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Article

Duet codes do not enhance neighbour recognition in two closely related species of duetting neotropical wrens

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Numerous studies have shown that territorial animals exhibit less aggression in response to neighbours than to strangers, a phenomenon known as dear enemy effect. The influence of acoustic features, such as song type sharing and repertoire sizes, in neighbour recognition has been widely documented in male songbirds. However, few studies have focused on duetting species, and particularly on those where pairs have pair-specific duet codes (consistent associations of their individual phrase types). Given that each pair in the population can have a unique repertoire of duet types, duet codes have been hypothesized to enhance discrimination. In this context, we tested for evidence of neighbour recognition and duet code discrimination in two closely related species of neotropical wrens, the riverside wren, *Cantorchilus semibadius*, and the canebrake wren, *C. modestus zeledoni*. Although both species have moderately large repertoires, riverside wrens have higher levels of phrase type and duet type sharing across the population. We compared the approach and vocal responses of focal pairs to three playback treatments: neighbours' correct duet type, neighbours' incorrect duet type and a strangers' duet type. We found that riverside wrens displayed a stronger response to the strangers' playback than to both neighbours' playbacks, whereas no differences among treatments were found in canebrake wrens. Given that both species exhibited similar levels of aggression during neighbour playbacks, regardless of whether the correct duet code was used, our findings suggest duet codes do not facilitate neighbour recognition. We conclude that the function of duet codes in these species might be more closely related to intra-pair communication. Finally, we suggest that the level of dear enemy effect a species exhibits depends on ecological factors that influence the perceived level of threat of territory intruders.

Keywords: canebrake wren, duet code, duet type, neighbour recognition, phrase type, riverside wren



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Introduction

In social animals, interactions between individuals are crucial for regulating conflict over limited resources, such as food, mates and space (Naguib 2005). Interactions are influenced by the ability of animals to distinguish among classes of individuals, and even among individuals (Snijders and Naguib 2017). For instance, several studies with amphibians, mammals and birds have shown that territory owners react less aggressively to intrusions by their neighbours than to intrusions by non-neighbours or strangers (Falls et al. 1982, Davis 1987, Temeless 1994, Frommolt et al. 2003, Wiley 2013). This difference in the responses was coined by Fisher (1954) as ‘dear enemy effect’. Here, territory holders stand to lose more with newcomers that might be looking for a territory or a mate, than they do with long-term neighbours that already have a territory and know their established boundaries (Beecher and Brenowitz 2005, Naguib 2005).

Within songbirds, song sharing and repertoire sizes have been suggested to influence neighbour recognition during aggressive encounters (Kroodsmma 1976, McGregor and Avery 1986, Stoddard et al. 1991). When individuals within a population have high levels of song type sharing or sing similar versions of song types, discrimination between local and foreign conspecific songs might be facilitated, as seen in many species including cardinals (*Cardinalis cardinalis*, Lemon 1974), white-crowned sparrows (*Zonotrichia leucophrys*, Verner and Milligan 1971), yellow-napped amazon parrots (*Amazona auropalliata*, Wright and Dorin 2001), skylarks (*Alauda arvensis*, Briefer et al. 2008) and great tits (*Parus major*, Mockford and Marshall 2009). Nevertheless, if neighbouring birds within a population sing similar versions of a song type or if they have high levels of song type sharing, then it can make these individuals harder to distinguish for the local residents, as seen in tufted titmice (*Baeolophus bicolor*, Schroeder and Wiley 1983), great tits (McGregor and Avery 1986) and song sparrows (*Melospiza melodia*, Beecher et al. 1994). As for repertoire sizes, initially it was suggested that bigger repertoires hinder neighbour recognition because they imply more songs to learn and remember, as well as leading to less exposure to each song, thereby reducing the familiarity of each song type of the neighbouring individuals (Kroodsmma 1976, Falls et al. 1982, Stoddard et al. 1991). However, a considerable number of studies have shown that males of species with moderate to large song repertoires not only can discriminate between neighbour and stranger vocalizations but also discriminate between the regular and opposite boundaries of neighbouring birds (Weary et al. 1992, Godard and Wiley 1995, Stoddard 1996, Briefer et al. 2008, Moser-Purdy and Mennill 2016).

A particularly complex behaviour that might also influence neighbour recognition during vocal encounters is duetting. Duetting involves two or more individuals, usually a mated pair, singing together to create a joint performance (Hall 2004, 2009). In some duetting species, each individual possesses a repertoire of sex-specific phrase types that they sing following specific rules with their mate (Mann et al.

2003, Logue 2006, Templeton et al. 2013). These sets of rules producing non-random duet types are defined as ‘duet codes’ (Logue 2006). Despite all of the research focused on the functions of duetting, it remains largely unknown whether duet codes play a role in between-pair interactions, within-pair interactions or in both contexts. However, species that present duet codes must be highly attentive because individuals have to make quick adjustments regarding their timing and phrase selection depending on their partner’s choice while duetting (Hall 2009). For instance, in an experiment with happy wrens, *Pheugopedius felix*, males were able to reply with the appropriate response according to their pair-specific duet code to a broadcast phrase of their mate in a fraction of a second (Templeton et al. 2013).

