own. We confirmed the behavioural pattern through the use of local stimuli, removing the confounding factor of familiarity which may have influenced response levels in an earlier study (Caro et al., 2013). Significant differences were found for vocal response patterns in fast mode and duet songs, but not for slow mode songs. However, *anachoreta* sang very few songs in slow

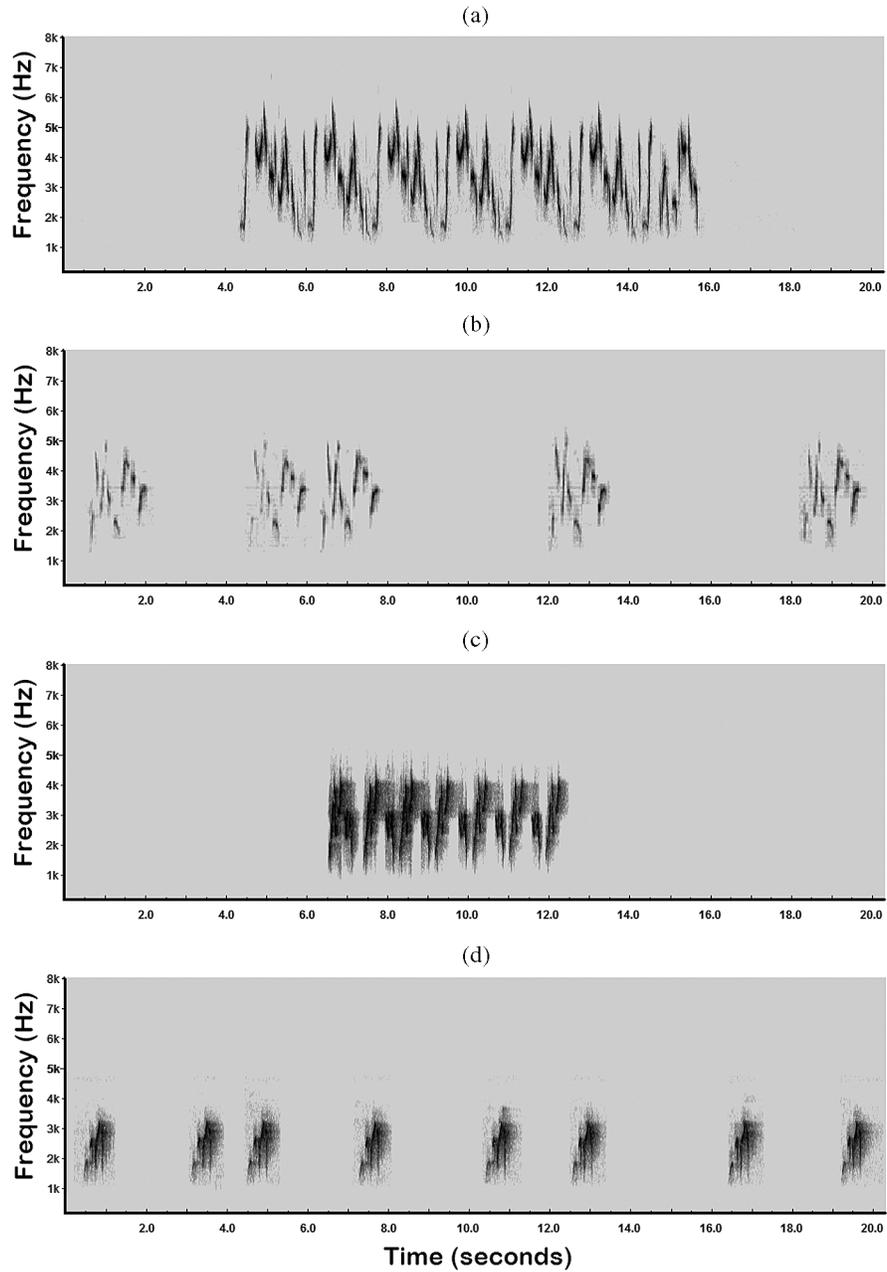


Figure 3. Examples of typical wood-wren songs: (a) *anachoreta* fast; (b) *anachoreta* slow; (c) *bangsi* fast; (d) *bangsi* slow.