Duet codes have been proposed to function in intra-pair communication by facilitating mate identification and mate location during non-agonistic and agonistic interactions (Farabaugh 1982, Logue and Gammon 2004, Logue 2007). Given the densely vegetated environments in which neotropical wrens live, having a system of coded answering might ensure communicating more reliably the location and identity of each bird to its mate, thus avoiding the possibility of misdirected aggression, and allowing the pair to coordinate and focus their efforts on intruding rivals (Farabaugh 1982, Logue 2006, 2007). Likewise, duet codes might participate in intra-pair communication by improving duet timing coordination (Logue et al. 2008, Mann et al. 2009). Considering that there is variation in the length of the different phrase types in each individual’s repertoire, being able to anticipate when the opposite sex’s contribution will end increases the chances that birds can begin their contribution at a precise time and maintain high levels of coordination (Logue et al. 2008, Mann et al. 2009). Finally, duet codes might be useful in between-pair interactions if individuals pay attention to the duet codes of their neighbouring pairs and are able to reliably discriminate between neighbours and strangers, or even between different neighbours (Logue 2006). Considering the limited number of studies that have investigated neighbour recognition on the basis of playback of duets and the contrasting results found to date (Wiley and Wiley 1977, Hall 2000, Grafe and Bitz 2004, Odom and Mennill 2010), it remains unclear how common neighbour recognition is in duetting birds. Furthermore, in species that present pair-specific duet codes, no study has been carried out to test whether individuals learn only the phrase types each neighbouring individual sings or if birds also learn the duet types their neighbours sing as a mated pair.

In this study, we tested for evidence of neighbour recognition in two closely related duetting species. Both have moderately large individual phrase type repertoires and pair-specific duet type repertoires, but present different levels of song sharing across the population. First, we investigated whether wrens pay attention and recognize the individual phrase types and/or the duet types used by their neighbours. Second, we evaluated if the species having higher levels of phrase and duet type sharing would have an increased difficulty in distinguishing neighbours from strangers. These questions were tested in two neotropical species, riverside wrens *Cantorchilus*

semibadius and canebrake wrens *C. modestus zeledoni*. The basic duet structure for both species is I(FM)_n, with the male introductory phrase (I) leading to a rapid cycle of repeated female (F) and male (M) song phrases. In these taxa, duet codes generate discrete duet types and mid-duet song type switching is not frequent. Individuals from both species select from a repertoire of sex-specific F and M phrases both to perform solo songs and to contribute to duets (Mann et al. 2009). In riverside wrens, I phrases are not always produced in duets, with duets also initiated with F or M phrases (Mann et al. 2009, Quirós-Guerrero et al. 2020a). Females and males have 20–30 phrase types, which they combine to form a repertoire of 25–35 duet types (Quirós-Guerrero et al. 2020a). In canebrake wrens, females and males have 15–25 phrase types, which they combine to form a repertoire of 20–30 duet types (Mann et al. 2003). While riverside wren individuals share on average 70% of their phrase types with other same-sex birds and pairs share on average 32% of their duet types with other pairs in the population (Quirós-Guerrero et al. 2020a), canebrake wrens share on average 50% of their phrase types with other same-sex birds and less than 20% of their duet types with other pairs in the population (Marshall-Ball and Slater 2008). We presented focal pairs of each species with three playback treatments: a correct duet type from a neighbouring pair, an incorrect duet type from the same neighbouring pair, and a duet type from a stranger pair. The neighbours' correct duet type and the strangers' duet type were used to test if birds display the 'dear enemy' effect and respond more aggressively to strangers' than to neighbours' vocalizations. The neighbours' correct and incorrect duet type were used to test if birds pay attention to the duet code used by their neighbours. Vocal recognition in territorial encounters of duetting species can occur at two levels, territorial pairs can recognize individual phrases (just as in non-duetting species) and/or they can recognize particular duet types sung by neighbouring pairs (unique to duetting species). If wrens rely only on individual recognition, then we expect that focal birds will respond similarly to the neighbour correct and incorrect duet types. However, if wrens use duet codes to facilitate pair recognition, then we expect that focal birds will respond differently to the neighbour correct and incorrect duet types. Finally, differences in the sharing levels between species allowed us to test whether phrase type and duet type sharing influence neighbour recognition. We predicted that if repertoire sharing hinders discrimination, riverside wrens would show weaker discrimination and more similar responses to the neighbour and stranger treatments, given that individuals have fewer unique songs in their repertoire.

Material and methods

Study sites and study species

We conducted the experiment with riverside wrens at Osa Conservation's Piro field station in southern Costa Rica (8°24'N, 83°20'W). The station is in lowland and wet

rainforest on the Osa Peninsula. Riverside wrens are common around the station, especially next to rivers and wetlands (Skutch 2001). We have studied this population since 2013 and we have colour banded almost 130 individuals at the study site. We conducted the experiment with canebrake wrens at La Selva Biological Station in northern Costa Rica (10°26'N, 83°59'W). The study site includes a mixture of lowland moist forest, swamps, scrub and cattle pasture, where canebrake wrens are common (Mann et al. 2003). We have studied this population since 2012 and we have colour banded almost 60 individuals at the study site.

We carefully mapped the territory boundaries of individuals in the two species prior to the experiments by following focal pairs while they sang and moved throughout their territories, especially when they participated in territorial disputes and interacted with their neighbours during the dawn chorus. Almost all pairs included in this study were monitored in two consequent years to be as accurate as possible with the borderlines between territories. We mapped the boundaries with a hand-held global positioning system (Garmin GPS-60SCx, Garmin, Olathe, KS, USA). Territory sizes are slightly different between species, with riverside wren territories averaging 0.61 ± 0.04 ha (1st author unpubl.) and canebrake wren territories averaging 0.4 ± 0.05 ha (2nd author unpubl.).

Playback stimuli

Prior to the playback trials we collected recordings to use as playback stimuli. For the neighbours' treatments we recorded a neighbouring pair of each focal pair on several different days. Neighbour correct was a duet that was recorded, and therefore sung, on at least three separate days, to ensure this combination was a correct and frequently used duet type in the neighbours' repertoire. Neighbour incorrect was a duet that we experimentally created by combining phrase types from two different duet types, so that the male contribution of one (I and M phrase) was paired with the female contribution of another (F phrase). We chose a combination not present in the recordings of all immediate neighbouring pairs of the focal pair, although this combination could exist in non-neighbouring pairs across the population. Finally, for the stranger stimuli we selected recordings of pairs that were at least 1 km away (more than seven territories) from the focal pair's territory centre to reduce the chance that our focal birds would have had any prior experience with the particular pair whose songs we broadcast. We recorded the songs using a Sennheiser ME66 directional microphone and a Marantz PMD670 solid-state digital recorder (Recording format PCM-16, sample rate 44.1 k at 16-bit quantization). We selected good quality songs with a high signal-to-noise ratio (a measure that compares the level of a signal, in our case the duets, compared to the background noise) and no other vocalizations in the background. Additionally, we selected songs where focal birds sang side by side ensuring that amplitude would be at natural levels when broadcast from the speakers and that the degree of coordination was relatively consistent across stimuli (Mennill and Vehrencamp 2008, Mann et al. 2009). Based on

our assessments in the field, natural amplitude for both species of wrens is 75–85 dB at 2 m of distance. To create stereo-duet playbacks (Mennill 2006), we duplicated the one-channel recordings and then carefully removed all of the phrases from one sex in one file and all of the phrases from the other sex in a second file with the frequency cursor filter function in Syrinx (J. Burt, Seattle, Washington, USA) using a Hanning window with a 512 pt FFT and a temporal resolution of 5.8 ms. We then used Audacity (<<http://www.audacityteam.org>>) to normalize each file so that the peak amplitude was 0 dB. We created a two-channel stereo sound file containing one channel with the female phrase types and one channel with the male introductory phrase and male phrase types, thus keeping the exact timing of the original duet. Phrases selected for the artificially created duets (neighbor incorrect treatment) maintained a similar, very short, gap between male and female phrases found in natural duets (average: 0.006 s; Mann et al. 2003, Mann et al. 2009). The contribution of each sex was randomly assigned to the left or right channel (with a coin flip). The stimuli consisted of 10 bouts of duets with one minute of silence after the first five bouts. Each bout consisted of one male introductory phrase and seven phrase types of each sex (I(FM)₇) (Fig. 1) and was separated from the next bout by 10 s. This song timing is consistent with the mean phrases per duet and mean inter-phrase duration previously reported for both species (Mann et al. 2009).

Playback design

We used two connected speakers (Foxpro Fury with FoxPro SP-55 External Speaker) to broadcast the female and male contributions as a stereo-duet playback (Mennill 2006). These two speakers produce standardized outputs (FoxPro Inc., PA, USA) and to our ears they sound equivalent in terms of quality and amplitude (Templeton et al. 2011, 2013). Because we had randomized which speaker played the female/male contributions for each trial, even if there were differences between speakers, they should not produce any bias in the data. We adjusted the speaker volume to simulate that of natural singing, approximately 80 dB at 1 m. The speakers were set 1.5 m above the ground and 2 m apart to facilitate accurate measurements of the approach from each individual to each speaker. Both riverside and canebrake wrens commonly sing duets at this height and from this distance (1st and 2nd authors pers. obs.). The speakers were placed at the same location for the three treatments, always positioned at the boundary between the two territories to simulate as accurately as possible the neighbours' behaviour and, if possible, where we had previously witnessed both pairs interacting and demarcating their territory boundaries. Each pair was subjected to three trials delivered in random order, with one day between each trial.

Data collection

The trials were conducted with 12 pairs of riverside wrens (20 out of the 24 individuals were previously captured and banded) from the 15th of April to the 4th of May 2016; and

with 13 pairs of canebrake wrens (21 out of the 26 individuals were previously captured and banded) from the 10th to the 28th of May 2016 (Fig. 2). All trials took place between 5:30 a.m. and 8 a.m. to minimize effects of time of day on the behavioural responses to playback. We chose this time period when birds are especially vocal, before temperature and humidity increased later in the day. We attempted to control the time of day and weather by testing the same pair at nearly the same time in each trial and by avoiding performing trials on rainy days. Each trial consisted of a 5 min pre-playback period, followed by 4 min of playback and a 5 min post-playback period. Riverside wrens were atypically inactive during this season (when compared with 2013, 2015 and 2017), and during the pre- and post-trial period very few behaviours were observed. Therefore, we focused our analyses on the 4-min playback period. Given that the trials lasted no more than 14 min, neighbour interference was rarely experienced. On the two occasions when neighbours approached or sang in response to our playback during the experiment, we aborted the trial and performed it two days later.

During the trial two observers monitored the behaviour of the focal pair. Both observers were positioned 5 m away from the speakers to maximize the accuracy of distance measurements while minimizing our overall influence on the birds' approach response. We have frequently seen that birds are not deterred by us at this distance during previous experiments (Rivera-Cáceres et al. 2016, Quirós-Guerrero et al. 2017). Focal birds were typically within sight and easy to track, but in some territories with especially dense vegetation, the location of the birds was sometimes estimated from their songs. We recorded all vocalizations from the focal individuals and continuously assessed the distance of each bird to both playback speakers. Distances were measured at 1 m intervals by comparing known distances of natural landmarks in the territories to where birds were located.