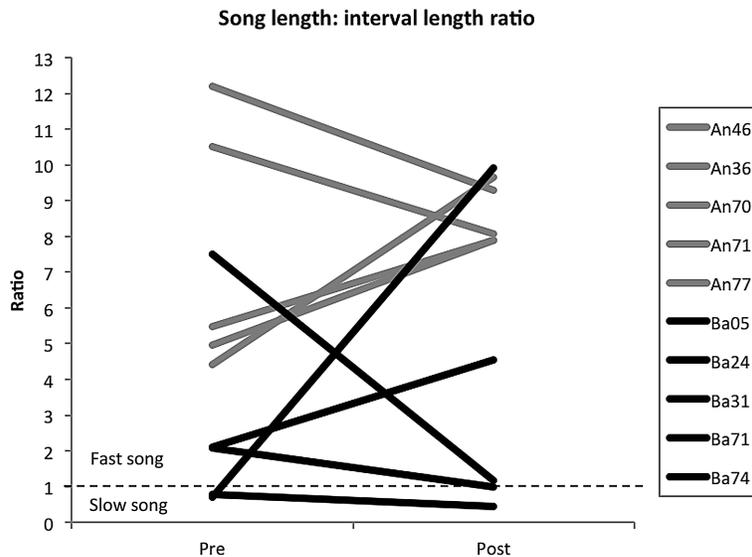


Figure 4. Average song length: interval length ratio for pre-playback compared to post-playback activity ($N = 10$, 5 *anachoreta* and 5 *bangsi* individuals). The song length/song interval length ratio gives a temporal measure of the singing speed. Measurements were taken from all the activities recorded for that individual, including response to both con- and heterospecific playback. Grey lines represent *anachoreta* individuals and black lines represent *bangsi* individuals. The dashed line indicates the division between fast and slow song, with a ratio < 1 indicating slow song and a ratio > 1 indicating fast song.

mode whereas *bangsi* sang a majority of songs in the slow mode, a clear difference in singing style between the two species. We also found for *bangsi* that singing birds may switch rapidly or gradually between modes during a playback trial, although we found examples of switching ‘from fast to slow’ as well as ‘from slow to fast’ when comparing pre- and post-playback periods.

4.1. Asymmetric response pattern

The fact that distinct songs affect behaviour in at least one species in a contact zone does not yet confirm but supports the hypothesis that behaviour may play a role in reducing gene flow between closely related lineages (e.g., Martin & Martin, 2001; Colbeck et al., 2010; Kirschel et al., 2011). In our study, we assume it was mostly the male that was vocally active, but the female may also have been active on occasions as we could not distinguish the sexes while moving around in the dense understory and they have been

active for certain in the duetting responses. For a full understanding of impact of the asymmetric response pattern on gene flow, we should study both male and female responses (see, e.g., Nelson & Soha, 2004; Patten et al., 2004; Danner et al., 2010) as addressed in Dingle et al. (2010).

The asymmetric response we observed in wood-wrens mirrors the situation observed for these birds in Ecuador, where high elevation *leucophrys* discriminate between conspecific songs and songs from the lower elevation *hilaris* (Dingle et al., 2008). In that study, a difference in spectral sensitivity, caused by selection or learning, was suggested as an explanation for the asymmetric discrimination. Tuning of the sensory system to the range of natural variation in a signal has been reported for other bird species (Slabbekoorn & ten Cate, 1998; Endler, 1999). We cannot exclude that this also applies to the Colombian wood-wrens. However, although the song frequency range of *anachoreta* covers the range of *bangsi* and not vice versa, *bangsi* may also generate calls that do cover the wider frequency range of *anachoreta*. Therefore, it remains to be investigated whether relative response strength to unfamiliar songs is dependent on whether their frequency ranges are partly or fully overlapping with frequency ranges of local songs and calls.

We further postulate that in Colombia the difference in temporal structure of song (i.e., the occurrence of fast and slow mode songs) may also influence the asymmetric response pattern. Although we did not use slow mode songs in playbacks, our discovery that individuals of the low elevation species *bangsi* commonly use the slow singing mode, whereas individuals of the high elevation species *anachoreta* almost never sing in this mode, agrees with the finding of Caro et al. (2013) that individuals at higher elevation generally sing faster. Future playbacks should explore the communicative value of singing mode within and across species.