We investigated focal pairs' responses by measuring approach and vocal variables designed to assess both individual and collective behaviours (Logue and Krupp 2016). The individual behaviours considered were: 1) female mean distance to the closest speaker, 2) male mean distance to the closest speaker, 3) female closest distance to any speaker, 4) male closest distance to any speaker, 3) female song initiation, 4) male song initiation, 5) female responsiveness and 6) male responsiveness. The collective behaviours considered were: 7) duet rate (duets/min) and 8) duet length (number of FM repetitions per duet). Initiation was measured as the number of instances an individual started singing (solos + initiated duets). Responsiveness was defined as the propensity of each sex to answer its partner's song. We assessed responsiveness by calculating the proportion of the partner's songs that were joined to form duets (e.g. female responsiveness = male led duets/(male solo songs + male led duets)).

Data analysis

For both approach and vocal traits, mixed-effects models were adopted, in which territory and trial order were set

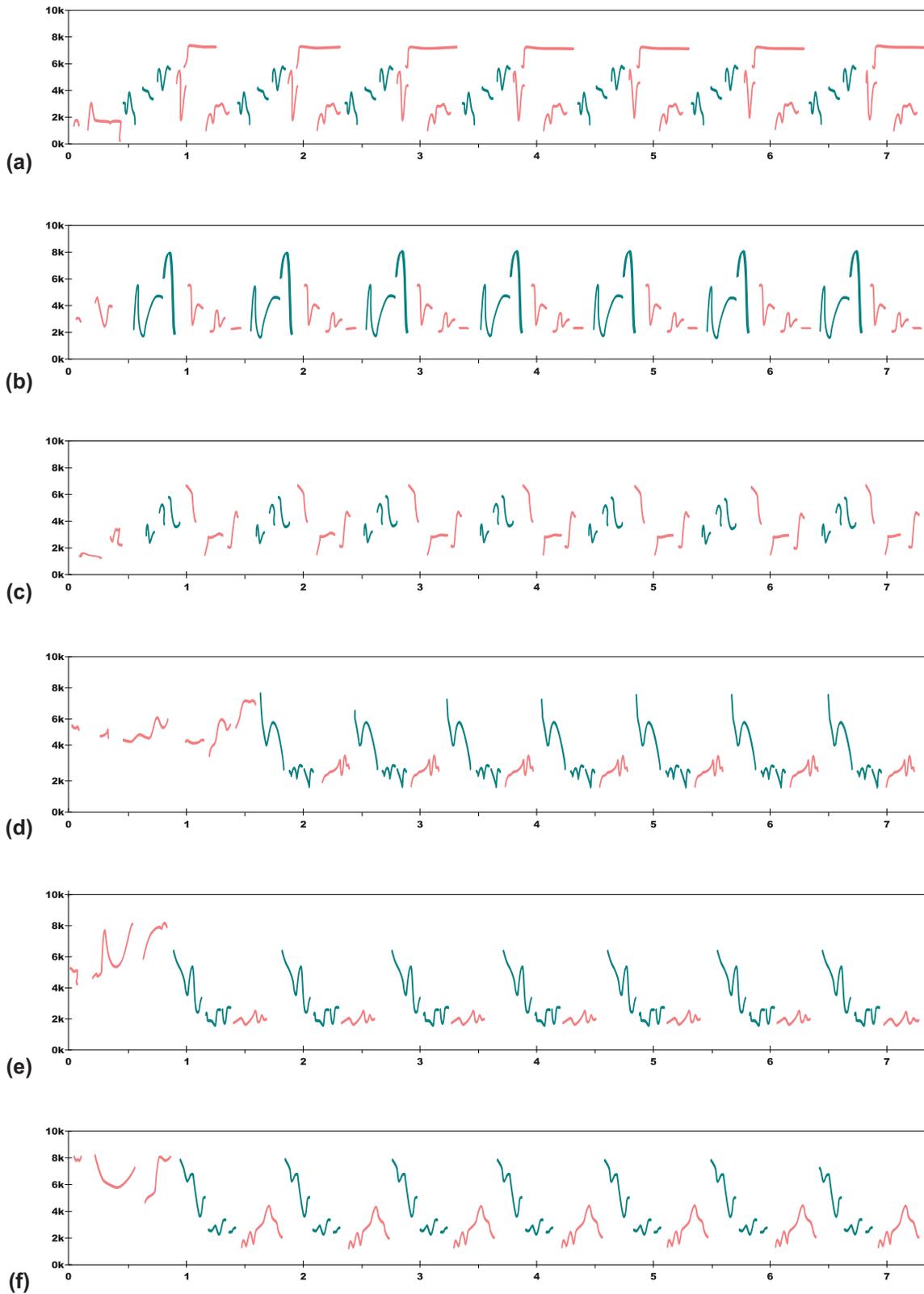


Figure 1. (a–c) Examples of three duet types used for the neighbour correct, neighbour incorrect and stranger treatments in riverside wrens. (d–f) Examples of three duet types used for the neighbour correct, neighbour incorrect and stranger treatments in canebrake wrens. Male introductory phrases and phrase types are depicted in coral and female phrase types are depicted in teal.

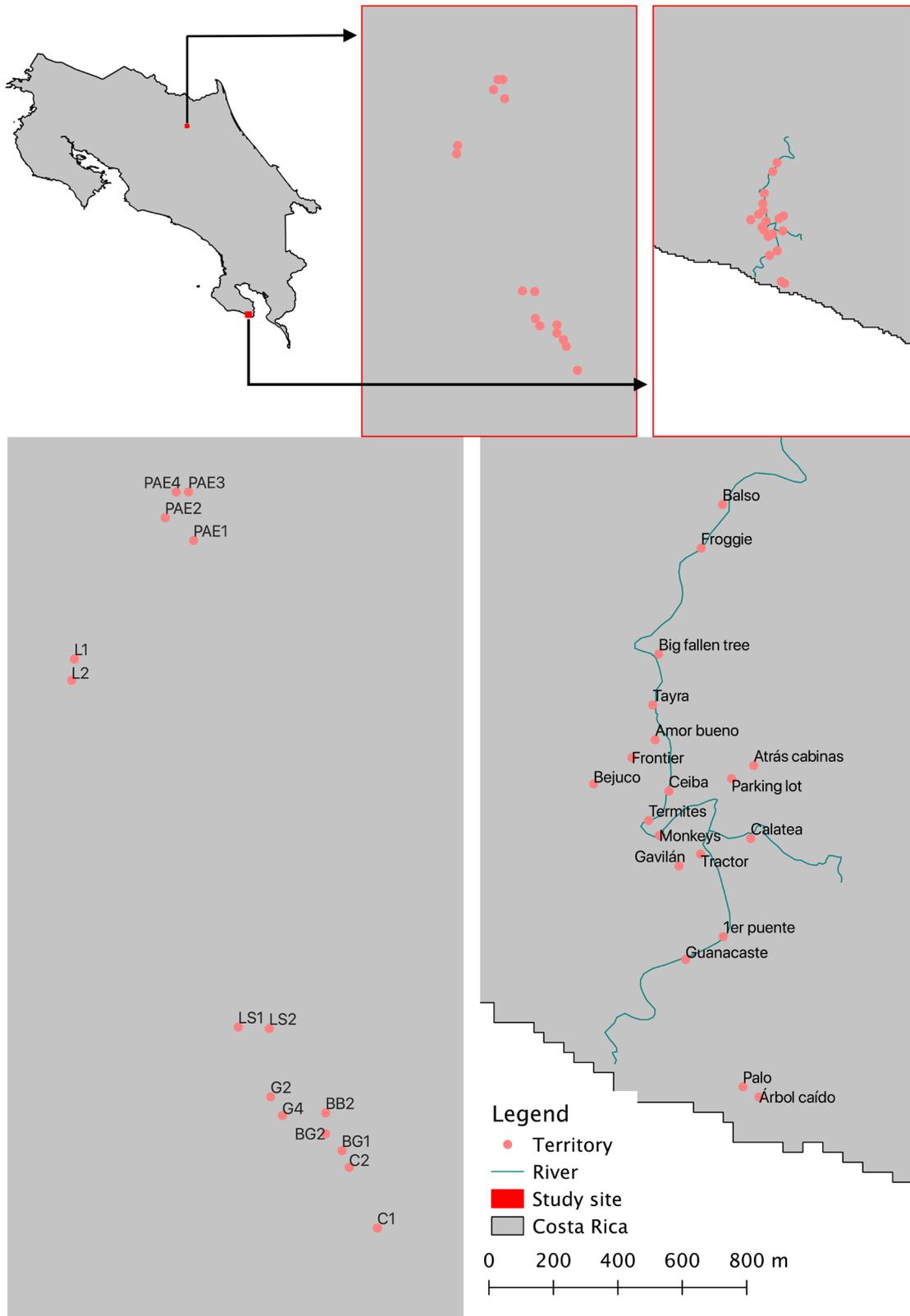


Figure 2. Study sites in Piro Osa Conservation and La Selva biological stations, Costa Rica. A detailed list of the focal and corresponding neighbouring pairs is provided in the Supporting information.

as random effects (in which the effect of order was nested within that of territory). Distance-related traits, as well as responsiveness, were modelled using Gaussian distributions, whereas duet rate, duet length and initiation were assumed to follow negative binomial distributions. The latter probability distribution was chosen for count data because it models overdispersion, which was found to characterize the data. In the models for duet rate, an offset for the duration of the trial was included, such that the estimated rate is in duets per minute rather than in duets per trial duration. We modelled linear mixed models with R package *lme4* (Bates et al. 2015) and negative binomial mixed models using R package *glmmADMB* (Skaug et al. 2013).

Results

Approach behaviour

Riverside wrens exhibited stronger approach responses during the stranger treatment than during both of the neighbour treatments (Fig. 3). Females' and males' closest distance to either of the two speakers and their mean distances to the

closest speaker were significantly smaller during the stranger playback than during the neighbours' playback (Supporting information). Canebrake wrens, on the other hand, showed more similar approach responses during the three types of simulated territorial intrusions (Fig. 3, Supporting information). Pairs approached quickly after the playback started and remained moving and flying very close to both speakers throughout the three treatments.

Vocal behaviour

Riverside wrens sang significantly more duets in response to the stranger treatment than in response to the two neighbour treatments (Fig. 4, Supporting information). Nevertheless, when comparing the length of the duets sung (number of FM cycles per duet), there was no significant difference across treatments (Fig. 4, Supporting information). Additionally, both sexes initiated more songs, either solos or duets, during the stranger treatment than during the neighbours' treatments (Fig. 5, Supporting information). However, although males tended to be more responsive than females during all trials, we found no difference in female or male responsiveness across treatments (Fig. 5, Supporting information).