Caro et al. (2013) showed that spectral variation in fast songs between *anachoreta* and *bangsi* individuals varied in parallel with morphological variation. Slower song in *bangsi* may therefore be a by-product of adaptive evolution in morphological traits (Slabbekoorn & Smith, 2000). The use of slow mode songs could lead to *bangsi* having a wider perceptual range of temporal differences, therefore allowing *bangsi* individuals to recognise *anachoreta* songs but not vice versa. Different singing rates have been shown to influence individual response to playback in other studies, both between

subspecies (Patten et al., 2004) and within the same subspecies when comparing current and historical songs (Derryberry, 2007). Further playbacks using a wider temporal range could test the importance of singing rate in contributing to the asymmetric response in the wood-wren populations.

Furthermore, an alternative explanation that should be explored further is the possibility of a behavioural dominance of *bangsi* over *anachoreta*, independent of any perceptual ranges (cf., Mc Entee, 2014). Although, we did not observe any obvious species differences in mean aggression levels, the biased occurrence of song modes may suggest a higher general activity level in agonistic interactions for *bangsi* compared to *anachoreta*. However, from the approach distance data it is clear that *anachoreta* is clearly responding very strongly to their own songs (18 out of 20 birds approaching to within 2 m of the speaker) and that the difference between the species is not a matter of inherent differences in species-specific boldness.

4.2. Division of song modes

In this study, we included the slower singing mode in the analysis of response for the first time. Previous work both in Ecuador and Colombia has focused on examining fast song because it is reported to be the predominant singing style and to have relatively high amplitude and therefore is presumed to function in communication over longer distances (Dingle et al., 2008). However, examining song lengths revealed a bimodal distribution for *bangsi*, with the shorter songs, sung at a slower tempo, being as common as the longer, faster-paced songs. Considering the frequency with which the slow mode songs were observed in *bangsi* post-playback, we believe that it could have a function in territorial defence behaviour and should not be dismissed.

Anachoreta showed a discriminatory pattern which was reflected in response differences for both fast solos and duets, whereas there were no significant differences between response to con- and heterospecific songs for any of the categories for *bangsi*. There was no significant difference for *anachoreta* for slow mode songs as they very rarely use this singing category. We believe, however, that all singing modes should be considered in analyses because counting only fast songs would reflect only part of the vocal repertoire, especially in *bangsi*. Furthermore, considering all songs in future studies may be vital for the understanding of song function because different singing modes may serve different purposes. Future studies are needed

because it is still unclear in what context individuals chose to sing fast or slow songs or whether they would respond differently to playback of the two categories.

Bangsi individuals switched regularly between both modes, with some birds responding to playback by changing from fast to slow mode and some from slow to fast mode. They were also observed to match song types on two occasions. Although other bird species are known to use song switching and song matching in territorial disputes as an aggressive tactic (Nelson & Vehrencamp, 1995; Molles, 2006; Fitzsimmons et al., 2007; Vehrencamp et al., 2007), we did not see a consistent pattern of *bangsi* individuals singing slower or faster after playbacks nor did we observe song matching in many trials. However, we do think follow-up studies are warranted because consistent switching or song matching may depend upon many factors, for example the stimulus and the receiver quality (e.g., Linhart et al., 2012). Variability in upward or downward shifts in response levels to different categories of playback stimuli depending on the relative threat have been reported for the closely related banded wrens (de Kort et al., 2009).

4.3. Conclusion

We have confirmed the asymmetric response pattern between *anachoreta* and *bangsi* wood-wrens, which verifies the potential role of song as a prezygotic mating barrier between the populations. Lack of song recognition reduces the potential for hybridization and is most likely contributing to the maintenance of distinct species (Caro et al., 2013; Cadena et al., data not shown). We have also revealed a novel type of vocal divergence in that slow mode songs are commonly used in territorial defence in one species but not in the other. We hope our attention to methodological rigor and experimental design, as well as the attention to the potential divergence in the use of vocal repertoire between closely related species will stimulate future studies in this discipline, as our findings may apply more widely to other avian systems.

Acknowledgements

We thank W. Halfwerk for valuable advice whilst planning the project, P. Pulgarin for assistance in the field and C. Dingle for sharing her knowledge and experiences from Ecuador. Funding was provided by KNAW Dobberke

Foundation for Comparative Psychology and four funds from Leiden University: Outbound Study Grant (OSG), Leids Universitair Fonds (LUF), LUS-TRA Scholarship and Curatorenfondsen.

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