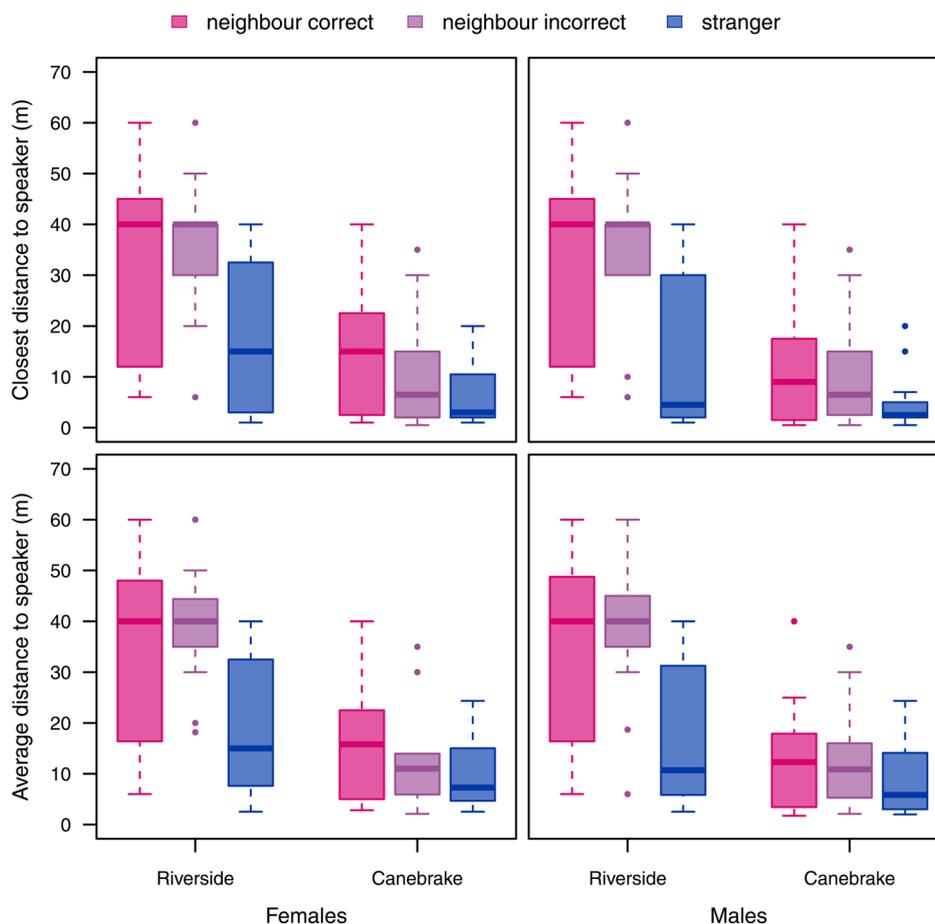


Figure 3. Closest distance to any speaker and mean distance to the closest speaker across treatments for female and male individuals in riverside and canebrake wrens. Median (horizontal line), quartiles (top and bottom of the box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (full circles) are shown for each boxplot.

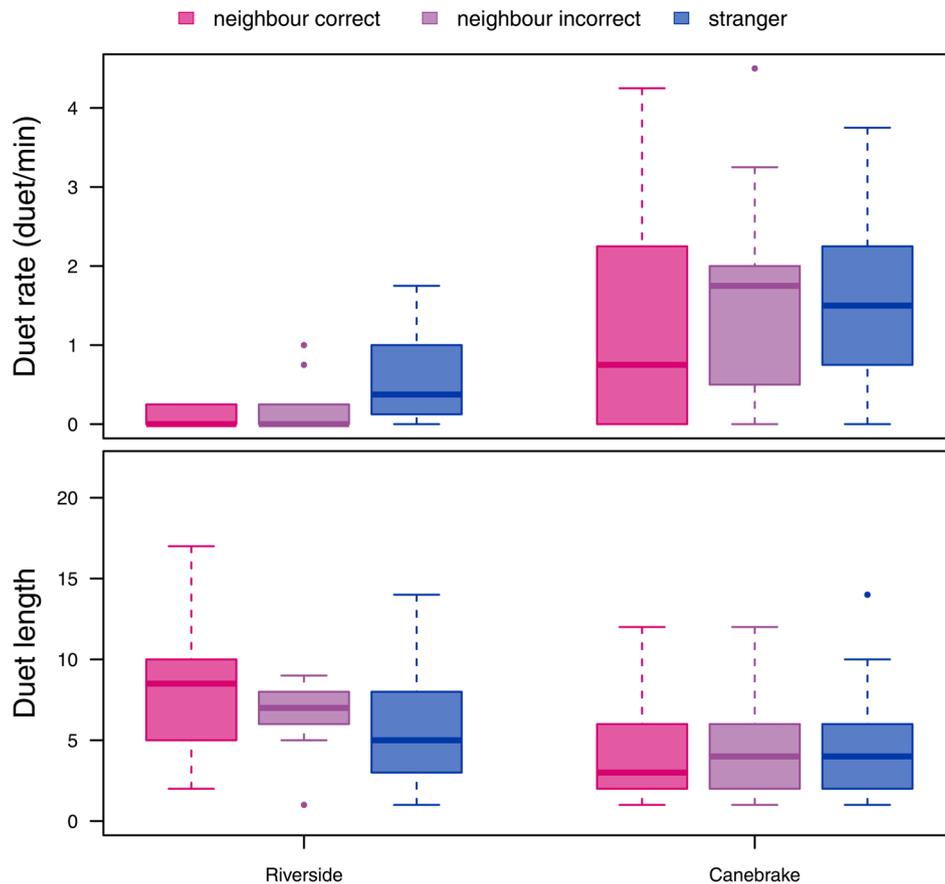


Figure 4. Duet rate (duets/min) and duet length (number of FM repetitions in each duet) across treatments in riverside and canebrake wrens.

Unlike riverside wrens, canebrake wrens exhibited high vocal activity in all of our playback simulations (Fig. 4). There was no significant difference in the duet rate or duet length sung across any of the treatments (Supporting information). Finally, there was also no significant difference in song initiation or responsiveness in neither of the sexes across the three trials (Fig. 5, Supporting information).

Discussion

We did not find support for the hypothesis that adherence to duet codes increases neighbour recognition in either species of neotropical wrens. Both riverside and canebrake wrens showed similar levels of aggression towards the two neighbour playbacks, regardless of whether the neighbours followed the appropriate duet code or not. This indicates that the function of duet codes in these species might not be related to facilitating pair identification. Additionally, we found that while riverside wrens present the dear enemy effect and show more aggressive responses towards strangers than towards neighbours, canebrake wrens did not respond significantly different to any of the three treatments. This indicates that, in contrast to our predictions, high levels of phrase type sharing and considerable levels of duet type sharing within the

population, does not prevent neighbour recognition in duetting species.

The apparent lack of attention to duet codes might arise from particular strategies during territorial defence in which individuals pay attention only to the phrases each neighbouring same-sex individual sings. Most animals focus on the same-sex individual because that is the one specifically endangering their territory ownership and mated status (Fedy and Stutchbury 2005, Logue 2005). Although this bias tends to be less pronounced in duetting species (Logue 2005), and it has been shown that in riverside wrens both pair members respond with equal levels of aggression to the female and male simulated intruders (Quirós-Guerrero et al. 2017), it is still possible that individuals focus primarily on the song phrases of their same-sex neighbours. In a study with canebrake wrens, Marshall-Ball and Slater (2004) showed that while females and males phrase type matched the playback, it was extremely rare for pairs to duet match it, even when there was a potential to do so, thus suggesting that matching the stimulus was mainly a same-sex defence strategy. Furthermore, the riverside wren population has a particularly high turnover rate unusual for tropical species (Stutchbury and Morton 2001), which results in neighbouring pairs frequently changing (more than 50% of adult birds are not seen again in the following season, Quirós-Guerrero et al. 2020a). Therefore,

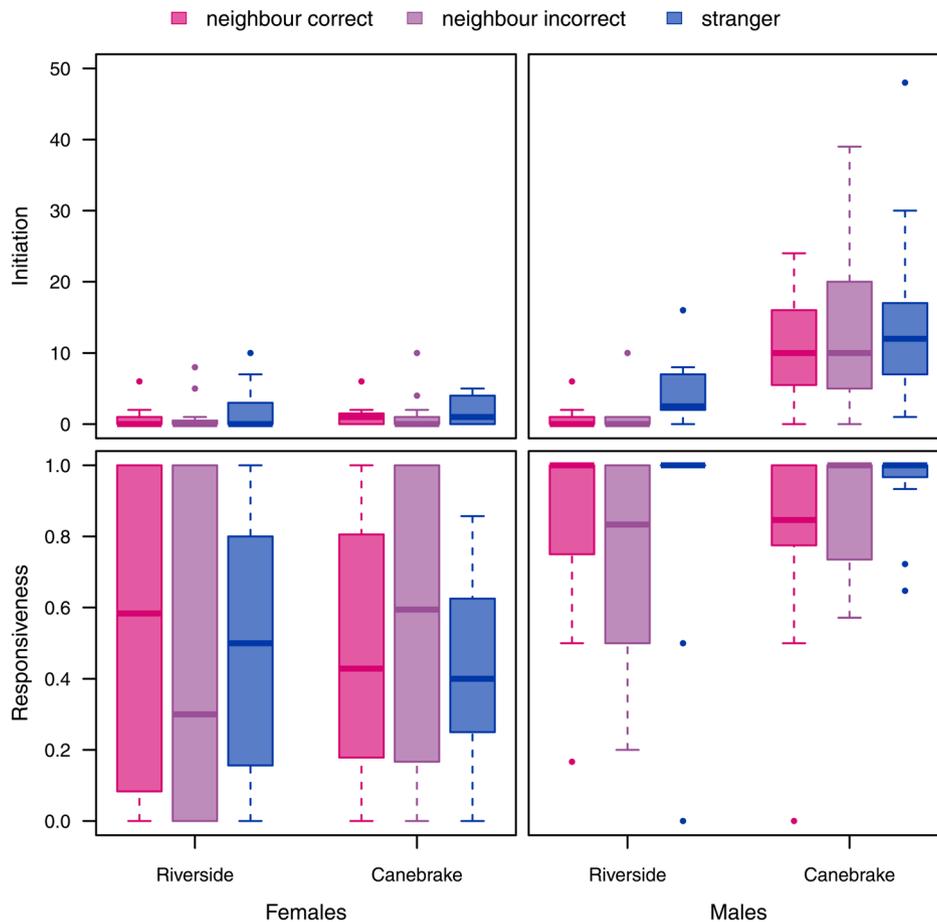


Figure 5. Initiation and responsiveness across treatments for female and male individuals in riverside and canebrake wrens.

perhaps for individual birds, learning the duet code of each new pair is a more difficult task than just learning the phrases of the same-sex neighbour, and it might then be possible that this behavior is not favoured given the high turnover rates.

An alternative explanation for why wrens responded similarly to the two neighbour playbacks, irrespective of whether the duet type followed the normal neighbours' duet code, is that repertoire composition is not the only cue used to discriminate between conspecifics. In some species, distinctive voice characteristics might also facilitate neighbour recognition (Weary and Krebs 1992, Blumenrath et al. 2007), as seen in stripe-backed wrens, *Campylorhynchus nuchalis* (Price 1999) and banded wrens, *Thryothorus pleurostictus* (Molles and Vehrencamp 2001). Voice characteristics are constant acoustic features (e.g. maximum frequency or frequency modulation) in an individual's songs that may result from individual morphological variation (i.e. body size or syrinx) (Weary and Krebs 1992, Gentner et al. 2000). Because territorial birds must recognize neighbouring vocalizations quickly in order to avoid escalated contests and unnecessary fights (Molles and Vehrencamp 2001), neighbour recognition based on voice quality might be valuable as individuals could then correctly identify opponents even if they are not familiar with the specific song that is being sung (Weary and

Krebs 1992). This would be advantageous, particularly in the riverside wren population in which immediate neighbouring same-sex birds have high levels of phrase type sharing (77%) and in which immediate neighbouring pairs have significant levels of duet type sharing (48%) (Quirós-Guerrero et al. 2020a).

Our riverside wren results suggest that individuals can discriminate between different intruders and selectively respond with increased levels of aggression towards strangers compared with neighbours. In the few studies that have investigated neighbour recognition on the basis of playback of duets, evidence of the dear enemy effect has been found in stripe-backed wrens (Wiley and Wiley 1977), magpie-larks, *Grallina cyanoleuca* (Hall 2000) and in tropical boubous, *Laniarius aethiopicus* (Grafe and Bitz 2004), but not in barred owls, *Strix varia* (Odom and Mennill 2010). During the stranger treatment, riverside wrens approached the speakers more closely, remained at a closer distance, initiated more songs, and had a higher duetting rate than during the neighbour treatments, in which pair mates rarely approached close to the speakers, sang very few duets, and if they approached, the majority of the times they quickly resumed foraging. In contrast, canebrake wrens did not show significant differences in the behaviours measured during any of the treatments and

displayed strong territorial responses to all simulated intruders. Individuals engaged with the three different treatments by approaching, singing and remaining close to the speakers, and the majority of pairs remained in the vicinity and continued duetting until the playbacks ended. This finding was contrary to our prediction in which the species with lower levels of song sharing would have more facility in discriminating between familiar and unfamiliar local intruders.

A potential explanation for why these two closely related species behaved differently in response to neighbours might be the differences in their habitat and territory features. First, while the riverside wren population is located in an area of continuous mature tropical forest where neighbours are contiguous, the canebrake population is located in an area of farm fields and cattle pastures, which in turn leads to territories being distributed in patches of vegetation. Since canebrake wren territories are often not contiguous, it is possible that a simulated intrusion right at the border might be perceived as highly aggressive. Additionally, species-level differences in territory sizes (1st and 2nd authors unpubl.) could also affect how threatening the same type of playback is perceived by each species. Because canebrake wrens have territories that are one third smaller than riverside wrens, even though the playback was being broadcast from the regular border in both cases, a ceiling effect could be occurring given that the signal is already too close to the territory centre. A ceiling effect can happen in studies measuring aggression when treatments' intensity goes beyond the point where every stimuli is perceived as maximum threat, leading to strong responses from subjects independently of the variable being tested (Templeton et al. 2012). Coupled together, these differences might explain why the simulated neighbours were not perceived as dear enemies in canebrake wrens. Finally, it is important to note that while we failed to detect a significant difference in the aggressive behaviours in canebrake wrens across treatments, our data indicates that both sexes approached and remained closer to the speakers during the stranger treatment. This suggests the potential that canebrake wrens might exhibit similar discrimination patterns to riverside wrens if they were tested using a lower-threat experimental design that was less likely to induce a ceiling effect (i.e. playback 2 m outside the border instead of at the boundary of the territory).

In conclusion, we have shown that in two neotropical duetting species, individuals do not seem to use the specific duet types sung by neighbouring pairs to facilitate neighbour recognition. In natural contexts, pairs of both species are often seen involved in duetting exchanges in which they are counter-matching each other (Marshall-Ball and Slater 2004, 1st author pers. obs.), which might suggest that duet types can be used to mediate territorial interactions even though they are not used for conspecific identification. Future experiments with riverside wrens will test if resident pairs duet-match during a simulated paired intrusion or if matching is an individual behaviour only, as previously shown in canebrake wrens (Marshall-Ball and Slater 2004). If riverside wrens, which have higher duet type sharing levels than canebrake

wrens, also fail to duet-match when there is a potential to do so and focus mainly on same-sex matching, the function of duet codes in neotropical wrens is likely to be related mostly to intra-pair communication, either by enhancing mate identification and mate location (Farabaugh 1982, Logue and Gammon 2004, Logue 2007) or by maintaining and improving duet coordination (Logue et al. 2008, Mann et al. 2009). Finally, while our results indicate that duetting species with large repertoires and significant levels of song sharing exhibit neighbour recognition, given that riverside wrens discriminated among conspecifics, this study has also shown that two closely related species behave very differently in response to neighbours. Canebrake wrens did not seem to exhibit the dear enemy effect, and this might have been due to ecological factors that influence the perceived level of threat of different intruders. To investigate the effect of ecological features in neighbour recognition, further work should increase the number of neotropical wrens sampled using a multi-species comparative approach.

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Conflicts of interest – We do not have any conflict of interest to declare.

Author contributions

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Data availability statement

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.5hqbzk4j>> (Quirós-Guerrero et al. 2020b).

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